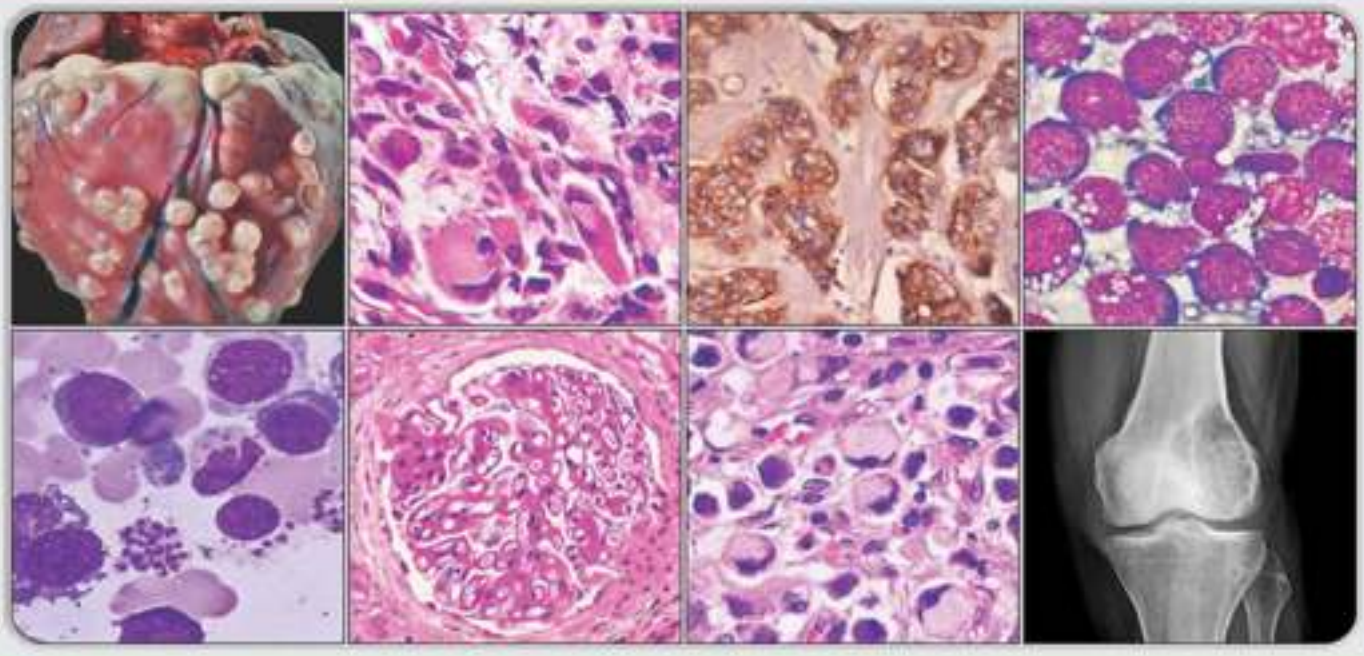
2nd Edition

Textbook of Pathology

Systemic Pathology & Molecular Diagnostics

Vinay Kamal

Volume II



Dedicated to Education
CBS Publishers & Distributors Pvt Ltd

Textbook of

Pathology

Second Edition

VOLUME II: Systemic Pathology and Molecular Diagnostics

Textbook of
Pathology

Second Edition

VOLUME II: Systemic Pathology and Molecular Diagnostics

Author

Vinay Kamal

Ex-Director Professor
Department of Pathology
Maulana Azad Medical College
New Delhi (India)

Co-Authors

Anubhav MD

Vigyat MD

Illustrations by

Ram Murti Senior Graphic Artist

Volume I: General Pathology and Hematology

Volume II: Systemic Pathology and Molecular Diagnostics

CBS Publishers and Distributors Pvt Ltd

New Delhi • Bengaluru • Chennai • Kochi • Kolkata • Lucknow • Mumbai
Hyderabad • Jharkhand • Nagpur • Patna • Pune • Uttarakhand

Disclaimer

Science and technology are constantly changing fields. New research and experience broaden the scope of information and knowledge. The authors have tried their best in giving information available to them while preparing the material for this book. Although, all efforts have been made to ensure optimum accuracy of the material, yet it is quite possible some errors might have been left uncorrected. The publisher, the printer and the authors will not be held responsible for any inadvertent errors, omissions or inaccuracies.

eISBN: **978-93-490-5709-8**

Copyright © Authors and Publisher

Second eBook Edition: 2025

All rights reserved. No part of this eBook may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system without permission, in writing, from the authors and the publisher.

Published by Satish Kumar Jain and produced by Varun Jain for
CBS Publishers & Distributors Pvt. Ltd.

Corporate O ice: 204 FIE, Industrial Area, Patparganj, New Delhi-110092
Ph: +91-11-49344934; Fax: +91-11-49344935; Website: www.cbspd.com; www.eduport-global.com;
E-mail: eresources@cbspd.com

Head O ice: CBS PLAZA, 4819/XI Prahlad Street, 24 Ansari Road, Daryaganj, New Delhi-110002, India.
Ph: +91-11-23289259, 23266861, 23266867; Fax: 011-23243014; Website: www.cbspd.com;
E-mail: publishing@cbspd.com; eduportglobal@gmail.com.

Branches

- **Bengaluru:** Seema House 2975, 17th Cross, K.R. Road, Banasankari 2nd Stage, Bengaluru - 560070, Karnataka Ph: +91-80-26771678/79; Fax: +91-80-26771680; E-mail: bangalore@cbspd.com
- **Chennai:** No.7, Subbaraya Street Shenoy Nagar Chennai - 600030, Tamil Nadu
  Ph: +91-44-26680620, 26681266; E-mail: chennai@cbspd.com
- **Kochi:** 36/14 Kalluvilakam, Lissie Hospital Road, Kochi - 682018, Kerala
  Ph: +91-484-4059061-65; Fax: +91-484-4059065; E-mail: kochi@cbspd.com
- **Mumbai:** 83-C, 1st floor, Dr. E. Moses Road, Worli, Mumbai - 400018, Maharashtra
  Ph: +91-22-24902340 - 41; Fax: +91-22-24902342; E-mail: mumbai@cbspd.com
- **Kolkata:** No. 6/B, Ground Floor, Rameswar Shaw Road, Kolkata - 700014
  Ph: +91-33-22891126 - 28; E-mail: kolkata@cbspd.com

Representatives

- **Hyderabad**
- **Pune**
- **Nagpur**
- **Manipal**
- **Vijayawada**
- **Patna**

Dedication

Dedication of this small work on Second Edition of Textbook of Pathology to the great scientists Juan Rosai (Rosai and Ackerman's Surgical Pathology), and Christopher DM Fletcher (Diagnostic Histopathology of Tumors), whose profound contribution to the field of Pathology has touched countless lives. Their legacy will continue to inspire and educate the generation of pathologists.

Dedication to our worthy teachers for teaching basics of pathology and excellence in medicine, Esteemed Faculty Members, and promising undergraduate and postgraduate Medical Students for enduring inspiration and encouragement, while working on Second Edition of Textbook of Pathology.

Dr Vinay Kamal
Dr Anubhav
Dr Vigyat

Dedication

Second Edition of Textbook of Pathology is dedicated to our family, who inspired, supported, encouraged, and patiently endured long hours of inattention and hearing from me during the creation of this final project to achieve an academic career. But for their understanding, it would be difficult to get away with the disproportionate amount of time that such projects consume.

Dr Mrs Manita Kamal

Siya Kamal

Dr Anubhav

Dr Vigyat

Dr Spriha Arun

and

Dr Mansi Siddharth Dhende

Contents

Preface to the Second Edition xiii
Preface to the First Edition xiv
Foreword by Dr Venkateswaran K Iyer xv
Foreword by Dr Nita Khurana xvi
Foreword by Dr Srivani N xvii
Foreword by Dr Hansa Goswami xviii
Foreword by Dr Ranjana Solanki xix
Foreword by Dr Reeni Malik xx
Foreword by Dr Roma Issacs xxi
Foreword by Dr Sunita Singh xxii
Foreword by Dr Syed Besina Yasin xxiii
Foreword by Dr Geeta Pachori xxiv
Foreword by Dr Rajeev Sen xxv
Foreword by Dr Kuldeep Kumar Kaul xxvi
Foreword by Dr Subhash Chander Bhardwaj xxvii
Foreword by Dr Soumitra Biswas xxviii
Foreword by Dr Pranita Medhi xxix
Foreword by Dr Naval Kishore Bajaj xxx

Section I: General Pathology

1. Cellular Pathology and Biology of Aging 3
Cell Unit of Life and Introduction to Pathology 4
Cellular Response to Stress and Harmful Stimuli 16
Cellular Adaptations 20
Cell Injury and Cell Death: Overview 34
Pathophysiology of Cell Injury, Morphologic Patterns and Outcome of Necrosis 45
Regulated Cell Death (RCD) 68
Apoptosis 80
Intracellular Accumulation of Substances 96
Extracellular Accumulation of Substances 109
Pathologic Calcification 113
Cellular Biology of Aging 116

2. Inflammation and Tissue Repair 127
Inflammation 128
Acute Inflammation 131
Chronic Inflammation 180
Tissue Repair 202

3. Hemodynamic Disorders, Thrombosis, Embolism and Shock 239
Edema and Effusions 239
Hyperemia and Congestion 247
Hemostasis 251
Hemorrhagic Disorders 268
Thrombosis 272
Embolism 289
Infarction 292
Shock 297

4. Immunopathology 305
Immune System 306
Innate Immune Response 315
Adaptive Immune Response 331
Vaccines and Immunization 376
Immunodeficiency Diseases 380
Hypersensitivity Reactions 404
Immunologic Tolerance 432
Autoimmune Diseases 440
Tissue/Organ Transplantation immunology 450
Amyloidosis 460

5. Genetic Disorders 471
Human Genome 472

Genetics and Molecular Biology 479
The Nucleus and DNA Replication 487
Gene Expression, Transcription, Translation and Protein Folding 492
DNA Damage and Repair Systems 503
Chromosomal Disorders 511
Human Gene Structure and Mutations 526
Mendelian (Monogenic) Disorders (Single-Gene Mutation) 536
Balanced Polymorphism 564
Polygenic and Multifactorial Disorders 567
Genetic Polymorphism 568
Disorders of Sexual Differentiation 570
Genome Imprinting Disorders 572
Diagnostic Approach of Genetic Disorders 573

6. Neoplasia 581

Neoplasia: General Considerations 582
Nomenclature and Classification of Tumors 602
Features used to Distinguish Benign Tumors from Malignant Tumors 651
Normal Processes that Regulate Cells and Inhibit Carcinogenesis 662
Epidemiology of Cancer 675
Preferential Routes of Metastasis of Tumors 698
Molecular Basis of Cancer 708
Carcinogenic Agents: Cellular Interaction and Carcinogenesis 779
Role of Oncogenes and Tumor Suppressor Genes in Carcinogenesis 809
Clinical Oncology 896
Molecular Diagnostics in Oncology 939

7. Nutritional and Infectious Diseases 963

Nutritional Disorders 964
Vitamins Deficiency 970
Bacterial Infections 984
Viral Infections 1014
Fungal Diseases 1034
Parasitic Infestations 1042

Section II: Hematology

8. Red Blood Cell Disorders 1055

Hematopoiesis 1056
Red Blood Cell, Hemoglobin, Anticoagulants and Anemias 1079
Iron Metabolism and Iron Deficiency Anemia 1090
Megaloblastic Anemia, Folic Acid and Vitamin B_{12} Deficiency 1097
Red Blood Cells Destruction and Classification of Hemolytic Anemia 1108
Hereditary Spherocytosis, Ovalocytosis and Stomatocytosis: RBC Membrane Defects 1115
Thalassemias 1120
Sickle Cell Disorders and Other Hemoglobinopathies 1129
Glucose-6-Phosphate Dehydrogenase Deficiency and Pyruvate Kinase Deficiency Disorders 1137
Immune-mediated Hemolytic Anemia 1140
Paroxysmal Nocturnal Hemoglobinuria 1146
Red Blood Cell Fragmentation Syndromes 1148
Diagnostic Approach of Hemolytic Anemia 1150
Bone Marrow Failure Syndromes 1154

9. White Blood Cell Disorders 1163

Leukopoiesis, Quantitative and Qualitative Disorders of Leukocytes 1164
Leukemias 1179
Acute Lymphoblastic Leukemia 1193
Acute Myelogenous Leukemia 1211
Myelodysplastic Syndromes 1238
Myelodysplastic/Myeloproliferative Neoplasms 1249
Chronic Myeloproliferative Neoplasms 1255
Chronic Lymphoproliferative Neoplasms 1300
Multiple Myeloma and Other Monoclonal Gammopathies 1313
Mononuclear Phagocytic System Disorders 1339
Hematopoietic Stem Cell Transplantation 1347

10. Platelet Disorders and Bleeding Diathesis 1355

Hemostasis 1355
Platelet Disorders 1366
Quantitative Platelet Disorders 1371
Qualitative Inherited Platelet Disorders 1375
Qualitative Acquired Platelet Disorders 1378
Diagnostic Approach and Treatment of Platelet Disorders 1380
Secondary Thrombocytosis and Essential Thrombocythemia 1381
Bleeding Associated with Vascular Disorders 1383

11. Coagulation Disorders and Diagnostic Approach of Bleeding Diathesis 1387

Blood Coagulation System 1387
Inherited Coagulation System Disorders 1392
Acquired Coagulation System Disorders 1403
Thrombophilia (Hypercoagulable State) 1407
Bleeding Diathesis: Diagnostic Approach 1410

12. Blood Banking and Transfusion Practices 1421
ABO and Hh Blood Group Systems 1422
Rh Blood Group System 1431
Bombay Phenotype and Para-Bombay Phenotype 1432
Other Blood Group Systems 1433
Blood Collection, Processing and Storage 1434
Blood Transfusion and Blood Component Therapy 1452
Adverse Blood Transfusion Reactions 1459
Blood Grouping 1466
Blood Cross-matching (Compatibility) Test 1470

13. Lymph Node, Spleen and Thymus Gland Disorders 1473
Disorders of Lymph Node 1474
Hodgkin's Disease 1488
Non-Hodgkin's Lymphomas 1500
Disorders of Spleen 1531
Disorders of Thymus Gland 1536

Index *i–xl*

Section III: Systemic Pathology and Molecular Diagnostics

14. Cellular–Molecular Diagnostic Techniques in Clinical Practice 1545
Frozen/Cryostat Microtomy Techniques 1545
Electron Microscopy and Polarizing Light Microscopy 1547
Tissue Fixation, Decalcification, Handling of Surgial Specimens and Histochemical Stains 1550
Immunohistochemistry Technique 1557
Immunofluorescence Microscopy 1589
Chemiluminescence Versus Chemifluorescence Techniques 1590
Molecular Diagnostics to Analyze Cell Proliferation 1592
Cytogenetic Analysis 1596
In situ Hybridization, Chromogenic *in situ* Hybridization and Fluorescence *in situ* Hybridization 1606
Enzyme-linked Immunosorbent Assay Technique 1612
DNA Sequencing Methods 1616
Tissue Microarray (TMA) Technique 1625
High-performance Liquid Chromatography 1627
Hemoglobin Electrophoresis and Gel Electrophoresis Techniques 1632

15. Blood Vessels 1637
Anatomy and Physiology of Blood Vessels 1637
Arteriosclerosis: Histologic Variants 1638
Atherosclerosis 1640
Aneurysms 1657
Vasculitis 1663
Venous System 1671
Lymphatic System 1673
Neoplastic Disorders of Blood Vessels 1674
Hypertension 1683

16. Heart 1695
The Normal Heart 1695
Ischemic Heart Disease 1696
Rheumatic Fever and Rheumatic Heart Disease 1715
Disorders of Myocardium 1724
Disorders of Endocardium 1731
Disorders of Cardiac Valves 1737
Disorders of Pericardium 1741
Cardiac Hypertrophy and Heart Failure 1747
Neoplastic Disorders of Heart 1751
Congenital Heart Diseases and Blood Vessel Malformations 1752

17. Nasal Cavity, Nasopharynx, Paranasal Sinuses, Ear, Larynx and Neck 1757
Disorders of Nasal Cavity, Nasopharynx and Paranasal Sinuses 1757
Disorders of Ear 1766
Disorders of Larynx 1769
Disorders of Neck 1772

18. Respiratory System 1777
Pneumonias and Lung Abscess 1782
Tuberculosis 1794
Chronic Obstructive Pulmonary Diseases (COPDs) 1803
Restrictive Pulmonary Diseases 1814
Pneumothorax and Atelectasis Lung 1828
Pulmonary Vascular Disorders 1831
Neoplastic Disorders of Lung 1837
Pleural Diseases 1872

19. Oral Cavity, Salivary Glands and Jaw 1879
Disorders of Oral Cavity 1879
Disorders of Salivary Glands 1886
Disorders of Jaw 1898

20. Gastrointestinal Tract and Peritoneum 1907
Disorders of Esophagus 1911
Disorders of Stomach 1922
Disorders of Small Intestine 1945
Disorders of Large Intestine 1963

Disorders of Appendix 1997
Disorders of Anus and Perianal Region 1999
Disorders of Peritoneum 2002
Gastrointestinal Tract Disorders: Diagnostic Approach 2004

21. Liver, Gallbladder and Exocrine Pancreas 2009
Disorders of Liver 2009
Jaundice and Cholestasis 2024
Hepatitis 2033
Bacterial Infections and Parasitic Infestations of Liver 2053
Liver Cirrhosis and Portal Hypertension 2059
Metabolic Liver Disorders 2076
Bile Duct Disorders 2092
Vascular Disorders of Liver 2095
Neoplastic Disorders of Liver 2098
Disorders of Gallbladder 2122
Disorders of Exocrine Pancreas 2137

22. Kidney and Ureter 2155
Renal Embryology, Anatomy and Physiology 2156
Glomerular Diseases of Kidney 2166
Tubular Diseases of Kidney 2239
Tubulointerstitial Diseases of Kidney 2244
Urolithiasis 2255
Vascular Diseases of Kidney 2262
Cystic Diseases of Kidney 2268
Congenital Anomalies of Kidney 2272
Neoplastic Disorders of Kidney 2274
Renal Function Tests 2297

23. Urinary Bladder and Male Reproductive System 2307
Disorders of Urinary Bladder 2308
Disorders of Testes 2322
Disorders of Prostate Gland 2351
Disorders of Penis 2363

24. Female Reproductive System 2371
Disorders of Vulva 2375
Disorders of Vagina 2381
Disorders of Uterine Cervix 2389
Disorders of Uterine Endometrium 2403
Disorders of Uterine Myometrium 2421
Disorders of Fallopian Tubes 2426
Disorders of Ovaries 2428
Neoplastic Disorders of Ovary 2431
Gestational Trophoblastic Neoplastic Disorders and Related Tumor-like Lesions 2468
Pregnancy Associated Non-Neoplastic Disorders 2475

25. Mammary Glands 2481
Female Breasts 2482
Inflammatory Disorders of Breast 2488
Fibrocystic Disease of Breast 2490
Revised 2019 WHO Classification of Breast Tumors 2494
Male Breasts 2547

26. Endocrine System: Pancreas, Thyroid, Parathyroid, Adrenal and Pituitary Glands 2549
Disorders of Endocrine Pancreas 2550
Disorders of Thyroid Gland 2586
Disorders of Parathyroid Glands 2625
Disorders of Adrenal Glands 2631
Disorders of Pituitary Gland 2641

27. Bones and Joints 2651
Disorders of Bones 2652
Osteomyelitis (Bone Infection) 2653
Metabolic Bone Diseases 2657
Bone Tumors 2661
Disorders of Joints 2723

28. Soft Tissue Tumors and Skeletal Muscle Non-Neoplastic Disorders 2731
Soft Tissue Tumors 2732
Non-Neoplastic Disorders of Skeletal Muscle 2796

29. Skin 2809
Skin Histology and Skin Disorders 2809
Infectious Dermatitis 2817
Inflammatory Dermatitis 2838
Non-Neoplastic Melanocytic Disorders and Melanocytic Neoplasms 2845
Nonmelanocytic Cutaneous Neoplasms 2853
Miscellaneous Skin Disorders 2877

30. Nervous System 2883
Central Nervous System 2883
Central Nervous System Infections 2887
Central Nervous System Tumors 2896
Hypoxia-induced Brain Injury 2933
Cerebrovascular Disorders 2934
Head Injury 2938
Demyelinating Disorders of Nervous System 2940
Congenital Disorders of Nervous System 2941

Index 2945

Preface to the Second Edition

Rudolph Virchow (1821–1902) is the father of 'Cellular Pathology' in terms of the dysfunction of cells. Many pathologic observations are being published in the Textbooks and Journals by great authors across world. Their outstanding contribution in the field of medical practice and education has been a great source of inspiration.

Second Edition of *Textbook of Pathology*, extensively revised, updated with recent developments as needed and amended as per the 'Competency-Based Medical Education Curriculum' is intended to provide undergraduate and postgraduate medical students with clear, and concise presentation of the pathologic basis of the human disorders.

The field of pathology emerged from the application of the scientific method to study of nature, etiology, molecular mechanism, and interrelated anatomical, functional, clinical manifestations and consequences of human diseases through examination of blood and tissue specimens.

The description of the human disorders evolved over time from gross morphology observation to the histologic examination of the diseased tissues based on the specific immunohistochemical stains, immunofluorescence microscopy, immunophenotyping, fluorescence *in situ* hybridization, molecular diagnostics and more recently to ultrastructural analysis of the disease with the advent of electron microscope. Thus, molecular pathology as a discipline represents the complementary intersection of medicine and basic science discoveries and the basis for the development of new strategies for disease prevention.

Textbook of Pathology has three sections: (i) General Pathology, (ii) Hematology including chapter on 'Blood Banking and Transfusion Practices', and (iii) Systemic Pathology and Molecular Diagnostics. This textbook contains approximately 1901 high-resolution colored figures and 1719 tables, which would complement quick revision of the chapters. It provides comprehensive coverage of etiology, molecular pathogenesis of human disorders, clinical manifestations, clinicopathological correlation and diagnostic approach.

Pathology is the fundamental bridging discipline linking the basic biomedical sciences to clinical medicine. Therefore, our basic aim is for our undergraduate as well as postgraduate medical students to use their knowledge of pathology to become scientifically grounded effective clinicians and healthcare professionals.

We hope that *Textbook of Pathology* will accomplish its purpose of providing medical students and researchers with in-depth coverage of molecular basis of a wide spectrum of human disorders in the context of understanding the molecular mechanisms of the disease and advancing the theory and practice of molecular medicine.

Author
Vinay Kamal
Email: vinaykamal@hotmail.com
Mobile: 09818001202

Co-Authors
Anubhav
Vigyat

Preface to the First Edition

The study of pathology is essential for safe clinical practice. In modern era, tremendous growth is in progress in diagnostic pathology with recent advances in molecular methods, cytogenetics and immunohistochemistry. Rapidly changing hematology needs integration of guidelines and diagnostic criteria of hematological disorders.

I joined India's premier institution in Department of Pathology, Maulana Azad Medical College, New Delhi. I puzzled over the fact that students admitted of very high caliber still had problems in grasping the teaching material. I interacted with students and eminent faculty members across country. I discussed problems faced by the students in reproducing text. I analyzed their inputs and then started working on Textbook of Pathology in 2007. The primary aim of this book is to discuss complex topics in a 'straightforward', clear and organized manner.

All the chapters have been shared with the eminent teachers and young budding students across country. Their valuable opinion on various aspects has been taken positively during drafting of the chapters to meet the need of the young generation of pathologists. Each chapter focuses on the essentials necessary to build a broad fundamentals backed up with numerous colored figures, gross morphology, and light microscopy photographs, tables and flow diagrams.

I trust, you will enjoy reading this book. I have a great deal of time and energy ensuring easy grasp and retaining the topics. I am inviting your comments, valuable suggestions and criticism.

Vinay Kamal
Email: vinaykamal@hotmail.com
Mobile: 09818001202

Foreword

It gives me great pleasure to write the Foreword for this wonderful Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor of Pathology, Maulana Azad Medical College, New Delhi. His vast teaching experience for undergraduate and postgraduate medical students is well-known in the corridors in the premier institution, where medical students have interacted to seek the advice.

I have gone through many chapters of this *Textbook of Pathology* and find his unique teaching experience that has been successfully transformed into wonderful method of writing and communication. Textbook abounds with revised WHO classification of neoplasms, approximately 1901 high-resolution colored figures and 1719 tables, and molecular diagnostics that explain everything in simple language—always the best way to teach.

I have no doubt that this *Textbook of Pathology* will help undergraduate and postgraduate medical students to achieve a better understanding and create strong foundation for medical education.

Dr Venkateswaran K Iyer
Senior Professor and Head
Department of Pathology
All India Institute of Medical Sciences
New Delhi

Foreword

It is with great enthusiasm, I look forward to Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor, Maulana Azad Medical College, New Delhi. The first edition of Textbook by him received a brilliant response from the undergraduate as well as postgraduate medical students throughout the country.

Bringing out the Second Edition of *Textbook of Pathology* within a short span of time, speaks volumes about the passion of the author to constantly update the information and share it in a presentable format to his medical students.

All the chapters of textbook have been updated with incorporation of revised WHO classification of neoplasms, new information on immunohistochemistry and molecular alterations. *Textbook of Pathology* contains around 1901 high-resolution colored figures and 1719 tables.

Enormous effort has been put in recreating the tables with additional content, making the flowcharts and illustrations as well as adding new and clear microphotographs. All these are the unique feature of this textbook to enable the medical students to learn pathology in context of etiology and pathogenesis making it a valuable source for gaining knowledge to build a solid foundation in pathology for the future doctors.

I hope the undergraduate and postgraduate medical students and teachers will benefit from the Second Edition of *Textbook of Pathology*.

Dr Nita Khurana
Director Professor and Head
Department of Pathology
Maulana Azad Medical College
New Delhi

Foreword

It is my privilege to write the Foreword to Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor of Pathology, Maulana Azad Medical College, New Delhi, releasing in 2025. The author of this *Textbook of Pathology* has more than three-decade long teaching experience at one of the country's finest and reputed medical institutes.

I had an opportunity to read the preprint of few topics, and I am immensely pleased to express that the latest Second Edition of *Textbook of Pathology* is in accordance with the 'Competency Based Medical Education Curriculum' recommended by the National Medical Commission.

Textbook of Pathology contains around 1901 high-resolution colored figures and 1719 tables, which makes the understanding of concepts very lucid for the undergraduate and postgraduate medical students.

Textbook of Pathology includes important topics like tissue fixatives, microscopy, frozen/cryostat section techniques, and histochemical stains which covers the much-needed practical aspects of the subject. Topics like recent updates, molecular pathogenesis, revised WHO classification of neoplasms, immunohistochemistry, hematology, flow cytometry, cytogenetic study, fluorescence *in situ* hybridization (FISH), enzyme-linked immunosorbent assay (ELISA), DNA sequencing, tissue microarray (TMA), high-performance liquid chromatography (HPLC), and electrophoresis have been presented in such a way that it would help the postgraduate medical students, teaching faculty and fellow practitioners also. Updated content on 'Blood Transfusion' being included, makes the *Textbook of Pathology* a very comprehensive read.

I wish Dr Vinay Kamal for all the success and hope that this latest Second Edition of *Textbook of Pathology* will receive wide acceptance by the undergraduate and postgraduate medical students and faculty members.

Dr Srivani N
Senior Professor and Head
Department of Pathology
Government Medical College
Nalgonda, Telangana

Foreword

I have known Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi for more than two decades, and have been interacting with him on various issues of pathology. The importance of pathology can be summarized in a quote by Great Sir Boyd "*As good is your pathology, so good will be your clinical practice*."

The undaunted association with teaching undergraduate and postgraduate medical students for more than three decades as faculty at Maulana Azad Medical College, New Delhi, distinguishes him from many other academicians by way of his integrated innovative approach to make the subject of pathology simple, and easy with clinico-pathological correlation of various diseases for medical students.

It is my proud privilege to write the Foreword to Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, which consists of the essence of his teaching experience in academics. This textbook contains revised WHO classification of neoplasms, molecular pathogenesis, and chapters on Blood Banking and Transfusion Practices and Cellular–Molecular Diagnostic Techniques in Clinical Practice. This textbook contains numerous high-resolution colored figures, tabulated data analysis, and methodology for diagnosis of various diseases accurately.

I am confident that this *Textbook of Pathology* by Dr Vinay Kamal will open a whole new avenue for all students in world of pathology, which will go long away in making undergraduate as well as postgraduate medical students to understand pathology and its relevance, and significance in medicine. I wish great success of new release Second Edition of *Textbook of Pathology*.

Dr Hansa Goswami
Senior Professor and Head
Department of Pathology
BJ Medical College
Ahmedabad, Gujarat

Foreword

I joined MAMC as a postgraduate student. He connects with the students and takes keen interest in their learning and tries to help them out in all their personal and academic problems. A similar helpful attitude towards staff with all help extended to them has been his nature at all times.

It is my distinct pleasure and honour to endorse the Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi, whom I have known for some years and interacted on various issues of pathology.

Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal covers various aspects of the subject in a systematic manner. The content in this textbook is in simple language, which makes it reader-friendly and is well illustrated by numerous gross and microscopic pictures.

The beauty of the *Textbook of Pathology* is that it highlights the clinicopathological correlation, diagnostic approach and includes revised WHO classification of neoplasms. Various illustrations in the form of figures, tables, flow diagrams and incorporation of basics of immunohistochemistry and molecular biology in this textbook provide a comprehensive understanding to medical students in pathology. The key highlights in every section of textbook also helps in quick revision of the topics.

I congratulate Dr Vinay Kamal for his dedication and immense passion of teaching medical students, who will enjoy reading this textbook and understand the basic concepts along with updated knowledge in the subject.

Dr Ranjana Solanki
Senior Professor
Department of Pathology
SMS Medical College
Jaipur, Rajasthan

Foreword

A seasonal writer—is the most appropriate word for Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi, which has more than three decades of teaching experience in the premier institution.

It gives me an immense pleasure to write the Foreword to Second Edition of *Textbook of Pathology* with recent updates. I have gone through many chapters penned down by him in this textbook. Nothing can be more informative and more simplified for undergraduate as well as postgraduate medical students than this version.

Beautifully integrating numerous high-resolution colored illustrations, images, flowcharts, tables and providing relevant knowledge in a well-structured and designed text is an art and his textbook is a perfect example of these qualities. The contents not only include the knowledge of basic *Textbook of Pathology* for undergraduate students but also integrates trivial topics of recent diagnostic techniques and advancements, and molecular and genetic pathology for postgraduate students as well.

I congratulate Dr Vinay Kamal for having the insight to produce a novel *Textbook of Pathology* that allows the undergraduate as well as postgraduate medical students of all levels to understand pathology with clinicopathological correlation and relish it while reading it cover to cover.

I wish and hope that this splendid contribution of Dr Vinay Kamal gets the well-deserved acclamation and applause.

Dr Reeni Malik
Senior Professor and Head
Department of Pathology
Gandhi Medical College
Bhopal, Madhya Pradesh

Foreword

I have this proud privilege to write the Foreword to Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi. He is a renowned Pathologist, who enjoys teaching undergraduate and postgraduate medical students and has put in dedicated efforts to facilitate teaching–learning methods.

Textbook of Pathology is in two volumes. The first volume deals with General Pathology and Hematology including chapter on Blood Banking and Transfusion Practices. The second volume deals with Systemic Pathology including a chapter on Cellular–Molecular Diagnostic Techniques in Clinical Practice.

This Second Edition of *Textbook of Pathology* is an integration of molecular pathology and routine histopathology and thorough in all aspects. The upcoming scientific discipline of molecular basis of disease development has been described in a simplified yet extensively detailed manner in this textbook. Numerous high-resolution colored illustrations are eye-catching and self-explanatory. All topics are covered comprehensively making it extremely student friendly for undergraduate and postgraduate medical students.

I congratulate Dr Vinay Kamal for his dedication and immense passion of teaching undergraduate and postgraduate medical students, I am confident that this splendid contribution of the author gets the well-deserved acclamation and applause.

Dr Roma Issacs
Senior Professor
Department of Pathology
Christian Medical College and Hospital
Ludhiana, Punjab

Foreword

It gives me an immense pleasure to write the Foreword to Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi, who brings out new concepts defining various learning domains for the undergraduate as well as postgraduate medical students of pathology.

As all of us know that pathology is a very vast subject but it forms the basis for understanding the disease process. This textbook covers all the aspects of pathology in a systemic manner. Every effort has been made to make the subject interesting and easy to understand by using numerous well illustrated diagrams, flowcharts, tables as well as key points placed separately in the boxes for quick revision.

An attempt has also been made to include revised WHO classification of neoplasms, recent advances in pathology, molecular and immunohistochemical correlations in various aspects of tumor diagnosis, which are aimed at individualized treatment.

I congratulate Dr Vinay Kamal for this new venture and wish him all the success in his future endeavors.

Dr Sunita Singh
Senior Professor and Head
Department of Pathology
Pandit Bhagwat Dayal Sharma Post Graduate
Institute of Medical Sciences
Rohtak, Haryana

Foreword

The first edition of *Textbook of Pathology* authored by Dr Vinay Kamal released in the year 2017, was well appreciated by undergraduate as well as postgraduate medical scholars, and eminent faculty all over India. Textbook of Pathology was the result of years of hard work and commitment by a professional teacher and experienced pathologist.

I congratulate Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi for bringing out the Second Edition of *Textbook of Pathology*. Keeping in line with the recent advances in the field of pathology, the focus in this new venture is on the molecular pathogenesis of various disorders and clinicopathological correlation. The revised WHO classification of neoplasms has been incorporated in the discussions on various entities. Updated information on immunohistochemical markers has also been provided.

I am sure that Second Edition of *Textbook of Pathology* will be of immense help to the budding undergraduate as well as postgraduate medical students, and pathologists in reproducing their knowledge.

I wish great success to the latest endeavor by Dr Vinay Kamal.

Dr Syed Besina Yasin
Senior Professor and Head
Department of Pathology
Sher-I-Kashmir Institute of Medical Sciences
Srinagar, Jammu and Kashmir

Foreword

Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi, is well known name in the field of pathology, who needs no introduction. He has more than three decades of teaching experience in nation's most prestigious institution at Maulana Azad Medical College, New Delhi. I am delighted to know about the new release of his Second Edition of *Textbook of Pathology* with recent updates.

This is a very well written textbook with numerous high-resolution figures and tables which make it a handy tool to understand the fine nuances of the subject. This textbook would make the subject interesting for both the undergraduate as well as postgraduate medical students. Seldom we come across a thorough professional who is also a person with a kind heart, always keen to go extra mile to help others.

I congratulate Dr Vinay Kamal all the best for new release of *Textbook of Pathology*. I am sure this textbook is going to be a milestone for all the medical fraternity in the times to come.

Dr Geeta Pachori
Senior Professor and Head
Department of Pathology
Jawahar Lal Nehru Medical College
Ajmer, Rajasthan

Foreword

Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi, is in your hands.

Dr Vinay Kamal has worked very hard and has rewritten all the chapters of textbook incorporating most of the contemporary knowledge and developments in the field as relevant within the scope of the book.

Many new tables, flow diagrams and illustrations have been added for easy remembrance. He has been constantly updating the content and has made tremendous efforts to present the content in a comprehensive yet concise style.

Revised WHO classification of neoplasms, concepts in the molecular pathogenesis, current Cellular–Molecular Diagnostic Techniques in Clinicale Practice of pathology and diagnostic approach have been incorporated.

I am sure the textbook is going to be useful not only to undergraduate but also to postgraduate medical students, and teachers of pathology.

Dr Rajeev Sen
Senior Professor and Head
Department of Pathology
Faculty of Medicine and Health Sciences
SGT University
Gurugram, Haryana

Foreword

Ever since the first edition of *Textbook of Pathology* by Dr Vinay Kamal was released in the year 2017, the author started working on its new edition as the book became so popular in just a couple of months that most of the undergraduate and postgraduate medical students, as well as faculty in pathology across the country procured this book for personal use and for the library of their institutions. This was due to numerous qualities of the textbook such as unique presentation, topics discussed in a simple, clear, and well-organized manner backed up with numerous colored figures, photographs of gross and microscopic lesions, tables, and diagrams along with the recent updates.

Second Edition of *Textbook of Pathology* by Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi, is releasing in 2025. In this textbook, all the chapters have been updated with incorporation of revised WHO classification of neoplasms, etiopathogenesis of the disease, clinical manifestations, clinicopathological correlation, and molecular diagnostics.

Textbook of Pathology contains around 1901 high-resolution colored figures, 1719 tables, chapters on Blood Banking and Transfusion Practices and Cellular–Molecular Diagnostic Techniques in Clinical Practice. All the chapters have been shared with eminent teachers and young budding residents across the country and their opinions have been given due weightage during drafting of the chapters.

While going through the chapters, the Author's honest, hard, and intelligent work is clearly felt by the reader. The book is designed to provide accurate, authoritative, and most current information and thus meets the need of the young generation of pathologists.

Dr Vinay Kamal's heart always beats for every medical student. I congratulate Dr Vinay Kamal on the Second Edition of *Textbook of Pathology* and wish good luck in his future endeavors.

Dr Kuldeep Kumar Kaul
Medical Advisor
J and K Thalassaemia Welfare Society
Former Professor and Head
Department of Pathology
Government Medical College
Jammu, Jammu and Kashmir

Foreword

It is indeed a pleasure and honour to write the Foreword to Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi. The book is an excellent compilation of knowledge of pathology in a very organized manner.

I have been associated with Dr Vinay Kamal for the last fifteen years and had the opportunity to review many of the chapters in this textbook. All the chapters in this new release *Textbook of Pathology*, have been updated with recent advances with strong emphasis on molecular pathogenesis, immunohistochemistry, flow cytometry and other molecular techniques. The book is easy to read and comprehend and shall prove student-friendly for undergraduate as well as postgraduate medical students.

I wish all the best to Dr Vinay Kamal for success of Second Edition of *Textbook of Pathology* and sincerely hope that he will keep on contributing more to the knowledge of medical students.

Dr Subhash Chander Bhardwaj
Senior Professor and Head
Department of Pathology
Government Medical College
Jammu, Jammu and Kashmir

Foreword

Books are the best to windows of the world as they are best source to gain knowledge and discipline. Pathology is the scientific study of the diseases. I have known Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi for many years by reading an excellent *Textbook of Pathology* authored by him in the year 2017.

It is my pleasure, and an honour to write the Foreword for the Second Edition of *Textbook of Pathology* by Dr Vinay Kamal releasing in 2025. The only thing that surpasses his knowledge is his humility about capabilities.

Textbook of Pathology contains around 1901 high-resolution colored figures and 1719 tables with recent updates including revised WHO classifications of neoplasms, molecular pathogenesis, immunohistochemistry, flow cytometry and molecular diagnostic techniques. This textbook contains two chapters on Cellular–Molecular Diagnostic Techniques in Clinical Practice and Blood Banking and Transfusion Practices.

Undergraduate and postgraduate medical students seeking a resource should consider Second Edition of *Textbook of Pathology* written by Dr Vinay Kamal. I congratulate Dr Vinay Kamal for grand success of the textbook releasing in 2025.

Dr Soumitra Biswas
Senior Professor and Head
Department of Pathology
Calcutta National Medical College
Kolkata, West Bengal

Foreword

The influence of a good teacher can never be erased. Dr Vinay Kamal, Ex-Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi, besides being a dedicated teacher and pathologist par excellence, is an awakener.

It is my proud privilege and I feel honored to be given an opportunity to write the Foreword to Second Edition of *Textbook of Pathology*. All the chapters of the book have recent updates including molecular pathogenesis. Many features such as revised WHO classification of neoplasms, immunohistochemistry, microscopy, histochemical stains, flow cytometric analysis, cytogenetic study, fluorescence *in situ* hybridization, enzyme-linked immunosorbent assay, DNA sequencing and tissue microarray.

This textbook contains around 1901 high-resolution colored figures and 1719 tables that will help undergraduate and postgraduate medical students to understand pathology of diseases and their clinicopathological correlation.

I convey my heartfelt appreciation for the untiring efforts and the hard work done by Dr Vinay Kamal for conceptualizing and executing his work. This new release Textbook of Pathology is a great boon to the undergraduate and postgraduate medical students, and will enjoy reading this book.

I wish Dr Vinay Kamal a grand success in his endeavor and happy to release this Second Edition of *Textbook of Pathology*.

Dr Pranita Medhi
Senior Professor and Head
Department of Pathology
Assam Medical College and Hospital
Dibrugarh, Assam

Foreword

I am privileged to write the Foreword to Second Edition of *Textbook of Pathology* written by a well experienced and excellent talented teacher Dr Vinay Kamal, Ex-Director Professor, Maulana Azad Medical College, New Delhi.

Textbook of Pathology contains 1901 high-resolution colored images, 1719 tables, chapters on 'Cellular–Molecular Diagnostic Techniques in Clinical Practice' which makes the understanding of concepts very lucid for the undergraduate and postgraduate medical students, which make the book unique.

The author has meticulously considered valuable suggestions and criticism after thorough discussions with many subject experts in upgradation of this Second Edition of *Textbook of Pathology*. Inclusion of recent advances in diagnostic pathology, cytogenetics, molecular pathology, and immunohistochemistry are the added advantages of the textbook. Recently revised WHO classification of neoplasms has also been included in the book. All the chapters of textbook are written, and edited with recent updates.

I am sure this Textbook of Pathology will prove to be an asset for undergraduate and postgraduate medical students in view of the 'Competency Based Medical Education' implemented by National Medical Commission. Salient features given in the boxes will help the medical students in quick revision of the course prior to their formative and summative assessments.

I wish Dr Vinay Kamal all the best in his endeavor.

Dr Naval Kishore Bajaj
Senior Professor and Head
Department of Pathology
Osmania Medical College
Hyderabad, Telangana

Acknowledgments

Our motivation in writing Second Edition of *Textbook of Pathology* continues to share recent scientific concepts, clinical applications, and molecular diagnostics with colleagues and undergraduate and postgraduate medical students. We have strived to provide explanatory text, 1901 photographs and 1719 tables to enhance pictorial insights into human diseases.

We acknowledge the support and encouragement of colleagues and undergraduate and postgraduate medical students, who have critically revied the draft chapters and provided photographs related to surgical pathology and hematology section. Their constructive invaluable suggestions have served to significantly improve the work.

We gratefully recognize the continual invaluable support of Dr Naval Kishore Bajaj (Senior Professor and Head, Department of Pathology, Osmania Medical College Hyderabad, Telangana), Dr Nita Khurana (Director Professor and Head, Department of Pathology, Maulana Azad Medical College, New Delhi), Dr Jyoti Priyadarshini (Professor, Department of Pathology, GR Medical College Gwalior, Madhya Pradesh), Dr Tushar Kalonia (Assistant Professor, Department of Pathology), Dr Neha Kumari (Assistant Professor, Department of Pathology), Rajiv Aggarwal, Sh PL Jayant, Smt Shanti Devi, Dr Satya Pal Jayant, Dr Vijaya Jayant, Mr Arjun Jayant, Dr Rohan Jayant, Dr Vimal Singh, Dinesh Kumar, and Dr Divyark Singh.

Words are less to express our gratitude to eminent faculty colleagues for showering their blessings by endorsing Second Edition of *Textbook of Pathology*. Comments from eminent faculty and undergraduate and postgraduate medical students are always welcome.

CBS PUBLISHERS AND DISTRIBUTORS PRIVATE LIMITED, NEW DELHI

We express our gratitude to Mr Satish Kumar Jain CHAIRMAN AND MANAGING DIRECTOR, Mr Varun Jain MANAGING DIRECTOR and Mr Sunil Dutt MANAGER PROMOTION AND DEVELOPMENT of CBS Publishers and Distributors Private Limited for extending their whole hearted support at every step in completion of this book.

A special thanks to Ram Murti SENIOR GRAPHIC ARTIST, Ananda Mohanty SENIOR PROOF READER and Mrs Sunita Rautela SENIOR DTP OPERATOR for their invaluable support in the completion of the book.

VISIONARIES AND FOUNDERS OF EDUCATIONAL INSTITUTIONS ACROSS NATION

Hon'ble Padmashree Dr DY Patil
Former Governor of Bihar, Tripura and West Bengal
Visionary and Founder of Educational Institutions
Navi Mumbai, Maharashtra

Hon'ble Dr PD Patil
Chancellor
Dr DY Patil Vidyapeeth
Pune, Maharashtra

Hon'ble Smt Bhagyshree Patil
Pro Chancellor
Dr DY Patil Vidyapeeth
Pune, Maharashtra

Hon'ble Dr SN Kadam MBBS, FRCS, MRCP (Edinburg)
Medical Director
Mahatma Gandhi Institute of Health Sciences
Navi Mumbai, Maharashtra

Hon'ble Dr Nitin Kadam MBBS, DCH, MD (Pediatrics Medicine)
Pro Vice Chancellor
Mahatma Gandhi Institute of Health Sciences
Navi Mumbai, Maharashtra

Hon'ble Dr HS Gill
Chancellor and Chairman
Adesh University and Adesh Group of Institutions
Bhatinda, Punjab

Hon'ble Shri PK Gupta
Chancellor and Chairman
Sharda University and Sharda Group of Institutions
Noida, Uttar Pradesh

Hon'ble Shri Suresh Kumar Jain
Chancellor
Teerthankar Mahavir University
Moradabad, Uttar Pradesh

Hon'ble Shri Tarsem Garg
Chancellor
Maharishi Markandeshwar University
Mullana Ambala, Haryana

Hon'ble G Dayanand
Chairman
Sapthagiri Institute of Medical Sciences and Research Center
Bengaluru, Karnataka

Hon'ble GD Manoj
Executive Director
Sapthagiri Institute of Medical Sciences and Research Center
Bengaluru, Karnataka

Hon'ble Suresh Singh Bhadoria
Chairman
Index Group of Institutions and Mayank Welfare Society
Indore, Madhya Pradesh

Hon'ble Mayankraj Singh Bhadoria
Chairman
Amaltas University
Indore, Madhya Pradesh

Hon'ble Mrs Aruna Bhadoria
Chancellor
Amaltas University
Indore, Madhya Pradesh

Hon'ble Mrs Gauri Singh
Chancellor
Malwanchal University
Indore, Madhya Pradesh

Hon'ble Dr Atul Krishna
Chairman
Subharti University
Merrut, Uttar Pradesh

Hon'ble Dr KM Navas
Chairman
KMCT Group of Institutions
Kozhikode, Kerala

Hon'ble Shri Man Mohan Chawla
Managing Trustee
Shree Guru Gobind Singh Tricentenary University
(SGT University)
Gurugram, Haryana

Hon'ble Dr Mansukh Shah
Managing Trustee
Suman Deep Vidyapeeth
Waghodia Vadodara, Gujarat

Hon'ble Dr Dixit Shah
Managing Trustee
Suman Deep Vidyapeeth
Waghodia Vadodara, Gujarat

Hon'ble JP Agarwal
Chairman
Geetanjali Medical College and Hospital
Udaipur, Rajasthan

Dr SS Lohchab
Professor of Cardiothoracic Surgery and Director
Pandit BD Sharma Post Graduate institute of
Medical Sciences
Rohtak, Haryana

Dr GS Narshetty
Professor of General Surgery and Dean
Mahatma Gandhi Institute of Health Sciences
Navi Mumbai, Maharashtra

Dr Jitendra Bhawalkar
Dean
Padmashree Dr DY Patil Medical College
Pune, Maharashtra

Dr Ashok Kshirsagar
Professor of General Surgery and Medical Director
Krisha Vishwa Vidyapeeth
Krishna Institute of Medical Sciences
Karad, Maharashtra

MEDICAL COLLEGES ACROSS NATION

ANDHRA PRADESH

Guntur Medical College Guntur, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr C Aparna

Professors of Pathology
• Dr M Rajani • Dr Prasad Uma • Dr B Sreedhar • Dr M Padma
• Dr G Neelima • Dr V Varuna Kumari

Rangaraya Medical College Kakinada, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr R Rajyalakhshmi

Associate Professors of Pathology
• Dr R Rajeshwari • Dr P Sridevi

Andhra Medical College Vishakhapatnam, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr CV Lakshmi

Government Medical College Eluru, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr P Satyanarayana Rao

Kurnool Medical College Kurnool, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr PV Ramanna Babu

Associate Professor of Pathology
• Dr G Revathi

Government Medical College Nandyal, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr G Baleswari

Professors of Pathology
• Dr V Jyothi • Dr D Anitha

Associate Professor of Pathology
• Dr T Ramakoteshwari

Assistant Professors of Pathology
• Dr Varalakshmi • Dr Lingeshwar • Dr Divya

Sidhartha Medical College Vijayawada, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr M Vijayshree

Government Medical College Machilipatnam, Andhra Pradesh
Associate Professor of Pathology
• Dr K Madhavi

All India Institute of Medical Sciences Manglagiri Vijayawada, Andhra Pradesh
Additional Professors of Pathology
• Dr Jyotsna Naresh Bharti Nigam • Dr Arunadhati Shashikala

Assistant Professors of Pathology
• Dr R Poongodi • Dr Jyoti Verma • Dr Prashant Pranesh Joshi

Government Medical College Ongole, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr K Murthi Devi

ACSR Medical College Nellore, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr BV Sai Prasad

Associate Professors of Pathology
• Dr K Srinivasulu • Dr Sushma K Chandulee

Government Medical College Madanapalle, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr M Koteshwari

Associate Professors of Pathology
• Dr B Spandana • Dr D Nagender Prasad • Dr K Suneetha

Assistant Professors of Pathology
• Dr D Bhargav • Dr B Maleswari • Dr D Amaravathi

Government Dharmapuri Medical College and Hospital Dharmapuri, Andhra Pradesh
Assistant Professor of Pathology
• Dr Saravana Kumar

Katuri Medical College and Hospital Guntur, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr Satya Narayana V

Professor of Pathology
• Dr Krushna Bharathi

Associate Professor of Pathology
• Dr Himbindu

PES Institute of Medical Sciences and Research (PESIMSR) Kuppum, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr Ramaswamy AS

Dr Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation Chinoutpalli, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr D Naveen Chandra Rao

Professors of Pathology
• Dr V Kalyan Chakraborty • Dr V Kalyan

GSL Medical College Rajahmundry, Andhra Pradesh
Senior Professor and Head of Pathology
• Dr Manisha Mahapatra

Alluri Sitaramaraju Academy of Medical Sciences (ASRAM) Visakhapatnam, Andhra Pradesh
Professor of Pathology
• Dr Sudheer

Gayatri Medical College Visakhapatnam, Andhra Pradesh
Associate Professor of Pathology
• Dr Aswani

Maharaja Institute of Medical Sciences (MIMS) Vizianagaram, Andhra Pradesh
Senior Tutor of Pathology
• Dr A Vijay Kumar

ARUNACHAL PRADESH

Tomo Riba Institute of Health and Medical Sciences (TRIHMS) Nahalagun, Arunachal Pradesh
Associate Professor and Head of Pathology
• Dr Hage Nobin

Assistant Professors of Pathology
• Dr Rubu Anu • Dr HIbu Yami

Senior Resident of Pathology
• Dr Padi Yassung

Tutor of Pathology
• Dr Tumli Lollen

ASSAM

Guwahati Medical College and Hospital Guwahati, Assam
Senior Professor and Head of Pathology
• Dr Manjula Choudhary

Professors of Pathology
• Dr Ena Dowerah • Dr Usha Sharma • Dr Dipankar Baruah

Associate Professors of Pathology
• Dr Junu Devi • Dr Leena Talukdar • Dr Naima Aziz
• Dr Bidyut Dutta • Dr Jabin Musfique

Assistant Professors of Pathology
• Dr Valina Brahma • Dr Rooprekha Das • Dr Rwishumwi Daimari
• Dr Surajit Kumar Roy • Dr Manoj Barma • Dr Daljeet Kaur
• Dr Jahnabi Sahara • Dr Debasmita Konwar • Dr Meghna Borgogoi

Demonstrators of Pathology
• Dr Wazhia Ahmed • Dr Hiramoni Haloi
• Dr Shailender Kumar Thakuria • Dr Parismrita Bora
• Dr Shima Shrestha • Dr Jayanta Kumar Das • Dr Jonali Das
• Dr Nanda Kanta Mahanta • Dr Rajib Das • Dr Krishma Bhuyan
• Dr Indusmita Borah • Dr Antriksha Sharma • Dr Nalay Talukdar

Assam Medical College and Hospital (AMCH) Dibrugarh, Assam
Senior Professor and Head of Pathology
• Dr Pranita Medhi

Professors of Pathology
• Dr Mondita Borgohain • Dr Adity Sarma

Associate Professors of Pathology
• Dr (Mrs) Rashmi Deori • Dr Asha Borah • Dr Aparna Dutta
• Dr Gayatri Gogoi • Dr (Mrs) Zarika Ahmed

Assistant Professors of Pathology
• Dr Sandeep Khakhlari • Dr Sima Sonowal • Dr Upam Kumar Sharma
• Dr Utpal Dutta • Dr Nilotpal Konwar • Dr (Mrs) Mousumi Baruah

Demonstrators of Pathology
• Dr Kaveri Neog • Dr Pranjal Kumar Gogoi • Dr Sudeshna Dutta

Post Graduate Residents of Pathology
• Dr Nandini Lahkar • Dr Bhanu Devi • Dr Nizam Priyakalita
• Dr Mridusmita Mech • Dr Namarta P Changmai • Dr Sanghamitra
• Dr Seminanasreen Ahmed

Fakhruddin Ali Ahmed Medical College and Hospital Barpeta, Assam
Senior Professor and Head of Pathology
• Dr Balmiki Datta

Professor of Pathology
• Dr Bobby Duarah

Associate Professors of Pathology
• Dr Rashmi Rekha Goswami • Dr Navanita Das • Dr Bibhuti Bhuyan

Assistant Professors of Pathology
• Dr Chandan Jyoti Saikia • Dr Michi Damary • Dr Susmita Sarma
• Dr Yogesh Kakati • Dr Apurba Baharali

Jorhat Medical College and Hospital Jorhat, Assam

Senior Professor and Head of Pathology
• Dr Projnan Saikia

Professor of Pathology
• Dr Aseema Das

Associate Professors of Pathology
• Dr Sarat Das • Dr Kusum Borsaikia • Dr Luckymoni Duara
• Dr Bidyut Kumar Gogai

Assistant Professors of Pathology
• Dr Anupam Gogai • Dr Amilee Gogoi • Dr Sangita Deka
• Dr Meher Abjun Mustakim

Silchar Medical College and Hospital Silchar, Assam

Senior Professor and Head of Pathology
• Dr Momota Naiding

Professors of Pathology
• Dr RN Choubey • Dr Shah Alam Sheikh

Associate Professors of Pathology
• Dr Manoj Kumar Deka • Dr Rajib Biswas

Assistant Professors of Pathology
• Dr Arindam Das • Dr Sumita Dutta Gupta • Dr Jyoti Choubey
• Dr Anirudha Debnath

Tezpur Medical College and Hospital Tezpur, Assam

Senior Professor and Head of Pathology
• Dr Geetanjali Gogoi

Professor of Pathology
• Dr Barnali Das

Associate Professors of Pathology
• Dr Pabir Hazarika • Dr Ranjana Deka • Dr Basanta Sonowal
• Dr Akash Bhuyan

Assistant Professors of Pathology
• Dr Bipil Kakti • Dr Priyanka Jain • Dr Abhinanda Barua
• Dr Kamal Kishore Chelleng • Dr Sampriti Barua • Dr Dimpee Lahkar
• Dr Anirban Goswami

All India Institute of Medical Sciences Guwahati, Assam

Additional Professor of Pathology
• Dr Prasad Sudhir Dange

Nagaon Medical College Nagaon Assam

Assistant Professor of Pathology
• Dr Pallabi Devi

BIHAR

All India Institute of Medical Sciences Patna, Bihar

Senior Professor and Head of Pathology
• Dr Poonam Prasad Bhadani

Additional Professors of Pathology
• Dr Ajeet Kumar • Dr Ruchi Sinha • Dr Shreekant Bharti

Associate Professor of Pathology
• Dr Surabhi

Indira Gandhi institute of Medical Sciences Patna, Bihar

Senior Professor and Head of Pathology
• Dr Bipin Kumar

Professors of Pathology
• Dr Vijayanand Chaudhary • Dr Mrs Reecha Singh • Dr Anju Singh

Additional Professor of Pathology
• Dr Kalpana Chandra

Associate Professors of Pathology
• Dr Zeenat M Imam • Dr Rashmi Rani Bharti • Dr Shuchismita
• Dr Satish Kumar • Dr Iffat Jamal • Dr Kaushal Kumar
• Dr Ravi Bhushan Raman • Dr Riwi Agrawal

Assistant Professors of Pathology
• Dr Richa Sharma • Dr Mamta Kumari • Dr Bharti Kumari
• Dr Guddi Rani • Dr Anushweta

Patna Medical College Patna, Bihar

Senior Professor and Head of Pathology
• Dr Naveen Kumar Bariar

Associate Professors of Pathology
• Dr Dilip Kumar • Dr Prakash Chander Jha • Dr Manish Kumar
• Dr Krishna Murari Prasad • Dr Vijay Kumar Gupta

Assistant Professors of Pathology
• Dr Ratnesh Kumar • Dr Pratibha Srivastava • Dr Pallavi Aggarwal
• Dr Rashmi Singh • Dr Mritunjay Kumar • Dr Rakesh Kumar Nirala
• Dr Arunadhati

Nalanda Medical College and Hospital Patna, Bihar

Senior Professor and Head of Pathology
• Dr CP Jaiswal

Associate Professors of Pathology
• Dr Sunil Kumar • Dr Ashish Kumar Gupta • Dr Kiran Kumari
• Dr Pawan Kumar Sah • Dr Sonal Verma • Dr Reena Sinha
• Dr Aamod Kumar

Assistant Professor of Pathology
• Dr Manohar Lal

Mata Gujri Medical College Kishanganj, Bihar

Senior Professor and Head of Pathology
• Dr Astitva Deb Roy

Professors of Pathology
• Dr Barnik Choudhary • Dr Sanjay Kumar • Dr Babai Halder
• Dr Nirvana Rasaily Halder • Dr Mala

Assistant Professors of Pathology
• Dr Anjani Kaushal • Dr Avinash Priyadarshi

CHANDIGARH

Government Medical College and Hospital, Chandigarh

Senior Professor and Head of Pathology
• Dr Uma Handa

Professors of Pathology
• Dr RPS Punia • Dr Anita Tahlan • Dr Anshu Palta
• Dr Ranjeev Bhagat

Associate Professor of Pathology
• Dr Phiza Aggarwal

Post Graduate Institute of Medical Education and Research, Chandigarh

Professor of Hematology
• Dr Prashant Sharma

CHHATTISGARH

Pandit Jawahar Lal Nehru Memorial Medical College Raipur, Chhattisgarh

Senior Professor and Head of Pathology
• Dr Arvind Neralwar

Professor of Pathology
• Dr Rabia Parveen Siddique

Associate Professors of Pathology
• Dr Chandrakala Joshi • Dr Amit Kumar Bhardwaj
• Dr Varsh Pandey

Assistant Professor of Pathology
• Dr Vanita Bhaskar

Government Medical College Raigarh, Chhattisgarh
Senior Professor and Head of Pathology
• Dr Manoj Kumar Minj

All India Institute of Medical Sciences Raipur, Chhattisgarh
Associate Professor of Pathology
• Dr Rakesh Gupta

Chhattisgarh Institute of Medical Sciences Bilaspur, Chhattisgarh
Associate Professor of Pathology
• Dr Jayanti Chandrakar

Atal Bihari Vajpayee Government Medical College Rajnandgaon, Chhattisgarh
Senior Professor and Head of Pathology
• Dr Reena Naik

Professor of Pathology
• Dr Renuka Gahine

DELHI

All India Institute of Medical Sciences, New Delhi
Senior Professor and Head of Pathology
• Dr Venkateswaran K Iyer

Professors of Pathology
• Dr Sandeep R Mathur • Dr Prasenjit Das

Additional Professors of Pathology
• Dr Shipra Agrawal • Dr Seema Kaushal • Dr Rajni Yadav

Associate Professor of Pathology
• Dr Ruchi Rathore

Additional Professor of Medicine
• Dr Arvind Kumar

Maulana Azad Medical College, New Delhi
Director Professor and Head of Pathology
• Dr Nita Khurana

Professors of Pathology
• Dr Sarika Singh • Dr Shramana Mandal • Dr Prerna Arora
• Dr Reena Tomar • Dr Nidhi Verma • Dr Meeta Singh
• Dr Varuna Ashwin Mallya

Assistant Professor of Pathology
• Dr Neelam Sahani

Lady Hardinge Medical College, New Delhi
Director Professor and Head of Pathology
• Dr Kiran Agarwal

Director Professors of Pathology
• Dr Sunita Sharma • Dr Shilpi Aggarwal • Dr Shailja Shukla
• Dr Anita Nangia

Professors of Pathology
• Dr Smita Singh • Dr Vandana Puri • Dr P Lalita Jyotsna • Dr Preeti

Assistant Professors of Pathology
• Dr Jenna Bhattacharya • Dr Shivali Sehgal • Dr Kavita Gaur
• Dr Deeksha • Dr Geetika

University College of Medical Sciences, New Delhi
Director Professor of Pathology
• Dr Richa Gupta

Assistant Professor of Pathology
• Dr Poonam Rani

Vardhaman Mahavir Medical College, New Delhi
Director Professor and Head of Pathology
• Dr Sunil Ranga

Professors of Pathology
• Dr Sufian Zaheer • Dr Sheetal Arora

PGIMER and RML Hospital, New Delhi
Professor and Head of Pathology
• Dr Arvind Ahuja

Associate Professor of Pathology
• Dr Khuraijam Bembem

Assistant Professor of Pathology
• Dr Garima Rakheja

Army College of Medical Sciences, New Delhi
Senior Professor and Head of Pathology
• Dr Sharmila Dudani

Associate Professor of Pathology
• Dr Archana Rautela

Assistant Professors of Pathology
• Dr Rachna Gulati • Dr Aditi Rawat

Hamdard Institute of Medical Sciences, New Delhi
Senior Professor and Head of Pathology
• Dr Sujata Jetley

Dr Baba Saheb Ambedkar Medical College and Hospital, New Delhi
Assistant Professors of Pathology
• Dr Nidhi Mahajan • Dr Khushboo Dewan

ESIC Hospital Basaidarapur, New Delhi
Senior Professor and Head of Pathology
• Dr Sujata Raychaudhuri

Janakpuri Super Specialty Hospital, New Delhi
Associate Professor of Pathology
• Dr Abhijit Das

GOA

Goa Medical College and Hospital Panaji, Goa
Senior Professor and Head of Pathology
• Dr RGW Pinto Professor of Pathology
• Dr Premila Rocha

GUJARAT

BJ Medical College Ahmedabad, Gujarat
Senior Professors of Pathology
• Dr Hansa Goswami

Professors of Pathology
• Dr Smita Shah • Dr Mahesh Patel • Dr Ina Shah

Associate Professors of Pathology
• Dr Sanjay Dhotre • Dr Hemina Desai • Dr Ami Shah
• Dr Monika Shah • Dr Meena Patel • Dr Purvi Patel
• Dr Hetal Jani

Assistant Professors of Pathology
• Dr Sakera Baji • Dr Urvi Parikh • Dr Viral Bhanvadiya
• Dr Vaishali Anand • Dr Hitendra P Barot

Smt NHL Municipal Medical College and SV Hospital Ahmedabad, Gujarat

Senior Professor and Head of Pathology
• Dr Cherry Shah

Professors of Pathology
• Dr Nanda Jagrit Shah • Dr Anjali Goyal

Associate Professors of Pathology
• Dr Minesh Gandhi • Dr Hinal Gajjar • Dr Preeti Jhaveri
• Dr Bijali Chakrabarti • Dr Gayatri Bamaniya

Assistant Professors of Pathology
• Dr Dipti Panat • Dr Krupali Patel • Dr Imran Shaikh
• Dr Suchita Mishra • Dr Saloni Sharma

NM Medical College Ahmedabad, Gujarat

Associate Professor of Pathology
• Dr Rajul Shah

Assistant Professor of Pathology
• Dr Jyoti Gadhvee

Pandit Deen Dayal Upadhyay (PDU) Medical College Rajkot, Gujarat

Senior Professor and Head of Pathology
• Dr Gaurvi Dhruva

Professors of Pathology
• Dr SR Talwelkar • Dr Shilpa H Gandhi • Dr Amit H Agrawat

Associate Professors of Pathology
• Dr Jagdish D Dalsania • Dr Deepa P Jethwani • Dr Rohit V Balhara
• Dr Milan B Purohit • Dr Payal M Shah

Assistant Professors of Pathology
• Dr PN Vekaria • Dr TM Purohit • Dr KR Bhojani • Dr DP Dhadania
• Dr Krupal M Pujara • Dr Khushboo Tilwa • Dr Shruti H Korvadia
• Dr Hiral Chauhan • Dr Mansi Mehta

Government Medical College Bhavnagar, Gujarat

Senior Professor and Head of Pathology
• Dr Pragnesh H Shah

Senior Professor of Pathology
• Dr Seema Baxi

Associate Professors of Pathology
• Dr Jitendra Chavda • Dr Alpeshpuri Goswami • Dr Mayuri Thaker

Assistant Professors of Pathology
• Dr Zalak Dholakiya • Dr Bharti Dholakiya • Dr Mayuri Gohil
• Dr Arohi P Parikh • Dr Anamika Navadiya • Dr Sejal R Kakadiya
• Dr Kavita R Valiya • Dr Khyati K Patel

Medical College Baroda Vadodara, Gujarat

Senior Professor and Head of Pathology
• Dr Manisha Singh

Professor of Pathology
• Dr Kirit Jadav

Associate Professors of Pathology
• Dr Meena Daveshkar • Dr Gena Ramchandani • Dr Heena Parikh
• Dr Manisha Shah • Dr Roopam Gidwani

Assistant Professors of Pathology
• Dr Jitendra Nasit • Dr Viren Vaghsiya • Dr Neema Rana
• Dr Shobhana Prajapati • Dr Yogita Kareliya • Dr Falguni Goswami

Surat Municipal Institute of Medical Education and Research Surat, Gujarat

Senior Professor and Head of Pathology
• Dr Arunima Banerjee

Professors of Pathology
• Dr Sudha Jain • Dr Ashwani Shukla • Dr Smita Alok Jhaveri

Associate Professors of Pathology
• Dr Ami Jagirdar • Dr Nikita P Roy • Dr Paras G Patel
• Dr Toral B Jiwani

Assistant Professors of Pathology
• Dr Suchi Niravkumar Godhani

Shri MP Shah Government Medical College and Research Centre Jamnagar, Gujarat

Senior Professor and Head of Pathology
• Dr Vijay C Popat

Professor of Pathology
• Dr Nandini J Desai

Associate Professor of Pathology
• Dr Dhara P Trivedi • Dr Neeru D Dave

All India Institute of Medical Sciences Rajkot, Gujarat

Associate Professor of Pathology
• Dr Tarang Patel

Assistant Professors of Pathology
• Dr Gyanendra Singh • Dr Parth Goswami • Dr Garima Anandani

GMERS Medical College Gandhinagar, Gujarat

Senior Professor and Head of Pathology
• Dr Meeta J Parikh

Associate Professors of Pathology
• Dr Nikunj U Suthar • Dr Bhaskar B Thakkar • Dr Gautam K Chauhan
• Dr Atul Srivastav

Assistant Professors of Pathology
• Dr Paragnesh J Patel • Dr Urvashi Mangar • Dr Vaidehi Patel

GMERS Medical College Gotri Vadodara, Gujarat

Senior Professor and Head of Pathology
• Dr Neena Doshi

Associate Professors of Pathology
• Dr Savitri Chauhan • Dr Dimple Darad • Dr Alaukika Rathwa
• Dr Jignasha Patel

Assistant Professors of Pathology
• Dr Aditi Dholakia • Dr Sheetal kher • Dr Vaibhavi Sharma
• Dr Mayuree Modi • Dr Piyush Vaghela

GMERS Medical College Vadnagar, Gujarat

Senior Professor and Head of Pathology
• Dr Harshid Patel

Associate Professors of Pathology
• Dr Pratik Desai • Dr Bipin Chawda

Assistant Professors of Pathology
• Dr Nirav Panchal • Dr Gamit Mittal • Dr Dimpal • Dr Kana Patel

Gujarat Cancer Society (GCS) Medical College and Hospital Ahmedabad, Gujarat

Senior Professor and Head of Pathology
• Dr Asha Purohit

Professors of Pathology
• Dr Anupama Dayal • Dr Rupal Shah

Associate Professors of Pathology
• Dr Puja Jarwani • Dr Madhur Modi

Assistant Professors of Pathology
• Dr Aishani Tiwari • Dr Jaya Patel • Dr Keval Patel • Dr Suhani Agrawal

Smt BK Shah Medical Institute and Research Center Vadodara, Gujarat

Senior Professor and Head of Pathology
• Dr Rakesh Tandon

Professors of Pathology
• Dr Swapan Goswami • Dr Jasmin Jasani • Dr Shashikant Mavadiya
• Dr Kuntal Patel

Associate Professors of Pathology
• Dr Jigna Patel • Dr Devanshi Gosai • Dr Trupti Jansari

Assistant Professors of Pathology
• Dr Mihir Bhalodia • Dr Khusbu Chhaya • Dr Dipali Shukla
• Dr Hemangini Parmar • Dr Shreem Vatsaraj • Dr Manan Bhesadadia

Gujarat Adani Institute of Medical Sciences Bhuj, Gujarat
Senior Professor and Head of Pathology
• Dr Bhushan M Waspe

Professor of Pathology
• Dr Shweta S Joshi

CU Shah Medical College and Hospital Surendranagar, Gujarat
Senior Professor and Head of Pathology
• Dr Nisha G Raval

Associate Professors of Pathology
• Dr Neelaba K Mori • Dr Siddhartha D Ghelani

Assistant Professors of Pathology
• Dr Bhavdish C Pandya • Dr Priyanka A Prasad
• Dr Kirtibahen V Jetapriya • Dr Poojaben M Vasoya
• Dr Anandkumar A Chauhan

Pramukhswami Medical College Karamsad, Gujarat
Senior Professor and Head of Pathology
• Dr Monica Gupta

HARYANA

Pandit Bhagwat Dayal Post Graduate Institute of Medical Sciences Rohtak, Haryana
Senior Professor and Head of Pathology
• Dr Sunita Singh

Senior Professor of Pathology
• Dr Nisha Marwah

Professors of Pathology
• Dr Rajneesh Kalra • Dr Sanjay Kumar • Dr Veena Gupta • Dr Meenu Gill
• Dr Sant Prakash Kataria • Dr Sumiti Gupta • Dr Sonia Chhabra
• Dr Monika Gupta • Dr Promil Jain

Associate Professors of Pathology
• Dr Renuka Verma • Dr Richa Pawar

Assistant Professor of Pathology
• Dr Usha Rani Narwal

MM Institute of Medical Sciences and Research Mullana Ambala, Haryana
Senior Professor and Head of Pathology
• Dr Vijay Shrawan Nijhawan

Professors of Pathology
• Dr Rupinder Kaur • Dr Prem Singh • Dr SP Khanna • Dr Charu Batra Atreja

Associate Professors of Pathology
• Dr Irbinder Kaur Bali • Dr Maitrayee Roy • Dr Jaskirat Singh
• Dr Neha Singh

Adesh Medical College and Hospital Mohri Kurukshetra, Haryana
Senior Professor and Head of Pathology
• Dr Ramesh Kumar Kundal

Professor of Pathology
• Dr Nasib Chand

Associate Professor of Pathology
• Dr Preeti Joseph

Assistant Professors of Pathology
• Dr Veerpal Kaur • Dr Akshita Rattan • Dr Ankit Mittal
• Dr Pushpinder Kaur

Kalpana Chawla Govt Medical College Karnal, Haryana
Professor and Head of Pathology
• Dr Hem Lata

Associate Professor of Pathology
• Dr Ajit Singh

Assistant Professor of Pathology
• Dr Atul Beniwal

BPS Government Medical College Khanpur, Haryana
Professor and Head of Pathology
• Dr Swaran Kaur Saluja

Professors of Pathology
• Dr Kulwant Singh • Dr Parveen Rana • Dr Ruchi Aggarwal

Associate Professor of Pathology
• Dr Monika Gathwal

Atal Bihari Vajpayee Government Medical College Faridabad, Haryana
Professor and Head of Pathology
• Dr Preeti Patni

Associate Professor of Pathology
• Dr Sweta Sinha

Assistant Professor of Pathology
• Dr Shweta Rana

SHKM Government Medical College Nalhar Mewat, Haryana
Professor and Head of Pathology
• Dr Puja

Professors of Pathology
• Dr Sheetal Baxi Gole • Dr Bhawna • Dr Shilpa Garg • Dr Sonia Hasija

Associate Professors of Pathology
• Dr Ashok Sangwaiya • Dr Shilpa Bairwa • Dr Shailesh Kumar Mishra

Assistant Professor of Pathology
• Dr Sapna Singla

Maharaja Agrasen Medical College Agroha, Haryana
Professor and Head of Pathology
• Dr Karandeep Singh

Professors of Pathology
• Dr Aditi Baghia • Dr Ajay Kumar Kochar

Associate Professors of Pathology
• Dr Monika • Dr Pravin Pawne

Assistant Professors of Pathology
• Dr Neha Garg • Dr Chetan • Dr Sarvek Bajaj • Dr Prachi Arun
• Dr Anjum Bhukal

ESIC Medical College Faridabad, Haryana
Professor of Pathology
• Dr Mukta Pujani

Faculty of Medicine and Health Sciences SGT University Gurugram, Haryana
Senior Professor and Head of Pathology
• Dr Rajeev Sen

Professor of Pathology
• Dr Pawan Singh

Assistant Professors of Pathology
• Dr Saloni Mahajan • Dr Komal Yadav • Dr Pooja Khanna
• Dr Snehil Aggarwal • Dr Roopali Sehrawat • Dr Anam Singh

Al-Falah School of Medical Sciences and Research Center Faridabad, Haryana

Senior Professor and Head of Pathology
• Dr Vinod Raghav

Professors of Pathology
• Dr Sunil Arora • Dr Naveen Chawla • Dr Wajid Ali Shah

Assistant Professors of Pathology
• Dr Mohd Talha • Dr Malika Mehta • Dr Samreen Naqsh
• Dr Shahnaz Parveen • Dr Anishika Srivastva • Dr Shafaque Zabin

Senior Residents of Pathology
• Dr Zohra Siddiqui • Dr Shujaat Khan • Dr Garima • Dr Meelad
• Dr Asbah Shams • Dr Neelaksh Kaul

Post Graduate Residents of Pathology
• Dr Priyanka • Dr Parveen Choudhary • Dr Nitesh Kumari Yadav
• Dr Ankita Saini • Dr Lalit Gupta • Dr Aliya Rahman
• Dr Diksha Sharma • Dr Chetna

NC Medical College and Hospital Israna Panipat, Haryana

Professor and Head of Pathology
• Dr Isha Gupta

World Medical College and Hospital Jhajjar, Haryana

Professor of Pathology
• Dr Jasneet Kaur Sandhu

HIMACHAL PRADESH

Indira Gandhi Medical College Shimla, Himachal Pradesh

Senior Professor and Head of Pathology
• Dr Sudarshan Kumar Sharma

Professors of Pathology
• Dr Neelam Sharma • Dr Kavita Mardi

Associate Professors of Pathology
• Dr Archana Gulati • Dr Lalita Negi

Dr Rajendra Prasad Government Medical College Tanda Kangra, Himachal Pradesh

Senior Professor and Head of Pathology
• Dr Rashmi Kaul

Associate Professors of Pathology
• Dr Bal Chander • Dr Suman Singh

Assistant Professors of Pathology
• Dr Aruna • Dr Saurabh Sharma • Dr Swati Agrawal
• Dr Monish Tomar

All India Institute of Medical Sciences Bilaspur, Himachal Pradesh

Associate Professor of Pathology
• Dr Manupriya Sharma

Maharishi Markandeshwar Medical College and Hospital Solan Himachal Pradesh

Senior Professor and Head of Pathology
• Dr Naveen Kakkar

Professors of Pathology
• Dr Rajni Kaushik • Dr Anuj Sharma

Associate Professor of Pathology
• Dr Nazma Khan

Assistant Professors of Pathology
• Dr Krishma Sarin • Dr Sarah Amestina • Dr Sourabh Kumar

JAMMU and KASHMIR

Sher-I-Kashmir Institute of Medical Sciences (SKIMS) Soura Srinagar, Jammu and Kashmir

Senior Professor and Head of Pathology
• Dr Syed Besina Yasin

Professors of Pathology
• Dr Rumana Makhdoomi • Dr Mohd Iqbal • Dr Zubaida Rasool

Associate Professors of Pathology
• Dr Aiffa Ayman • Dr Rukhsana Riyaz • Dr Sumyra Qadri

Assistant Professors of Pathology
• Dr Tazeen Jeelani • Dr Zafirah Zahir

SKIMS Medical College Srinagar, Jammu and Kashmir

Senior Professor and Head of Pathology
• Dr Aafiya Shafi

Associate Professors of Pathology
• Dr JB Singh • Dr Bushra Rashid

Government Medical College Srinagar, Jammu and Kashmir

Senior Professor and Head of Pathology
• Dr Bilal Ahmad Sheikh

Professors of Pathology
• Dr Lateef Ahmed Wani • Dr Mehnaaz Sultan Khuroo • Dr Salma Bhat
• Dr Sheema Shekh

Associate Professors of Pathology
• Dr Roohi Wani • Dr Adil Hassan Siddique • Dr Nusrat Bashir

Assistant Professors of Pathology
• Dr Faheem Manzoor • Dr Summiya Farooq • Dr Gousia Rahim

Lecturers of Pathology
• Dr Sharifa Akhter • Dr Nuzhat Samon • Dr Ambreen Beigh • Dr Zafirah
• Dr Mir Wajahat

Government Medical College Jammu, Jammu and Kashmir

Senior Professor and Head of Pathology
• Dr Subhash Bhardwaj

Professors of Pathology
• Dr Jyotsna Suri • Dr Ruchi Khajuria • Dr Deepti Mahajan
• Dr Sindhu Sharma • Dr Rupali Bargotra

Associate Professor of Pathology
• Dr Deepa Hans

Assistant Professors of Pathology
• Dr Ameet Kaur • Dr Deepti Gupta • Dr Rajat Gupta • Dr Bharti Thaker

Lecturers of Pathology
• Dr Anu Gupta • Dr Roopali Jandial • Dr Suby Singh
• Dr Abhimanyu Sharma

Acharya Shri Chander College of Medical Sciences Sidhra Jammu, Jammu and Kashmir

Senior Professor and Head of Pathology
• Dr Arvind Khajuria

Professors of Pathology
• Dr Aneeta Singh • Dr KC Goswami • Dr Bhawana Raina
• Dr Anuja Sharma • Dr Archana Gupta

Associate Professors of Pathology
• Dr Nitin Gupta • Dr Swati Gupta

Assistant Professors of Pathology
• Dr Shailja Kotwal • Dr Prableen Kour

Post Graduate Residents of Pathology
• Dr Madiha Mir • Dr Maureen • Dr Sheeba Parvez • Dr Shivani
• Dr Tufail Ahmed Khan • Dr Atisha • Dr Avantika Kailu • Dr Chakshu
• Dr Farishta • Dr Gazala Shamim • Dr Huraira

JHARKHAND

All India Institute of Medical Sciences Deogarh, Jharkhand

Senior Professor and Head of Pathology
- Dr Sanjeet Kumar Singh

Associate Professor of Pathology
- Dr Harish Kumar Bohra

Assistant Professors of Pathology
- Dr Lisa Mona • Dr Nishi • Dr Ranwir Sinha • Dr Nikhil Kumar
- Dr Nidhi Priya Allie Barla • Dr Prima Suchita Lakra

Rajendra Institute of Medical Sciences Ranchi, Jharkhand

Associate Professor and Head of Pathology
- Dr Sunil Kumar Mahto

Associate Professors of Pathology
- Dr Manoj Kumar Paswan • Dr Anshu Jaimyar

Assistant Professor of Pathology
- Dr Deepali Tirkey

MGM Medical College and Research Institute Jamshedpur, Jharkhand

Professor and Head of Pathology
- Dr RK Mandhan

Associate Professors of Pathology
- Dr Umashankar Singh • Dr SN Paul • Dr Arun Kumar Jha

KARNATAKA

Bangalore Medical College and Research Institute Bengaluru, Karnataka

Senior Professor and Head of Pathology
- Dr Siddique Mukami Ahmed

Senior Professors of Pathology
- Dr Natrajan M • Dr Sreelakshmi K • Dr Ashalatha N • Dr Nirmala C
- Dr Latha B

Associate Professors of Pathology
- Dr Lakshmi Bai Mallapa • Dr Shulbha V Sejekan

Assistant Professors of Pathology
- Dr Ramya BS • Dr Ashwini BR • Dr Sujata Metan • Dr Divya Rani MN

St John's Medical College Bengaluru, Karnataka

Senior Professor and Head of Pathology
- Dr Julian Alphonse Crasto

Sapthagiri Institute of Medical Sciences and Research Centre (SIMSRC) Bengaluru, Karnataka

Senior Professor and Head of Pathology
- Dr Vijaya C

Professors of Pathology
- Dr Suguna BV • Dr Vamshidhar • Dr Shridhara

Associate Professor of Pathology
- Dr Suraj Jain

Assistant Professors of Pathology
- Dr Shubha HV • Dr Pushpa HR • Dr Ganraj Bhat S • Dr Varsha S
- Dr Komala HM • Dr Chandana N • Dr Spoorthy M

Senior Residents of Pathology
- Dr Umme Romaan • Dr Meghana • Dr Greeshma

Adichunchanagiri Institute of Medical Sciences Bellur Mandya, Karnataka

Senior Professor and Head of Pathology
- Dr Abhishek MG

Professors of Pathology
- Dr S Vijay Shankar • Dr Amita K Yagish • Dr Sanjay M

Associate Professors of Pathology
- Dr Nanjundaswamy D • Dr Prakash VB • Dr Rakshitha HB
- Dr Kusuma KN • Dr Rajini T

Assistant Professors of Pathology
- Dr Prajwala Nagaraju • Dr Sumaya • Dr Apoorva Pandit
- Dr Jayshree HK • Dr Indira CS

Kasturba Medical College Manipal, Karnataka

Senior Professor and Head of Pathology
- Dr Kanthilatha Pai

Professors of Pathology
- Dr Ranjini Kudwa • Dr Mary Mathew

Additional Professors of Pathology
- Dr Sushma Belurkar • Dr Geetha V

Associate Professors of Pathology
- Dr Vidya Monappa • Dr Deepa HC • Dr Ruchee Khanna
- Dr Sindhura Lakshmi KL • Dr Deepak M Nayak • Dr Bhawna Nayal
- Dr Srilatha PS • Dr Swati Sharma

Assistant Professor of Pathology
- Dr Seemitr Verma

Kasturba Medical College Mangalore, Karnataka

Senior Professor and Head of Pathology
- Dr Jyoti R Kini

Professors of Pathology
- Dr Hema Kini • Dr Sharada Rai

Additional Professors of Pathology
- Dr Padmapriya J • Dr Pooja Suresh • Dr Sri Devi HB

Associate Professors of Pathology
- Dr Chaithra GV • Dr Ranjitha Rao • Dr Cheryl Sara Philipose
- Dr Saraswathy Sreeram • Dr KB Vatsala

Assistant Professors of Pathology
- Dr Rakshatha Nayak • Dr Shikha Jayasheelin • Dr Sneha Rao
- Dr Sunita Hegde

Srinivas Institute of Medical Sciences and Research Center Mukka Mangalore, Karnataka

Senior Professor and Head of Pathology
- Dr Sukesh Nair

Professor of Pathology
- Dr Kuladeep Vaidya

JSS Academy of Higher Education and Research Mysore, Karnataka

Senior Professor and Head of Pathology
- Dr Sheeladevi CS

Professor of Pathology
- Dr Nandini N Manoli

SS Institute of Medical Sciences and Research Center Davangere, Karnataka

Senior Professor and Head of Pathology
- Dr P Shashikala Krishnamoorthy

Professors of Pathology
- Dr Gowda Kavitha Umapathy • Dr Deepthi Pruthvi

Associate Professors of Pathology
- Dr Neetha Y • Dr Rajashree K • Dr Balaji TG

JJM Medical College Davangere, Karnataka

Senior Professor and Head of Pathology
- Dr Vardendra Kulkarni

Professor of Pathology
- Dr Suresh Hanagavdi

KS Hegde Medical Academy Mangalore, Karnataka
Senior Professor and Head of Pathology
• Dr Jayaprakash Reddy K
Professors of Pathology
• Dr Teerthanath S • Dr (Mrs) K Padma Shetty
• Dr (Mrs) Michelle Mathias • Dr Sunil Kumar Y • Dr Kishan Parsad HL
Additional Professor of Pathology
• Dr Shubha P Bhat
Associate Professors of Pathology
• Dr Chandrika Rao • Dr Sajitha K
Assistant Professor of Pathology
• Dr Shruti

Basaveshwara Medical College and Hospital Chitradurga, Karnataka
Senior Professor and Head of Pathology
• Dr Narayana Murthy C
Professors of Pathology
• Dr Ramu R • Dr Sonam S Nandyal
Associate Professors of Pathology
• Dr Bindu BJ • Dr Sharvani Setty
Assistant Professors of Pathology
• Dr Ruhi Salma Naagar • Dr Prajna SK • Dr Prajna KS
• Dr Farheen Kauser

Belgaum Institute of Medical Sciences (BIMS) Belgaum, Karnataka
Senior Professor and Head of Pathology
• Dr Rekha V Harvi
Associate Professors of Pathology
• Dr Aruna Sharanappa • Dr Meena N Jadhav • Dr Rashmi K Patil
Assistant Professors of Pathology
• Dr Ameet G • Dr Priya Patil • Dr Datar Rujuta Anand
• Dr K Geetha Mala

Jawaharlal Nehru Medical College Belgaum, Karnataka
Senior Professor and Head of Pathology
• Dr Vijayalakshmi M Dhorigol
Professors of Pathology
• Dr Hema Pardeep Shantagiri • Dr Ramesh Chavan
• Dr Ranjit Kangle • Dr Reshma Karishetti • Dr Bhagyashri Hungund
• Dr Sunita Y Patil • Dr Manasi Gosavi
Associate Professor of Pathology
• Dr Ashwani Ratnakar
Assistant Professors of Pathology
• Dr Adarsh Sanikop • Dr Suvarna Inumella • Dr Vithal Mane
• Dr Shantala M

Raja Rajeshwari Medical College Kambipura Bengaluru, Karnataka
Senior Professor and Head of Pathology
• Dr Jyoti A Raj

MVJ Medical College Hoskote, Karnataka
Senior Professor and Head of Pathology
• Dr Raja Parthiban SR

Gadag Institute of Medical Sciences Gadag, Karnataka
Senior Professor and Head of Pathology
• Dr Basavaraj P Bommanahalli
Associate Professors of Pathology
• Dr Manika Alexandar • Dr Harish S Permi

Gulbarga Institute of Medical Sciences (GIMS) Gulbarga, Karnataka
Senior Professor and Head of Pathology
• Dr Rajesh Patil
Professors of Pathology
• Dr Anita A Mahanta • Dr Anuradha Patil • Dr SB Shivashetty
• Dr Shilpa U
Associate Professor of Pathology
• Dr Meenakshi S Nigudgi
Assistant Professors of Pathology
• Dr Reshma Devani • Dr Priyanka Patil • Dr Shreya AS
• Dr Sneha Sheelwanth • Dr Asha Patil
Senior Resident of Pathology
• Dr Rohini Mokshi

Khaja Bandanawaz Institute of Medical Sciences (KBNIMS) Gulbarga, Karnataka
Senior Professor and Head of Pathology
• Dr Mandakini BT
Professors of Pathology
• Dr Zeenath • Dr Pratima Sambrani
Associate Professor of Pathology
• Dr Geetanjali
Assistant Professors of Pathology
• Dr Vishal V • Dr Sudharani • Dr Afra • Dr Sara Siddique
• Dr Tahniyat • Dr Arpita • Dr Summaiya

Kempegowda Institute of Medical Sciences Bengaluru, Karnataka
Senior Professor and Head of Pathology
• Dr SR Niveditha

BGS Global Institute of Medical Sciences Bengaluru, Karnataka
Senior Professor and Head of Pathology
• Dr Manjunath HK

Vydehi Institute of Medical Sciences Bangaluru, Karnataka
Senior Professor and Head of Pathology
• Dr Prathima S

Atal Bihari Vajpayee Medical College and Research Institute Bengaluru, Karnataka
Senior Professor and Head of Pathology
• Dr Sharmila PS

Dr BR Ambedkar Medical College Bengaluru, Karnataka
Senior Professor and Head of Pathology
• Dr Shaista Choudary

Oxford Medical College Hospital and Research Center Bengaluru, Karnataka
Senior Professor and Head of Pathology
• Dr Naveen Kumar BJ

Akash Medical College Bengaluru, Karnataka
Senior Professor and Head of Pathology
• Dr Vidyavathi
Professor of Pathology
• Dr Adishesha Shankar
Associate Professors of Pathology
• Dr Supriya Sandeepa • Dr Shilpa S Biradar

KERALA

Government Medical College Kozhikode, Kerala
Senior Professor and Head of Pathology
• Dr Supriya KN

Government Medical College Manjeri, Kerala
Senior Professor and Head of Pathology
• Dr Prabhalakshmy KK

Government Medical College Palakkad, Kerala
Senior Professor and Head of Pathology
• Dr Magadalene KF

Government Medical College Thrissur, Kerala
Associate Professor of Pathology
• Dr Subitha K

Jubilee Mission Medical College and Research Institute Thrissur, Kerala
Senior Professor and Head of Pathology
• Dr Lincy Joseph

Associate Professor of Pathology
• Dr Usha Pai KN

Government Medical College Pariyaram Kannur, Kerala
Senior Professor and Head of Pathology
• Dr Jeena Sam K

Assistant Professor of Pathology
• Dr Prathibha PP

Government Medical College Palakkad, Kerala
Senior Professor and Head of Pathology
• Dr Magadalene KF

KMCT Medical College Kozhikode, Kerala
Senior Professor and Head of Pathology
• Dr Sathi PP

Professor of Pathology
• Dr Benzy Paul

Associate Professors of Pathology
• Dr Anupama Raj • Dr Mary Nandini • Dr Reslin A Khade

Assistant Professors of Pathology
• Dr Janaky Ramakrishnan • Dr Athira Sasidharan • Dr Fazna N
• Dr Shamla P • Dr Sneha Ravindran • Dr Nazneen Abdul Kader
• Dr Dhanya V • Dr Naisana Nasar • Dr Rameeza A
• Dr Swathy Shanker • Dr Greeshma P • Dr Fathima Roofa CH

Malankara Orthodox Syrian Church (MOSC) Medical College Kochi, Kerala
Senior Professor and Head of Pathology
• Dr Usha Poothiode

Amrita Institute of Medical Sciences and Research Center Kochi, Kerala
Senior Professor and Head of Pathology
• Dr Seethahlekshmy NV

Associate Professor of Pathology
• Dr Vidya Jha

Senior Resident of Pathology
• Dr Reshma Jeladharan

Travancore Medical College Kollam, Kerala
Associate Professor of Pathology
• Dr Anjali S Arun

Dr Moopen's Medical College Wayanad, Kerala
Senior Professor and Head of Pathology
• Dr TG Konapur

Associate Professors of Pathology
• Dr Gitanjali MM • Dr Girija C • Dr Divya Prakash

MADHYA PRADESH

Gaja Raja Medical College, Gwalior, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Sudha Iyengar

Professors of Pathology
• Dr Jyoti Priyadarshini • Dr Arun Jain • Dr Reena Jain

Associate Professors of Pathology
• Dr Arti Gupta • Dr Shilpi Sikharwar • Dr Gajender Pal Singh
• Dr Sunita Rai • Dr Amit Niranjan

Assistant Professors of Pathology
• Dr Reema Bhushan • Dr Saurabh Shrivastava

Gandhi Medical College Bhopal, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Reeni Malik

Professors of Pathology
• Dr RK Nigam • Dr Pramila Jain

Associate Professors of Pathology
• Dr Rajni Choudhary • Dr Ashish Koshti • Dr Archana Shrivastava
• Dr Manish Sulya • Dr Sharda Balani • Dr Sonal Gupta

Assistant Professors of Pathology
• Dr Varsha Rampuri • Dr Upasna Uniya • Dr Archana Romale
• Dr Priyanka Patangia

All India Institute of Medical Sciences Bhopal, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Vaishali Walke

Professor of Pathology
• Dr Garima Goyal

Additional Professor of Pathology
• Dr Jaishankar E

Associate Professor of Pathology
• Dr Ujjawal Khurana

Netaji Subhash Chandra Bose Medical College Jabalpur, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Sanjay Totade

Professors of Pathology
• Dr Ranu Tiwari Mishra • Dr Radhika Punshi Nandwani

MGM Medical College Indore, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Ashok Panchonia

Professors of Pathology
• Dr Meena Mittal • Dr Shikha Ghanghoria

Associate Professors of Pathology
• Dr Rakesh Mehar • Dr Ravi Jain • Dr Pankaj Shinde
• Dr Chandra Shekhar Chatrasal • Dr Shailendra S Thakur

Assistant Professors of Pathology
• Dr Poonam Nanwani • Dr Aksharaditya Shukla • Dr Priyanka Kiyawat
• Dr Radhika Rai

Shyam Shah Medical College Rewa, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr SK Sutrakar

Senior Professor of Pathology
• Dr UR Singh

Assistant Professors of Pathology
• Dr Lokesh Tripathi • Dr Jagannath Jatav

Index Medical College, Hospital and Research Center Indore, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Sanjeev Narang

Professors of Pathology
• Dr VK Jain • Dr Anjali Singh • Dr Pawan Bhambani
• Dr Romi Srivastava

Assistant Professors of Pathology
• Dr Rahul Kishore • Dr Chhaya Dhangar

Amaltas institute of Medical Sciences Indore, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Roopam Kumar Jain

Professors of Pathology
• Dr Shrikant Nema • Dr M Gupta • Dr Abhiraj Ramchandani

Associate Professor of Pathology
• Dr SK Hora

Assistant Professors of Pathology
• Dr Amarnath Yadav

Sri Aurobindo Medical College and Post Graduate Institute Indore, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Amit Varma

Professors of Pathology
• Dr Kamal Malukani • Dr Garima Malpani

Associate Professor of Pathology
• Dr Sanjana Ahuja

Lakshmi Narayan Medical College (LNMC) Bhopal, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Vivek Khare

Professors of Pathology
• Dr Vandana Aggarwal • Dr Arun Maity • Dr Parul Gupta
• Dr Nandkishore Pehladani

Associate Professors of Pathology
• Dr Pragya Saxena • Dr Vibha Trichal • Dr Pooja Katiyar
• Dr Syed Sarfaraz Ali

Assistant Professors of Pathology
• Dr Neelambara Bidwai • Dr Vani Swapanil Gadre • Dr Deepika Agrawal
• Dr Kavita Jain • Dr Shubhra Prasad

RD Gargi Medical College Ujjain, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Manju Purohit

Professors of Pathology
• Dr Swati Patel • Dr Kavita Jain

Associate Professors of Pathology
• Dr Rajesh Golash • Dr Mala Patidar • Dr Dhananjay Naik

Assistant Professors of Pathology
• Dr Sanchita Sanghai Kothari • Dr Gautam Bhagwat • Dr Preeti Singh

People's College of Medical Sciences and Research Center Bhopal, Madhya Pradesh
Senior Professor and Head of Pathology
• Dr Varsha Jadhav

Associate Professors of Pathology
• Dr Sushil Kumar Sharma

MAHARASHTRA

GS Seth Medical College and KEM Hospital, Mumbai, Maharashtra
Senior Professors of Pathology
• Dr Pradeep Vaideeshwar • Dr Daksha Prabhat Piyush

Associate Professor of Pathology
• Dr Rachana Chaturvedi

Topiwale National Medical College and Dr BYL Charitable Hospital Mumbai, Maharashtra
Additional Professor of Pathology
• Dr Rima Kamat

Associate Professor of Pathology
• Dr Varsha Dhume

MGM Institute of Health Sciences Navi Mumbai, Maharashtra
Senior Professor and Head of Pathology
• Dr Shilpi Sahu

Senior Professor of Pathology
• Dr Ujwala Maheshwari

Professor of Pathology
• Dr Manisha Tambekar

Associate Professors of Pathology
• Dr Priyanka Jadhav • Dr Meera Methe • Dr Trupti Patil • Dr Niraj Rathi

Dr DY Patil Medical College Hospital and Research Nerul Mumbai, Maharashtra
Senior Professor and Head of Pathology
• Dr S Sudhamani

Professors of Pathology
• Dr Rajiv Rao • Dr Pragati Upasham • Dr Anita Sharan
• Dr Surekha Bhalekar

Associate Professor of Pathology
• Dr Snigdha Mukherjee

Assistant Professors of Pathology
• Dr Prakash Dive • Dr Shirish Khatu • Dr Sneha Chavarkar
• Dr Divya Shetty • Dr Kritika Singh • Dr Anand Ambekar

Senior Residents of Pathology
• Dr Saloni Patra • Dr Atithi Bansal

Tata Memorial Centre Mumbai, Maharashtra
Senior Professor of Pathology
• Dr Sumeet Gujral

Terna Medical College Navi Mumbai, Maharashtra
Senior Professor and Head of Pathology
• Dr Shanu Srivastava

Professor of Pathology
• Dr Kanchan Mala Ghorpade

BJ Government Medical College Pune, Maharashtra
Senior Professor and Head of Pathology
• Dr Leena A Nakate

Professors of Pathology
• Dr Sharada R Rane • Dr Suvemkar SV

Dr DY Patil Medical College, Hospital and Research Pimpri Pune, Maharashtra
Senior Professor and Head of Pathology
• Dr Gore Charushila R

Professors of Pathology
• Dr SS Chandanwale • Dr Archana C Buch • Dr Arpana Dharwadhkar
• Dr Rupali Bavikar • Dr Tushar Kambale • Dr MB Iqbal
• Dr Komal Sawaimul

Associate Professors of Pathology
• Dr Sushma G Gurwale • Dr Yamini G Gurwale • Dr Mangesh M Londhe

Assistant Professors of Pathology
• Dr Tanmayi Kulkarni • Dr Mayur R. Ambekar • Dr Neha V. Kamble
• Dr Madhuri Singh • Dr Akshi Raj

Senior Residents of Pathology
• Dr Rutika M Ghatge • Dr Suraj Desai • Dr Pooja Naik • Dr Ashima Binny
• Dr Ashwani Sunil Sonwane

Tutors of Pathology
• Dr Deshpande Anjali H • Dr Wrunda C Raut • Dr Advait A Gaikwad
• Dr Kanchan Dattatray Gaikwad • Dr Satyajit Bajarang Paymal

Bharati Vidyapeeth (Deemed to be University, Pune) Medical College Pune, Maharashtra
Senior Professor and Head of Pathology
• Dr Reena Bhardwaj

Professors of Pathology
• Dr NS Mani • Dr Ravinder C. Nimbargi • Dr Manjiri Karandikar
• Dr Kunda A Jagdale • Dr Preeti Doshi • Dr Amita Nisal

Associate Professors of Pathology
• Dr Rachana Lakhe • Dr Parineeta Shelke

Assistant Professors of Pathology
• Dr Niranjan Nayak • Dr Prajakta Shinde • Dr Anuja Patil • Dr R Karthik
• Dr Purva Kulkarni

Senior Residents of Pathology
• Dr Amrutraj Patil • Dr Payal Telkar

Tutors of Pathology
• Dr Avantika Jain • Dr Suraj Kumbhar • Dr Dhara Patel
• Dr Bhumika Patel • Dr Ashwani Somani • Dr Shraddha Mulkar

Krishna Institute of Medical Sciences Karad, Maharashtra
Senior Professor and Head of Pathology
• Dr Sujata R Kanetkar

Professors of Pathology
• Dr SV Jagtap • Dr (Mrs) Nanda J Patil

Associate Professor of Pathology
• Dr Sujata Kumbhar

Prakash Institute of Medical Sciences and Research Islampur, Maharashtra
Senior Professor and Head of Pathology
• Dr Jyotsna V Wadar

Dr DY Patil Medical College, Hospital and Research Kolhapur, Maharashtra
Senior Professor and Head of Pathology
• Dr Mahadeo Mane

Professor of Pathology
• Dr Meena N Jadhav

Associate Professors of Pathology
• Dr Rajender S Patil • Dr Ravindra M Shinde • Dr Suchita V Deshmukh
• Dr Sapna S Deshpande

Assistant Professors of Pathology
• Dr Purva N Shinde • Dr Sandeep S Yadav

Senior Residents of Pathology
• Dr Amruta K Thomake • Dr Pradnya M Chimankar • Dr Vishal Raut
• Dr Sonal Jain

Tutors of Pathology
• Dr Sunil Jagdale • Dr VA Mane • Dr Sharvari Patil • Dr Akansha Patil
• Dr Pramod P Khot

Rajiv Gandhi Medical College Kalwa Thane, Maharashtra
Senior Professor and Head of Pathology
• Dr BB Kore

Professor of Pathology
• Dr Kavita Sane

Government Medical College Akola, Maharashtra
Senior Professor and Head of Pathology
• Dr Dilip Sarate

Associate Professors of Pathology
• Dr Ajay Jungare • Dr Suwarna Patil • Dr Manjiri Khade • Dr Rajesh Kate

Assistant Professors of Pathology
• Dr Shreeram Chopade • Dr Swarada Kangate • Dr Pranav Shirbhate
• Dr Ashish Shinde • Dr Anup Gosavi

Senior Residents of Pathology
• Dr Akshata Chaturkar • Dr Pooja Talokar • Dr Aishwarya Mantri
• Dr Shweta Nemade • Dr Shalaka Tupkari • Dr Snehal Tale
• Dr Dnyaneshwar Doiphode

Post Graduate Residents of Pathology
• Dr Shital Thorat • Dr Pratiksha Badapurkar • Dr Snehal Bhomble
• Dr Anubhav Uraon • Dr Suraj Pawar • Dr Prerna Dindokar
• Dr Niharika Lokhande • Dr Neha Hussani • Dr Minal Nikhade
• Dr Haritha Pillai • Dr Shubhashri Ilpate • Dr Anmol Pachpande
• Dr Ashish Singh

Government Medical College Jalgaon, Maharashtra
Associate Professor of Pathology
• Dr Deepak Shejwal

Assistant Professor of Pathology
• Dr Kunal Deore

Lecturer of Pathology
• Dr Pooja Luxman Khandwa

Ulhas Patil Medical College Jalgaon, Maharashtra
Senior Professor and Head of Pathology
• Dr Dhananjay Shridhar Borole

Government Medical College Miraj, Maharashtra
Senior Professor and Head of Pathology
• Dr Yasmin Altaf Momin

Professors of Pathology
• Dr Prashant Shankarrao Murarkar • Dr Medha Pradeep Kulkarni

Dr Vaishampayan Memorial (VM) Government Medical College Solapur, Maharashtra
Senior Professor and Head of Pathology
• Dr Sumita S Dantkale

Dr Shankarrao Chavan Government Medical College Nanded, Maharashtra
Senior Professor and Head of Pathology
• Dr MA Sameer

Associate Professor of Pathology
• Dr PS Muley

Assistant Professors of Pathology
• Dr DS Sadhu • Dr SB Narwade • Dr SM Patil • Dr SS More
• Dr RP Wattamwar

Senior Residents of Pathology
• Dr Pooja Nagargoje • Dr Ankita Lohiya • Dr Smita Hilalpure

Post Graduate Residents of Pathology
• Dr Sushma • Dr Mohibul • Dr Sanskruti • Dr Pooja L • Dr Pratichi Parida

Government Medical College Aurangabad, Maharashtra
Senior Professor and Head of Pathology
• Dr BR Sonwane

Associate Professors of Pathology
• Dr SN Gawai • Dr PP Phulgirkar • Dr AS Kulkarni • Dr DG Jaiswal
• Dr Vaishali Nagose • Dr SN Pore

Assistant Professors of Pathology
• Dr Shazia Anjum • Dr SK Shere • Dr VS Wane • Dr SM Munde
• Dr SD Ganokar • Dr Amrin Fatima • Dr Pramod Sarwade

MGM Institute of Health Sciences Aurangabad, Maharashtra
Senior Professor and Head of Pathology
• Dr CP Bhale

Professors of Pathology
• Dr SS Kale • Dr AA Vare • Dr Suparna Bindu

Associate Professors of Pathology
• Dr Reeta Taksali • Dr Archana Bidari • Dr Meera Mahajan
• Dr Ganesh Kadam • Dr Anuradha Patil • Dr Neha Borde

Assistant Professors of Pathology
• Dr Rohini Shewale • Dr Praveen Kumar R • Dr Pranita Sontakke

Senior Residents of Pathology
• Dr Snehal Patil • Dr Kalyani Deshmukh

Tutor of Pathology
• Dr Sangeeta Kulkarni

Indira Gandhi Government Medical College and Hospital Nagpur, Maharashtra
Senior Professor and Head of Pathology
• Dr Balwant D Kowe

Associate Professors of Pathology
• Dr Nitin Y. Shende • Dr Rekha Patil • Dr Saroj Gaikwad Meshram
• Dr Jayshree Joshi Sathe • Dr Amit Agrawal

Assistant Professors of Pathology
• Dr Anjali Bode • Dr Sonali Datar • Dr Amrapali Gaiwad
• Dr Pradnya Bhadarje • Dr Sayyed Wasim

Government Medical College and Hospital Nagpur, Maharashtra
Head of Pathology
• Dr Satish Halwatkar

Associate Professors of Pathology
• Dr Poornima Kodake • Dr Tulsi Chadi • Dr Pradeep Butle
• Dr Prajakta Gaddewar • Dr Supreeta Nayak • Dr Rajna Pohekar
• Dr Anjali Bode

Assistant Professors of Pathology
• Dr Vedita Golhar • Dr Shailender Yadav • Dr Sanjay Chauhan
• Dr Shubhangi Jibkate • Dr Shubhangi Khamankar • Dr Archana Khade
• Dr Manjiri Makde • Dr Fatema Kamal • Dr Renuka Badnale
• Dr Nikhil Charmode • Dr Pravin Meshram • Dr Aniket Meshram
• Dr Anushree Wagh • Dr Sonal Meshram • Dr Shalakha Khade

NKP Salve Institute of Medical Sciences Nagpur, Maharashtra
Associate Professors of Pathology
• Dr Anne R Winkinson

Mahatma Institute of Medical Sciences Sewagram, Maharashtra
Director Professor and Head of Pathology
• Dr Anupama Gupta

Director Professor of Pathology
• Dr VB Shiv Kumar

Professor of Pathology
• Dr Anshu

Associate Professors of Pathology
• Dr Mansi Bhalavi • Dr Bharat Patil • Dr Abhay Deshmukh

Assistant Professors of Pathology
• Dr Shubhangi Mangam • Dr Vina Dhruve • Dr Chetna Pangul
• Dr Shikha Singh

Jawaharlal Nehru Medical College Sawangi, Maharashtra
Senior Professor and Head of Pathology
• Dr Sunita Wagh

Professor of Pathology
• Dr Arvind Bhake

Vasantrao Pawar Medical College Nashik, Maharashtra
Senior Professor and Head of Pathology
• Dr Preeti Bajaj

Professors of Pathology
• Dr Jyoti Sonawane (Kasture) • Dr Devangana Rajyaguru

Associate Professors of Pathology
• Dr Rairikar Shubhangi • Dr Nikhat Anjum Sayyed
• Dr Sheetal Sameer Dharrao

Assistant Professors of Pathology
• Dr Bharati Deore • Dr Nathe Neha S • Dr Mugdha R Deshmukh
• Dr Kale Vidya Gaurav • Dr Purva More

SMBT Institute of Medical Sciences and Research Center Nashik, Maharashtra
Senior Professor and Head of Pathology
• Dr Supriya Deelip Joshi

Swami Ramanand Teerath Rural (SRTR) Medical College Ambajogai, Maharashtra
Senior Professor and Head of Pathology
• Dr Arvind N Bagate

Associate Professor of Pathology
• Dr Dnyaneshwar Shivajirao Jadhav

Shree Bhausaheb Hire Government Medical College Dhule, Maharashtra
Senior Professor and Head of Pathology
• Dr MS Vasaikar

Associate Professors of Pathology
• Dr Dhiraj B Nikumbh • Dr SS Chavan • Dr RP Damble • Dr MP Tambse
• Dr SA Bora • Dr BM Patil

ACMS Medical College Dhule, Maharashtra
Senior Professor and Head of Pathology
• Dr Newadkar Dhananjay V

Professors of Pathology
• Dr Deore Prashant N • Dr Amale Anagha P

Associate Professors of Pathology
• Dr Ahirrao Bhagyashree M • Dr Sonawane Nilesh R

Assistant Professors of Pathology
• Dr Shah Ashish D • Dr Pagare Poonam S • Dr Patil Rajshri A
• Dr Ubhale Sarika B • Dr Agrawal Sakshi S • Dr Jain Sapna P

Senior Residents of Pathology
• Dr Gadre Arunadhati S • Dr Jajare Megha P • Dr Oswal Sandeep J
• Dr Desale Shruti S • Dr Newadkar Neha D • Dr Chaudhari Shubhangi R

Post Graduate Residents of Pathology
• Dr Patil Poonam B • Dr Patil Priyanka U • Dr Hekare Shubham B
• Dr Shende Ketaki R • Dr Saoji Shreeya M

Tutors of Pathology
• Dr Wadile Sarika V • Dr Borse Tejaswini S • Dr Jadhav Mohit V
• Dr Patil Yash H

Vilasrao Deshmukh Government Medical College Latur, Maharashtra
Senior Professor and Head of Pathology
• Dr Umesh S Kanade

Associate Professors of Pathology
• Dr Sunil Yogiraj Swami • Dr Suresh Arjunrao Chaware
• Dr Rajharsh Dattatray Hanmate • Dr Smita Shashikant Pudale
• Dr Rahul Suryakant Rao Abhange • Dr Kacharu Tukaram Dalve

Assistant Professors of Pathology
• Dr Shivraj Nagnath Kanthikar • Dr Manisha Biradar
• Dr Khule Subhangi Kamlakar

Maharashtra Institute of Medical Sciences and Research (MIMSR) Latur, Maharashtra
Senior Professor and Head of Pathology
• Dr Sheela N Kulkarni

Professors of Pathology
• Dr RV Kulkarni • Dr Jayshree B Patil

Symbiosis Medical College Pune, Maharashtra
Senior Professor and Head of Pathology
• Dr Anand A Bhosale

Professors of Pathology
• Dr RS Bindu • Dr Shruti Vimal

Associate Professor of Pathology
• Dr Shruti Vimal

ODISHA

MKCG Medical College Berhampur, Odisha
Senior Professor and Head of Pathology
• Dr Sulata Choudhary

Professors of Pathology
• Dr Samira Kumar Behera • Dr Manoj Kumar Patro • Dr Subrat Burma

Associate Professors of Pathology
• Dr Anita Choudhury • Dr Sunita Rani Senapati

SCB Medical College Cuttack, Odisha
Senior Professor and Head of Pathology
• Dr Lity Mohanty

Faqir Mohan Medical College Balasore, Odisha
Senior Professor and Head of Pathology
• Dr Asaranti Kar

Kalinga Institute of Medical Sciences Bhubaneshwar, Odisha
Senior Professor and Head of Pathology
• Dr Ranjana Giri

Professor of Pathology
• Dr Urmila Senapati

All India Institute of Medical Sciences Bhubaneshwar, Odisha
Assistant Professor of Pathology
• Dr Sarojini Raman

PUDUCHERRY (PONDICHERRY)

Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER), Puducherry
Professor and Head of Pathology
• Dr Pakhee Kar

Senior Professors of Pathology
• Dr Surender Kumar Verma • Dr Bhavna A Badhe • Dr Neelaiah Siddaraju

Thiruvarur Government Medical College, Puducherry
Professor and Head of Pathology
• Dr OA Meharaj Banu

Government Medical College Nagapaitnam, Puducherry
Professor and Head of Pathology
• Dr G Sudha

Assistant Professor of Pathology
• Dr KM Aadithya

Vinayaga Mission Medical College Karaikal, Puducherry
Professor and Head of Pathology
• Dr N Anusha

Assistant Professor of Pathology
• Dr Kaviya Priya

Raja Muthiya Medical College Chidhambaram, Puducherry
Professor and Head of Pathology
• Dr Krishnaswamy

Assistant Professor of Pathology
• Dr Thamariselvi

Mahatma Gandhi Medical College and Research Institute, Puducherry
Professor and Head of Pathology
• Dr KR Umadevi

Aurupadi Veedu Medical College, Puducherry
Professor and Head
• Dr Jawahar

Associate Professor of Pathology
• Dr J Jeya Shambavi

Pondicherry Institute of Medical Sciences, Puducherry
Professor and Head of Pathology
• Dr Kiran

Associate Professor of Pathology
• Dr Gayathri

Indira Gandhi Medial College and Research Institute, Puducherry
Professor and Head of Pathology
• Dr Banushree

Assistant Professor of Pathology
• Dr Chaithra R Jadhan

Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry
Professor and Head of Pathology
• Dr Partho Barman

Associate Professor of Pathology
• Dr G Jeevana Nikund

Sri Venkateswara Medical College, Hospital and Research Center, Puducherry
Professor and Head of Pathology
• Dr Govindaraj Thimmarayan

Assistant Professor of Pathology
• Dr Meera

Sri Manakula Vinayagar Medical College, Puducherry
Professor and Head of Pathology
• Dr Erli Amel Ivan

Associate Professor of Pathology
• Dr Ramaya

Melmaruvathur Adhiparasakthi Institute of Medical Sciences, Puducherry
Professor and Head of Pathology
• Dr S Sumathi

Assistant Professor of Pathology
• Dr K Pooja

PUNJAB

Government Medical College Amritsar, Punjab
Senior Professor and Head of Pathology
• Dr Permeet Kaur Bagga

Professor of Pathology
• Dr Jaspreet Singh

Associate Professors of Pathology
• Dr Navjot Kaur • Dr Mandeep Randhawa

Assistant Professors of Pathology
• Dr Hardeep Singh Sethi • Dr Vinay Sharma • Dr Poonam Kattru

Government Medical College Patiala, Punjab
Senior Professor and Head of Pathology
• Dr Harpal Singh

Professor of Pathology
• Dr Vijay Kumar

Associate Professors of Pathology
• Dr Navneet Kaur • Dr Mohanvir Kaur • Dr Monika Garg

Assistant Professors of Pathology
• Dr Ramandeep Kaur • Dr Gurvinder Kaur

Guru Govind Singh Medical College Faridkot, Punjab
Senior Professor and Head of Pathology
• Dr Saria

Professors of Pathology
• Dr Amarjit Singh (Contractual) • Dr Vaneet Kaur Sandhu

Associate Professor of Pathology
• Dr Ekta Rani

Assistant Professors of Pathology
• Dr Saveena Jindal • Dr Shilpa Bairagi • Dr Arshdeep Kaur
• Dr Navjyot Kaur

Christian Medical College Ludhiana, Punjab
Senior Professor and Head of Pathology
• Dr Preethi Anni Mercy Paul

Professor of Pathology
• Dr Roma Isaacs

Associate Professors of Pathology
• Dr Vandana Bhatti • Dr Ashima • Dr Saurabh Donald

Assistant Professor of Pathology
• Dr Prabhjot Kaur

Senior Resident of Pathology
• Dr Ravneet Kaur

Dayanand Medical College and Hospital Ludhiana, Punjab
Senior Professor and Head of Pathology
• Dr Harpreet Kaur

Professors of Pathology
• Dr Bhavna Garg • Dr Vikram Narang • Dr Aminder Singh
• Dr Sumit Grover

Associate Professor of Pathology
• Dr Ruchita Tyagi

Assistant Professor of Pathology
• Dr Saloni Arora

Sri Guru Ram Das Institute of Medical Sciences Amritsar, Punjab
Senior Professor and Head of Pathology
• Dr Karamjit Singh Gill

Professors of Pathology
• Dr Menka Khanna • Dr Harjot Kaur • Dr Sanjay Piplani
• Dr Manisha Sharma • Dr Manas Madan

Associate Professor of Pathology
• Dr Neha Sharma

Assistant Professors of Pathology
• Dr Arshdeep Kaur • Dr Nimrat Kaur

Adesh Institute of Medical Sciences and Research Bhatinda, Punjab
Senior Professor and Head of Pathology
• Dr Arun Puri

Professors of Pathology
• Dr Vijay Suri • Dr Kuljeet Sigh

Associate Professors of Pathology
• Dr Harsh Dhingra • Dr Arnav Roy • Dr Shikha

Assistant Professors of Pathology
• Dr Sahil Chhabra • Dr Shilp Rani • Dr Gunjot Kaur

Tutors of Pathology
• Dr Sahil Paul • Dr Vikas Garg • Dr Ishani Gupta • Dr Amanpreet Kaur
• Dr Divya Abbhi

Punjab Institute of Medical Sciences Jalandhar, Punjab
Senior Professor and Head of Pathology
• Dr Kanwal Masih

Professor of Pathology
• Dr Vaneeta Bhardwar

Associate Professors of Pathology
• Dr Neha Batra • Dr Shaffy Thukral

Assistant Professors of Pathology
• Dr Maninder Kaur • Dr Silky Mahajan • Dr Simarpreet Kaur

All India Institute of Medical Sciences Bhatinda, Punjab
Additional Professor of Pathology
• Dr Manjit Kaur

Dr BR Ambedkar State Institute of Medical Sciences Mohali, Punjab
Associate Professor of Pathology
• Dr Rashi Garg

Assistant Professor of Pathology
• Dr Neha Kumari

RAJASTHAN

SMS Medical College Jaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Arpita Jindal

Senior Professors of Pathology
• Dr Ajay Yadav • Dr Ranjana Solanki • Dr Sandhya Gulati
• Dr Karuna Gupta • Dr Sangita Sehgal • Dr Dilip Ramrakhiani
• Dr Dipika Mishra • Dr Anita Harsh

Professors of Pathology
• Dr Devki Keshwani • Dr Kamlesh Yadav • Dr Sapna Gandhi
• Dr Dipika Hemrajani • Dr Saroj Pachori • Dr Kalpana Mangal
• Dr Priti Saini • Dr Neeraj Verma • Dr Ashish Bagadiya
• Dr Mukesh Khinchi • Dr Subhash Sharma • Dr Shruti Bhargav

Associate Professors of Pathology
• Dr Ruchi Agrawal • Dr Nidhi Sharma • Dr Garima Sharma
• Dr Alpana Jain • Dr Namrata Aggarwal • Dr Jayanti Mala
• Dr Mukta Meel • Dr Pratibha Maan • Dr Prerana Choudhary
• Dr Batti Lal Meena • Dr Vandna Yadav • Dr Swapna Devi
• Dr Neha Gupta

Assistant Professors of Pathology
• Dr Shweta Agrawal • Dr Vijeta Tomar • Dr Utsav Joshi
• Dr Rimjhim Shrimal • Dr Sunita Meena • Dr Sarla Saini
• Dr Sweta Meena

Senior Demonstrator of Pathology
• Dr Parul Gautam

Dr Sampurna Nand Medical College Jodhpur, Rajasthan
Senior Professor and Head of Pathology
• Dr Madhu Gupta

Senior Professors of Pathology
• Dr AR Kalla • Dr SR Negi • Dr Rajni Joshee

Professors of Pathology
• Dr Yogi Raj Joshi • Dr Meeta Deval • Dr Kiran Meena

Associate Professors of Pathology
• Dr Kishore Khatri • Dr Shilpa Agarwal • Dr Taruna Choudhary
• Dr Tarun Sharma

Assistant Professors of Pathology
• Dr Omveer Singh • Dr Arpita Singhvi • Dr Kanchan Rathore
• Dr Swamlata • Dr Neha Bagga • Dr Mamta Choudhary
• Dr Shreya Agrawal • Dr Amrit Raj Kalla • Dr Bharti Varshney
• Dr Rishita Gehlot • Dr Anubha Gupta

Senior Demonstrator of Pathology
Dr Parveen Verma

Jawahar Lal Nehru Medical College Ajmer, Rajasthan

Senior Professor and Head of Pathology
• Dr Geeta Pachori

Senior Professors of Pathology
• Dr Neena Kasliwal • Dr Vandana Porwal

Professors of Pathology
• Dr Swaranlata Ajmera • Dr Seema Gupta • Dr Rashmi Sharma
• Dr Kalpana Sharma • Dr Deepali Jain

Associate Professors of Pathology
• Dr Shweta Khandelwal • Dr Shilpa Jalan • Dr Kalpana Singh Beniwal

Assistant Professors of Pathology
• Dr Aparana Rathi • Dr Sonia Tanwar • Dr Leena Verma
• Dr Soniya Sanaadhya • Dr Pushpa Bai Bairwa • Dr Anita Kumari
• Dr Om Prakash Pachori • Dr Aditi Sirohi • Dr Neha Vijay
• Dr Hansa Choudhary • Dr Pankaj Kumar Jain • Dr Vimlesh
• Dr Sunil Kasana • Dr Sanju Bhati

Government Medical College Kota, Rajasthan

Senior Professor and Head of Pathology
• Dr RK Singh

Senior Professors of Pathology
• Dr Rajshree Bhati • Dr Sudha Pankaj Meena

Professors of Pathology
• Dr Akta Rasania • Dr Shaida Riyaz

Associate Professor of Pathology
• Dr Deepti Sukheeja

Assistant Professor of Pathology
• Dr Lakshmi Aggarwal

Sardar Patel Medical College Bikaner, Rajasthan

Senior Professor and Head of Pathology
• Dr SP Vyas

Professors of Pathology
• Dr Vanita Kumar • Dr Dharam Pal Soni • Dr Nilima Arora
• Dr Nidhi Chitlangia • Dr Sunita Bika • Dr Sharda Dawan

Associate Professors of Pathology
• Dr Sunita Kulhari • Dr Vijayta Modi • Dr Saroj Ola
• Dr Nikita Manoj

Assistant Professors of Pathology
• Dr Ritika Agrawal • Dr Madhusudan Rankawat • Dr Ankita
• Dr Om Prakash Singh • Dr Durga Shankar Jaipal
• Dr Ajit Singh Beniwal

Rabinder Nath Medical College (RNT) Udaipur, Rajasthan

Senior Professor and Head of Pathology
• Dr Sandhya Bordia

Senior Professor of Pathology
• Dr Namita Goyal

Associate Professors of Pathology
• Dr Noratan Jatiya • Dr Sonal Bhati • Dr Pooja Kanwat
• Dr Gunja Dwivedi • Dr Vijay Rajak • Dr Priyanka Kumari

Assistant Professors of Pathology
• Dr Manjulata Gupta • Dr Seema Meena • Dr Jigyashu Joshi
• Dr Priya Parmar • Dr Dharmendra Kumar Garg • Dr Gunjan Bhatia
• Dr Samay Singh Meena • Dr Laxmi Kumari • Dr Abhilasha Garg
• Dr Jubeda Bano • Dr Hemant Kumar

All India Institute of Medical Sciences Jodhpur, Rajasthan

Senior Professor and Head of Pathology
• Dr Poonam Elhence

Additional Professors of Pathology
• Dr Abhishek Purohit • Dr Aasma Nalwa • Dr Meenakshi Rao
• Dr Sudeep Khera

Associate Professors of Pathology
• Dr Deepak Vedant • Dr Vikram Vishwajeet • Dr Divya Aggarwal

Assistant Professor of Pathology
• Dr Vikrant Verma

Jhalawar Medical College, Jhalawar, Rajasthan

Senior Professor and Head of Pathology
• Dr Sumit Prakash Rathore

Professors of Pathology
• Dr Rishi Diwan • Dr Richa Sharma • Dr Chetna Jain • Dr Manish Kumar
• Dr Brajendra Shakyawal

Associate Professor of Pathology
• Dr Yogendra Madan

Assistant Professors of Pathology
• Dr Shahdt Hussian • Dr Prashant Gupta • Dr Ratna Rekha Fagna
• Dr Deeksha Buliwal • Dr Deepika Jain

Senior Demonstrators of Pathology
• Dr Hanif Khan • Dr Bhagwati Prasad Meena • Dr Ajay Kalwa
• Dr Shruti kelwa

Rajasthan University of Health Sciences (RUHS) College of Medical Sciences, Jaipur, Rajasthan

Senior Professor and Head of Pathology
• Dr Yogesh Gupta

Professor of Pathology
• Dr Hema Udawat

Associate Professor of Pathology
• Dr Alka Mittal

Assistant Professors of Pathology
• Dr Radha Sharma • Dr Asha Bansal • Dr Nidhi Priya • Dr Seema Singh

Government Medical College Bharatpur, Rajasthan

Professor and Head of Pathology
• Dr Manish Kumar Singhal

Professor of Pathology
• Dr Madhuri Agarwal

Associate Professors of Pathology
• Dr Neil Sharma • Dr Shweta Goel

Assistant Professor of Pathology
• Dr Himanshu Jain

Government Medical College Chittorgarh, Rajasthan

Assistant Professor of Pathology
• Dr Savita Soni

Geetanjali Medical College Udaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Geeta Mukhiya
Professor of Pathology
• Dr Sunita Bhargava
Associate Professors of Pathology
• Dr Ashumi Gupta • Dr Manisha Jain
Assistant Professors of Pathology
• Dr Poonam Woike • Dr Nehal Minda • Dr Harish Kamboz
• Dr Abhijit Rajawat

Pacific Medical College Udaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Bhawana Garg
Professors of Pathology
• Dr Abha Patni • Dr ML Gupta • Dr Sunanda Chauhan • Dr Kavita Gupta
Assistant Professors of Pathology
• Dr Priyanka Sharma • Dr Swapnil Kumawat • Dr Amandeep Kaur
• Dr Sarita Tatawat • Dr Mansi Khamesara

Mahatma Gandhi University Medical Sciences and Technology Jaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Manju Raghav
Professors of Pathology
• Dr Surabhi Tyagi • Dr Anjana Mittal • Dr Ram Mohan Jaiswal
• Dr Abha Mathur
Associate Professors of Pathology
• Dr Chetna Mehrol • Dr Deepak Garg • Dr Indu Chaudhary
• Dr Sony Mandal • Dr Sonia Agarwal
Assistant Professors of Pathology
• Dr Priyanka Agarwal • Dr Neeraj • Dr Meenu Gupta • Dr Himaxi
• Dr Shalini Gupta • Dr Sangita Hudda

Jaipur National University Institute of Medical Sciences and Research Center Jaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Naresh N Rai
Professors of Pathology
• Dr BRN Srivastav • Dr Kusum Mathur
Associate Professors of Pathology
• Dr Indu Saxena • Dr Charu Tripathi • Dr Murari Lal Dhanetwal
Assistant Professors of Pathology
• Dr Ajay Gupta • Dr Aradhana Gupta • Dr Sumit Kumar • Dr Swati Setia
• Dr Anuprita Kulkarni • Dr Avadh Kishore
Senior Residents of Pathology
• Dr Divya Jain • Dr Manjari • Dr Lilita Sharma • Dr Manish Raut
• Dr Ritu Meena
Post Graduate Residents of Pathology
• Dr Shivangi Shukla • Dr Princy Patel • Dr Bhaswati Talukdar
• Dr Nikunj Bhimani • Dr Amit Yadav • Dr Anita Choudary
• Dr Anurag Choudary • Dr Kajal Godara • Dr Nikita Chauhan
• Dr Urvashi Bishnoi

National Institute of Medical Sciences and Research Center Jaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Ashish Gupta
Senior Professor of Pathology
• Dr Parnab Dey

American International Institute of Medical Sciences Udaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Preeti Agrawal
Professors of Pathology
• Dr Megha Pandey • Dr Ashish Pandey
Assistant Professor of Pathology
• Dr Prachi Gupta • Dr Priyanka Tiwari • Dr Swarneet Bhamra
• Dr Lavanya Raghavan
Senior Residents of Pathology
• Dr Sidhi Periwal • Dr Ashwani S Deshmukh
Post Graduate Residents of Pathology
• Dr Desai Payal Sanjaybhai • Dr Thakor Tosha Umed Sinh
• Dr Ashima Johari • Dr Bhajiyawala Feny Chetan • Dr Dona Kalita
• Dr Nidhi Patel

Ananta Institute of Medical Sciences and Research Centre Udaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Syed Javed

Pacific Institute of Medical Sciences Udaipur, Rajasthan
Senior Professor and Head of Pathology
• Dr Chandra Mathur

TAMIL NADU

Sri Ramachandra Medical College and Research Institute Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Leena Dennis Joseph
Professor of Pathology
• Dr Sandhya Sundram
Associate Professor of Pathology
• Dr Pavithra Vittal Raj
Assistant Professor of Pathology
• Dr Arthi

ACS Medical College Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Vidhya Subramanium

All India Institute of Medical Sciences Ananthapuram, Tamil Nadu
Additional Professor and Head of Pathology
• Dr Rajeshwari

Annapurna Medical College Salem, Tamil Nadu
Associate Professor of Pathology
• Dr C Sashikala

Ariyalur Medical College Ariyalur, Tamil Nadu
Assistant Professor of Pathology
• Dr Nazia

Arunai Medical College Thiruvannamalai, Tamil Nadu
Associate Professor of Pathology
• Dr Kavitha

Bhaarath Medical College and Hospital Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Jamuna Rani Shaktesh
Assistant Professors of Pathology
• Dr S Manjani • Dr Rejal

Chengalpattu Medical College Chennai, Tamil Nadu
Associate Professor of Pathology
• Dr Ashok Kumar

Chettinad Medical College Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Vijayashree

Dhanalakshmi Srinivasan Medical College Perambalur, Tamil Nadu
Associate Professor of Pathology
• Dr Monica

Dindigul Government Medical College Dindigul, Tamil Nadu
Senior Professor and Head of Pathology
• Dr S Dhamayanthi

Government Dharmapuri Medical College, Tamil Nadu
Professor of Pathology
• Dr Shruthi

Government Sivaganagai Medical College Sivaganagai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Sharmila Thilagavathy

Kallkururichi Medical College Kanyakumari, Tamil Nadu
Assistant Professor of Pathology
• Dr Anand C

KAP Vishwanathan Medical College Trichy, Tamil Nadu
Professor of Pathology
• Dr Lalitha

Karpaga Vinayaga Medical College Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Vishwanathan

Kilpauk Medical College Chennai, Tamil Nadu
Professor of Pathology
• Dr Jamila

KMCH Institute of Medical Sciences Coimbatore, Tamil Nadu
Professor of Pathology
• Dr Karthikeyan

Krishnagiri Medical College Krishnagiri, Tamil Nadu
Associate Professor of Pathology
• Dr Palaniappan

Lalithambkai Medical College Chennai, Tamil Nadu
Professor of Pathology
• Dr Gayathri

Madha Medical College Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Sushma Nair

Assistant Professor of Pathology
• Dr Brinda

Madurai Medical College Madurai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Rani

Mohan Kumarmangalam Medical College Salem, Tamil Nadu
Professors of Pathology
• Dr Duraiswamy

Ramanathapuram Government Medical College, Tamil Nadu
Additional Professor and Head of Pathology
• Dr Rajeshwari

Shri Sathya Sai Medical College and Research Institute Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Revathish

Professor of Pathology
• Dr Ravichander Kumaresan

SRM Medical College Hospital and Research Center Kattankulathur Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Jaison Jacob John

Assistant Professor of Pathology
• Dr Shamili

Christian Medical College Vellore, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Meera Thomas

Professor of Pathology
• Dr Elanthemral Singamani,

Associate Professor of Pathology
Dr Vikram Raj Gopinathan

Thanjavur Medical College Thanjavur, Tamil Nadu
Associate Professors of Pathology
• Dr Senthilkumar • Dr Padmanabhan

Assistant Professor of Pathology
• Dr G Sudha • Dr Kayalvizhi

St Peter Medical College Hosur, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Seema

Stanley Medical College Chennai, Tamil Nadu
Assistant Professor of Pathology
• Dr Kiruthika

Tagore Medical College Chennai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr CP Luck

The Institute of Road Transport Perunthurai Medical College Perunthurai, Tamil Nadu
Assistant Professor of Pathology
• Dr Vinuta

Theni Government Medical College Theni, Tamil Nadu
Senior Professor and Head of Pathology
• Dr G Meenakumari

Thiruvanamalai Medical College Thiruvanamalai, Tamil Nadu
Assistant Professor of Pathology
• Dr KAT Miruna

Thoothukudi Medical College Thoothukudi, Tamil Nadu
Assistant Professor of Pathology
• Dr John Raj Suresh

Tirunelveli Medical College Tirunelveli, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Swaminathan

Tirupur Medical College Tirupur, Tamil Nadu
Assistant Professor of Pathology
• Dr Lavanya

Trichy SRM Medical College Trichy, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Pramila

Velammal Medical College Hospital and Research Institute Madurai, Tamil Nadu
Senior Professor and Head of Pathology
• Dr Yogumuthu

Vels Medical College Chennai, Tamil Nadu
Assistant Professor of Pathology
• Dr Ramya M

Villupuram Medical College Villupuram, Tamil Nadu
Senior Professor and Head of Pathology
• Dr S Prabha

Vinayak Missions Medical College Salem, Tamil Nadu
Associate Professor of Pathology
• Dr Devi

Virudhunagar Government Medical College Virudhunagar, Tamil Nadu
Professor and Head of Pathology
• Dr German Pasha

TELANGANA

Osmania Medical College Hyderabad, Telangana
Senior Professor and Head of Pathology
• Dr Naval Kishore Bajaj

Professors of Pathology
• Dr Kota Devender Reddy • Dr Rajrikam Nagarjuna Chary
• Dr BS Nithyananda • Dr Arunayi Jesthadi • Dr Ather Fatima
• Dr OH Radha Krishna

Associate Professors of Pathology
• Dr Mohd Chand Moula • Dr Zaheda Kauser • Dr M Mamatha

Assistant Professors of Pathology
• Dr S Vandana • Dr T Mahima • Dr Zu Afshan Sultana
• Dr R Manasa Reddy • Dr V Soumya • Dr A Swetha • Dr M Supriya
• Dr Akifa Zahid • Dr Tulsi V • Dr G Soumya Reddy • Dr K Shailaja
• Dr K Josephain • Dr K Aleksha • Dr Lanka Yatish Sai • Dr Rubina Hassan

Senior Residents of Pathology
• Dr Fatima • Dr Hafsa Mahnaz • Dr Kadali Sunada • Dr PA Sanjukatha
• Dr P Pushpa Kumari • Dr Tasneem Farooqui • Dr Suvaitha
• Dr Anapindi Pranitha • Dr V Swetha • Dr Neethal B

First Year Postgraduate Residents of Pathology
• Dr Dareen Pasha • Dr K Keerthi Preethy • Dr Manisha Gaikwad
• Dr Shaik Afia Sultana • Dr VK Shalini • Dr Mudavath Jayalaxmi
• Dr Vadeeha Zeba • Dr G Sushmitha • Dr Vanaja • Dr Aditya Nair
• Dr P Pirahi Nila • Dr P Kavipriyaa • Dr S Manisha • Dr L Haritha
• Dr B Mohitha • Dr B Mrunalini • Dr K Saroja Kalyankar
• Dr M Gnana Prasuna • Dr K Sri Sahithi • Dr Farah Naaz • Dr N Umadevi
• Dr Syeda Hafsa Razvi • Dr Machuveri Aaron • Dr S Sravani
• Dr J Susuhmitha Shaifali • Dr Tejaswini Muddam
• Dr G Sony Siyona Priya • Dr G Lavanya • Dr MD Juberia Tabassum
• Dr B Parshiya

Second Year Postgraduate Residents of Pathology
• Dr K Nazma • Dr Wajeha Tazeen • Dr Humera Tabassum
• Dr G Venkatesh • Dr Madhavi Divvala • Dr Sakshi Borker
• Dr Varsha Prabhu • Dr Keerthika Pandian • Dr GS Shilpa
• Dr Nimrah Aaymann • Dr Summaya Begum • Dr Gubba Pranitha
• Dr Kavuru Jyotshna • Dr Deekonda Harshad • Dr Amatul Kareem Naela
• Dr Mounika Chintha • Dr Yaganganddla Harsha Vardhan
• Dr Aksh Rathod • Dr Mohammad Waseemuddin • Dr Bairi Anusha
• Dr Deepshikha Lakra • Dr Uppala Jhansi • Dr Nakka Pranitha Kanthi
• Dr G Muni Sreenivasulu • Dr Mahendravada • Dr Nisha Samreen
• Dr Tumojhu Praveen Kumar • Dr Bandela Rajani
• Dr Somapudi Srivishalakha • Dr Nafisa Parveen

Third Year Postgraduate Residents of Pathology
• Dr S Haripriya • Dr S Balamithra • Dr Shaikh Shahedah • Dr G Monika
• Dr Sadaf Bari • Dr K Nishkala • Dr P Amulya • Dr Sowndarya
• Dr Vishnu • Dr K Bhargavi • Dr Anju Singh • Dr Dusra Manasa
• Dr Syeda Busra • Dr Saranga Sahithi • Dr Doli Ravali • Dr Neerati Anjali
• Dr T Mrudula • Dr Faiza Mahreen • Dr Maddela Pratyusha
• Dr Nasesha Iffath • Dr CP Sneha • Dr B Sai Vineela • Dr Saggala Mahala
• Dr Neha Fathima • Dr Sarraff Gouripriya • Dr Mourya Prashamsa
• Dr Sabiha Akhter • Dr Vasanta Leela

Gandhi Medical College and Hospital Secunderabad, Telangana
Senior Professor and Head of Pathology
• Dr Chander Shekhar

Senior Professor of Pathology
• Dr Devojee Molothe Nayak

Nizam's Institute of Medical Sciences Hyderabad, Telangana
Senior Professor of Pathology
• Dr Shantveer G Uppin

Additional Professor of Pathology
• Dr Megha Shantveer Uppin

Associate Professors of Pathology
• Dr Pramod Kumar Pamu • Dr Navatha Vangala

Kakatiya Medical College Hanumakonda Warangal, Telangana
Senior Professor and Head of Pathology
• Dr Bookya Kishan

Senior Professor of Pathology
• Dr S Sandhya

All India Institute of Medical Sciences Bibinagar, Telangana
Additional Professor and Head of Pathology
• Dr Shailaja Prabhala

Additional Professors of Pathology
• Dr Gunvanti Rathod • Dr Sriniwas Bheemrao Somalwar

Associate Professor of Pathology
• Dr Jitender Singh Nigam

Senior Resident of Pathology
• Dr Sai Swetha

Government Medical College Nalgonda, Telangana
Senior Professor and Head of Pathology
• Dr S Srivani

Associate Professor of Pathology
• Dr B Vijaya Nirmala

Government Medical College Medak, Telangana
Senior Professor and Head of Pathology
• Dr Vani Padmaja

Bhaskar Medical College Yenkepally Moinabad Rangareddy, Telangana
Senior Professor and Head of Pathology
• Dr Vijay Sreedhar

Professors of Pathology
• Dr Sailaja V • Dr MNP Charan Paul • Dr K Radhika

Associate Professor of Pathology
• Dr V Anuradha

Assistant Professors of Pathology
• Dr S Nageshwar Rao • Dr G Yamini Priya • Dr CH Divya
• Dr G Venkata Lakshmi • Dr Y Chandana Chowdhary
• Dr S John Emmanuel Raju

Senior Residents of Pathology
• Dr G Lalitha Reddy • Dr T Lasya • Dr K Poornima • Dr M Sulochana

RVM institute of Medical Sciences and Research Center Siddipet, Telangana
Senior Professor and Head of Pathology
• Dr Byram Bheeshma

Senior Professor of Pathology and Dean
• Dr O Shravana Kumar

Chelmeda Anand Rao Institute of Medical Sciences Karimnagar, Telangana
Senior Professor and Head of Pathology
• Dr P Vidhyadhara Rani

Professors of Pathology
• Dr T Aruna • Dr P Aruna Kumari

Prathima Institute of Medical Sciences Karimnagar, Telangana
Senior Professor and Head of Pathology
• Dr G Anandam

Professor of Pathology
• Dr Shradrutha Prasad

Associate Professor of Pathology
• Dr N Devayani

Malla Reddy Institute of Medical Sciences, Telangana
Senior Professor and Head of Pathology
• Dr S Srikanth

Professor of Pathology
• Dr M Ramani

Malla Reddy Medical College for Women Hyderabad, Telangana
Senior Professor and Head of Pathology
• Dr Balasheb Ramling Yaliker

Professor of Pathology
• Dr P Jijiya Bai

Mamata Medical College Khammam, Telangana
Senior Professor and Head of Pathology
• Dr Yalavarthi Sushma

Professor of Pathology
Dr Thommurothu Vasudeva Rao

Deccan College of Medical Sciences Hyderabad, Telangana
Senior Professor and Head of Pathology
• Dr Idrees Akhtar

Professor of Pathology
• Dr Atiya Begum

Associate Professor of Pathology
• Dr Khatija Shameem

MediCiti Institute of Medical Sciences Ghanpur, Telangana
Senior Professor and Head of Pathology
• Dr B Rajashekar Reddy

Professors of Pathology
• Dr S Mrudula • Dr NNS Srinivas

Associate Professor of Pathology
• Dr Sama Snehaja Reddy

Assistant Professors of Pathology
• Dr P Viswanath Sai • Dr Sabbineni Ramya • Dr Kalyani Vemavarapur

SVS Medical College Mahabubnagar, Telangana
Senior Professor and Head of Pathology
• Dr K Suresh

Professors of Pathology
• Dr Y Srikant Babu • Dr Parimala Devi • Dr Florescence Nightingale
• Dr Deepthi KN • Dr Rasheed Fatima

Associate Professors of Pathology
• Dr V Manaja • Dr K Shilpa

Assistant Professors of Pathology
• Dr M Pramod Kumar Reddy • Dr M Sravani

TRIPURA

Agartala Government Medical College and GB Pant Hospital Agartala, Tripura
Senior Professor and Head of Pathology
• Dr Abhijit Datta

Associate Professors of Pathology
• Dr Arup Bhoumik • Dr Kulashekhar Bhattacharjee • Dr Alpana Banerji

Assistant Professors of Pathology
• Dr Sushanta Chakma • Dr Sumana Ghosh • Dr Kamal Kali Das

Senior Resident of Pathology
• Dr Haimanti Sarkar

Tutors of Pathology
• Dr Partha Sarathi Chaudhuri • Dr Sudeshna Debnath

Tripura Medical College and Dr BRAM Teaching Hospital Agartala, Tripura
Senior Professor and Head of Pathology
• Dr Nabaneet Majumder

Associate Professors of Pathology
• Dr Sayandeep Nath • Dr Swarupa Chakma

Assistant Professors of Pathology
• Dr Sutanuka Khasnabish • Dr Maharshi Debnath • Dr Parijat Datta

Senior Residents of Pathology
• Dr Saurabh Das • Dr Rajarshi Sen • Dr Pragyamita Datta
• Dr Dibyendu Majumder

Junior Resident of Pathology
• Dr Sutapa Bhattacharjee

UTTAR PARDESH

King George Medical University Lucknow, Uttar Pradesh
Senior Professor and Head of Pathology
• Dr Uma Shankar Singh

Professors of Pathology
• Dr Suresh Babu • Dr Rashmi Kushwaha

Additional Professors of Pathology
• Dr Madhu Kumar • Dr Chanchal Rana

Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS) Lucknow, Uttar Pradesh
Professor of Pathology
• Dr Ram Naval Rao

Dr Ram Manohar Lohia Institute of Medical Sciences Lucknow, Uttar Pradesh
Senior Professor and Head of Pathology
• Dr Nuzhat Hussain

Professor of Pathology
• Dr Anurag Gupta

Jawahar Lal Nehru Medical College Aligarh Muslim University Aligarh, Uttar Pradesh
Chairman Professor and Head of Pathology
• Dr Mahboob Hasan

Principal of Institution and Professor of Pathology
• Dr Veena Maheshwari

Professors of Pathology
• Dr Kiran Alam • Dr Nishat Afroz Abbasi • Dr Kafil Akhtar

Sarojini Naidu Medical College (SNMC) Agra, Uttar Pradesh
Senior Professor and Head of Pathology
• Dr Garima Dundy

Professors of Pathology
• Dr Harendra Yadav • Dr Deepa Rani

GSVM Medical College Kanpur, Uttar Pradesh
Professor of Pathology
• Dr Lubna Khan

Muzaffarnagar Medical College and Hospital Muzaffarnagar, Uttar Pradesh
Senior Professor and Head of Pathology
• Dr Anupam Varshney

All India Institute of Medical Sciences Raebareli, Uttar Pradesh
Professor and Head of Pathology
• Dr Neeraj Kumar

Associate Professor of Pathology
• Dr Shruti Gupta

All India Institute of Medical Sciences Gorakhpur, Uttar Pradesh
Additional Professor and Head of Pathology
• Dr Vikas Shrivastav

Assistant Professors of Pathology
• Dr Brijnandan Gupta • Dr Mithlesh Bhargav • Dr Deepika
• Dr Mimna VM

Senior Residents of Pathology
• Dr Priyanka Gautam • Dr Meena • Dr Ishita Das • Dr Neha Singh
• Dr Varuni Yadav • Dr Jasleen Kaur Oberoi • Dr Jyotsna Singh
• Dr Mohd Asif Khan • Dr Shivam Gupta

Post Graduate Residents of Pathology
• Dr Sonu Lohchib • Dr Manu • Dr Valentina • Dr Thoudam
• Dr Sarvesh Dhammi • Dr Lokesh Vaibhav

Moti Lal Medical College (MLN) Allahabad, Uttar Pradesh
Professor and Head of Pathology
• Dr Kachnar Varma

Professor of Pathology
• Dr Anshul Singh

Associate Professor of Pathology
• Dr Varsha Gupta

Assistant Professor of Pathology
• Dr Poornima Mishra

Lala Lajpat Rai Memorial (LLRM) Medical College Merrut, Uttar Pradesh
Professor and Head of Pathology
• Dr Preeti Singh

Professor of Pathology
• Dr Nidhi Verma

Associate Professors of Pathology
• Dr Monika • Dr Priya Gupta

Assistant Professor of Pathology
• Dr Deepali Mittal

Maharani Laxmibai Medical College (MLB) Jhansi, Uttar Pradesh
Professor and Head of Pathology
• Dr Mayank Singh

Professor of Pathology
• Dr Pallavi Agrawal

Baba Raghav Das Medical College Gorakhpur, Uttar Pradesh
Professor and Head of Pathology
• Dr Shilpa Vahikar

Professors of Pathology
• Dr Shaila Kumari Mitra • Dr Rajesh Kumar Rai • Dr Kanchan Srivastava

Associate Professors of Pathology
• Dr Archana Bundela • Dr Alpana Bundela

Assistant Professors of Pathology
• Dr Vibha Singh • Dr Amit Kumar Gupta • Dr Sonal Ratnakar
• Dr Sunita Bharti • Dr Sudha Chakarvarty • Dr Mayank Jain
• Dr Archana Tripathi

Senior Residents of Pathology
• Dr Deval Brajesh Dubey • Dr Swati Dubey

Post Graduate Residents of Pathology
• Dr Akriti Gupta • Dr Alyuza Saman • Dr Priti Yadav • Dr Saumya Verma
• Dr Shweta Tripathi • Dr Shilpi Raj Singh • Dr Nikki Tanya
• Dr Saumya Garg • Dr Maryam • Dr Dheerendra Chaudhary
• Dr Ajita Singh • Dr Rakesh Yadav • Dr Shivani Sharma • Dr Laxman
• Dr Vandana Sharma • Dr Puntu • Dr Raj Bahadur • Dr Rushab Singh
• Dr Jay Prakash Verma • Dr KM Priyanka • Dr Vipin Ranjan • Dr Punit
• Dr Shilpi Priyadarshini • Dr Vivaswan Pradhan • Dr Pallvi Verma
• Dr Priyambada • Dr Manish • Dr Neelam • Dr Mohd. Adil • Dr Puja Rani
• Dr Ashutosh Tripathi • Dr Shweta Singh

Rani Durgawati Medical College Banda, Uttar Pradesh
Senior Professor and Head of Pathology
• Dr Rajni Bharti

Associate Professor of Pathology
• Dr Fahimuddin

Assistant Professors of Pathology
• Dr Arpita Singh • Dr Pritika Kushwaha • Dr Tarun Mishra

Homi Bhabha Cancer Hospital Varanasi, Uttar Pradesh
DM Oncology Resident of Pathology
Dr Pallavi Majumdar

Maharshi Vishwamitra Autonomous State Medical College Ghazipur, Uttar Pradesh
Associate Professor and Head of Pathology
• Dr Manoj Bind

School of Medical Sciences and Research Sharda University Noida, Uttar Pradesh
Professor and Head of Pathology
• Dr Anshu Gupta Devra

Professors of Pathology
• Dr Seema Goel • Dr Salony Mittal

Associate Professor of Pathology
• Dr Atul Verma

Assistant Professors of Pathology
• Dr Tushar Kalonia • Dr Prachi Saxena • Dr Vatsala Gupta
• Dr Kangana Senger • Dr Devyani Pendharker

Subharti Medical College Merrut, Uttar Pradesh
Professor and Head of Pathology
• Dr Anjali Khare

Professors of Pathology
• Dr Rani Bansal • Dr Vinay Bharat • Dr Sangeeta Sharma
• Dr Devender Swarup

Associate Professor of Pathology
• Dr Shubhangi Gupta

Assistant Professors of Pathology
• Dr Navya Jaiswal • Dr Archita • Dr Kavita Chauhan • Dr Preeti Gupta

Senior Resident of Pathology
• Dr Purbita Chatterjee

Rohilkhand Medical College and Hospital and Research Bareilly, Uttar Pradesh
Professor and Head of Pathology
• Dr Nitesh Aggarwal
Professors of Pathology
• Dr Nitesh Mohan • Dr Anjana Arya
Associate Professor of Pathology
• Dr Mithila Bisht
Assistant Professor of Pathology
• Dr Purnima Mittra

Rajshree Medical Research Institute Bareilly, Uttar Pradesh
Professor and Head of Pathology
• Dr Ranjan Aggarwal

Rama Medical College Hospital and Research Kanpur, Uttar Pradesh
Professor and Head of Pathology
• Dr Sanjay Nigam
Associate Professor of Pathology
• Dr Nisha Ahuja

Hind Institute of Medical Sciences (HIND) Barabanki, Lucknow, Uttar Pradesh
Professor and Head of Pathology
• Dr Vivek Gupta
Professors of Pathology
• Dr Aarti Bhattacharya • Dr Sangita Tripathi
Assistant Professor of Pathology
• Dr Prakriti Shukla

Hind Institute of Medical Sciences (HIND) Sitapur, Uttar Pradesh
Professor and Head of Pathology
• Dr Vijay Singh
Associate Professor of Pathology
• Dr Shambhavi

Heritage Institute of Medical Sciences Varanasi, Uttar Pradesh
Senior Professor and Head of Pathology
• Dr Ajmal Singh Bhayal
Professors of Pathology
• Dr Neeraj Kumar Singh • Dr Vatsala Kishore
Assistant Professor of Pathology
• Dr Saumya Pandey

Shri Ram Murti Smarak Institute Medical Science (SRMSIMS) Bareilly, Uttar Pradesh
Professor and Head of Pathology
• Dr Tanu Agarawal
Professors of Pathology
• Dr Hema Pant • Dr Surabhi Pandey
Associate Professor of Pathology
• Dr Subhra Narayan

Tirthankara Medical College Moradabad, Uttar Pradesh
Senior Professor and Head of Pathology
• Dr Seema Avasthi

Maharishi Devaraha Baba Autonomous State Medical College Deoria, Uttar Pradesh
Associate Professor and Head of Pathology
• Dr Pawan Trivedi
Associate Professor of Pathology
• Dr Priyanka Rai

UTTARAKHAND

All India Institute of Medical Sciences Rishikesh, Uttarakhand
Senior Professor and Head of Pathology
• Dr Sanjeev Kishore
Professors of Pathology
• Dr Shalinee Rao • Dr Neha Singh

Veer Chandra Singh Garhwali Government Institute of Medical Science and Research Srinagar, Uttarakhand
Senior Professor and Head of Pathology
• Dr Gazala Rizvi
Professors of Pathology
• Dr Sheela Chaudhary • Dr Deepa Hathwal
Associate Professors of Pathology
• Dr Sachan Bhat • Dr Satish Kumar • Dr Pawan Raj Bhat
Assistant Professor of Pathology
• Dr Srijan Srivastav

Government Doon Medical College Dehradun, Uttarakhand
Senior Professor and Head of Pathology
• Dr Naveen Chandra Thapliyal

Himalayan Institute of Medical Sciences Jolly Grant Dehradun, Uttarakhand
Professors of Pathology
• Dr Anuradha Kusum • Dr Meena Harsh

Shri Guru Ram Rai Institute of Medical Sciences Dehradun, Uttarakhand
Senior Professor and Head of Pathology
• Dr Seema V Acharya
Assistant Professor of Pathology
• Dr Sucheta Bansal

Gautam Buddha Chikitsa Mahavidyalaya Dehradun, Uttarakhand
Senior Professor and Head of Pathology
• Dr Vivek Vikram Bharosay

WEST BENGAL

All India Institute of Medical Sciences Kalyani, West Bengal
Senior Professor and Head of Pathology
• Dr Santosh Kumar Mondol
Additional Professor of Pathology
• Dr Indranil Chakrabarty

Institute of Post Graduate Medical Education Research and SSKM Hospital Kolkata, West Bengal
Senior Professor and Head of Pathology
• Dr Uttra Chatterjee

Medical College Kolkata, West Bengal
Senior Professor and Head of Pathology
• Dr Anuradha De (Pati)

Calcutta National Medical College Kolkata, West Bengal
Senior Professor and Head of Pathology
• Dr Soumitra Biswas
Associate Professors of Pathology
• Dr Sarmila Sen • Dr Aditi Bhattacharyya
Assistant Professors of Pathology
• Dr Arindam Karmakar • Dr Kajari Mandal • Dr Piyali Ghosh

RG Kar Medical College Kolkata, West Bengal
Senior Professor and Head of Pathology
• Dr Tushar Kanti Das

Professors of Pathology
• Dr Anjali Bandopadhyay • Dr Palash Kumar Mandal

Midnapore Medical College Midnapore, West Bengal
Senior Professor and Head of Pathology
• Dr Mallika Pal

North Bengal Medical College Siliguri, West Bengal
Senior Professor and Head of Pathology
• Dr Bidyut Krishna Goswami

Associate Professors of Pathology
• Dr Pranati Bera • Dr Mimi Ganguly • Dr Rama Das • Dr Biswajit Haldar

Bankura Sammilani Medical College and Hospital Bankura, West Bengal
Senior Professor and Head of Pathology
• Dr Swapan Pathak

KPC Medical College Jadavpur, West Bengal
Senior Professor and Head of Pathology
• Dr Ranu Sarkar

Nilratan Sircar Medical College Kolkata, West Bengal
Senior Professor and Head of Pathology
• Dr Gopi Nath Barui

Burdwan Medical College Burdwan, West Bengal
Senior Professor and Head of Pathology
Dr Rupam Karmarkar

Malda Medical College and Hospital Malda, West Bengal
Senior Professor and Head of Pathology
• Dr Suchandra Ray Saha

Associate Professors of Pathology
• Dr Arindam Karmakar • Dr Tarak Banik • Dr Prasenjit Gayan
• Dr Karabi Konar (Sanyal)

Murshidabad Medical College Berhampur, West Bengal
Associate Professor and Head of Pathology
• Dr Suman Ghosh

Senior Professor of Pathology and MSVP
• Dr Anadi Roy Chowdhury

Assistant Professors of Pathology
• Dr Swapan Kumar Sarkar

Santiniketan Medical College Bolpur, West Bengal
Senior Professor and Head of Pathology
• Dr Amita Majumdar Giri

Professor of Pathology
• Dr Mamta Guha Mallick Sinha

Associate Professor of Pathology
• Dr Rajarshi Bhattacharya

College of Medicine and Sagore Dutta Hospital Kamarhati, Kolkata, West Bengal
Senior Professor and Head of Pathology
• Dr Srabani Chakrabadi

Harbour Diamond Government Medical College and Hospital Kolkata, West Bengal
Associate Professor and Head of Pathology
• Dr Seema Mondal

Deben Mahata Government Medical College Purulia, West Bengal
Senior Professor and Head of Pathology
• Dr Debashis Chakraborty

Section III

SYSTEMIC PATHOLOGY

14. Cellular–Molecular Diagnostic Techniques in Clinical Practice
15. Blood Vessels
16. Heart
17. Nasal Cavity, Nasopharynx, Paranasal Sinuses, Ear, Larynx and Neck
18. Respiratory System
19. Oral Cavity, Salivary Glands and Jaw
20. Gastrointestinal Tract and Peritoneum
21. Liver, Gallbladder and Exocrine Pancreas
22. Kidney and Ureter
23. Urinary Bladder and Male Reproductive System
24. Female Reproductive System
25. Mammary Glands
26. Endocrine System: Pancreas, Thyroid, Parathyroid, Adrenal and Pituitary Glands
27. Bones and Joints
28. Soft Tissue Tumors and Skeletal Muscle Non-Neoplastic Disorders
29. Skin
30. Nervous System

CHAPTER

14

Cellular–Molecular Diagnostic Techniques in Clinical Practice

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

FROZEN/CRYOSTAT MICROTOMY TECHNIQUES

- Frozen section technique
- Cryostat section technique
- Cutting of tissue section
  - Ultramicrotomy
  - Laser microtomy

ELECTRON MICROSCOPY AND POLARIZING LIGHT MICROSCOPY

- Electron microscopy
- Polarizing light microscopy

TISSUE FIXATION, DECALCIFICATION, HANDLING OF SURGICAL SPECIMENS AND HISTOCHEMICAL STAINS

- Tissue fixatives
- Tissue decalcification
- Handling of surgical specimens
- Histochemical stains

IMMUNOHISTOCHEMISTRY TECHNIQUE

- Immunohistochemistry technique: overview
- Detection systems of immunohistochemistry
- Immunohistochemistry protocol

IMMUNOFLUORESCENCE MICROSCOPY

- Immunofluorescence microscopy: overview
- Types of immunofluorescence microscopy
  - Direct immunofluorescence microscopy
  - Indirect immunofluorescence microscopy

CHEMILUMINESCENCE VERSUS CHEMIFLUORESCENCE TECHNIQUES

- Chemiluminescence technique
- Chemifluorescence technique

MOLECULAR DIAGNOSTICS TO ANALYZE CELL PROLIFERATION

- Ki-67 (MB-1) analysis
- Mitotic cell count analysis
- Flow cytometry technique

CYTOGENETIC ANALYSIS

- Cytogenetic analysis: overview
- Cytogenetic analysis techniques
  - Metaphase fluorescence *in situ* hybridization (FISH) for chromosomal karyotyping technique
  - Multiplex metaphase FISH for chromosomal karyotyping technique
  - Comparative genomic hybridization (CGH) technique
  - Microarray analysis technique

IN SITU HYBRIDIZATION, CHROMOGENIC *IN SITU* HYBRIDIZATION AND FLUORESCENCE *IN SITU* HYBRIDIZATION

- *In situ* hybridization: overview
- Chromogenic *in situ* hybridization: overview
- Fluorescence *in situ* hybridization: overview

ENZYME-LINKED IMMUNOSORBENT ASSAY TECHNIQUE

- Enzyme-linked immunosorbent assay (ELISA) technique: overview

DNA SEQUENCING METHODS

- Polymerase chain reaction (PCR) technique
- Southern blot technique
- Northern blot technique

TISSUE MICROARRAY (TMA) TECHNIQUE

- Tissue microarray technique: overview

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

- HPLC technique

HEMOGLOBIN ELECTROPHORESIS AND GEL ELECTROPHORESIS TECHNIQUES

- Hemoglobin electrophoresis technique
- Gel electrophoresis technique

FROZEN/CRYOSTAT MICROTOMY TECHNIQUES

FROZEN SECTION TECHNIQUE

Frozen section technique is used for rapid diagnosis, study of margins of malignant neoplasm, enzyme histochemistry, demonstration of lipid and molecular studies. Drawbacks of frozen section technique include distortion of morphology of tissue and cellular cytomorphologic details and poor staining. Tissue sample must reach the histopathology laboratory immediately. It should be kept in normal saline to prevent drying of sample. The tissue can directly be taken to the cryostat or can be fixed with 10% formalin or formalin-alcohol.

- Frozen sections are stained normally, because this is faster for one or a few individual sections. Toluidine blue stain generally used as a progressive stain to demonstrate adipose tissue in which the section is left in contact with the stain until the desired tint is achieved.

Table 14.1 Examples of fresh frozen section evaluation

Anatomic Sites	Indications	Comments
Skin	▪ Squamous cell carcinoma (eyelid, nose or ear) ▪ Basal cell carcinoma (eyelid, nose or ear) for evaluation of margins	Not indicated for melanoma skin
Breast	Breast carcinoma	Evaluation of margins is sometimes required
Lymph nodes	Metastases of breast carcinoma in lymph nodes (sentinel node)	Sensitivity (60%)
Thyroid	Papillary carcinoma of thyroid (imprint smear cytology for nuclear groove)	Not suitable for follicular neoplasms
Female genital system	Intraoperative ovarian neoplasm (most important)	Not suitable for cervical carcinoma
Pancreas	Intraoperative pancreatic carcinoma to look for margins	Accuracy 98%

- The Oil red O (ORO) stain can identify neutral lipids and fatty acids in touch smears and tissues.
- Fresh touch smears or cryostat sections of tissue are necessary because fixatives containing alcohols, or routine tissue processing with clearing will remove lipids.
- The Oil red O stain is used to identify lipid-laden macrophages in bronchoalveolar lavage (BAL) cytologic samples to diagnose fat embolic syndrome (FES).
- Other fat stains are Sudan black, Sudan III and Sudan IV. Examples of fresh frozen section evaluation are given in Table 14.1.

CRYOSTAT SECTION TECHNIQUE

Cryostat is a device by which temperature can be maintained at a low level. In histopathology, cryostat consists of a microtome within its chamber for cutting sections of frozen tissue. The cryostat is essentially an ultrafine 'deli-slicer', called a microtome, placed in a freezer. It is usually stationary upright freezer, with an external wheel for rotating the microtome.

- The temperature within cryostat can be varied, depending on the tissue being cut—usually – 20° to –30°C. Low temperature may be maintained within cryostat by using various refrigeration methods, most commonly using cryogenic fluid bath such as liquid helium or nitrogen.
- Although cryosections are physically less stable than paraffin-embedded tissue sections. Cryosections are generally superior for the preservation of antigenicity and therefore the detection of antigens by immunohistochemistry. The preparation of cryosections does not involve the dehydration process. Rapid freezing of tissue reduces the formation of ice crystals, thus minimizes morphologic damage.

CUTTING OF TISSUE SECTION

ULTRAMICROTOMY

Ultramicrotomy is a technique for cutting tissue specimens into extremely thin transparent sections. The ultrathin sections can be studied at different magnifications in a transmission electron microscope (TEM).

- Ultrathin sections must be very thin because the 50–125 kV electrons of the standard electron microscope cannot pass through biological material much thicker than 150 nm. For best resolutions, tissue sections must be 30–60 nm in thickness.
- Prior to the cutting ultrathin sections, the biological materials are usually placed in a more rigid fixative used in the process known as embedding. This is achieved by the inflow of a liquid substance around the sample, such as paraffin or epoxy.

LASER MICROTOMY

Recent development in the microtomy is the use of a laser microtome, which has a capacity to cut in few seconds. No sample preparation is required. The thickness of tissue sections varies 10–100 nm. Using a vibrating knife improves the results when dealing with soft materials. This is the only technique yielding directly 30 nm sections of constant thickness. Laser microtomy technique cannot be applied to very heterogenous, very hard, very soft and very fragile samples.

ELECTRON MICROSCOPY AND POLARIZING LIGHT MICROSCOPY

ELECTRON MICROSCOPY

Electron microscope (EM) was invented by Ernst Ruska in 1933, for which he was awarded the Nobel Prize in Physics in 1986.

- Electron microscopes are scientific instruments that use a beam of energetic electrons to examine objects on a very fine scale. It was developed due to the limitations of resolution power of light microscope that is limited by physics of light.
- Electron microscopes are basically of two types: (a) transmission electron microscopy (TEM); (b) scanning electron microscopy (SEM).
- Principle of light microscopy, transmission electron microscopy and scanning electron microscopy is shown in Fig. 14.1A to C. Technical processes used for electron microscopy is given in Table 14.2.

Table 14.2 Technical processes used for electron microscopy

Process	Methodology
Fixation of tissue	2% glutaraldehyde
Processing of tissue	▪ Application of tetraoxide in post-fixed tissue ▪ Dehydration done in a graded ethanols ▪ Infiltration of tissue with EM epoxy resin
Embedding	Tissue embedded in mold
Sectioning of tissue	▪ Heating of resin infiltrated tissue hardened into blocks ▪ Sections obtained with microtome ▪ Sections but on a specimen grid
Electron microscopy	▪ Section image studied under electron microscope ▪ Digital image obtained and analyzed

TRANSMISSION ELECTRON MICROSCOPY

In transmission electron microscopy (TEM) source is a beam of high velocity electrons accelerated under vacuum, focused by condenser lens (electromagnetic bending of electron beam) onto specimen, which causes loss and scattering of electrons by individual parts of specimen. Emergent electron beam is focused by objective lens. Final image is formed on a fluorescent screen for viewing.

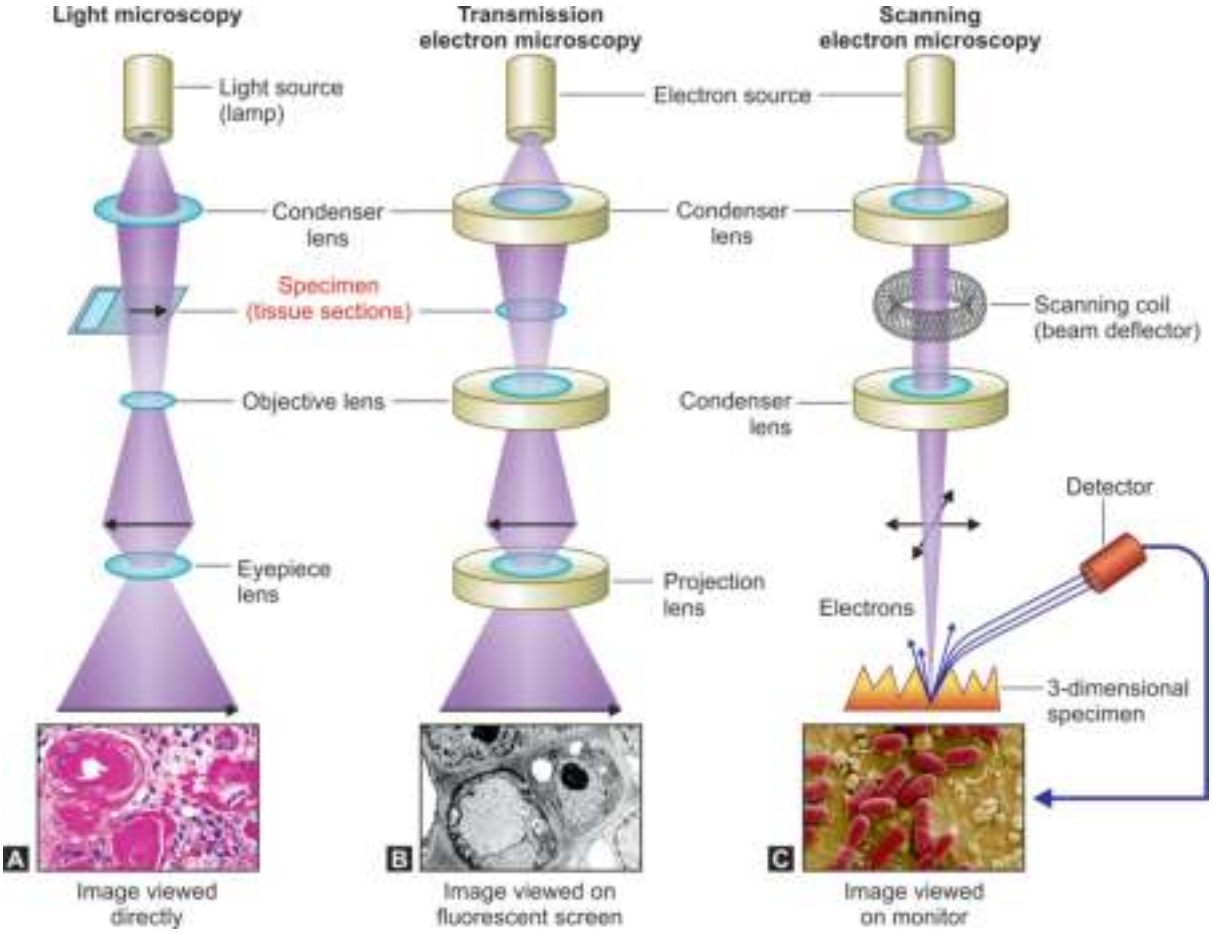


Fig. 14.1A to C: Principle of light microscopy, transmission electron microscopy and scanning electron microscopy.

- Standard transmission electron microscope has a resolving power to about 15–30 angstroms.
- Magnified images are typically formed from 1000X to 50,000X. By using fine grain photographic film, it is possible for further enlargements of images from 150,000X to 1,000,000X.

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) differs from standard transmission electron microscopy in that the electrons are bounced off the surface of an object with SEM, not passed through it. The major advantages of SEM are the great depth of field (more of the specimen can be in focus at once) and simple specimen preparation.

Principle of Scanning Electron Microscopy

The basic principle of scanning electron microscopy is that a beam of electrons is generated by a suitable source, typically a tungsten filament or a field emission gun.

- The electron beam is accelerated through a high voltage and passes through a system of apertures and electromagnetic lenses to produce a thin beam of electrons. Then electron beam scans the surface of the specimen.
- Electrons are emitted from the specimen by the action of the scanning electron beam and collected by a suitably positioned detector.
- Comparison between light microscope and electron microscope is given in Table 14.3. Comparison between transmission electron microscopy and scanning electron microscopy is given in Table 14.4.

Applications of Scanning Electron Microscopy in Surgical Pathology

Scanning electron microscopy is a useful diagnostic technique to supplement morphologic, immunohistochemical, cytogenetic and molecular analysis of tissues, although immunoperoxidase techniques have largely replaced EM for tumor diagnosis in surgical pathology.

- Scanning electron microscopy is used to diagnose alcoholic liver disease, genetic disorders, neoplasms, glomerulonephritis and identification of microorganisms.
- Diagnostic utility of electron microscopy in various disorders is given in Table 14.5.

POLARIZING LIGHT MICROSCOPY

Some materials such as crystals or fibers have the property of birefringence which is the ability to pass light in a particular plane. Such materials (crystals or fibers) are called anisotropic substances because of this property.

- Normally, most materials are isotropic substances because any light that passes through them will be scattered in all directions.

Table 14.3 Comparison between light microscope and electron microscope

Light Microscope	Electron Microscope
Small and portable	Very large and operated in a special room
Resolution is 200 nm	Resolution is 0.4 nm (in SEM) or 0.5 Å (in TEM)
Magnification is 1000X	Magnification is 2 million (in SEM) or 50 million (in TEM)
It is unaffected by magnetic fields	It is affected by magnetic fields
Preparation of material is quick and simple	Preparation of material is lengthy and requires expertise
Preparation rarely distorts the material	Preparation may distort the material
Cheap to purchase and operate	Expensive to purchase and operate
Natural color of object is observed	Images are in black and white

Table 14.4 Comparison between transmission electron microscopy and scanning electron microscopy

Transmission Electron Microscopy (TEM)	Scanning Electron Microscopy (SEM)
TEM is based on transmitted electrons	SEM is based on scattered electrons
Electrons are directly pointed towards the sample in TEM	The scattered electrons in SEM produce the image of sample after the microscope collects and counts the scattered electrons
TEM shows the sample as a whole	SEM shows the sample bit by bit
TEM has up to 50 million magnification	SEM has up to 2 million magnification
TEM provides a two-dimensional image	SEM provides a three-dimensional image
The resolution in TEM is 0.5 Å (1 Å = 10^{-10} m)	The resolution in SEM is 0.4 nm (1 nm = 10^{-9} m)

Table 14.5 Diagnostic utility of electron microscopy in various disorders

Subcellular Features in Cell or Tissue	Diagnostic uses in Clinical Practice
Genetic disorders	
▪ Cilia in epithelial cells	▪ Primary ciliary dyskinesis
▪ Lysosomes in neurons	▪ Demonstration of lipids and mucopolysaccharides in tissues
▪ Absence of peroxisomes in liver and kidneys	▪ Zellweger syndrome and neonatal adrenoleukodystrophy
Acquired non-neoplastic disorders	
▪ Increased number of peroxisomes in hepatocytes	▪ Alcoholic liver disease, chronic venous congestion, oral contraceptives
▪ Siderosomes in mitochondria	▪ Sideroblastic anemia
Neoplasms	
▪ Intercellular junctions in epithelial neoplasms and lymphoma	▪ Distinction between carcinomas and lymphomas (intercellular junctions present in carcinomas and absent in lymphomas)
▪ Intracellular or intercellular in glandular epithelium	▪ Diagnosis of adenocarcinoma
▪ Microvillous core rootlets in glandular epithelium of intestines	▪ Diagnosis of metastatic deposits from gastrointestinal carcinoma
▪ Cytoplasmic tonofibrils in squamous epithelium	▪ Diagnosis of squamous differentiation of epithelial neoplasms
▪ Premelanosomes and melanosomes in melanotic cells	▪ Diagnosis of melanoma
▪ Neurosecretory granules in neuroendocrine and neuroectodermal cells	▪ Diagnosis of neuroendocrine and neuroectodermal neoplasms
▪ Birbeck granules in Langerhans' cells	▪ Diagnosis of Langerhans' cell histiocytosis
Miscellaneous disorders	
▪ Viruses and parasites in solid tissues, fecal specimens and body fluids	▪ Identification of infectious agents in tissues
▪ Electron dense deposits in glomeruli	▪ Identification and classification of glomerulonephritis
▪ Electron dense deposits present adjacent to vascular smooth muscle	▪ Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) syndrome

- When viewed under polarized light, however, anisotropic materials will be brightly visible in one plane (birefringent), but will be dark in a plane turned 90°. Principle of polarizing microscope is shown in Fig. 14.2A and B.

POSITIVE/NEGATIVE BIREFRINGENCE

The birefringence is categorized into positive or negative based on the property of birefringence in which rays of light travelling through the anisotropic material

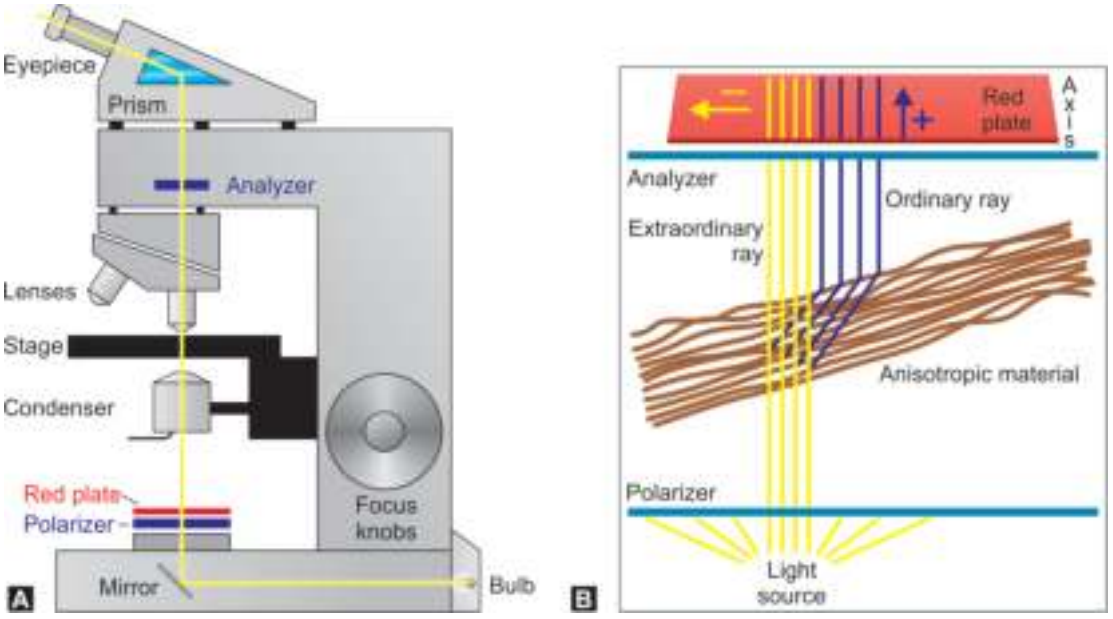


Fig. 14.2A and B: Principle of polarizing microscope.

in perpendicular planes (at right angles) will travel at different velocities through the material.

BIREFRINGENT MATERIAL REFRACTIVE INDEX

Birefringent material has two refractive indices: high for fast rays and small for slow rays. Fast rays travel in parallel with the crystalline structure of the birefringent material than the extraordinary's low rays traverses the material. Negative birefringence occurs when the ordinary ray becomes the slow ray when it is reflected and travels across the crystalline structures.

POLARIZER AND ANALYZER FILTERS IN POLARIZING MICROSCOPE

There are two polarizing filters made of glass or plastic material in polarizing microscope: polarizer and analyzer. These filters pass light in only one plane.

APPLICATIONS OF POLARIZING MICROSCOPY

Exogenous crystalline material, such as talc crystals, is demonstrated by polarizing microscopy found in subcutaneous injection site, lungs, and organs of the mononuclear phagocyte system of persons engaging in injection drug use. Applications of polarizing microscopy in clinical practice are given in Table 14.6.

Table 14.6 Applications of polarizing microscopy in clinical practice

Disorders	Characteristics
Sodium urate crystals gout	Negative birefringence
Calcium pyrophosphate crystals	Positive birefringence
Amyloid deposits in various organs	Apple green birefringence
Silicosis in organs	Insignificant finding in lungs and lymph nodes
Formalin-heme pigment (artefact of poor fixation)	Bright white birefringence
Collagen: Collagen fibrils	Dull yellow-white

TISSUE FIXATION, DECALCIFICATION, HANDLING OF SURGICAL SPECIMENS AND HISTOCHEMICAL STAINS

TISSUE FIXATIVES

Tissue fixatives preserve tissues in their natural state and fixes all components by denaturing or precipitating proteins. The ideal tissue fixative would prevent autolysis and bacterial decomposition. Microorganisms are also fixed and killed during tissue fixation. Most tissue fixatives act by denaturing or precipitating proteins which then form a sponge or meshwork tending to hold the other cell constituents. Formaldehyde is tissue fixative of choice.

CLASSIFICATION OF TISSUE FIXATIVES

Tissue fixatives are classified according to mechanism of action: aldehydes (formaldehyde and glutaraldehyde), mercurials, alcohols, oxidizing agents and picrates (Table 14.7), routine 10% formalin saline tissue fixative (Table 14.8), routine 10% buffered formalin tissue

Table 14.7 Tissue fixatives used in histopathology

Categories of Tissue Fixatives	Examples of Tissue Fixatives	Advantages of Tissue Fixatives	Disadvantages of Tissue Fixatives
Aldehyde fixatives	▪ Neutral formalin (10%) ▪ Buffered formalin (10%) ▪ Buffered glutaraldehyde (%)	▪ Immunoperoxidase techniques ▪ Immunoperoxidase techniques ▪ Prevents formalin-heme pigment ▪ Electron microscopy	▪ Formalin-heme pigment ▪ Nil ▪ Not good for immunoperoxidase techniques ▪ Nil
Mercurial fixatives	Zenker's fixative	Hematopoietic and reticuloendothelial tissues, i.e. lymph nodes, spleen, thymus, and bone marrow	Marked shrinkage and hardness of tissues. It reduces the amount of demonstrable glycogen
Alcohol fixatives	Carnoy's fluid	▪ Suitable for small biopsies giving nuclear details ▪ Preserves glycogen	Causes lysis of RBCs, dissolution of lipid and myelin; and shrinkage of tissue

Contd...

Table 14.7 Tissue fixatives used in histopathology (*Contd...*)

Categories of Tissue Fixatives	Examples of Tissue Fixatives	Advantages of Tissue Fixatives	Disadvantages of Tissue Fixatives
Chromate fixatives	Helly's fluid	Used to demonstrate chromaffin tissue (adrenal medulla), mitochondria and preservation of phospholipids	Glycogen preservation poor
Picrate fixatives	Bouin's fluid fixative (40% formalin, picric acid and glacial acetic acid)	▪ Testicular, gastrointestinal tract, and endocrine tissue biopsies ▪ Gives yellow color to the tissue and gives good nuclear details	▪ Makes tissue hard and brittle ▪ Lysis of RBCs, reduce amount of demonstrable ferric iron

Table 14.8 Routine 10% formalin saline tissue fixative

Constituents	Composition
Formalin	100 ml
Water	900 ml
Sodium chloride	8.5 g

Disadvantage: Routine 10% formalin saline fixative forms formalin-heme pigment giving black discoloration to tissues.

fixative (Table 14.9), composition of Zenker's tissue fixative (mercurial fixative) (Table 14.10), Schaudinn's sublimated alcohol tissue fixative (mercurial tissue fixative) (Table 14.11), composition of Carnoy's fluid (alcohol tissue fixative) (Table 14.12), Orth's fluid tissue fixative (chromate tissue fixative) (Table 14.13) and Regaud's (Moller's) fluid (chromate tissue fixative) (Table 14.14).

Table 14.9 Routine 10% buffered formalin tissue fixative

Constituents	Composition
Formalin	100 ml
Water	900 ml
NaH_2PO_4	3.5 g
Na_2HPO_4	6.5 g

Advantage: 10% buffered formalin prevents formation of formalin-heme pigment.

Table 14.10 Composition of Zenker's tissue fixative (mercurial tissue fixative)

Constituents	Composition
$HgCl_2$	50 g
Potassium dichromate	25 g
Sodium sulfate	10 g
Distilled water	1000 ml

Mercurial fixatives are used to fix lymph nodes, spleen, thymus, and bone marrow.

Table 14.11 Schaudinn's sublimated alcohol tissue fixative (mercurial tissue fixative)

Constituents	Quantity
$HgCl_2$	3 g
Distilled water	50 ml

Schaudinn's sublimated alcohol fixative is most useful fixative for making smears of loose cells on a slide.

Table 14.12 Composition of Carnoy's fluid (alcohol tissue fixative)

Constituents	Quantity
Absolute ethyl alcohol	60 ml
Chloroform	30 ml
Glacial acetic acid	10 ml

Table 14.13 Orth's fluid tissue fixative (chromate tissue fixative)

Constituents	Composition
Potassium dichromate (2.5%)	100 ml
Formalin solution	10 ml

Formalin solution is added just before using as fixative.
Chromate fixatives are used to demonstrate chromaffin tissue (adrenal medulla), mitochondria, Golgi apparatus and mitotic figures.

Table 14.14 Regaud's (Moller's) fluid (chromate tissue fixative)

Constituents	Composition
Potassium dichromate (3%)	80 ml
Formalin	20 ml

Formalin solution is added just before using it as fixative.
Chromate fixatives are used to demonstrate chromaffin tissue (adrenal medulla), mitochondria, Golgi apparatus and mitotic figures.

Table 14.15 Factors affecting fixation of tissue

Parameters	Characteristics
Concentration of fixative	▪ Formalin 10% used for tissue fixation ▪ Glutaraldehyde 0.25–4% used for electron microscopy
Volume of fixative	10–20 times of tissue sample
Agitation of specimen	Enhances tissue fixation
Buffers used to prevent formalin-heme pigment	▪ Neutral buffered formalin prevents excessive acidity and formation of formalin-heme pigment ▪ Common buffers (phosphate, bicarbonate, cacodylate and veronal)
Penetration of tissue fixative	▪ Formalin and alcohol fixatives (best penetration) ▪ Glutaraldehyde (worst penetration) ▪ Mercurial fixatives somewhat in between ▪ Sectioning of specimen 2–3 cm enhances fixation
Temperature	Increasing temperature of formalin enhances the speed of tissue fixation
Time interval between fixation and removal of tissue	Tissue should be fixed faster and removed within 6–8 hours. Longer tissue fixation leads to nuclear shrinkage and loss of organelles

Formalin used to fix tissue forms formalin-heme pigment.

FACTORS AFFECTING TISSUE FIXATION

There are a number of factors that will affect the fixation process: volume, buffering, penetration, temperature, concentration and time interval. Fixation is best carried out close to neutral pH, in the range of 6–8. Commercial formalin is buffered with phosphate at a pH of 7. Factors affecting fixation of tissue are given in Table 14.15.

TISSUE DECALCIFICATION

Bone and other tissues containing calcified areas and calcium deposits are extremely firm and which will not section properly with paraffin embedding owing to the difference in densities between calcium and paraffin. Hence, calcium must be removed prior to embedding to allow tissue sectioning. Mineral acids (nitric acid and hydrochloric acid), organic acids (acetic acid and formic acid), EDTA, and electrolysis are used to decalcify such tissues.

STRONG ACIDS

Strong acids, i.e. nitric acid and hydrochloric acid are used to decalcify dense cortical tissue at a rapid rate; but also damage cellular details. These are not recommended for delicate tissues such as bone marrow.

ORGANIC ACIDS

Organic acids such as acetic acid and 10% formic acid are better suited to bone marrow, because these act more slowly on dense cortical bone. Formic acid in 10% concentration is the best all-around decalcifier.

ETHYLENEDIAMINETETRA-ACETIC ACID

Ethylenediaminetetra-acetic (EDTA) acid can remove calcium slowly but is expensive in large amounts. Electrolysis removes calcium slowly, hence not suited for routine purpose.

COMMERCIAL DECALCIFYING SOLUTIONS

Commercial decalcifying solutions containing formic acid with formalin is available to decalcify tissues.

HANDLING OF SURGICAL SPECIMENS

Under normal circumstances, the specimens are received in 10% neutral buffered formalin. Tissue fixation process prevents autolysis and decomposition of specimen. Gross processing of surgical specimens comprises description of size, shape, color and overall appearance. The tissues placed in cassettes are designated number. When a malignancy is suspected, then the specimen is often covered with ink in order to mark the margins of the specimen.

AUTOMATIC TISSUE PROCESSOR

This multistep process is done by an automatic machine which has multiple jars containing different chemicals, i.e. formalin, ascending series of alcohols, xylene and paraffin through which tissue is passed on a preset time scale. Automatic tissue processor with digitally programmed schedule is given in Table 14.16.

PARAFFIN EMBEDDING OF PROCESSED TISSUE

Properly fixed and processed tissue in the tissue processor are embedded in the molds containing paraffin with melting point 54–62°C to prepare them for cutting in microtome. During paraffin embedding, the tissue is oriented with the surface placed down in the mold becoming the face of tissue block.

MICROTOMY

Tissue embedded in paraffin can be sectioned at 4–8 microns in thickness with microtome having thick metallic knife.

- Tissue ribbon is then floated on warm water bath at a temperature 6–8°C below the melting point of paraffin, which aids in mounting tissue sections on a glass slide properly labelled for light microscopy.

Table 14.16 Automatic tissue processor (histokinette) with digitally programmed schedule

Jar Numbers	Chemical Used	Time Variable Depending on Convenience and Fixation of Tissues	Step
1.	70% alcohol	½ hour	Dehydration of tissue is done to replace fixative and water by a dehydrating fluid
2.	95% alcohol	½ hour	
3.	100% alcohol	1 hour	
4.	100% alcohol	1 hour	
5.	100% alcohol	1 hour	
6.	100% alcohol	1 hour	
7.	100% alcohol	1 hour	
8.	100% alcohol + xylene	1 hour	Clearing is performed to replace dehydrating fluid with a medium totally miscible with embedding medium (molten paraffin wax)
9.	Xylene	1 hour	
10.	Xylene	2 hours	
11.	Molten paraffin wax	2 hours	A vacuum can be applied inside the tissue processor to assist penetration of the embedding paraffin agent. This process replaces water content of tissue and clearing agent by paraffin wax used in molten state
12.	Molten paraffin wax	2 hours	

- Thin tissue sections for electron microscopy are best obtained with a diamond knife which is very expensive. After embedding tissues in epoxy resin, a microtome equipped with a glass or diamond knife is used to cut very thin sections (typically 60–100 nm). Thin tissue sections are stained and examined with a transmission electron microscope.

HEMATOXYLIN AND EOSIN STAINING

The staining process makes use of a variety of dyes that have been chosen for their ability to stain various cellular components of tissue. Hematoxylin and eosin (H&E) stain is routinely done.

- Hematoxylin had been derived from the logwood tree in the past. Eosin at pH 4.6–5.0 has high affinity for positively charged tissue protein group.
- However, special histochemical stains are employed in specific situations according to the diagnostic need.
- Commercially ripened synthetic hematoxylin is commonly used due to less availability of logwood tree. Hematoxylin is the oxidized/ripened product of the logwood tree known as hematein.
- Hematoxylin, a basic dye has an affinity for the nucleic acids of the cell. Eosin, an acidic dye has affinity for cytoplasmic components of the cell. Problem with eosin may occur due to overstaining of decalcified tissues.
- Routine hematoxylin and eosin staining technique in surgical pathology is given in Table 14.17. Applications of hematoxylin stains for different purpose in surgical pathology are given in Table 14.18.

Table 14.17 Routine hematoxylin and eosin staining technique in surgical pathology

Steps	Purpose	Procedure
Step 1	Deparaffinization of tissue section	Deparaffinize the section with xylene (three changes)
Step 2	Rehydration of tissue section in graded alcohol	Rehydration is done by putting section in graded alcohol and then water for 1–2 minutes (2 changes)
Step 3	Nuclear staining by hematoxylin	Put hematoxylin on section and keep for 5–15 minutes
Step 4	Differentiation of tissue section	Differentiation of section is done by putting in 1% acid alcohol (2 dips and then wash section in tap water
Step 5	Bluing of tissue section in alkaline solution (ammonia water)	Wash in running tap water for 5 minutes until section becomes blue
Step 6	Counterstaining of tissue section by eosin	1% eosin Y for 2–3 minutes
Step 7	Dehydration of tissue section	Put section in graded alcohol (70%, 95%, 100%) for 3–5 minutes in each graded alcohol or until excess of eosin is removed
Step 8	Clearing of stained section	Clearing of section in xylene two charges of two minutes each
Step 9	Mounting of stained section	Mount section in DPX (mixture of distyrene, a plasticizer and xylene)

Table 14.18 Applications of hematoxylin stains for different purpose in surgical pathology

Hematoxylin Types	Purpose
Harris hematoxylin	▪ Routine stain on histologic sections and cytology smears (nuclei blue, cytoplasm pink) ▪ Stain acidophilic in nature and lacks alcohol
Mayer's hematoxylin	▪ Carbohydrates ▪ Stain contains alcohol
Baker's acid-hematein	Phospholipid
Tungsten hematoxylin	▪ Fibrin ▪ Cross striations of skeletal muscle
Verhoeff's iron hematoxylin	Strong affinity for connective tissue fibers
Iron hematoxylin	▪ *Entamoeba histolytica* ▪ *Wuchereria bancrofti*
Gill's hematoxylin	▪ Conventional stain used on histologic sections, cytology smears and immunohistochemistry ▪ Nuclear chromatin
Heidenhain's iron hematoxylin	▪ Photomicrography ▪ Skeletal muscle striations ▪ Mitotic structures blue black
Ehrlich's hematoxylin	▪ Counterstaining in immunohistochemistry and cytochemistry ▪ Nuclear stain

Mordant to Tissues

Hematoxylin will not directly stain tissues, but needs a mordant or link to the tissues, i.e. metal cation iron, aluminum, or tungsten. Depending on the mordant used, there is variety of hematoxylins, which vary in intensity.

Progressive Hematoxylin Staining

Hematoxylin and eosin staining can be either progressive or regressive. With a progressive staining the slide is dipped in the hematoxylin until the desired intensity of staining is achieved such as with a frozen section. This is simple for a single slide, but lends itself poorly to batch of slides processing.

Regressive Hematoxylin Staining

With a regressive hematoxylin and eosin staining, the slides are left in the solution for a set period of time and then taken back through a solution such as acid alcohol that removes part of the stain. This method works best for large batches of slides to be stained and is more predictable on a day-to-day basis.

HISTOCHEMICAL STAINS

Commonly used stains in histopathology are given in Table 14.19. Carbohydrate classification for the purpose of histochemical staining is given in Table 14.20. Mucin types and their origin for the purpose of histochemical staining are given in Table 14.21.

Table 14.19 Commonly used stains in histopathology

Special Stains	Uses	Result
Hematoxylin and eosin stain	Routine stain	Nucleus blue, cytoplasm pink, bone blue, mucoid and cartilaginous area pale blue, RBC and eosinophil granules orange red
Stains for carbohydrates		
Periodic acid–Schiff's stain	▪ Glycogen ▪ Fungi ▪ Neutral mucin ▪ Amyloidosis ▪ Basement membrane ▪ Glycoproteins ▪ Glycolipids	▪ Red color ▪ Red color ▪ Red color ▪ Red color ▪ Red color ▪ Red color ▪ Red color
Periodic acid–Schiff with diastase stain	Neutral mucin	
Alcian blue stain at pH 2.5	▪ All acidic mucopolysaccharides ▪ Sulphated mucin	▪ Blue color ▪ Blue color
Mucicarmine stain	▪ Acidic epithelial mucin ▪ Cryptococcus capsule	▪ Blue color ▪ Blue color

Contd...

Table 14.19 Commonly used stains in histopathology (*Contd...*)

Special Stains	Uses	Result
Colloidal iron stain pH 1	Prussian blue reaction (Perls reaction) to demonstrate hemosiderin	Blue color
Congo red	Amyloid	▪ Pink color under light microscope ▪ Green birefringence for under polarizing microscope
Methyl violet (metachromatic stain)	Amyloid	Rose pink color
von Gieson stain	Amyloid	Khaki color
Crystal violet (metachromatic stain)	Amyloid	Pink color
Thioflavin T and thioflavin S	Amyloid	Yellow to yellow green color under immunofluorescent microscopy
Stains for microorganisms		
Bacteria		
Ziehl-Neelsen (ZN) stain (20% H_2SO_4)	*Mycobacterium tuberculosis*	Red bacilli
Auramine-rhodamine	*Mycobacterium tuberculosis*	Yellow fluorescence in auramine-rhodamine stain
Fite acid-fast stain (5% H_2SO_4)	*Mycobacterium leprae*	Red bacilli
Papanicolaou's stain, May-Grünwald Giemsa stain and Gomori methenamine silver stain	Actinomycosis	Clustered bacilli with sunray appearance
May-Grünwald Giemsa stain, Gram stain, Modified acid-fast stain	Nocardia	Thin slender bacilli
May-Grünwald Giemsa stain, hematoxylin and eosin stain, Gram stain, Steiner's stain	*Helicobacter pylori*	Flying bird-like bacilli in row
Warthin-Starry stain	*Treponema pallidum*	Black color against yellow to pale brown background
Fungi		
Papanicolaou's stain, May-Grünwald Giemsa stain, Gomori methenamine stain and periodic acid–Schiff (PAS) stain	*Candida albicans*	Thin slender-like pseudohyphae and spores
Papanicolaou's stain, periodic acid–Schiff (PAS), hematoxylin and eosin stain, Gram stain	Aspergillus	Slender bacilli with acute angle branching
Papanicolaou's stain, hematoxylin and eosin stain, Gomori methenamine silver stain	Mucormycosis	Broad wide-angle branching, nonseptate
Mucicarmine stain, Masson-Fontana silver stain	*Cryptococcus neoformans*	Small 3–5 µm round structure
Gomori methenamine silver stain, periodic acid–Schiff (PAS) stain	*Histoplasma capsulatum*	Small 2–3 µm round structure
Gomori methenamine silver stain	*Pneumocystis jiroveci*	Organism appears as ping pong balls or folded spheres
Parasites		
Periodic acid–Schiff (PAS) stain, hematoxylin and eosin stain, iron hematoxylin and eosin stain	*Entamoeba histolytica*	Round-shaped small organism
Hematoxylin and eosin stain	*Giardia lamblia*	Pear-shaped small organisms
Hematoxylin and eosin stain, Gomori methenamine silver stain, periodic acid–Schiff (PAS) stain	*Echinococcus granulosus*	Hooklets demonstrated on fine needle aspiration cytology

Contd...

Table 14.19 Commonly used stains in histopathology (*Contd...*)

Special Stains	Uses	Result
Stains for connective tissue		
Reticulin stain (used to highlight outlines of parenchyma of organs and highlighting growth pattern of neoplasia)	Reticulin fibers	Black against clear background
Jones methenamine silver stain	Similar to reticulin stain	
Masson's trichrome stain (used in chronic active hepatitis with hepatocytes collapse, cerebral abscess and scleroderma with fibrosis of gastric submucosa)	▪ Elastic fibers ▪ Collagen fibers ▪ Background	▪ Black color ▪ Red color ▪ Yellow color
Verhoeff-van Gieson (VVG) stain (used to outline arteries)	Elastic fibers	Black color
PTAH (phosphotungstic acid hematoxylin)	Cross striations in muscle fibers	Deep purple color
Pentachrome stain	▪ Elastic fibers ▪ Collagen fibers ▪ Fibrin ▪ Muscle ▪ Mucin	▪ Black color ▪ Red color ▪ Red color ▪ Red color ▪ Red color
Oil red O	Fat stain	Red color
Stains for nervous system		
Bielschowsky's stain	Nerve endings, neuron fibrils, tangles and plaques	Black color
Luxol fast blue	Myelin	Blue color
Pigments and minerals		
Perls Prussian blue reaction	Iron	Blue color
Hall's bile	Bile pigments	Emerald green color
von Kossa stain	Calcium	Black color
Alzarin red S (pH 4.8)	Calcium	Red color
Rhodamine stain	Copper	Red to orange color
Gomori's methenamine silver stain	Urate crystals	Black color
Masson-Fontana stain (used in melanoma)	Melanin	Black color
Churukian-Schenk stain	Argyrophil granules	Black color

PAS reactive tissue components are glycogen, starch, mucin, α_1-antitrypsin, fungal capsule, pancreatic zymogen granules, thyroid, colloid, corpora amylacea and plasma cell of Russell bodies.

Table 14.20 Carbohydrate classification for the purpose of histochemical staining

Parameters	Simple Carbohydrates	Glycoconjugates
Composition	Composed of pure carbohydrate	Composed of carbohydrates and other molecules such as protein and lipid
Examples	▪ Monosaccharides (glucose, mannose, galactose) ▪ Oligosaccharides (sucrose, maltose) ▪ Polysaccharides (glycogen, starch)	▪ Proteoglycans ▪ Mucins ▪ Other glycoproteins

Table 14.21 Mucin types and their origin for the purpose of histochemical staining

Parameters	Neutral Mucins	Acidic Simple, Nonsulfated Epithelial Mucins (Sialomucins)	Acidic Complex, Sulfated Epithelial Mucins (Sulfomucins)	Acidic Simple, Mesenchymal Mucins Contain Hyaluronic Acid	Acidic Complex, Connective Tissue Mucins
Location of mucin	▪ Surface epithelia of gastric mucosa ▪ Brunner's glands and prostatic epithelia	▪ Bronchial submucous glands ▪ Salivary glands ▪ Goblet cells	Bronchial mucous glands	Sarcomas	Tissue stroma, cartilage, and bone (chondroitin sulfate or keratan sulfate)
PAS stain	Positive	Positive	Positive	Positive	Negative
Alcian blue at pH	Negative	Positive (pH 2.5)	Positive (pH 1)	Negative	Selectively positive at pH 0.5
Colloidal iron	Negative	Positive	Positive	Negative	Negative
Mucicarmine	Negative	Positive	Positive	Negative	Negative
Metachromatic stain	Negative	Positive	Positive	Negative	Negative

PAS stain is used to demonstrate mucin and glycogen. Mucicarmine stain is specific for epithelial mucins. Acidic sulfomucins resist hyaluronidase digestion.

IMMUNOHISTOCHEMISTRY TECHNIQUE

IMMUNOHISTOCHEMISTRY TECHNIQUE: OVERVIEW

Immunohistochemistry (IHC) technique is the most powerful and widely used ancillary methods in surgical pathology to detect antigens in tissue sections by use of labeled antibody through antigen–antibody reactions. These are visualized by using fluorescent dye, enzyme, radioactive element or colloidal gold.

- Immunofluorescence microscopy is still used in the evaluation of medical renal biopsies. Currently, antigens are detected in formalin fixed paraffin-embedded tissue sections by immunoperoxidase technique.
- Common indications for immunohistochemistry technique are characterization of neoplasms, detection of infectious organisms and evaluation of prognostic or predictive factors.

TISSUE FIXATION

Tissue should be fixed in neutral pH formalin for 12–48 hours at room temperature. It is worth mentioning that formalin induces crosslinks that may mask some epitopes resulting in loss of immunoreactivity. Decalcification of bone samples also reduces immunoreactivity.

TISSUE SECTION USED FOR IMMUNOSTAINING

Unstained tissue sections are cut from paraffin block and placed on charged slides coated with poly-L-lysine or gelatin or albumin. Immunostaining should be performed immediately because unstained sections exposed to air for prolonged time may lose antigen immunoreactivity.

Frozen Section

If the unfixed tissue is frozen, the tissue sections obtained may need to be used for immunoassays. Tissue antigens in frozen sections are not altered. Frozen tissue sections may fall off the slides.

Formalin-fixed Paraffin-embedded Section

If the tissue is formalin-fixed paraffin-embedded, deparaffinize tissue sections to remove the infiltrated paraffin wax by using organic solvents. The tissue section then needs to be rehydrated by sequential immersion in graded alcohols (100%, 70%, 50% and then PBS, i.e. phosphate buffered saline). The deparaffinized tissue section may need to be treated to expose buried antigenic epitopes with either proteases or by heating in low pH citrate buffer or high pH EDTA buffer (antigen retrieval).

CLASSIFICATION OF ANTIBODIES

Antibodies can also be classified as primary or secondary antibodies used in immunohistochemistry technique.

PRIMARY ANTIBODIES

Primary antibodies are immunoglobulin molecules that bind to the target antigen in the tissue sections. Antibodies used for specific detection can be

Table 14.22 Comparison between polyclonal antibody and monoclonal antibody

Parameters	Polyclonal Antibody	Monoclonal Antibody
Synthesis	Mixture of synthesis of different antibodies to a single antigen	Synthesis by single clone commonly raised in mice
Specificity	Multiple epitopes	Single epitope

monoclonal derived via the hybridoma technique or polyclonal obtained from an antiserum (Table 14.22). Primary antibodies are unconjugated antibodies. In general, monoclonal antibodies are considered to exhibit greater specificity than polyclonal antibodies.

SECONDARY ANTIBODIES

Secondary antibodies are raised against primary antibodies, which recognize immunoglobulin of a particular species. These secondary antibodies are not visible with standard microscopy, hence labeled with fluorochrome (fluorescein, rhodamine), enzymes (horseradish peroxidase, alkaline phosphatase). Labeling secondary antibody is done in such a manner that it does not interfere with their binding specificity. For electron microscopy study, scattering compounds such as ferritin and colloidal gold are used for labeling.

BACKGROUND STAINING

Background staining occurs due to binding of nonspecific antibody or endogenous enzymes to the chromogenic substrate. Therefore, working dilution of primary antibody must be done, that yields contrast between specific staining and background staining. Endogenous enzymes present in red blood cells, neutrophils, eosinophils, monocytes/macrophages and neoplastic cells yield background staining. Endogenous peroxidase activity can be blocked by incubation with hydrogen peroxide.

DETECTION SYSTEMS OF IMMUNOHISTOCHEMISTRY TECHNIQUE

The principle of immunohistochemistry technique is to localize antigens in tissue sections by the use of labeled antibodies as specific reagents through antigen–antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold.

DIRECT CONJUGATE-LABELED ANTIBODY METHOD FOR IMMUNOSTAINING

Direct conjugate-labeled antibody method is the application of labeled nonspecific antibody directly to tissue section obtained from skin or renal biopsies. It is one step immunohistochemical staining technique, that utilizes only one antibody.

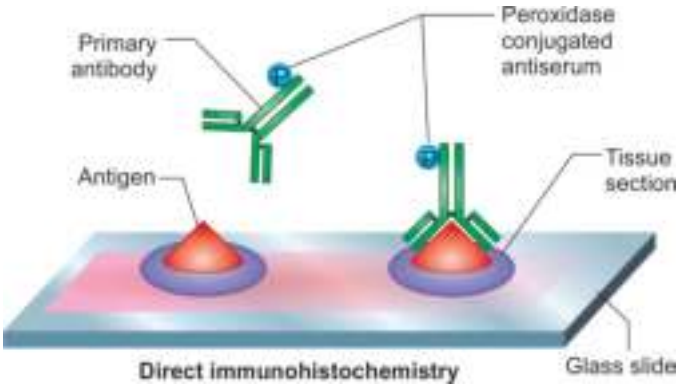


Fig. 14.3: Direct method. Enzyme-labeled primary antibody reacts with tissue antigen.

- The immunomarker then demonstrates the cellular location of the antigen in the tissue section. The label used can be an enzyme, biotin, fluorochrome or colloidal gold.
- There are several procedures that are being used to enhance the direct conjugate-labeled antibody method. The most common one is the biotin label to conjugate the antibody, and then the biotin will bind to the receptor of either the labeled avidin or streptavidin.
- With the second layer of labeling, the staining becomes simplified, which gives quick results, however, labeling intensity is low. Drawback of this procedure is requirement of large amount of antibody for labeling. Secondly, there is lack of signal amplification (Fig. 14.3).

INDIRECT OR SANDWICH METHOD FOR IMMUNOSTAINING

Indirect or sandwich method includes two major procedures: (a) indirect labeled procedure, and (b) indirect unlabeled antibody procedure.

- Indirect or sandwich medhod for immunostaining is more sensitive due to signal amplification through secondary reactions with different antigens. In addition, this method is also economical. Since one labeled second layer of antibody can be used with many first layer antibody to different antigens.
- Indirect labeled procedure has two layers of antibodies. The primary unlabeled antibody is specific

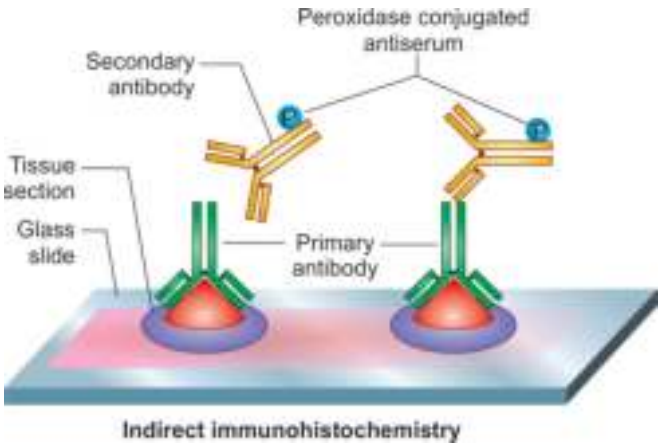


Fig. 14.4: Two-step indirect method of immunostaining. Enzyme-labeled secondary antibody reacts with primary antibody bound to tissue antigen, if the primary antibody is prepared in rabbit or mouse, the secondary antibody must be directed against rabbit or mouse immunoglobulins, respectively.

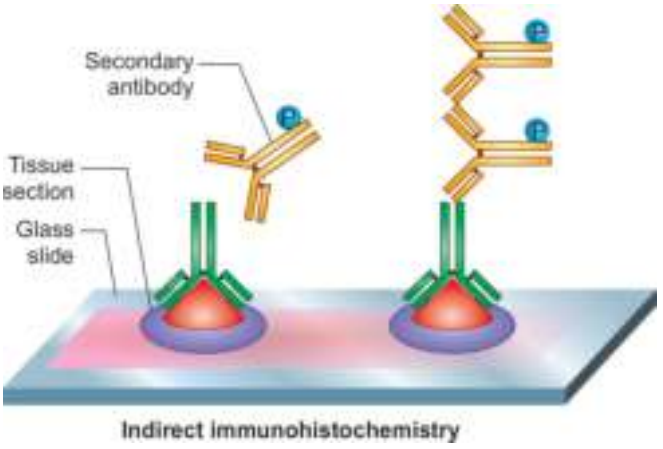


Fig. 14.5: Peroxidase–antiperoxidase (PAP) indirect method of immunostaining. In this three-step indirect method, enzyme-labeled tertiary antibody reacts with enzyme-labeled secondary antibody. Three-step indirect method provides a simple way to further increase the staining intensities compared to the two previous techniques.

for a particular tissue antigen. The secondary labeled antibody with fluorescent dye or peroxidase enzyme is an anti-immunoglobulin antibody. The marker then identifies the sites of the tissue antigen.

- The unlabeled antibody method has three layers of antibodies. The primary antibody is specific for a particular antigen. The secondary unlabeled antibody is an anti-immunoglobulin that reacts to both the primary antibody and to the tertiary antibody. The tertiary antibody is an anti-enzyme antibody, which reacts to a particular enzyme is demonstrated by its reaction with the substrate. Two-step indirect method of immunostaining is shown in Fig. 14.4.

Peroxidase–Antiperoxidase (PAP) Indirect Method

Unlabeled antibody technique is also known as peroxidase–antiperoxidase (PAP) indirect method, which uses an unlabeled primary antibody, an unlabeled bridge antibody and an antiperoxidase antibody and peroxidase molecule.

- Unlabeled bridge antibody directed against both primary antibody and the antiperoxidase antibody links the primary antibody to tissue antigen via signal generated by peroxidase. The sensitivity is about 100–1000 times higher since peroxidase molecule is not chemically conjugated with anti-IgG.
- Although peroxidase molecule is immunologically bound, yet it does not lose its enzymatic activity. It also permits much higher dilution of primary antibody resulting in elimination of many unwanted antibodies and reducing nonspecific background staining. Peroxidase–antiperoxidase (PAP) indirect method of immunostaining is shown in Fig. 14.5.

Pathology Pearls: Immunoperoxidase Staining

Tissue antigen markers that can be demonstrated by immunoperoxidase staining of formalin fixed paraffin sections as mentioned below:

- CD45 is a common hematopoietic lineage marker.
- S-100 is a neurological and neural crest cell marker. It is a calcium-binding protein soluble in 100% saturated ammonium sulfate.
- Synaptophysin is a neuroendocrine cell marker.
- Desmin is a skeletal muscle tissue marker.
- HMB-45 is human melanoma, black protein is common marker for melanoma and other tumors with melanocytic differentiation. It stains immature melanosomes.

Avidin–Biotin Complex (ABC) Indirect Method

Abiotinylated secondary antibody is used to recognize primary antibody. Avidin complexed with biotinylated peroxidase is then bound to secondary antibody. The peroxidase is then developed by DAB (3,3′-diaminobenzidine) used as chromogen in immunostaining or other substrate to produce different calorimetric end products. This reaction delivers several peroxidase molecules to the primary antibody sites and boosts its sensitivity. Disadvantage of this avidin–biotin complex method is background staining (Fig. 14.6).

Labeled Streptavidin–Biotin (LSAB) Indirect Method

Streptavidin derived from *Streptococcus avidin* is recent substitute of avidin. A biotinylated secondary antibody is used to recognize primary antibody. Streptavidin complexed with biotinylated peroxidase is then bound to secondary antibody. This labeled streptavidin–biotin technique is similar to standard avidin–biotin complex method. This reaction delivers several peroxidase molecules to the primary antibody

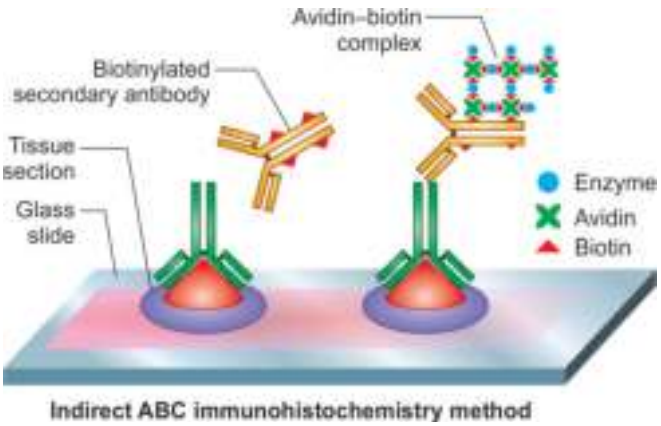


Fig. 14.6: Indirect avidin–biotin complex (ABC) immunohistochemistry method: in the ABC technology, the avidin or streptavidin–biotin enzyme complex reacts with the biotinylated secondary antibody.

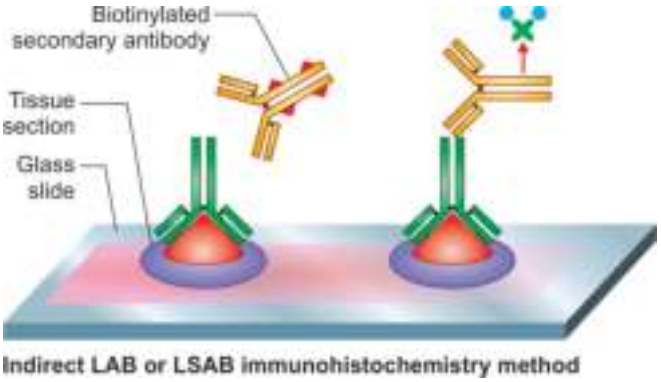


Fig. 14.7: Indirect labeled streptavidin-biotin (LSAB) immunohistochemistry method: Labeled streptavidin–biotin method. Labeled streptavidin reacts with biotinylated secondary antibody.

sites and boosts its sensitivity. The enzyme is then visualized by application of the substrate chromogen solutions to produce different calorimetric end products. Streptavidin–biotin method has decreased background staining (Fig. 14.7).

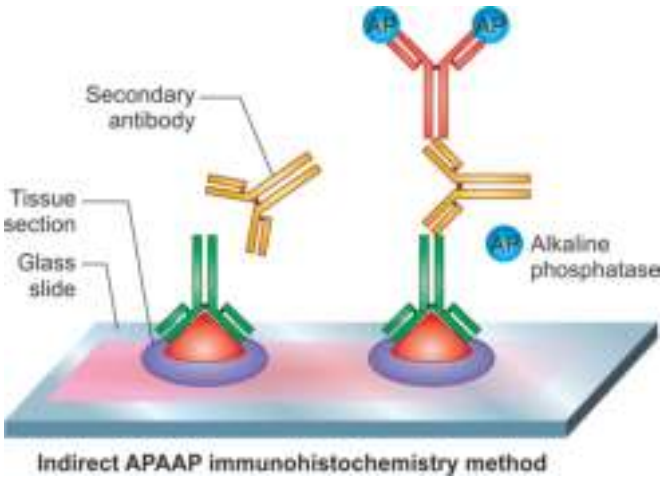


Fig. 14.8: Indirect alkaline phosphatase and antiphosphatase (APAAP) and immunohistochemistry method.

Alkaline Phosphatase and Antiphosphatase (APAAP) Indirect Method

Enzyme alkaline phosphatase and anti-alkaline phosphatase instead of peroxidase is widely used, which gives most sensitive results and can be applied on paraffin, cryostat sections or on smears (Fig. 14.8).

IMMUNOHISTOCHEMISTRY TECHNIQUE PROTOCOL

In order to visualize enzyme-substrate reactions, various chromogens, which get converted into visible colored end products, are used. Commonly used chromogens are diaminobenzidine (DAB), 3-amino-9-ethylcarbazole (AEC) and 4-chloro-1-naphthol (CN) producing brown, red and dark blue colors, respectively. Immunohistochemistry (IHC) technique protocol is given in Table 14.23.

Table 14.23 Immunohistochemistry (IHC) technique protocol

Protocol of IHC	Steps	Procedure
Tissue preparation	▪ Tissue fixation	▪ Immersion or perfusion fixation (neutral formalin, paraformaldehyde and Bouin's solution)
	▪ Tissue sectioning	▪ Microtome for paraffin blocks; cryostat for frozen tissue and vibratome for fixed hard tissue
Pre-incubation steps	▪ Deparaffinization of tissue section	▪ Paraffin is removed from tissue section
	▪ Removing the fixative	▪ Washing of tissue section in buffer solutions (phosphate buffer, tris-HCl buffer, HEPES, i.e. hydroxyethyl piperazine-ethane-sulfonic acid buffer, etc.)
	▪ Neutralization of endogenous peroxidase	▪ Endogenous peroxidase is removed
	▪ Blocking	▪ Covering the nonimmunological sticky sites on tissue section (bovine serum albumin, nonimmune normal serum, gelatin, milk)
	▪ Blocking the surface tension	▪ Triton X-100 and NaCl are used to block surface tension

Contd...

Table 14.23 Immunohistochemistry (IHC) technique protocol (*Contd...*)

Protocol of IHC	Steps	Procedure
Incubation with antibody	▪ Primary antibody application ▪ Secondary antibody application	▪ Used in a solution at different dilutions with the blocking agent. The incubation time changes according to the properties of the antigen or antibody as well as depending on the temperature ▪ Must be raised in the species other than the species of which the cells or tissues are taken or the primary antibody is raised. It should specifically recognize the immunoglobulin of the species in which the primary antibody is raised
Labeling	Labeling of antibody	Immunoenzyme, immunofluorescence, immunogold
Microscopical analyses	Microscopic study of tissue section	Slides are examined under microscope

TISSUE POSITIVE AND NEGATIVE CONTROL IN IMMUNOHISTOCHEMISTRY TECHNIQUE PROTOCOL

Tissue positive control is to test for immunohistochemistry technique protocol or procedure used. It will be ideal to use known tissue positive as a control. Tissue negative control is used to test for the specificity of the antibody involved.

APPLICATIONS OF IMMUNOHISTOCHEMISTRY

Immunohistochemistry is used for detection of intercellular antigens, cell surface antigens, tissue antigen for diagnosing autoimmune diseases, soluble antigens of the cell, protein hormones in histologic diagnosis, diagnosis of the endocrine tumors, small amounts of peptides in endocrine or neuroendocrine cells, immunodeposits and tumor markers and tumor typing.

IMMUNOPROFILE AS DIAGNOSTIC TOOL

Panel of immunohistochemical markers is applied on formalin-fixed paraffin-embedded sections in surgical pathology (Tables 14.24 to 14.67 and Figs 14.9 to 14.11). Kindly *refer* to immunohistochemical markers of soft tissue tumors and central nervous system in Chapters 28 and 30, respectively.

Table 14.24 Panel of immunohistochemical markers for various tissues differentiation as diagnostic tool

Markers	Expression
Epithelial tissue differentiation	
Basic cytokeratins	CK1 to CK8 (type B/class II) detected by AE3 antibody
Acidic cytokeratins (type A/class I) (HMWCK)	▪ HMWCK (CK9, CK10, CK11, CK16, CK17) is detected by CK903 and CK5/6 antibodies for demonstration of squamous keratins in squamous cell carcinoma ▪ LMWCK (CK18, CK19, CK20) is detected by CAM 5.2 antibody in colon carcinoma, prostatic carcinoma, renal cell carcinoma and hepatocellular carcinoma
Epithelial membrane antigen (EMA) common epithelial marker	Meningioma, breast adenocarcinoma, colorectal adenocarcinoma, pancreatic adenocarcinoma, Paget's disease of breast, choriocarcinoma, desmoplastic small round cell tumor, perineuroma, epithelioid sarcoma, plasma cell neoplasms, anaplastic large cell lymphoma, diffuse large B cell lymphoma, Hodgkin disease (lymphocytic predominance), renal cell carcinoma, thymic carcinoma, mesothelioma (epithelioid variant) carcinoid tumor, chordoma
Carcinoembryonic antigen (CEA)	Hepatocellular carcinoma (50–90%), colorectal carcinoma (70–80%), gastric carcinoma, lung adenocarcinoma (97%), pancreatic adenocarcinoma (90%), breast carcinoma, urinary bladder carcinoma, cervical carcinoma and another tumor (synovial sarcoma biphasic variant)
p53 protein	▪ TP53 tumor suppressor gene located on chromosome 17p13 encodes p53 protein that repairs damaged DNA before cell division by inducing cell cycle arrest to allow time for DNA repair or force the cell to undergo apoptosis via activation of BAX gene. ▪ Mutation in TP53 is detected in human neoplasia, however, its absence does not rule of malignancy. Germline heterogenous mutation in TP53 is demonstrated in Li-Fraumeni syndrome.

Contd...

Table 14.24 Panel of immunohistochemical markers for various tissues differentiation as diagnostic tool (*Contd...*)

Markers	Expression
	▪ The p53 immunomarker differentiates malignant conditions, which are most often p53 positive (carcinoma *in situ* and invasive carcinoma) from p53 negative conditions (reactive and metaplastic conditions) ▪ The p53 immunomarker can distinguish uterine serous carcinoma, which is p53 positive from p53 negative endometrioid carcinoma ▪ The p53 immunomarker may be useful as serum tumor marker
B72.3	Adenocarcinomas and epithelioid angiosarcoma
β-Catenin	Colon carcinoma, familial adenomatous polyposis (FAP) and other tumors (hepatoblastoma, pancreatoblastoma, craniopharyngioma, tubular adenoma)
α-Fetoproteins	Yolk sac tumor, hepatocellular carcinoma (except fibrolamellar variant) and another tumor (hepatoblastoma)
BerEP4/EpCAM	Metastatic adenocarcinoma to liver, cholangiocarcinoma, lung adenocarcinoma and another tumor (synovial sarcoma biphasic variant)
E-Cadherin	E-cadherin (present in ductal carcinoma and absent in lobular carcinoma of breast), E-cadherin loss in nonepithelial malignancies
Calretinin	Some carcinomas and other tumors (epithelial mesothelioma, synovial sarcoma, sex–cord stromal tumors, cardiac myxoma, adrenocortical tumors, adenomatoid tumors)
Desmoplakin	Epithelial tumors in general and other tumors (meningioma, Ewing sarcoma)
HBME-1	Adenocarcinomas (some cases), other tumors (mesothelioma, synovial sarcoma, chordoma, chondrosarcoma)
Mesothelin	Ovarian serous cystadenocarcinoma, adenocarcinomas (some others) and other tumors (mesothelioma, synovial sarcoma biphasic variant)
MOC31	Adenocarcinoma (most common)
TTF-1 (thyroid transcription factor 1)	Thyroid papillary carcinoma, thyroid follicular carcinoma, thyroid medullary carcinoma, and other tumors (small cell lung carcinoma in 90%, lung adenocarcinoma and small cell carcinomas of many organs)
Calcitonin	Thyroid medullary carcinoma and neuroendocrine tumors
Villin and CDX2	Intestinal differentiation (e.g. colon carcinoma), other tumors (carcinoid tumors, adenocarcinoma of urinary bladder)
Napsin A	Lung carcinoma, renal cell carcinoma (papillary variant), and thyroid carcinoma (weak positivity)
Gross cystic disease fluid protein (GCDFP)	Breast carcinoma (low sensitivity and specificity), and salivary gland carcinoma (low sensitivity and specificity)
Mammaglobin	Breast carcinoma, gynecological tumors (cross reactivity)
PAX8	Renal cell carcinoma (pan-renal marker broader than PAX2), pan-Müllerian tumors of uterus and ovary, thyroid carcinoma (papillary > follicular >> medullary variants)
PAX2	Renal cell carcinoma (pan-renal marker in 50%)
DPC4 (decapentaplegic homolog 4)	DPC4 negative in pancreatic carcinoma and cholangiocarcinoma (50%) due to loss of expression, cholangiocarcinoma (loss of expression in 50%), but positive staining is noncontributory
PSA (prostate-specific antigen), PSAP (prostate-specific acid phosphatase), PSMA (prostate-specific membrane antigen +ve), prostein	Prostatic carcinoma (note: PSAP reactivity in rectal carcinoids)
HepPar1	Hepatocellular carcinoma
Arginase 1 (new marker)	Hepatocellular carcinoma, hepatoid variants of carcinoma at other sites (also positive)
CAIX (carbonic anhydrase)	Renal cell carcinoma (clear cell variant), renal cell carcinoma (papillary variant) in areas of necrosis

Contd...

Table 14.24 Panel of immunohistochemical markers for various tissues differentiation as diagnostic tool (*Contd...*)

Markers	Expression
WT1 (Wilm's tumor 1) protein	Desmoplastic small round cell tumor, Wilm's tumor (nephroblastoma), malignant mesothelioma, ovarian serous cystadenocarcinoma
Vascular endothelial differentiation	
CD31 (PECAM1) endothelial marker	Blood vessel and lymphatic channels
CD34 (hematopoietic stem cell antigen)	Blood vessels
D2–40 (podoplanin) endothelial marker	Lymphatic channels, Kaposi sarcoma, angiosarcoma, lymphangioma
vWF VIII-related antigen (endothelial marker)	Epithelioid hemangioblastoma, angiosarcoma (some cases), vascular invasion in thyroid carcinoma
Ulex europaeus 1 (endothelial marker)	Angiosarcoma
CD141 (thrombomodulin) endothelial marker	Blood vessels and lymphatic channels
Fli-1 (endothelial marker)	Blood vessels and lymphatic channels, angiosarcoma
Flt-4 (VEGFR3) endothelial marker	Kaposi sarcoma, angiosarcoma and hemangioma
Smooth muscle differentiation	
Desmin (intermediate filament)	Pan-muscle marker (smooth muscle, skeletal muscle); and myofibroblasts, leiomyoma, leiomyosarcoma (70%), rhabdomyosarcoma
MSA (muscle-specific actin)	Pan-muscle marker (smooth muscle, skeletal muscle); and myofibroblasts, leiomyoma, leiomyosarcoma, rhabdomyosarcoma
SMA (smooth muscle actin also known as α-actin)	Smooth muscle, leiomyoma, leiomyosarcoma and myoblastic tumors (e.g. nodular fasciitis) but absent in skeletal muscle
SMMHC (smooth muscle myosin heavy chain) contractile apparatus associated protein encoded by CALD-1 gene	Smooth muscle, leiomyoma, leiomyosarcoma; but absent in skeletal muscle
Calponin (contractile apparatus associated protein encoded by CALD-1 gene)	Smooth muscle, leiomyoma, leiomyosarcoma, myoepithelial cells. myofibroblasts, myofibroblastic tumors; but absent in skeletal muscle
Caldesmon (contractile apparatus associated protein encoded by CALD-1 gene)	Smooth muscle, leiomyoma, leiomyosarcoma; but absent in skeletal muscle including skeletal muscle tumors (rhabdomyoma and rehabdomyosarcoma)
Skeletal muscle differentiation	
Desmin	Smooth muscle and skeletal muscle, leiomyoma, leiomyosarcoma (70%), rhabdomyosarcoma; and myofibroblasts (variable staining)
MSA (muscle-specific actin)	Smooth muscle, skeletal muscle, leiomyoma, leiomyosarcoma, rhabdomyosarcoma
MyoD1 (transcriptional factor)	Skeletal muscle, alveolar rhabdomyosarcoma (diffuse staining), embryonal rhabdomyosarcoma (focal staining), leiomyosarcoma but negative in normal smooth muscle and leiomyoma
Myogenin (transcriptional factor)	Skeletal muscle, alveolar rhabdomyosarcoma (diffuse staining), embryonal rhabdomyosarcoma (focal staining), leiomyosarcoma but negative in normal smooth muscle and leiomyoma
Myoglobin	Regenerating skeletal muscle and well-differentiated cells in rhabdomyosarcoma and tumors with heterologous skeletal muscle differentiation
Melanocytic lineage differentiation	
Melan A (A103)	Epithelioid melanoma positive (80–100%), but negative in spindle cell melanoma and desmoplastic melanoma
MART-1 (melanoma antigen recognized by cells)	Epithelioid melanoma positive (80–100%), but negative in spindle cell melanoma and desmoplastic melanoma
HMB-45 (human melanoma black 45) marker of immature melanocytes	Epithelioid melanoma positive (60–90%), but negative in spindle cell melanoma and desmoplastic melanoma, expressed in other tumors (clear cell sarcoma and angiolipoma family member PEComa)

Contd...

Table 14.24 Panel of immunohistochemical markers for various tissues differentiation as diagnostic tool (*Contd...*)

Markers	Expression
S-100 (calcium binding protein)	Epithelioid melanoma, spindle cell melanoma and desmoplastic melanoma and other tumors
MITF (microphthalmia transcription factor)	Epithelioid melanoma, and other tumors neurothekeoma and osteoclastic giant cells
SOX 100	Epithelioid melanoma
PNL2	Epithelioid melanoma
MUM1	Epithelioid melanoma
Tyrosinase	Malignant melanoma
CD63	Nevi and malignant melanoma, and also positive in other tumors (some carcinomas, neurothekeoma, alveolar soft part sarcoma)
PNL2	Melanoma
MUM1	Melanoma
Neural and neuroendocrine-specific markers	
Synaptophysin	Neuroblastoma, medulloblastoma, Ewing sarcoma/PNET, paraganglioma, neuroendocrine carcinoma, pheochromocytoma, thyroid medullary carcinoma
Chromogranin A	Neuroblastoma, medulloblastoma, Ewing sarcoma/PNET, paraganglioma, neuroendocrine carcinoma, pheochromocytoma, thyroid medullary carcinoma and another tumor (malignant melanoma)
NSE (neuron-specific enolase)	Neuroblastoma, medulloblastoma, Ewing sarcoma/PNET, paraganglioma, neuroendocrine carcinoma, pheochromocytoma, thyroid medullary carcinoma
Neurofilament proteins	Neuroblastoma, paraganglioma, Merkel cell carcinoma
α-Internexin	Neuroblastoma, paraganglioma, Merkel cell carcinoma
Myofibroblastic tissue differentiation	
Immunomarkers for myofibroblastic tissue differentiation	Calponin (+), smooth muscle actin (+), desmin (variable staining), caldesmon (–)
Myoepithelial differentiation (polyphenotype)	
Immunomarkers for myoepithelial differentiation (polyphenotype)	GFAP (+ glial fibrillary acidic protein), S-100 protein (+), p53 (+), cytokeratin (+), SMMHC (+ smooth muscle myosin heavy chain), calponin (+), caldesmon (+)
Histiocytic differentiation	
CD163 (high specificity)	Histiocytes, histiocytic sarcoma, juvenile xanthogranuloma
XIIIa (high-specificity)	Histiocytes especially dendritic cells
CD68 (low-specificity)	Histiocytes, melanoma, paraganglioma, schwannoma, granular cell tumor
Lysozyme also called muramidase (low-specificity)	Histiocytes, myelomonocytic cells, goblet cell carcinoid, breast lactating lobules, acinic cell carcinoma, acute myelomonocytic leukemia (AML-M4), acute monocytic leukemia (AML-M5), adenocarcinoma (various sites), chordoma, oncocytoma of conjunctiva, histiocytoma, histiocytic sarcoma, Rosai-Dorfman disease, Langerhans cell histiocytosis, mastocytosis, myeloid sarcoma, renal collecting duct/Bellini duct carcinoma, spleen angiosarcoma, xanthogranuloma
HAM56	Histiocytes, vascular endothelium and adenocarcinoma
MAC387	Histiocytes
α-Antitrypsin	Histiocytes
Neuroendocrine (NE) differentiation	
Immunomarkers for neuroendocrine differentiation	Synaptophysin, chromogranin A, NSE (neuron-specific enolase), CD56 (neuronal cell adhesion molecule)
Glial differentiation	
Immunomarker for glial differentiation	Glial fibrillary astrocytic protein (GFAP)

Contd...

Table 14.24 Panel of immunohistochemical markers for various tissues differentiation as diagnostic tool (*Contd...*)

Markers	Expression
Neuronal differentiation	
Immunomarker for neuronal differentiation	Sm31 (pan-NF), Sm32 (cell body) in glioma or meningioma, Sm31 (axon), NeuN (neuronal nuclei)
Schwann (nerve sheath) cells	
Immunomarker for Schwann (nerve sheath) cells	S-100 protein, Leu-7 (CD57)
Hematopoietic tissue	
Pan-hematopoietic marker	CD45/Leukocyte common antigen
Pan-T cell markers	CD3, cCD3 (flow cytometry only)
Pan-B cell markers	CD20, CD79a, PAX5, CD138 and κ/λ light chains
Myeloblast	Lineage-specific markers (CD13, CD15, CD33, CD11b)
B lymphoblast	Lineage-specific markers (CD19, CD79a), and TdT (terminal deoxynucleotidyl transferase); and variable expression of CD20, CD34, CD10, HLA-DR
T lymphoblast	CD33 and TdT (terminal deoxynucleotidyl transferase); and variable expression of CD10, CD34
Serous acinar cells	
PAS (periodic acid–Schiff) stain	General serous marker
Trypsin	Pancreas serous acinar cells
Chymotrypsin	Pancreas serous acinar cells
Lipase	Pancreas serous acinar cells
S-100 (calcium binding protein) and other multispecific markers	
S-100 protein	Epithelioid melanoma, spindle cell melanoma, desmoplastic melanoma, schwannoma, neurofibroma, nerve sheath myxoma, chondroma, chondrosarcoma, extraskeletal myxoid chondrosarcoma (<30%), liposarcoma, synovial sarcoma (20–30%), rhabdomyosarcoma, myoepithelioma/mixed tumor, Rosai-Dorfman disease, Langerhans cell histiocytosis, interdigitating reticulum cell sarcoma, ossifying fibromyxoid tumor (>90%)
p75 nerve growth factor	Nerve sheath tumors and dermatofibrosarcoma protuberans and other sarcomas
CD66 (NCAM)	Neuroendocrine carcinoma, rhabdomyosarcoma and other sarcomas
Other important tumor markers	
Anaplastic lymphoma kinase (ALK)	Large cell anaplastic lymphoma, inflammatory myoblastic tumors (variable expression)
Basement membrane proteins	Schwannoma, angiosarcoma
BCL-2 (B cell lymphoma 2)	Follicular lymphoma, CLL/SLL, MCL (mantle cell lymphoma), MZL (marginal zone lymphoma)
BCL-6 (B cell lymphoma 6)	Follicular lymphoma, Burkitt lymphoma and synovial sarcoma
ALK (anaplastic lymphoma kinase) expression as a result of t(2;5)/NMP-ALK	Anaplastic large cell lymphoma, lung adenocarcinoma with EML4-ALK rearrangement
CD10	Endometrial stromal tumors, myoblastic tumors
CD99	Ewing sarcoma, widespread metastasis in different tumors
CD117	Gastrointestinal stromal tumors (GISTs), Ewing sarcoma, mast cell neoplasms, angiosarcoma, neuroblastoma, seminoma/ dysgerminoma, clear cell sarcoma, adenoid cystic carcinoma of salivary gland, thymic carcinoma, melanoma (some cases)
ER/PR (estrogen/ progesterone receptors)	Breast carcinoma (prognostic and therapeutic markers), uterine leiomyoma, retroperitoneal leiomyoma, angiomyoblastoma, aggressive angiomyxoma, endometrial carcinoma (some cases), ovarian carcinomas (some cases)
GFAP (glial fibrillary acidic protein)	Glial tumors, schwannoma, myoepithelial tumors
Inhibin	Granulosa cell tumor of ovary, adrenal cortical carcinoma, granular cell tumor
Osteocalcin	Osteosarcoma, osteoid tissue

Contd...

Table 14.24 Panel of immunohistochemical markers for various tissues differentiation as diagnostic tool (*Contd…*)

Markers	Expression
Brachyury	Chordoma, hemangioblastoma
Cathepsin K	Xp translocation renal cell carcinoma, chordoma, PEComas
Vimentin	Mesenchymal tumors, renal cell carcinoma (clear cell variant), endometrial carcinoma, mesothelioma, salivary gland carcinoma, sweat gland carcinoma, spindle cell carcinoma of any site
CD1a	Langerhans cell histiocytosis, T cell lymphoblastic lymphoma (some cases), thymoma with admixed thymocytes
CD2	T cell lymphoma, NK cell lymphoma/leukemia
cCD3 (flow cytometry only)	Most lineage-specific marker for T cell differentiation
CD3	Pan-T cell marker, T cell leukemia/ lymphoma
CD4 and CD8	T cell lymphoma/leukemia
CD5	CLL/SLL, MCL, blastoid marginal cell lymphoma, DLBCL (diffuse large B cell lymphoma in some cases)
CD7	T-ALL (100%), B cell lymphoma
CD10 (CALLA, common acute leukemia antigen)	T cell ALL (100%)
CD11b	Myelogenous leukemias with differentiation, NK cell tumors
CD11c	Hairy cell leukemia
CD13, CD14, CD33	Myelogenous leukemias
CD15	AML differentiation, adenocarcinoma, Reed-Sternberg cells in classic Hodgkin disease
CD19, CD20, CD22 (pan B cell markers)	B cell lymphoma, B-precursor ALL, DLBCL (diffuse large B cell lymphoma)
CD21, CD35	Follicular lymphoma, mantle cell lymphoma, angioimmunoblastic T cell lymphoma
CD23	Low-grade lymphoma (CLL/SLL), follicular lymphoma, mantle cell lymphoma
CD25 (IL-2 receptor)	Hairy cell leukemia, adult T cell leukemia/lymphoma, anaplastic large cell lymphoma, neoplastic mast cells
CD30 (Ki-1)	Reed-Sternberg cells in classic Hodgkin disease, anaplastic large cell lymphoma, embryonal carcinoma
CD38	Mantle cell lymphoma, chronic lymphocytic leukemia
CD41, CD42b, CD61 (flow markers)	AML-M7 with megakaryocytic differentiation
CD43	Low-grade lymphoma (e.g. CLL/SLL, mantle cell lymphoma, marginal zone lymphoma; but not follicular lymphoma), myeloid sarcoma (chloroma)
CD44	Small cell carcinoma of prostate
CD45 (leukocyte common antigen—LCA)	Classic Hodgkin disease Lymphoblastic lymphoma, anaplastic large cell lymphoma (some cases)
hCG-β (human chorionic gonadotropin-β)	Choriocarcinoma
Cell cycle progression inhibitors	
$P16^{INK4a}$ and $P14^{ARF}$ protein (CDKN2A gene) indirectly activate p53 gene	Familial melanoma, breast carcinoma (sporadic), pancreatic carcinoma (sporadic), esophageal carcinoma (sporadic)
RB1 (RB1 gene codes for RB1 protein, master break in cell cycle, that inhibits nuclear transcription factor)	Retinoblastoma (familial/sporadic), osteosarcoma (familial/sporadic), breast carcinoma (sporadic), colon carcinoma (sporadic), prostate carcinoma (sporadic), urinary bladder carcinoma (sporadic), lung carcinoma (sporadic)
Genome stability enabler proteins	
p53 protein transcriptional factor encoded by TP53 gene	Li-Fraumeni syndrome (cancers of breast and adrenal gland; sarcoma, leukemia and brain tumors) in more than 50% of cases

**Cytokeratin-positive other tumors include synovial sarcoma, epithelioid sarcoma, epithelioid angiosarcoma, yolk sac tumor, embryonal carcinoma, trophoblastic tumors, mesothelioma, chordoma, thymoma, leiomyosarcoma, malignant peripheral nerve sheath tumor.*

Table 14.25 Applications of immunohistochemistry in surgical pathology

Features	Applications
Diagnosis of neoplasms	
Diagnosis of poorly differentiated neoplasms such as carcinoma, lymphoma and melanoma	▪ Carcinoma (cytokeratin +) ▪ Lymphoma (CD45 +) ▪ Melanoma (melanocytic markers, e.g. S-100 calcium-binding protein), HMB-45 (human melanoma black 45) positive for epithelioid melanoma, melan A (A-203), MART1 (melanin antigen recognized by T cells 1), MITF (microphthalmic transcription factor), tyrosinase, SOX 100, PNL2, MUM1)
Diagnosis of carcinoma of unknown primary	▪ Colon carcinoma (CDX2 +) ▪ Lung carcinoma (TTF-1 +) ▪ Prostatic carcinoma (PSA +)
Diagnosis of invasion of neoplasm	▪ Loss of myoepithelial cells (breast carcinoma) ▪ Loss of basal cells (prostatic carcinoma) ▪ Loss of basement membrane/collagen type 4 (various carcinomas, but rarely used)
Analysis of prognostic markers	
Progniostic markers examples	▪ Ki-67/MIB1 (general proliferative marker) ▪ p53 (general apoptosis marker) ▪ HER2/neu expression in breast carcinoma has adverse prognosis ▪ CD38 expression in chronic lymphocytic leukemia has adverse prognosis
Analysis of markers reflecting a therapeutic response	
Predictive markers	▪ ER/PR (ER/PR positive breast carcinoma treated by 'Tamoxifen therapy') ▪ HER2/neu positive breast carcinoma treated by 'Herceptin therapy' ▪ c-KIT (Gleevec for gastrointestinal stromal tumor, chronic myelogenous leukemia)
Detection of micrometastases	
Detection of micrometastases by immunohistochemistry	▪ Melanoma (melanocytic markers, e.g. S-100 calcium binding protein), HMB-45 (human melanoma black 45) positive for epithelioid melanoma, melan A (A-203), MART1 (melanin antigen recognized by T cells 1), MITF (microphthalmic transcription factor), tyrosinase, SOX 100, PNL2, MUM1) ▪ Breast carcinoma (cytokeratins)

Table 14.26 Primers on location of antigens in the cells demonstrated by immunohistochemical markers

Primer on Location of Antigens in the Cells	Immunohistochemical Markers
Cell membrane reactivity	
Cell surface receptors	Epidermal growth factor (EGFR), HER2/neu
Cell adhesive molecule protein	E-cadherin
Cluster of differentiation (CD)	CD3 (T cell marker), CD20 (B cell marker), CD45, CD30 (Golgi region), CD15 (Golgi region), CD138, pan-T cell antigens
Cytoplasmic reactivity	
Intermediate filaments of cytoskeleton	Cytokeratins, desmin, vimentin, GFAP (glial fibrillary acidic proteins), neurofilament
Contractile proteins	SMA (smooth muscle actin also known as α-actin), calponin, caldesmon, SMMHC (smooth muscle myosin heavy chain)
Cytoplasmic proteins	S-100 protein, α_1-antitrypsin, NSE (neuron-specific enolase)
Melanocyte associated proteins in melanosomes	HMB-45 (human melanoma black 45), S-100 protein, MITF (microphthalmic transcription factor), SOX-100, PNL2, tyrosinase
Secretory products	ACTH
Mitochondria	BCL-2

Contd...

Table 14.26 Primers on location of antigens in the cells demonstrated by immunohistochemical markers (*Contd...*)

Primer on Location of Antigens in the Cells	Immunohistochemical Markers
Cytoplasmic granules and vesicles	Chromogranin, synaptophysin
Lysosome	MPO (myeloperoxidase)
Nuclear reactivity	
Steroid hormones	ER/PR (estrogen and progesterone receptors)
Transcriptional factors	BCL-1, BCL-6, TdT (terminal deoxynucleotidyl transferase), EBER, PAX-5, BOB-1, OCT-2, HHV-8, TTF-1 (thyroid transcription factor 1), CDX2, myogenin, WT1, p53, p63RB1, p21, p27
Proliferative marker	Ki-67
Nuclear as well as cytoplasmic reactivity	
Nuclear and cytoplasmic reactivity	S-100 protein, calretinin, ALK-1, ZAP70, β-catenin (only nuclear reactivity is specific)
Patterns of cytoplasmic reactivity	
Cytoplasmic granular reactivity pattern	Racemase, prostein (P501S), napsin A, Hep Par 1
Cytoplasmic dot-like reactivity pattern	CD30 and CD15 (Hodgkin disease), cytokeratins in neuroendocrine tumors

Table 14.27 Reactivity code of immunohistochemical markers

Expression	Interpretation
Positive (+++)	Overexpressed or consistently diffuse
Positive (+)	Positive
Positive/negative (+/–)	Usually, positive
Negative/positive (–/+)	Usually, negative
Negative (–)	Negative

Table 14.28 Expression of vimentin in various epithelial and mesenchymal human tumors

Vimentin Positive Tumors	Vimentin Negative Tumors
Mesenchymal tumors	Renal cell carcinoma (chromophobe variant)
Renal cell carcinoma (clear cell variant)	Lung carcinoma
Endometrioid carcinoma	Breast carcinoma
Mesothelioma	Endocervical carcinoma
Salivary gland carcinoma	Ovarian carcinoma
Serous ovarian carcinoma	Prostatic adenocarcinoma
Spindle cell carcinoma of any site	Colorectal adenocarcinoma
Malignant mixed Müllerian tumor	Hepatocellular carcinoma

Vimentin is a type 3 intermediate filament protein expressed in mesenchymal cells. It has been used in the past to differentiate sarcoma (vimentin positive) from carcinoma (vimentin negative). But is variably expressed in various carcinomas. Current application of vimentin is to confirm immune-reactivity, when all markers are negative.

Table 14.29 Applications of immunohistochemistry technique in analysis of cytokeratins

Monoclonal Antibody Used	Analysis of Various Types of Cytokeratins	Comments
AE1 antibody	All acidic cytokeratins except CK9, CK12, CK17, CK18	Not applicable
AE3 antibody	All basic cytokeratins	Not applicable
AE1/AE3 antibody (pan-CK)	▪ First line mark broad-spectrum pan-cytokeratin antibody to identify both LMWCK and HMWCK in various carcinomas ▪ Variable in neuroendocrine tumors should be taken in interpretation of results	Squamous cell carcinoma, colon adenocarcinoma, prostatic carcinoma, hepatocellular carcinoma, renal cell carcinoma, adrenocortical neoplasms

Contd...

Table 14.29 Applications of immunohistochemistry technique in analysis of cytokeratins (*Contd...*)

Monoclonal Antibody Used	Analysis of Various Types of Cytokeratins	Comments
OSCAR, PANK (MNF-116) antibody	Broad spectrum cytokeratins similar to AE1/AE3 antibody identify both LMWCK and HMWCK	Squamous cell carcinoma, colon adenocarcinoma. prostatic carcinoma, hepatocellular carcinoma, renal cell carcinoma, adrenocortical neoplasms
CAM 5.2 (34bH11) antibody	CAM 5.2 (34bH11) antibody is a good complement to AE1/AE3 antibody (pan-CK). It detects those carcinomas missed by AE1/AE3 antibody (pan-CK)	▪ Simple/nonsquamous keratins in glandular epithelia (colon adenocarcinoma, prostatic adenocarcinoma) ▪ Visceral parenchyma (hepatocellular carcinoma, renal cell carcinoma
CK903 (K903; 34bE12) antibody and CK5/6 antibody	Demonstrates HMWCK (CK1, CK5, CK10, CK14) without LMWCK (CK1, CK5, CK10, CK14) expressed in keratinizing squamous epithelia and in majority of squamous cell carcinoma; however, some nonkeratinizing poorly differentiated carcinomas show positivity for both HMWCK and LMWCK	Keratinizing squamous epithelia (squamous cell carcinoma positive for HMWCK, and some nonkeratinizing poorly differentiated carcinomas show positive for both HMWCK and LMWCK)

HMWCK (CK9, CK10, CK11, CK16, CK17) detected by CK903 and CK5/6 antibodies for demonstration of squamous keratins in squamous cell carcinoma.

LMWCK (CK18, CK19, CK20) detected by CAM 5.2 antibody in colon carcinoma, prostatic carcinoma, renal cell carcinoma and hepatocellular carcinoma

Expression of cytokeratins and EMA is key diagnostic feature of carcinomas. Cytokeratin positivity may also be seen synovial sarcoma, epithelioid sarcoma, epithelioid angiosarcoma, nonseminomatous germ cell tumors (yolk sac tumor, embryonal carcinoma), trophoblastic tumors, mesothelioma, chordoma and thymoma; and aberrant expression of leiomyosarcoma, malignant peripheral nerve sheath tumor.

Table 14.30 Immunohistochemical markers expression profile of CK7 and CK20 in human cancers

CK7 and CK20 Immunohistochemistry	Human Cancers
CK7 +ve/CK20 +ve	Gastric carcinoma, pancreatic adenocarcinoma (some cases), biliary tree malignancy, urinary bladder carcinoma
CK7 +ve/CK20 –ve	Breast carcinoma, lung carcinoma, mesothelioma, well-differentiated thyroid carcinoma, salivary gland malignancy, including renal cell carcinoma (papillary variant), pancreatic carcinoma, gynecologic endometrioid carcinomas, gynecologic serous carcinoma
CK7 –ve/CK20 +ve	Colorectal carcinoma, ovarian mucinous adenocarcinoma, urinary bladder carcinoma, some appendiceal carcinomas (some cases)
CK7 –ve/CK20 –ve	Hepatocellular carcinoma, renal cell carcinoma, prostatic carcinoma, adrenocortical malignancy

Table 14.31 Expression of epithelial membrane antigen (EMA) in various neoplasms

Meningioma	Plasma cell neoplasms
Breast adenocarcinoma	Anaplastic large cell lymphoma
Colorectal adenocarcinoma	Diffuse large B cell lymphoma
Pancreatic adenocarcinoma	Hodgkin disease (lymphocyte-rich)
Paget's disease of breast	Renal cell carcinoma
Choriocarcinoma	Thymic carcinoma
Desmoplastic small round cell tumor	Mesothelioma (epithelioid variant)
Perineuroma	Carcinoid tumor
Epithelioid sarcoma	Chordoma

Contd...

Table 14.32 Expression of carcinoembryonic antigen (CEA) in human cancers

Colorectal carcinoma (70–80%)
Hepatocellular carcinoma (50–90%)
Lung adenocarcinoma (97%)
Pancreatic adenocarcinoma (90%)
Gastric carcinoma
Breast carcinoma
Urinary bladder carcinoma
Cervical carcinoma

Variable expression of carcinoembryonic antigen (CEA), urothelial carcinoma, breast carcinoma and cervical carcinoma.

Table 14.33 TP53 gene mutation and associated cancers

Li-Fraumeni syndrome
Perineuroma
Epithelioid sarcoma
Plasma cell neoplasm
Anaplastic large cell lymphoma
Diffuse large B cell lymphoma (DLBCL)
Hodgkin disease (lymphocytic predominance)
Renal cell carcinoma
Thymic carcinoma
Mesothelioma (epithelioid variant)
Carcinoid tumor
Chordoma

Table 14.34 Vascular endothelial immunomarkers in normal human tissues and some human cancers

CD31 (PECAM1): Blood vessel and lymphatic channels
CD34 (hematopoietic stem cell antigen): Blood vessels
D2–40 (podoplanin): Lymphatic channels
vWF VIII-related antigen: Epithelioid hemangioblastoma, angiosarcoma (some cases), vascular invasion in thyroid carcinoma
Fli-1: Blood vessels and lymphatic channels including angiosarcoma
Flt-4 (VEGFR3): Kaposi sarcoma, angiosarcoma and hemangioma
CD141 (thrombomodulin): Blood vessels and lymphatic channels
Ulex europaeus 1: Endothelial marker in vascular tumors

Table 14.35 Desmin expression in various human tumors

Leiomyoma
Leiomyosarcoma
Angiolipoma
Benign stromal spindle cell tumor of breast
Phyllodes tumor of breast
Chondrosarcoma dedifferentiated
Desmoplastic small round cell tumor
Giant cell tumor of tendon sheath
Endometrial stromal tumor
Atypical fibroxanthoma of skin
Epithelioid sarcoma
Malignant fibrous histiocytoma
Malignant mixed Müllerian tumor
Inflammatory myofibroblastic tumor
Lymphangioleiomyomatosis

Table 14.36 Melanocytic lineages differentiation immunomarkers

HMB-45 (human melanoma black 45): Epithelioid melanoma
Melan A (A-203)
MART1 (melanin antigen recognized by T cells)
MITF (microphthalmic transcription factor)
S-100 protein
Tyrosinase
SOX 100
PNL2
MUM1
The melanocyte lineage is derived from the neural crest, which has its origin in the neural tube.
Melanocytes produce melanin in the skin
LDH is prognostic serum marker for melanoma that has been validated for metastatic disease

Table 14.37 Intermediate filaments, microtubules, cytokeratins and their locations

Component	Location
Microfilaments (actin, nonmuscle myosin) and intermediate filaments	Cytoskeleton of all cells
Glial fibrillary acidic protein (GFAP)	Astrocytes
Neurofilament	Neural tissue
Vimentin	Mesenchymal tissue
Desmin	Smooth and skeletal muscles
Microtubules	Neural derivatives (e.g. neuroblastoma)
Cytokeratins (CK)	Epithelial cells

Table 14.38 Tissue specificity of MUC genes and their expression in tumors

MUC Gene	Expression of MUC in Various Tumors
MUC1	Adenocarcinoma (diffuse staining), hepatocellular carcinoma
MUC2	Breast carcinoma (mucinous variant), pancreatic carcinoma (mucinous variant), ovarian mucinous cystadenocarcinoma, gastric adenocarcinoma (mucinous variant), but downregulation in colon carcinoma
MUC3	Colon carcinoma, pancreatic carcinoma
MUC4	Colon carcinoma, pancreatic carcinoma, lung carcinoma, ovarian carcinoma
MUC5	Endocervical adenocarcinoma, gastric carcinoma, but downregulation in colon carcinoma
MUC6	Gastric carcinoma (diffuse staing)
MUC7	Urothelial carcinoma, micrometastases in lymph node from urothelial carcinoma of urinary bladder

Table 14.39 Distribution of S-100 (calcium binding protein) in normal cells and selected soft tissue neoplasms analyzed by immunohistochemistry

S-100 Protein Expression in Normal Cell	S-100 Protein Expression in Soft Tissue Tumors
Melanocyte	Nevi, malignant melanoma (epithelioid, spindle cell and desmoplastic variants)
Schwann cell	Schwannoma, neurofibroma nerve sheath myxoma
Cartilage	Chondroma, chondrosarcoma, extraskeletal myxoid chondrosarcoma (<30%)
Adipocyte	Liposarcoma (variable expression)
Regenerating skeletal muscle	Rhabdomyosarcoma
Myoepithelial cells	Myoepithelioma/mixed tumor
Langerhans cell/interdigitating reticulum cell	Rosai-Dorfman disease, Langerhans cell histiocytosis, interdigitating reticulum cell sarcoma
Unknown normal cell	Ossifying fibromyxoid tumor (>90%), synovial sarcoma (20–30%)

Table 14.40 Panel of immunohistochemical markers of myoepithelial cells in breast in assessment of benign noninvasive lesion and invasive breast neoplasms

Myoepithelial Cell Markers Feature	Immunohistochemistry (Benign/Noninvasive Breast Lesion)	Immunohistochemistry (Invasive Breast Neoplasm)
Myoepithelial cells	Present	Absent
SMMHC (smooth muscle myosin heavy chain)	Positive	Negative
Calponin	Positive	Negative
Caldesmon	Positive	Negative
GFAP (glial fibrillary acidic protein)	Positive	Negative
S-100 protein	Positive	Negative
p53 protein	Present	Negative

Table 14.41 Panel of immunohistochemical markers applied in breast carcinoma

Tumor	Immunohistochemical Markers
Ductal carcinoma of breast (*in situ*/invasive)	ER/PR (+ 50%), GCDFP-15 (+ 50%), mammaglobin (+ 50%), HER2/neu (+ 50%), Ki-67 (+ 10–50%), p53 (+ poor prognosis), CK7 (+), GATA3 (+)
Lobular carcinoma of breast (*in situ*/invasive)	GCDFP-15 (+100%), E-cadherin (–)
Medullary carcinoma of breast	Triple negative (ER/PR, HER2/neu)
Metaplastic carcinoma of breast	CK903.p63, CAM 5.2 (most reliable marker), CK7 (most reliable marker)
Undifferentiated carcinoma of breast	NY-BR1 (highly specific marker)
Typical ductal hyperplasia	E-cadherin (+), CK903 (–), CK5/CK6 (–), ER (+), p120 catenin (+)
Atypical ductal hyperplasia/ductal carcinoma *in situ*	E-cadherin (+), CK903 (–), CK5/CK6 (–), ER (+), p120 catenin (+)
Atypical lobular hyperplasia/lobular carcinoma *in situ*	E-cadherin (–), CK903 (+), CK5/CK6 (+), ER (+), p120 catenin (+)

Table 14.42 Panel of immunohistochemical markers for vulva and vagina tumors of female genital system

Tumor	Immunohistochemical Markers
Vulva Tumors	
Angiomyoblastoma of vulva	Desmin (+), CD34 (+), ER (+), PR (+)
Extra-mammary Paget disease	CK7 (+), CK20 (+), CEA (+), EMA (+)
Vagina Tumors	
Botryoid rhabdomyosarcoma	Desmin (+), vimentin (+), myogenin (+), MyoD1 (+), α-actin (+)
Leiomyosarcoma	SMA (+), S-100 protein (+)
Melanoma	HMB-45 (+), S-100 protein (+), melan A (+), MART1 (+), tyrosinase (+), SOX 100 (+), PNL2 (+), MUM1 (+)

Table 14.43 Panel of immunohistochemical markers for tumors of uterus and cervix

Tumor	Immunohistochemical Markers
Uterine cervical tumors	
Cervical adenocarcinoma	HPV (+), p16 (+), Ki-67 (+), p53 (–/+ focal staining)
Uterine endometrial tumors	
Type 1 endometrioid carcinoma (ER dependent)	ER/PR (+ >90%), PAX8 (+), CA125 (+), PTEN (+), p16 (–/+ patchy staining)
Type 2 endometrial carcinoma (serous papillary variant)	PAX8, ER/PR (+ >50%), p53 (+), p16 (+), HER2/neu (+), EGFR (+)
Type 2 endometrial carcinoma (clear cell variant)	HNF-1b (+), HER2/neu (+), EGFR (+)
Endometriosis of any site	ER/PR (+ glands and stroma), CD10 (+ stroma)
Uterine smooth muscle tumors	
Leiomyoma	SMA (+), desmin (+), MSA (+), h-caldesmon (+), SMMHC, calponin (+), ER/PR (+)
Leiomyosarcoma	SMA (+), desmin (+), MSA (+), h-caldesmon (+), SMMHC, calponin (+), ER/PR (+)
Endometrial stromal tumors of the uterus	CD10 (+), ER/PR (+), JAZF1-JJAZ1 gene fusion protein (+), SMA (–/+), desmin (–/+), h-caldesmon (–)

Table 14.44 Role of immunohistochemistry technique in differential diagnosis of uterine endometrioid carcinoma, serous carcinoma and clear cell carcinoma

Immunohistochemical Markers	Endometrioid Carcinoma (Type 1)	Serous Carcinoma of Endometrium (Type 2)	Clear Cell Carcinoma of Endometrium (Type 2)
Estrogen/progesterone receptors (ER/PR)	Positive	Positive (50%)	Negative/positive
Ki-67 (proliferative marker)	Positive (low expression)	Positive (high expression)	Positive (variable expression)
p53	Negative/positive (patchy/ rare weakly cells)	Positive (strong diffuse staining)	Negative
p16	Negative/positive (patchy)	Positive (strong diffuse staining)	Positive/negative
HNF-1β	Negative	Negative	Positive
WT1	Negative	Negative/positive	Negative

WT1 is positive in ovarian carcinoma, but negative/positive in serous carcinoma of endometrium.
PTEN is positive in endometrioid carcinoma.

Table 14.45 Panel of immunohistochemical markers for ovarian surface epithelial tumors of female genital system

Tumor	Immunohistochemical Markers
Borderline ovarian surface epithelial tumor	S-100 protein (+)
Serous cystadenocarcinoma of ovary	CA125 (+), SMO47 (+), CK7 (+), CK20 (+), CEA (+), B72.3 (anti-TAG72) (+), Ber EP4 (+), PAX2 (+++ strong staining) PAX8 (+), PLAP (+), ER/PR (+), WT1 (+), BRAC1 (+ indicates high-grade), p53 (+ indicates low-grade), p53 (– indicates high-grade)
Mucinous cystadenocarcinoma of ovary	CK7 (+), CEA (+), CD29 (–/+ focal staining), CDX2 (–/+ focal staining)
Endometrioid cystadenocarcinoma of ovary	ER/PR (++++ in >90%)
Clear cell carcinoma of ovary	HNF-1β (+), CA125 (+), CEA (+), EMA (+), PAX8 (+), CK7 (+), CAM (+), CD15/LeuM1 (+), vimentin (+), BCL-2 (+), p53 (+), ER/PR (variable staining)
Brenner tumor of ovary	CK (+), CEA (+), EMA (+)

Table 14.46 Panel of immunohistochemical markers for ovarian germ cell tumors, sex-cord tumors and Krukenberg tumor of female genital system

Tumor	Immunohistochemical Markers
Ovarian germ cell tumors	
Dysgerminoma	PLAP (+), CD117/c-KIT (+), SALL4 (+ nuclear staining), D2–40 (podoplanin) (+ membranous staining), PAS (+), OCT4/POU5F1 (+), CAM 5.2 (–), AE1/AE3 (–), EMA (–),
Embryonal carcinoma	PLAP (+), OCT4/POU5F1 (+), SALL4 (+), CD30 (+), D2–40 (podoplanin) (+/–), CD117/c-KIT (–)
Yolk sac tumor	PLAP (+), SALL4 (+), CAM 5.2 (+), AE1/AE3 (+), AFP (+), α_1-antitrypsin (+), CD117/c-KIT (–), D2–40/ podoplanin (–)
Choriocarcinoma	CAM 5.2 (+), β-HCG (+++ specific marker), inhibin (+), human placental lactogen (+ hPL), CK7 (+), LK26/folate binding protein (+), SALL4 (+/–), PLAP (+/–), CD117/c-KIT (–), OCT4 (POU5F1) (–), D2–40 (podoplanin) (–)
Struma ovarii (monodermal teratoma)	EMA (+), TTF-1 (+), thyroglobulin (+)
Sex cord-stromal tumors of the ovary	
Ovarian granulosa cell tumors/Leydig cell tumors	CD99 (+), inhibin (+), calretinin (+ most sensitive), (+), melan A (Mart-1), clone A103 (+), ER/PR (+)
Krukenberg tumor (primary tumors metastasizing to ovary)	
Gastric adenocarcinoma	CK (+), EMA (+), CEA (+)
Colon adenocarcinoma	CK7 (+++), CK20 (+++), CDX (+++), MUC1 (+++), CEA (+), β-catenin (+), SAT2 (+), p53 (+)
Breast lobular carcinoma	ER/PR (+), E-cadherin (–), HER2/neu (–)

Table 14.47 Panel of immunohistochemical markers applied in differential diagnosis of ovarian germ cell tumors of female genital tract

Immunohistochemical Markers	Dysgerminoma (Counterpart to Testicular Seminoma)	Embryonal Carcinoma	Yolk Sac Tumor	Choriocarcinoma
OCT4 (POUC5F1) (nuclear staining)	Positive	Positive	Negative	Negative
SALL4 (nuclear staining)	Positive	Positive	Positive	Positive/negative
Placental alkaline phosphatase (PLAP)	Positive	Positive	Positive	Negative
c-KIT	Positive	Negative	Negative	Negative
D2–40 (podoplanin) membrane staining	Positive	Negative/positive	Negative	Negative
CAM 5.2 and AE1/3 (epithelial marker)	Negative	Positive	Positive	Positive
EMA (epithelial membrane antigen)	Negative	Negative	Negative	Positive (50%)
PAS positive	Positive	Negative	Negative	Negative
CD117 (c-KIT)	Positive (membranous staining)	Negative	Negative	Negative
CD30	Negative	Positive	Negative	Negative
α-Fetoproteins positive	Negative	Negative	Positive (nonspecific)	Negative
β-hCG	Negative	Negative	Negative	Positive
Inhibin	Negative	Negative	Negative	Positive
hPL (human placental lactogen)	Negative	Negative	Negative	Positive
CD146	Negative	Negative	Negative	Positive
CK7 (cytokeratin 7)	Negative	Negative	Negative	Positive
LK26 (folate binding protein)	Negative	Negative	Negative	Positive

Table 14.48 Panel of immunohistochemical markers for tumors of kidney

Tumors	Immunohistochemical Markers
Renal cell carcinoma (clear cell variant)	CAIX (+++++), PAX8 (+), PAX2 (+), EMA (+)
Renal cell carcinoma (papillary variant)	CK7 (+), racemase (+), PAX8 (+), PAX2 (+), CAIX (– except areas of necrosis)
Renal cell carcinoma (chromophobe variant)	Hale's colloidal iron (+), EpCam (epithelial cell adhesive molecule) (+), c-KIT (CD117) (+), TFE3 (+), cathepsin K (+), PAX8 (+)
Renal cell carcinoma with Xp11 chromosomal translocation	TFE3 (+), TFEB transcription factor (+), cathepsin K transcription factor (+)
Renal clear cell sarcoma	Vimentin (+)
Wilms' tumor	WT1 (+ epithelial component), CK (+), EMA (+)
Renal mixed epithelial stromal tumor (MEST)	ER/PR (+), desmin (+), SMA (+), CD10 (+)
Renal angiomyolipoma	HMB-45 (+), melan A (+), SMA (+), vimentin (+), TFE3 (–/+), cathepsin K (–/+)

Table 14.49 Role of immunohistochemistry in differential diagnosis of renal tumors

Immunohistochemical Markers	Renal Cell Carcinoma Clear Cell Variant (Chromosome 3p Loss)	Renal Cell Carcinoma Papillary Variant with Clear Cell (Trisomy 7 and 17)	Chromophobe Renal Cell Carcinoma	Xp11 Translocation Renal Cell Carcinoma
CAIX	Positive (diffuse staining)	Negative	Negative	Negative/Positive
Vimentin	Positive	Negative	Negative	Positive
CD10	Positive	Positive/Negative	Negative/positive	Positive
Cytokeratin 7 (CK7)	Negative	Positive	Positive	Negative/positive
Racemase	Negative	Positive	Negative	Positive
c-KIT, Hale's colloidal iron stain	Negative	Negative	Positive	Negative
TFE3	Negative	Negative	Negative	Positive
Cathepsin K	Negative	Negative	Negative	Positive

Renal cell carcinoma (clear cell and papillary variants) is a low-grade tumor occurring in end stage renal disease, which shows positivity for CAIX and CK7

Table 14.50 Panel of immunohistochemical markers for urinary bladder tumors

Tumors	Immunohistochemical Markers
Normal urothelium or reactive urothelial atypia	CK20 (+ umbrella cells), E-cadherin (+ all layers), CD44 (+), p53 (–)
Urothelial carcinoma *in situ*	p53 (+ 89%), CK20 (+ all layers), E-cadherin (–/reduced), CD44 (–/reduced)
Urothelial carcinoma	CK903 (+), p63 (+ more specific marker), thrombomodulin (+), uroplakin (+), GATA3 (+), CK7 (+++), CK20 (+++), PSA (–), PSAP (–), prostein (–), PSMA (–)
Adenocarcinoma of urinary bladder	β-Catenin (–), PSA (–), PSAP (–), prostein (–), PSMA (–), racemase (–), CDX 2 (variable staining), CK20 (variable staining), CK7 (variable staining), thrombomodulin (variable staining)
Inflammatory myoblastic tumor	Vimentin (+), ALK (+ 66%) SMA (+/–), desmin (+), CK (variable)

Table 14.51 Panel of immunohistochemical markers for germ cell tumors and Sertoli-Leydig cell tumors of testis

Tumor	Immunohistochemical Markers
Germ cell tumors of testis	
Seminoma and intratubular germ cell neoplasm	LIN28 (++++), OCT4 (+++), SALL4 (+++), PLAP (+), D2–40 (+), PAS (+), CD117/c-KIT (+), LDH (+), CAM 5.2 (–), AE1/AE3 (–), EMA (–)
Embryonal carcinoma	OCT4 (POU5F1) (+), SALL4 (+), CAM 5.2 (+), AE1/AE3 (+), CD30 (+), PAS (–), PLAP (–), D2–40 (podoplanin) c-KIT (CD117) (–)
Yolk sac tumor	LIN28 (+), SALL4 (+), PLAP (+), CAM 5.2 (+), AE1/AE3 (+), AFP (+), Glypican-3 (+), D2–40 (–), CD117/c-KIT (–)
Choriocarcinoma	CAM 5.2 (+), AE1/AE3 (+), EMA (+ 50%), β-hCG (+++), inhibin (+), human placental lactogen (hPL) (+), HLA-G (+), glypican 3 (+), SALL4 (+), PLAP (+/–), D2–40/podoplanin (–)
Teratoma	CAM 5.2 (+), AE1/AE3 (+), EMA (+), PLAP (+), OCT4 (POU5F1) (–), SALL4 (+/–), D2–40 (podoplanin) (–)
Sertoli-Leydig cell tumors of testis	
Sertoli-Leydig cell tumors	Inhibin (+), melan A (+), CD10 (+), CD99 (+), calretinin (+), OCT4 (POU5F1) (+), SALL4 (–), EMA (–), PLAP (–),

CD117 (c-KIT) shows membrane staining in seminoma; and cytoplasmic staining in other germ cell tumors. Epithelial membrane antigen (EMA) is always negative in germ cell tumors of testes. Cytokeratins may be positive in some Sertoli cell tumors and granulosa cell tumors of the ovary.

Table 14.52 Panel of immunohistochemical markers in the assessment of benign/noninvasive prostatic lesions

Prostatic Cells	Immunohistochemistry (Benign/Noninvasive Prostatic Lesion)	Immunohistochemistry (Invasive Prostatic Neoplasm)
Prostatic basal cells	▪ Prostatic basal cells present ▪ CK903+ (cytoplasm) ▪ p63+ (nucleus)	▪ Prostatic basal cells absent ▪ CK903 negative ▪ p63 negative
Prostatic acinar cells	▪ Prostatic acinar cells present ▪ Recemase negative	▪ Prostatic acinar cells present ▪ Recemase positive (cytoplasmic staining)

Table 14.53 Panel of immunohistochemical markers for tumors of prostate gland

Tumors of Prostate	Immunohistochemical Markers
Prostatic adenocarcinoma	Prostate specific antigen (PSA) (+++), prostate-specific acid phosphatase (PSAP) (+++), racemase (+++), prostein (P501S) (+), prostate-specific membrane antigen (PSMA) (+), p63 (+), racemase (P504S, AMACR) (+)
Prostatic stromal tumor of uncertain malignant potential (STUMP)	CD34 (+), PR (+), ER (–), desmin (+/–), SMA (+/–)
Prostatic stromal sarcoma	CD34 (+), ER (–), PR (+), SMA (–), desmin (–)
Prostatic and urinary bladder inflammatory myoblastic tumor (IMT)	Vimentin (+), ALK (+ 66%) SMA (+/–), desmin (+), CK (variable)

Basal cell markers: p63 (nuclear staining), CK903 (cytoplasmic staining) negative/positive.

Table 14.54 Panel of immunohistochemical markers for tumors of sinonasal tract

Tumors	Immunohistochemical Markers
Nasopharyngeal carcinoma (NPC)	CK (HMW) (+), EBV (+ FISH), p63 (+), synaptophysin (–)
Sinonasal undifferentiated carcinoma (SNUC)	CK (LMW) (+), EMA (+), synaptophysin (+ 25%), p53 (+ patchy/–)
NUT-midline translocation carcinoma	NUT (+), NUT translocation (+ diagnostic marker) CK (+), EMA (+), p63 (+), CD34 (+/–), synaptophysin (–), EBV (–)
Olfactory neuroblastoma/esthesioneuroblastoma	Synaptophysin (++++ 100%), chromogranin A (+ 25%), neuron-specific enolase (NSE) (+), calretinin (+), CD56 (+), CD57 (+), CK (–/+ focal staining), EMA (–/+), EBV (–)
Nasal NK/T cell lymphoma (lethal midline granuloma)	HBV analyzed by FISH (+), CD45 (+), CD2 (+), CD3ε (+), cytotoxic protein perforin (+), CK (–), EMA (–)

Table 14.55 Panel of immunohistochemical markers for lung (bronchopulmonary) tumors and mesothelioma

Tumors	Immunohistochemical Markers
Squamous cell lung carcinoma	p40 (+), p63 (+), CK5/CK6 (+ diffuse staining), CK903 (+ diffuse staining), CEA (+), CK7 (+/–), CK20 (–), TTF-1 (–), napsin A (–), synaptophysin (–), chromogranin A (–), neuron-specific enolase (NSE) (–), CD56 (neuronal cell adhesion molecule—NCAM) (–)
Small cell lung carcinoma	TTF-1 (+), synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), CAM 5.2 (+), AE1/AE3 (+), CK7 (–/+), CK20 (–), p63 (–), CK5/CK6 (–), CK903 (–), napsin A (–)
Adenocarcinoma (mucinous variant) of lung	TTF-1 (+10–20%), CDX2 (+ focal staining), napsin A (+/–), p63 (+/–), CK20 (+/–), CK5/K6 (+/–), CK903 (+/–), synaptophysin (–), chromogranin A (–), neuron-specific enolase (NSE) (–), CD56 (neuronal cell adhesion molecule—NCAM) (–)
Adenocarcinoma (non-mucinous variant) of lung	TTF-1 (+), napsin A (+), CK7 (+/–), p63 (+/–), synaptophysin (–), chromogranin A (–), neuron-specific enolase (NSE) (–), CD56 (neuronal cell adhesion molecule—NCAM) (–), CK20 (–)
Bronchopulmonary carcinoids	TTF-1 (+ 50%), synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), napsin A (–), CK7 (–), CK20 (–), p63 (–)
Mesothelioma	Calretinin (+), WT1, CK5/CK6 (+), CK7 (+), CK903 (+), thrombomodulin (CD141) (+), HBME-1, N-cadherin (+), D2–40 (+), mesothelin (+/–), thyroid transcription factor 1 (TTF-1) (–), napsin A (–), CK20 (–), p63 (–), synaptophysin (–), chromogranin A (–), neuron-specific enolase (NSE) (–), CD56 (neuronal cell adhesion molecule—NCAM) (–)

Thyroid transcription factor 1 (TTF-1) is positive in small cell lung carcinoma (90%), lung adenocarcinoma (mucinous variant, 20%) and lung adenocarcinoma (non-mucinous variant), extrapulmonary small cell carcinoma (40%).
It is difficult to differentiate metastatic adenocarcinoma of pancreas and upper gastrointestinal tract from mucinous adenocarcinoma of lung. Both metastatic tumors are CK7 positive, CK20 positive but TTF-1 negative. DPC4 is deleted in 55% cases of adenocarcinoma of pancreas but not lung origin.

Table 14.56 Panel of immunohistochemical markers used in differential diagnosis of bronchopulmonary neuroendocrine neoplasms

Immunohistochemical Markers	Typical Carcinoid Tumor	Atypical Carcinoid Tumor	Small Cell Lung Carcinoma	Large Cell Neuroendocrine Carcinoma
TTF-1 (thyroid transcription factor 1)	Positive (<30% weak staining)	Positive (<30% weak staining)	Positive (90%)	Positive (50%)
Cytokeratins	Positive (<80%)	Positive (<80%)	Positive (dot-like)	Positive
Synaptophysin	Positive (strong diffuse staining)	Positive (strong diffuse staining)	Positive (weak focal staining)	Positive (diffuse or focal staining)
Chromogranin A	Positive (strong diffuse staining)	Positive (strong diffuse staining)	Positive (weak focal staining)	Positive (diffuse or focal staining)
Neuron-specific enolase (NSE)	Positive (strong diffuse staining)	Positive (strong diffuse staining)	Positive (weak focal staining)	Positive (diffuse or focal staining)
CD56 (neuronal cell adhesion molecule)	Positive (strong diffuse staining)	Positive (strong diffuse staining)	Positive (weak focal staining)	Positive (diffuse or focal staining)
Ki-67	Positive (<2%)	Positive (<20%)	Positive (>20%)	Positive (>20%)

Ki-67 expression is helpful in evaluation of small biopsy specimens, particularly if crushed.

Table 14.57 Panel of immunohistochemical markers and histochemical stains applied in the assessment of tumors of salivary glands

Tumors	Immunohistochemical Markers and Histochemical Stains
Tumors with epithelial glandular and myoepithelial component ▪ Pleomorphic adenoma ▪ Adenoid cystic carcinoma ▪ Epithelial-myoepithelial carcinoma	▪ Epithelial glandular component: CK (+), EMA (+), CEA (lumen: c-KIT) (+) ▪ Myoepithelial cell component: calponin (+), caldesmon (+), glial fibrillary acidic protein (GFAP) (+), SMMHC (+), S-100 protein (calcium binding protein) (+), p63 (+)
Mucoepidermoid carcinoma	▪ Goblet cells: mucicarmine (+) ▪ Intermediate cells: p63 (+) ▪ Myoepithelial cell component: calponin (–), caldesmon (–), glial fibrillary acidic protein (GFAP) (–), S-100 protein (calcium binding protein) (–), p63 (–)
Acinic cell carcinoma	PAS (+), PAS/diastase (+)
Tumors with oncocytic cells (Warthin's tumor, oncocytoma and oncocytic carcinoma)	Phosphotungstic acid hematoxylin (PTAH) (+), PAS/diastase (+)
Polymorphous low-grade adenocarcinoma	CK (+ 100%), vimentin (+ 100%), S-100 protein (calcium binding protein) (+ 97%), CEA (+ 55%), glial fibrillary acidic protein (GFAP) (+), MSA (+), EMA (+), BCL-2 (+)
Myoepithelioma	Myoepithelial cell component: calponin (+), caldesmon (+), glial fibrillary acidic protein (GFAP) (+), SMMHC (+), S-100 protein (calcium binding protein) (+), p53 (+)
Clear cell adenocarcinoma	▪ PAS/Diastase (+) ▪ Myoepithelial cell component: calponin (–), caldesmon (–), glial fibrillary acidic protein (GFAP) (–), SMMHC (+), S-100 protein (calcium binding protein) (–), p63 (–)
Salivary duct carcinoma	Androgen receptors (+), gross cystic disease fluid protein (GCDFP) (+), HER2/neu amplification (–/+)
Low-grade cribriform cystadenocarcinoma	S-100 (calcium binding protein) (+)

Immunohistochemistry technique has a limited role (except to confirm myoepithelial differentiation); diagnosis of salivary gland tumors is primarily based on hematoxylin and eosin histological examination. Proper sampling of tumor will reveal a component of the tumor that shows classic features of the tumor (i.e. not dominated by clear cells).

Tumors with clear cells include epithelial-myoepithelial carcinoma, mucoepidermoid carcinoma, acinic cell carcinoma, oncocytoma, oncocytic carcinoma, Warthin's tumor, myoepithelioma, myoepithelial carcinoma, clear cell adenocarcinoma and metastatic clear cell renal cell carcinoma.

Table 14.58 Immunohistochemistry technique of gastrointestinal tract tumors

Tumors	Immunohistochemical Markers
Barrett's esophagus (intestinal metaplasia at the gastroduodenal junction)	CK7 (surface and deep region) (+), CK20 (surface region) (+), Das (+), MUC1 (+), MUC2 (+)
Gastric adenocarcinoma	CDX2 (transcription factor, specific marker) (+), CK (+), EMA (+), CEA (+)
Colon adenocarcinoma	CDX transcription factor (+ specific marker associated with poor prognosis), CK7 (+), CK20 (+), EMA (+), CEA (+), SAT transcription factor (+), β-catenin (+), p53 (+), villin (+)
Gastrointestinal stromal tumors (GISTs)	DOG1 (+ most specific marker), c-KIT (CD117) (+ in 05%), PDGFα (+), CD171 (+), vimentin (+), SMA (+), desmin (+)
Gastrointestinal tract carcinoids (neuroendocrine tumors)	Synaptophysin (+), chromogranin A (+), NSE (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), CDX2 (+ 50%)
Gastrointestinal tract mucosa-associated lymphoid tissue (MALT) lymphoma	CD20 (+), CD43 (+), IgM > IgA > IgG (+), IgD (–), CD5 (–), CD10 (–), BCL-16 (–), cyclin D1

There is no definite immunohistochemical marker for gastric MALT lymphoma. Panel of immunohistochemical markers is applied to assess architecture of infiltration lymphoid cells, immunophenotype and chain restriction.

Table 14.59 Panel of immunohistochemical markers for tumors of liver

Tumors	Immunohistochemical Markers
Hepatocellular carcinoma (conventional variant)	Hepatocyte paraffin 1 (Hep Par 1) (+95%), p-CEA (highly specific canalicular marker) (+ 80%) α-fetoproteins (AFP) (+30–60%), CD10 (canalicular) (+), villin (canalicular) (+), CK20 (+ ≤20%), AE1/AE3 (+), CAM 5.2 (+ in 5–20%), CK903 (HMWCK) (+), glypican 3 (+ promising new marker of hepatocellular differentiation) (+), arginase 1 (new marker of hepatocellular differentiation) (+), α_1-antitrypsin (+ up to 50%), thyroid transcription factor 1 (TTF-1) (+), CK7 (–), epithelial membrane antigen (EMA) (–), MOC-31, mucicarmine (–)
Hepatocellular carcinoma (fibrolamellar variant)	CK7 (+), α-fetoproteins (AFP) (–)
Hepatoblastoma	α-fetoproteins (AFP) (+), hepatocyte paraffin 1 (Hep Par 1) (+), delta-like protein (DLK) (+), hCG (+), CK (+), CEA (+), glypican 3 (+), EMA (+), vimentin (+)
Cholangiocarcinoma	CK19 (+70–100%) (+), carcinoembryonic antigen monoclonal (m-CEA) (+), p-CEA (+), CK7 (+), CK20 (+), AE1/AE3 (+), CAM 5.2 (+), CK903 (+), α-fetoproteins (AFP) (–/+), hepatocyte paraffin 1 (Hep Par 1) (–), CD10 (–), villin (–), glypican 3 (–) arginase 1 (–)

Primary tumors metastasizing to liver include breast carcinoma, lung carcinoma, gastric carcinoma, colorectal carcinoma, pancreatic adenocarcinoma, renal cell carcinoma, prostatic carcinoma, endometrial carcinoma and thyroid carcinoma.

Table 14.60 Panel of immunohistochemical markers for tumors of pancreas

Tumors	Immunohistochemical Markers
Pancreatic adenocarcinoma*	CK7 (+), CK20 (+), CA19.9 (+), DPC4 (+) (SMAD4, MADH4 allelic loss), CEA (+), MUC1 (+), MUC5 (+), EMA (–).
Pancreatoblastoma (pancreatic element with ductal, acinar and neuroendocrine differentiation)	Trypsin (+ acinar marker), chymotrypsin (+ acinar marker), lipase (+ acinar marker), CK (+ ductal marker), CEA (+ ductal marker), synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+)
Pancreatic endocrine neoplasm (formerly known as islet cell tumor)	Synaptophysin (+), chromogranin A (+), NSE (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), AE1/AE3 (+), CAM 5.2 (+), insulin hormone (variable staining), glucagon hormone (variable staining), somatostatin hormone (variable staining)
Mucinous cystic neoplasm	ER/PR (+), inhibin (+)
Intraductal papillary mucinous neoplasm (IPMN)	DPC4 (+), MUC1 (+), MUC4 (+), MUC2 (–), CDX2 (–)
Serous cystadenoma	CK (+), EMA (+ 33%), inhibin (+), MUC6 (+), calponin (+), CD31 (+), factor VIII (+)
Lymphoplasmacytic sclerosing (autoimmune) pancreatitis	IgG4 + plasma cells infiltration (>20 per high power field)

Gene mutation and loss of expression of DPC is demonstrated in 55% of pancreatic adenocarcinoma.
**Serum tumor markers for pancreatic adenocarcinoma include CA 19-9, CEA, glucagon, proinsulin, C-peptide, IGFBP-1, pancreatic polypeptide and serotonin.*

Table 14.61 Panel of immunohistochemical markers for tumors of thyroid, parathyroid, adrenal, pituitary and thymus glands

Tumors	Immunohistochemical Markers
Thyroid gland	
Papillary thyroid carcinoma	TTF-1 (+), thyroglobulin (+), PAX8 (+), CAM 5.2 (+), AE1/AE3 (+), CK903 (+), CK10 (+), HBME-1, CK903, CK19, HBME-1, and galectin 3 favors thyroid papillary carcinoma over benign follicular carcinoma.
Follicular thyroid carcinoma	TTF-1 transcription factor (+), thyroglobulin (+), PAX8 (+), CK (+ CAM 5.2, AE1/AE3) (+), Ki-67 (+)
Medullary thyroid carcinoma	TTF-1 transcription factor (+), CAM 5.2 (+ 70%), AE1/AE3 (+ 75%), calcitonin (+), CEA (+), synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), Congo red (+ amyloid deposit in the tumor, PAX8 (–/+), thyroglobulin (–)
Thyroid anaplastic carcinoma	CAM 5.2 (+), AE1/AE3 (+ 75%), PAX8 (–/+), TTF-1 (–), thyroglobulin (–)
Parathyroid glands	
Parathyroid adenoma	CAM 5.2 (+), AE1/AE3 (+), parathormone (PTH) (+), synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), GATA3 (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), parafibromin (CDC73) (+), TTF-1 (–), thyroglobulin (–), PAX8 (–)

Contd...

Table 14.61 Panel of immunohistochemical markers for tumors of thyroid, parathyroid, adrenal, pituitary and thymus glands (*Contd...*)

Tumors	Immunohistochemical Markers
Parathyroid carcinoma	CK903 (+), CK19 (+), HBME-1 (+), galectin 3 (+), parafibromin (–)
Parathyroid neuroendocrine tumors	Synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), CK (AE1/AE3 and CAM 5.2) (+), PTH (+), S-100 protein (–)
Adrenal glands	
Adrenocortical neoplasms	Melan A (+), inhibin (+), calretinin (+), S-100 protein (–), HMB-45 (–), CK (AE1/AE3 and CAM 5.2) (–/+), synaptophysin (variable staining), chromogranin A (variable staining), neuron-specific enolase (NSE) (–), EMA (–)
Pheochromocytoma	Synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), S-100 protein (+), CK (–)
Neuroblastoma/ganglio-neuroblastoma	Synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), S-100 protein (+), CD56 (neuronal cell adhesion molecule) (–), CD99, CK (AE1/AE3 and CAM 5.2) (–)
Pituitary gland	
Pituitary neuroendocrine neoplasms	Synaptophysin (+), chromogranin A (+), neuron-specific enolase (NSE) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), S-100 protein (–)
Thymus gland	
Thymoma	Terminal deoxynucleotidyl transferase (TdT+), CD99 (+), CD1a (+), CD5 (+)
Thymic carcinoma	CD5 (+), glucose transporter 1 (GLUT-1+), CK (+)

Table 14.62 Panel of immunohistochemical markers applied in assessment of selected skin tumors

Tumors	Immunohistochemical Markers
Melanoma *in situ*	Melan A (A103) (80–100%). MART-1 (melanoma antigen recognized by cells (+80–100%). HMB-45 (human melanoma black 45) (+ 60–90%), MITF (microphthalmia transcription factor) (+), S-100 protein (+)
Melanoma (epithelioid variant)	Melan A (A103) (80–100%), MART-1 (melanoma antigen recognized by cells (+80–100%). HMB-45 (human melanoma black 45) (+60–90%), MITF (microphthalmia transcription factor) (+), S-100 protein (+), SOX (+), PNL2 (+), MUM1 (+)
Melanoma (desmoplastic variant)	S-100 protein (+), melan A (A103) (–), MART-1 (melanoma antigen recognized by cells) (–), HMB-45 (human melanoma black 45) (–), MITF (microphthalmia transcription factor) (–), SOX 100 (–), PNL2 (–),
Mycosis fungoides (MF)	T cell markers: CD45 (+), CD3 (+), CD20 (–), CD2 (–), CD5 (–), CD7 (–)
Squamous cell carcinoma *in situ*	CK903 (HMWCK) (+)
Squamous cell carcinoma (spindle cell variant)	CKs (+), p63 (+), desmin (–/+), SMA (smooth muscle actin) (–/+)
Sebaceous carcinoma	BerEP4 (+), CK7 (+), EMA (epithelial membrane antigen) (+)
Merkel cell carcinoma	CK20 (+), neurofilament (100%) (+), synaptophysin (+), chromogranin-A (+), NSE (neuron-specific enolase) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), cytokeratins (AE1/AE3, CAM 5.2) (+), merkel cell polyomavirus
Metastatic small cell carcinoma	TTF-1 (+), cytokeratins (AE1/AE3, CAM 5.2) (+), synaptophysin (+), chromogranin A (+), NSE (neuron-specific enolase) (+), CD56 (neuronal cell adhesion molecule—NCAM) (+), cytokeratins (AE1/AE3, CAM 5.2) (+), CK20 (–), neurofilament (–)
Dermatofibroma	Factor XIIIa (+), D2–40 (+), CD34 (–)

Contd...

Table 14.62 Panel of immunohistochemical markers applied in assessment of selected skin tumors (*Contd...*)

Tumors	Immunohistochemical Markers
Dermatofibrosarcoma protuberans (DFSP)	CD34 (+), factor XIIIa (–), D2–40 (podoplanin) (–)
Atypical fibroxanthoma	CD10 (+)
Primary cutaneous marginal zone lymphoma	CD20 (+), CD79a (+), BCL-2 (+), IgM (+/–), CD43 (+/–), CD5 (+/–), CD23 (–)
Primary cutaneous follicular cell lymphoma	CD20 (+), CD79a (+), BCL-2 (–/+)
Primary cutaneous diffuse large B cell lymphoma	CD20 (+), CD79a (+), BCL-2 (+), MUM1 (+), monotypic k or l (+), IgM (+), BCL-6 (+/–)

Table 14.63 Immunohistochemistry of round blue cell tumors (SRBCT)

Tumors	Immunohistochemical Markers
Wilm's tumor, blastema predominant	Cytokeratin (CK) (+), epithelial membrane antigen (EMA) (+), WT1 (+), WT2 (+), desmin (blastemal component) (+)
Ewing sarcoma/PNET	CD99 (+), synaptophysin (+), neuron-specific enolase (NSE) (+), periodic acid–Schiff (PAS) (+), cytokeratin (CK) (+ focal staining), epithelial membrane antigen (EMA) (focal staining) (+)
Lymphoblastic lymphoma	CD45 (+), CD99 (+), Terminal deoxynucleotidyl transferase (TdT) (+), CD4 (+), CD3 (+)
Rhabdomyosarcoma (solid alveolar variant)	Desmin (+), smooth muscle actin (SMA) (+), myogenin (+), MyoD1 (+), cytokeratin (CK) (+ focal staining), epithelial membrane antigen (EMA) (focal staining) (+), CD99 (focal staining) (+), synaptophysin (focal staining)

Table 14.64 Immunophenotyping of myeloblasts, B lymphoblasts and T lymphoblasts

Tumors	Immunophenotyping Markers
Myeloblast	Lineage-specific markers (CD13, CD15, CD33, CD11b) (+), CD34 (+/–), c-KIT (CD117) (+/–), HLA-DR (+/–)
B lymphoblasts	Lineage markers (CD19, CD79a) (+), terminal deoxynucleotidyl transferase (TdT) (+), CD20 (+/–), CD34 (+/–), CD10 (+/–), HLA-DR (+/–)
T lymphoblasts	Lineage marker (CD3), terminal deoxynucleotidyl transferase (TdT) (+), CD34 (+/–), CD10 (+/–), HLA-DR (–/+), c-KIT (CD117) (–/+), HLA-DR (–/+)

Table 14.65 Comparison of fibroblasts, smooth muscle cells and myofibroblasts and correlation of ultrastructure with hematoxylin and eosin staining

Characteristics	Fibroblasts	Smooth Muscle Cells	Myofibroblasts
Ultrastructure features	Rich in endoplasmic reticulum	Rich in myofilaments	Rich in both endoplasmic reticulum and myofilaments
Hematoxylin and eosin (H&E) stain	Cytoplasm stains—blue	Cytoplasm stains—deep blue	Cytoplasm stains—purple

Table 14.66 Commonly used immunohistochemical stains in surgical pathology

Immunohistochemical Markers	Expression and Cellular Localization	Classic use for Diagnosis
α_1-Antitrypsin (AAT-1)	Positive (cytoplasmic staining)	▪ Globules of α_1-antitrypsin (which accumulate in liver in α_1-antitrypsin deficiency) ▪ Hepatocellular carcinoma (Hep Par 1 is more specific) ▪ Histiocytic lesions (but CD68 is more specific)
α-Fetoproteins	Positive (cytoplasmic staining)	Yolk sac tumor (not specific), hepatocellular carcinoma except fibrolamellar variant, hepatoblastoma

Contd...

Table 14.66 Commonly used immunohistochemical stains in surgical pathology (*Contd...*)

Immunohistochemical Markers	Expression and Cellular Localization	Classic use for Diagnosis
ALK (anaplastic lymphoma kinase) expression as a result of t(2;5)/NMP-ALK	Positive (nuclear, cytoplasmic, membranous staining)	Anaplastic large cell lymphoma, lung adenocarcinoma with EML4-ALK rearrangement
Arginase 1 (new marker)	Positive (cytoplasmic staining)	Hepatocellular carcinoma
B72.3	Positive (cytoplasmic, membranous staining)	Adenocarcinoma
β-Catenin	Positive (nuclear staining)	Colon carcinoma, hepatoblastoma, pancreatoblastoma, craniopharyngioma, tubular adenoma, familial adenomatous polyposis
BCL-2 (B cell lymphoma 2)	Positive (cytoplasmic, membranous staining)	Follicular lymphoma, CLL/SLL, MCL (mantle cell lymphoma), MZL (marginal zone lymphoma)
BCL-6 (B cell lymphoma 6)	Positive (nuclear staining)	Follicular lymphoma, Burkitt lymphoma, synovial sarcoma
Ber-EP4	Positive (membranous staining)	Adenocarcinoma, lung adenocarcinoma
Brachyury	Positive (nuclear staining)	Chordoma, hemangioblastoma
CAIX (carbonic anhydrase)	Positive (membranous staining)	Renal cell carcinoma (clear cell variant), renal cell carcinoma (papillary variant in areas of necrosis)
CA125	Positive (luminal staining)	Serum tumor marker for monitoring ovarian carcinoma
CA 19-9 (carbohydrate antigen)	Positive (cytoplasmic staining)	Serum tumor marker for monitoring pancreatic carcinoma
Calcitonin	Positive (cytoplasmic and extracellular staining)	Medullary thyroid carcinoma, neuroendocrine tumors
Caldesmon (h-caldesmon)	Positive (cytoplasmic staining)	Leiomyosarcoma
CALLA (common acute leukemia antigen) CD10	Positive (membranous staining)	T cell ALL (100%)
Calponin	Positive (cytoplasmic staining)	Smooth muscle, myoepithelial cells, myofibroblasts (variable)
Calretinin (CRT)	Positive (cytoplasmic and nuclear staining)	Epithelial mesothelioma, sex–cord stromal tumors, cardiac myxoma, adrenocortical tumors, adenomatoid tumors
Cathepsin K	Positive (cytoplasmic staining)	Xp translocation renal cell carcinoma, chordoma, PEComas
CD1a	Positive (membranous staining)	Langerhans cell histiocytosis. T cell lymphoblastic lymphoma (some cases), thymoma with admixed thymocytes
CD2	Positive (membranous staining)	T cell lymphoma, NK cell lymphoma, leukemia
cCD3 (flow cytometry only)	Positive (cytoplasmic staining)	Most lineage-specific marker for T cell differentiation
CD3	Positive (membranous and cytoplasmic staining)	Pan-T cell marker, T cell lymphomas/leukemias
CD4, CD8	Positive (membranous staining)	T cell lymphomas/leukemias
CD5	Positive (membranous staining)	Low-grade lymphomas: CLL/SLL and MCL, high-grade lymphoma (blastoid marginal cell lymphoma, occasional DLBCL)

Contd...

Table 14.66 Commonly used immunohistochemical stains in surgical pathology (*Contd...*)

Immunohistochemical Markers	Expression and Cellular Localization	Classic use for Diagnosis
CD7	Positive (membranous staining)	T-ALL (100%), B cell lymphoma
CD10 (CALLA, common acute leukemia antigen)	Positive (membranous staining)	T cell ALL (100%)
CD11b—a flow marker	Positive (membranous staining)	Myelogenous leukemias with differentiation, NK cell tumors
CD11c—a flow marker	Positive (membranous staining)	Hairy cell leukemia
CD13, CD14, CD33	Positive (membranous staining)	Myelogenous leukemias
CD15	Positive (membranous and paranuclear dot-like staining)	AML differentiation, adenocarcinoma, Reed Sternberg cells in classic Hodgkin disease
CD19, CD20, CD22 (pan-B cell markers)	Positive (cytoplasmic, membranous staining)	B cell lymphoma, B precursor ALL (CD19), variable CD20, CD22; diffuse large B cell lymphoma (DLBCL) constantly CD19 positive), and CD20 reactivity is lost after treatment with rituximab
CD21	Positive (membranous staining)	Follicular lymphoma, mantle cell lymphoma, angioimmunoblastic T cell lymphoma
CD35	Positive (membranous staining)	Follicular lymphoma, mantle cell lymphoma, angioimmunoblastic T cell lymphoma
CD23	Positive (membranous staining)	Low-grade lymphoma (CLL/SLL), follicular lymphoma, mantle cell lymphoma
CD25 (IL-2 receptor)	Positive (membranous and cytoplasmic staining)	Hairy cell leukemia, adult T cell leukemia/lymphoma, anaplastic large cell lymphoma, neoplastic mast cells
CD30 (Ki-1)	Positive (membranous and paranuclear dot-like staining)	Reed-Sternberg cells in classic Hodgkin disease, anaplastic large cell lymphoma, embryonal carcinoma
CD31 (more sensitive and specific than CD34)	Positive (cytoplasmic and membranous staining)	Endothelial cell differentiation (e.g. vascular tumors like angiosarcoma, Kaposi sarcoma)
CD34	Positive (cytoplasmic and membranous staining)	Angiosarcoma, Kaposi sarcoma, hemangiopericytoma, dermatofibrosarcoma protuberans, gastrointestinal stromal tumors (70%), solitary fibrous tumor, nerve sheath tumors (e.g. schwannoma, neurofibroma, malignant peripheral nerve sheath tumor), epithelioid sarcoma (50%), adipocytic tumors
CD38	Positive (membranous staining)	Plasma cell differentiation, poor prognosis in CLL/SLL
CD41, CD42b, CD61 (flow markers)	Positive (membranous staining)	AML with megakaryocytic differentiation (AML-M7)
CD43	Positive (cytoplasmic staining)	Low-grade lymphoma (e.g. CLL/SLL, mantle cell lymphoma, marginal zone lymphoma; but not follicular lymphoma), myeloid sarcoma (chloroma)
CD44	Positive (cytoplasmic staining)	Small cell carcinoma of prostate

Contd...

Table 14.66 Commonly used immunohistochemical stains in surgical pathology (*Contd...*)

Immunohistochemical Markers	Expression and Cellular Localization	Classic use for Diagnosis
CD45 (leukocyte common antigen—LCA)	Cytoplasmic, membranous staining	Classic Hodgkin disease, lymphoblastic lymphoma, anaplastic large cell lymphoma (some cases)
CDX2	Positive (nuclear staining)	Gastrointestinal tract especially colon
CK (AE1/AE3, CAM 5.2)	Positive (cytoplasmic staining)	Epithelial cells, carcinoma
Desmin	Positive (cytoplasmic staining)	Smooth and striated muscle differentiation
E-cadherin	Positive (membranous staining) (mainly) and cytoplasmic (some) staining	Favors ductal over lobular breast carcinoma
ER/PR (estrogen and progesterone receptors)	Positive (nuclear staining)	Breast carcinoma—prognostic and therapeutic factor
HER2/neu	Positive (membranous staining)	Breast carcinoma—prognostic and therapeutic factor
GFAP (glial fibrillary astrocytic protein)	Positive (cytoplasmic staining)	Astrocytes and neural tumors
Human chorionic gonadotropin-β (hCG-β)	Positive (cytoplasmic staining in syncytiotrophoblastic component)	Choriocarcinoma
Hep Par 1	Positive (cytoplasmic staining)	Hepatocellular carcinoma
Melan A (MART1)	Positive (cytoplasmic staining)	Melanoma
HMB-45	Positive (cytoplasmic staining)	Melanoma
Ki-67 proliferative marker	Positive (nuclear staining)	Mitotic activity
p63	Positive (nuclear staining)	Epithelial cells (squamous, transitional)
Placental alkaline phosphatase (PLAP)	Positive (cytoplasmic staining)	Seminoma
Prostatic specific antigen (PSA)	Positive (membranous staining)	Prostatic carcinoma
S-100 (calcium binding protein)	Positive (cytoplasmic and nuclear staining)	Melanoma, neural differentiation
Synaptophysin	Positive (cytoplasmic staining)	Neuroendocrine differentiation (carcinoid)
Chromogranin A	Positive (cytoplasmic staining)	Neuroendocrine differentiation (carcinoid)
Thyroid transcription factor 1 (TTF-1)	Positive (nuclear staining)	Thyroid carcinoma, lung carcinoma
Vimentin	Positive (cytoplasmic staining)	Mesenchymal marker to confirm antigenicity

Table 14.67 Monoclonal antibodies used in immunohistochemistry in leukemias and lymphomas

CD Antigen (Cluster Designation)	Cell Specificity	Clinical Application
Anaplastic large cell lymphoma		
ALK (anaplastic lymphoma kinase)	ALCL cell	Anaplastic large cell lymphoma (ALCL)
B cell derived lymphomas/leukemias		
▪ BCL-2 ▪ BCL-6	▪ B cell ▪ B cell	▪ B cell NHL ▪ B cell NHL
▪ CD20 ▪ CD23 ▪ CD79a ▪ CD79b	▪ B cell ▪ B cell ▪ B cell ▪ B cell	B cell NHL

Contd...

Table 14.67 Monoclonal antibodies used in immunohistochemistry in leukemias and lymphomas (*Contd...*)

CD Antigen (Cluster Designation)	Cell Specificity	Clinical Application
▪ Cyclin D1 ▪ DBA-44 ▪ CD10	▪ B cell ▪ B cell ▪ Immature B cell	▪ Mantle cell lymphoma ▪ Hairy cell leukemia ▪ ALL (acute lymphoblastic leukemia) and lymphoma of follicular cell origin
B cell-derived classic variants of Hodgkin's disease		
▪ CD30 ▪ CD15	▪ Reed-Sternberg cell ▪ Reed-Sternberg cell	Classic variants of Hodgkin's disease
Activated T cell		
▪ CD3 ▪ CD5 ▪ CD4 ▪ CD8	▪ T cell ▪ T cell ▪ T-helper cell ▪ T-suppressor cell	T cell leukemia/T cell lymphoma
T cell/B cell subset derived lymphomas		
▪ CD43 ▪ CD45 RA ▪ CD45 RO	T cell, B cell subset	▪ T/B cell lymphoma/myeloid sarcoma ▪ T/B cell lymphoma ▪ T/B cell lymphoma
Natural killer cell (NK cell)-derived leukemias/lymphomas		
▪ CD56 ▪ CD57	NK cell (natural killer cell)	NK cell leukemias/lymphomas
Precursor T cell/B cell-derived lymphomas		
TdT (terminal deoxynucleotidyl transferase)	Percursor T cell/B cell	Precursor T cell/B cell lymphoma
Thymocyte/Langerhans cell derived leukemias/lymphomas		
CD10	Thymocyte, Langerhans cell	Precursor T cell/B cell leukemias/lymphomas
Follicular dendritic (T) C cell-derived tumor		
CD21	FDC (follicular dendritic cell)	FDC (follicular dendritic cell tumor and follicle differentiation)
Hematopoietic stem cell-derived leukemias		
▪ CD34 ▪ CD117	Hematopoietic stem cell	▪ Acute myelogenous/lymphoid leukemias ▪ Acute myelogenous leukemia
All leukocytes derived leukemias/lymphomas		
CD45	All leukocytes	Leukemias/lymphomas
Monocyte/histiocytic derived tumors		
CD68	Monocyte/histiocyte	Monocytic/histiocytic tumors
Platelet/megakaryocyte derived leukemia		
▪ CD42b ▪ CD61	Platelet/megakaryocyte	Acute megakaryoblastic leukemia
Proliferation fraction		
Ki-67	Proliferation fraction	High-grade lymphoma

Adapted from flow cytometry, immunohistochemistry and molecular genetics for hematolymphoid malignancies.

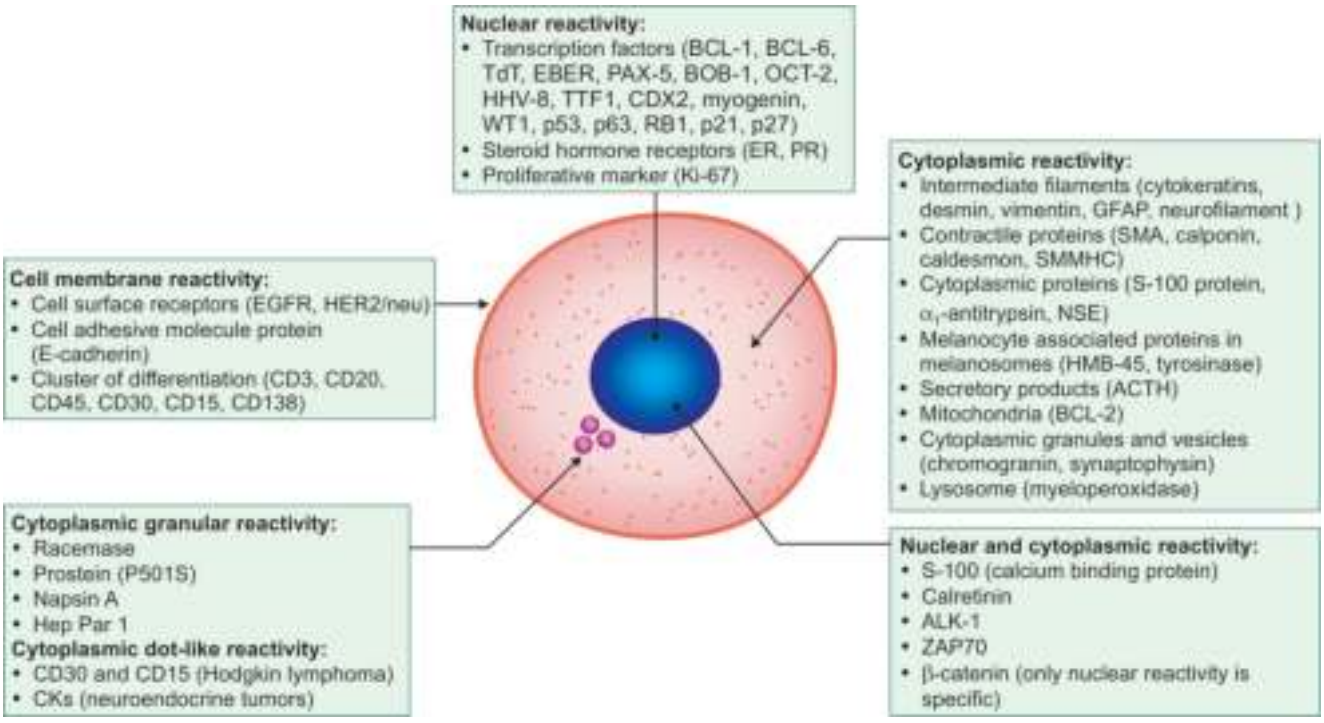


Fig. 14.9: Primers on location of antigens in the cells demonstrated by immunohistochemical markers.

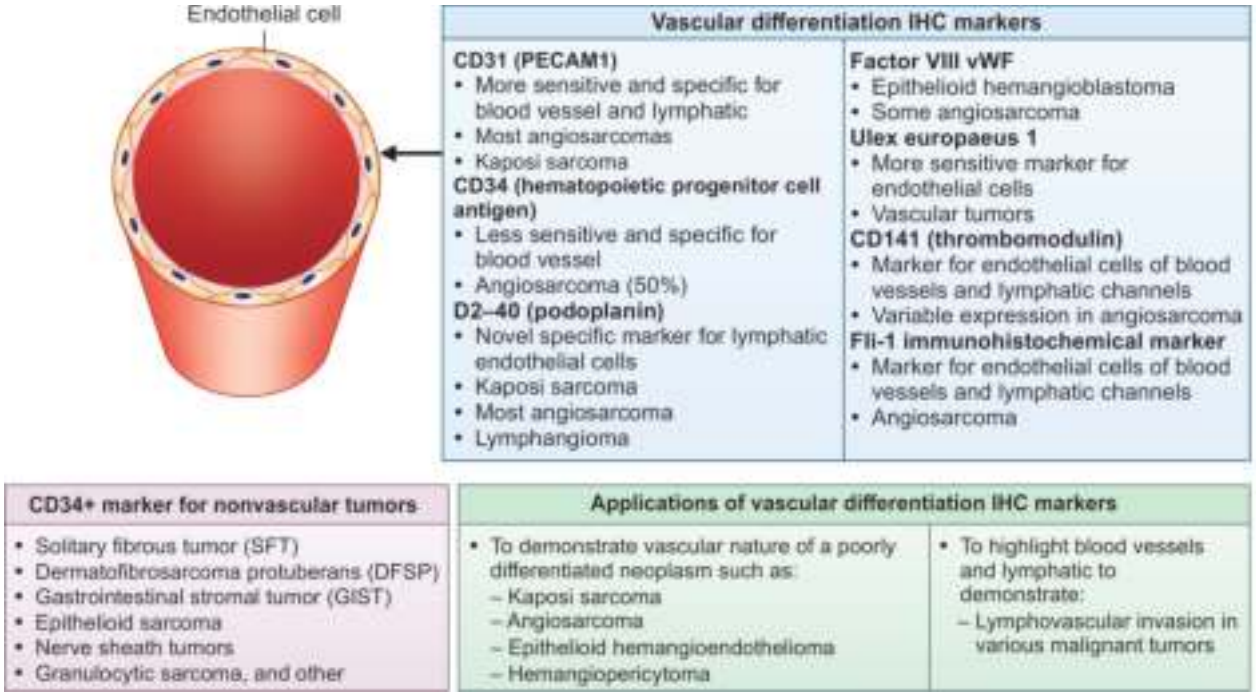
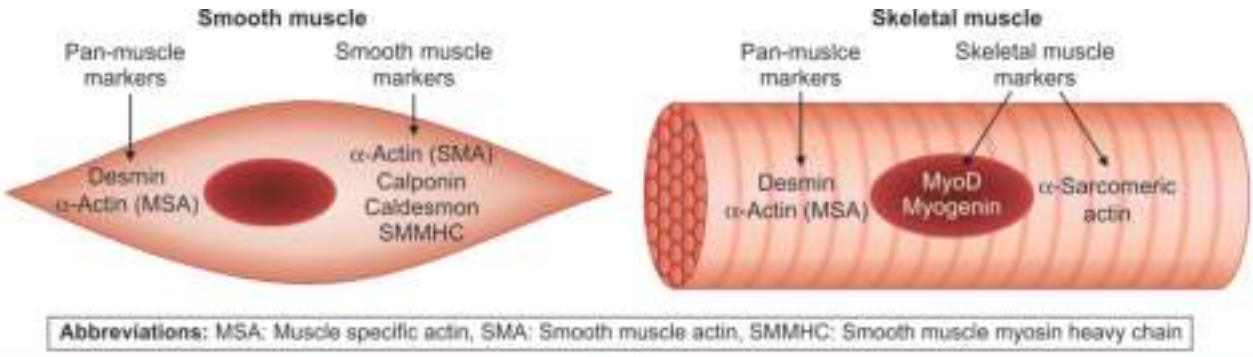


Fig. 14.10: Vascular endothelial immunomarkers.

EPITHELIAL TISSUE DIFFERENTIATION

Cytokeratins

Cytokeratins are cytoskeleton proteins, which belong to a family of intermediate filaments. These are detected by immunohistochemistry technique in tumors showing epithelial differentiation. Cytokeratins exist as heterodimers formed by two protein dimers and composed of acidic and basic subunits. Therefore, certain pairs of cytokeratins are expressed jointly, e.g. CK8/CK18 and CK1/CK10. Based on the nature, cytokeratins are categorized into basic (type B/class II) and acidic (type A/class I). Applications of immunohistochemistry in analysis of cytokeratins are given in Table 14.29.



Abbreviations: MSA: Muscle specific actin, SMA: Smooth muscle actin, SMMHC: Smooth muscle myosin heavy chain

IHC markers for smooth muscle differentiation

Desmin (intermediate filament)
- Universal pan-muscle marker for smooth muscle and striated muscle and also expressed variable in myofibroblasts
- Leiomyoma
- Leiomyosarcoma (70%)
- Rhabdomyosarcoma
- Also expressed in various epithelial neoplasms, e.g. renal cell carcinoma (clear cell variant), endometrioid carcinoma, thyroid carcinoma, mesothelioma, salivary gland carcinoma, sweat gland carcinoma and spindle cell carcinoma of any site

Muscle specific actin (MSA)
- Pan-muscle marker for smooth muscle and striated muscle

Smooth muscle actin (also known as actin)
- Expressed in smooth muscle, but absent in striated muscle
- Smooth muscle tumors (e.g. leiomyoma, leiomyosarcoma)
- Myoblastic tumors like lesion (nodular fasciitis)

Contractile apparatus associated proteins
- Expressed in smooth muscle, but absent in skeletal muscle
- Smooth muscle myosin heavy chain (SMMHC)
- Calponin (contractile apparatus associated proteins encoded by CALD-1 gene) expressed in smooth muscle, leiomyoma, leiomyosarcoma, , myofibroblasts, myofibroblastic tumors and myoepithelial cells
- Caldesmon (encoded by CALD-1 gene) expressed in normal smooth muscle, leiomyoma and leiomyosarcoma

IHC markers for skeletal muscle differentiation

Desmin (intermediate filament)
- Universal pan-muscle marker for smooth as well as striated muscle (antibody clone D33)
- Expression of desmin variable in myofibroblasts
- Leiomyoma
- Leiomyosarcoma (70%)
- Rhabdomyosarcoma
- Also expressed in various epithelial neoplasms, e.g. renal cell carcinoma (clear cell variant), endometrioid carcinoma, thyroid carcinoma, mesothelioma, salivary gland carcinoma, sweat gland carcinoma and spindle cell carcinoma of any site

Muscle specific actin (MSA)
- Like desmin, muscle specific antigen is pan-muscle marker for smooth muscle and striated muscle. HHF-35 antibody recognizes epitope common to α-skeletal, α-cardiac and γ-smooth muscle actin

MyoD1 and myogenin (transcriptional factors)
- Expressed in striated muscle but absent in smooth muscle
- Expressed in leiomyosarcoma but absent in leiomyoma
- Embryonal rhabdomyosarcoma shows focal positivity staining pattern
- Alveolar rhabdomyosarcoma shows diffuse positivity staining pattern

Fig. 14.11: Immunomarkers for smooth muscle and skeletal muscle differentiation.

Immunohistochemical markers expression profile of CK7 and CK20 in human cancers is given in Table 14.30. Based on gel electrophoresis, cytokeratins are categorized into high-molecular weight cytokeratin (HMWCK) and low-molecular weight cytokeratin (LMWCK).

- **High molecular weight cytokeratins (HMWCKs):** These are also known as squamous keratins, which form a dense cytoplasmic network of filaments and account for resistance to mechanical stress of surface epithelia. These are also known as tonofilaments demonstrated in squamous epithelia and squamous cell carcinoma. HMWCKs (CK9, CK10, CK11, CK16, CK17) are detected by CK903 and CK5/6 antibodies for demonstration of squamous keratins in squamous cell carcinoma.
- **Low molecular weight cytokeratins (LMWCKs):** These are also known as 'nonsquamous keratins' or simple keratins. These are loosely distributed in the cytoplasm but unable to form bundle. LMWCK experiences little mechanical stress as seen in liver, kidney and various glandular epithelia. It is worth mentioning that glandular epithelia of breast, high-grade squamous cell carcinoma, pancreatic adenocarcinoma and endometrial adenocarcinoma co-express both LMWCK and HMWCK cytokeratins. LMWCK (CK18, CK19, CK20) is detected by CAM 5.2 antibody in colon carcinoma, prostatic carcinoma, renal cell carcinoma and hepatocellular carcinoma.

Epithelial Membrane Antigen

Epithelial membrane antigen (EMA) is common epithelial cell marker demonstrated in meningioma, breast adenocarcinoma, colorectal adenocarcinoma, pancreatic adenocarcinoma, Paget's disease of breast, choriocarcinoma and desmoplastic small round cell tumor. Expression of epithelial membrane antigen (EMA) in various neoplasms is given in Table 14.31.

Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA) is a protein normally present in very low levels in the blood of adults. Its blood level may be increased in certain types of human cancers and benign conditions. CEA analysis is most commonly performed in patient of colorectal carcinoma, in which CEA level is elevated. After treatment, if elevated CEA level returns to normal, it indicates that the colorectal carcinoma has responded to treatment. CEA level that rises steadily after treatment of complete excision of colorectal carcinoma often indicates that the cancer has recurred. Expression of carcinoembryonic antigen (CEA) in human cancers is given in Table 14.32.

TP53 Gene Product (p53 Protein)

TP53 is a tumor suppressor gene mapped at 17p13 that encodes p53 protein. The p53 protein ensures that cells repair any damaged DNA before cell division by inducing cell cycle arrest to allow time for DNA repair or to force the cell to undergo apoptosis via activation of proapoptotic BAX gene. Mutation in TP53 gene is most commonly demonstrated in human cancers. TP53 gene product analysis can differentiate human cancers, which as often p53 positive (carcinoma *in situ* and invasive epithelia of different sites) from metaplastic conditions, which are usually p53 negative. Germline heterozygous mutations in TP53 gene cause Li-Fraumeni syndrome. TP53 gene mutation and associated cancers are given in Table 14.33.

VASCULAR ENDOTHELIAL CELL DIFFERENTIATION

Many studies have been published comparing the expression and distribution of vascular endothelial cell markers such as CD31, CD34, D2–40, von Willebrand factor-related antigen, Fli-1, Fli-4, CD141 and Ulex europaeus 1 in different vascular beds in normal human tissues. Formalin fixed paraffin-embedded sections are applied vascular endothelial immunomarkers to study angiogenesis in human cancers. Vascular endothelial immunomarkers are shown in Fig. 14.10.

SMOOTH AND SKELETAL MUSCLE DIFFERENTIATION

Immunomarkers for smooth muscle and skeletal muscle differentiation are given in Fig. 14.11.

Smooth Muscle Differentiation Immunomarkers

Desmin

Desmin is intermediate filament present near the Z line in sarcomeres in smooth muscle, skeletal muscle and cardiac muscle. There is variable expression of desmin in myofibroblasts. Desmin stain is used in panel of immunomarkers for mesenchymal tissue, which helps to differentiate smooth muscle tumors (desmin positive) from gastrointestinal stromal tumors (c-KIT positive but always desmin negative). In serous effusion, reactive mesothelial cells are desmin positive (84%). However, desmin is expressed in mesothelioma (8%) or carcinoma (2%).

Muscle-specific Actin

Muscle-specific actin (MSA) is expressed in smooth muscle and skeletal muscle. Nonmuscle sarcomas, carcinomas, lymphomas and melanomas do not express muscle specific actin. MSA expression is demonstrated in soft tissue tumors with muscle differentiation such as leiomyomas, leiomyosarcomas and rhabdomyosarcomas subtypes.

Contractile Apparatus Associated Proteins

Contractile apparatus associated proteins are demonstrated in the cytoplasm of smooth muscle fibers that consist of the myofilaments, myosin and actin.

- Examples of contractile apparatus associated proteins are SMMHC (smooth muscle myosin heavy chain), calponin (contractile apparatus associated protein) and caldesmon (contractile apparatus associated protein).
- SMMHC is expressed in smooth muscle, leiomyoma, leiomyosarcoma; but absent in skeletal muscle.
- Calponin is expressed in smooth muscle, leiomyoma, leiomyosarcoma, myoepithelial cells, myofibroblasts, myofibroblastic tumors; but absent in skeletal muscle.
- Caldesmon is demonstrated in smooth muscle, leiomyoma, leiomyosarcoma; but absent in skeletal muscle.

Skeletal Muscle Differentiation Immunomarkers

Desmin

Desmin is intermediate filament present near the Z line in sarcomeres in smooth muscle, skeletal muscle and cardiac muscle. It is expressed in myoblasts. Desmin stain is used in panel of immunomarkers for mesenchymal tissue. Desmin is expressed in leiomyoma, leiomyosarcoma (70%), rhabdomyosarcoma; and myofibroblasts (variable staining).

Muscle-specific Actin

Muscle-specific actin (MSA) is expressed in smooth muscle and skeletal muscle. MSA expression is demonstrated in soft tissue tumors with muscle differentiation such as leiomyomas, leiomyosarcomas and rhabdomyosarcomas of various subtypes.

MyoD1 (Transcriptional Factor)

MyoD1 gene encodes a nuclear protein MyoD1 that belongs to the basic helix-loop-helix (bHLH) family of transcription factors. MyoD1 regulates muscle

cell differentiation by inducing cell cycle arrest, a prerequisite for myogenic initiation. MyoD1 is expressed in skeletal muscle, alveolar rhabdomyosarcoma (diffuse staining), embryonal rhabdomyosarcoma (focal staining), leiomyosarcoma but negative in normal smooth muscle and leiomyoma.

Myogenin (Transcriptional Factor)

Myogenin is expressed in skeletal muscle, alveolar rhabdomyosarcoma (diffuse staining), embryonal rhabdomyosarcoma (focal staining), leiomyosarcoma but negative in normal smooth muscle and leiomyoma.

Myoglobin

Myoglobin is expressed in regenerating skeletal muscle and well-differentiated cells in rhabdomyosarcoma and tumors with heterologous skeletal muscle differentiation.

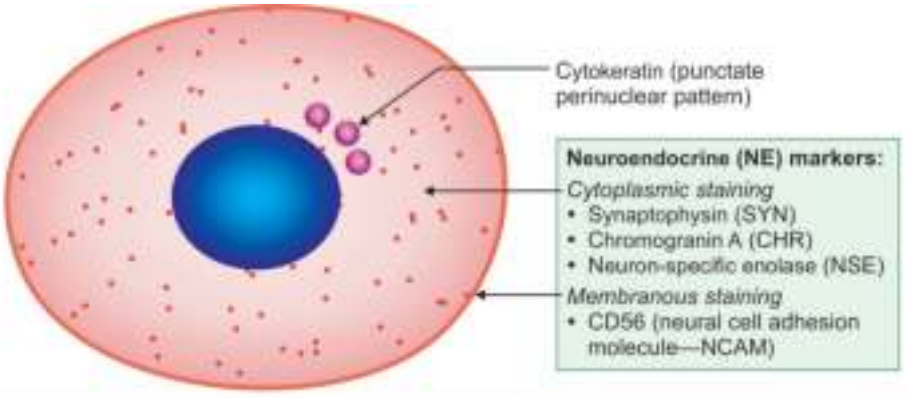
NEUROENDOCRINE DIFFERENTIATION

Neuroendocrine differentiation marks a structural and functional feature of certain benign and malignant tumors. Neuroendocrine tumors may arise at almost any anatomical site, including paraganglia and are distributed throughout the body in organs including soft tissues.

- Malignant neuroendocrine tumors contain a significant proportion of cells displaying neuronal, endocrine or mixed features. Neuroendocrine tumors may contain higher than normal quantity of hormones into the circulation in response to signal from the nervous system which can cause various clinical manifestations.
- Some examples of neuroendocrine tumors are prostatic neuroendocrine tumor, carcinoid tumors, islet cell tumors, medullary thyroid carcinoma, pheochromocytoma, neuroendocrine carcinoma of the skin (Merkel cell carcinoma), small cell lung carcinoma and large cell neuroendocrine carcinoma of lung.
- Three grading parameters of prognostic relevance in neuroendocrine tumors are the mitotic index, Ki-67 labeling index and presence or absence of necrosis defined by morphologic criteria.
- Immunohistochemical stains for neuroendocrine differentiation include neuron-specific enolase (NSE), chromogranin A, chromogranin B, synaptophysin and CD56 as well as site-specific markers such as hormones and transcription factor. Panel of immunohistochemical markers of neuroendocrine and neuroectodermal neoplasms is shown in Fig.14.12.

MYOFIBROBLASTIC TISSUE DIFFERENTIATION

Myofibroblasts are activated fibroblasts, essential for wound healing and tissue remodeling following insult. These can also be derived from epithelial cells, endothelial cells, and mononuclear cells. Local resident



Immunohistochemical markers of neuroendocrine (NE) and neuroectodermal neoplasms

- Carcinoid tumors (SYN, CHR, NSE, CD56, CK, TTF1)
- Medullary carcinoma of thyroid (SYN, CHR, NSE, CD56, CK, TTF1, calcitonin, CEA, amyloid)
- Pancreatic endocrine neoplasm (SYN, CHR, NSE, CD56, CK)
- Pheochromocytoma (SYN, CHR, NSE, CD56, CK, S-100 protein)
- Paraganglioma (SYN, CHR, NSE, CD56, S-100 protein)
- Small cell neuroendocrine carcinoma (SYN, CHR, NSE, CD56, TTF1)
- Merkel cell carcinoma (SYN, CHR, NSE, CD56, CK, neurofilament, Merkel cell polyomavirus)
- Pituitary neoplasms (SYN, CHR, NSE, CD56, CK, GH, ACTH, TSH, LH, FSH)
- Parathyroid neoplasms (SYN, CHR, NSE, CD56, CK, parathormone)
- Neuroblastoma (SYN, CHR, NSE, CD56, S-100 protein)
- PNET/Ewing sarcoma (NSE, CK, CD99, PAS)

Fig. 14.12: Flow chart represents panel of immunohistochemical markers of neuroendocrine and neuroectodermal neoplasms

fibroblasts are activated by mechanical stretch such as TGF-β. Myofibroblasts and activated fibroblasts proliferate and synthesize extracellular matrix. Infiltrating macrophages synthesize cytokines that contribute to many of cellular processes. Myofibroblasts express calponin, smooth muscle actin, desmin (variable expression), however do not express caldesmon.

MYOEPITHELIAL DIFFERENTIATION (POLYPHENOTYPE)

Myoepithelial cells are usually present in glandular epithelium as thin layer above the basement membrane but generally beneath the luminal cells, which may be positive for smooth muscle actin (also called α-actin), p53, glial fibrillary acidic protein (GFAP), S-100 protein, CK14, smooth muscle myosin heavy chain (SMMHC), calponin and caldesmon. Smooth muscle actin can contract and expel the secretions of exocrine glands.

MELANOCYTIC LINEAGE DIFFERENTIATION

Melanocyte lineage is derived from neural crest. The neural crest has its origin in the neural tube. Following neural crest formation, neural crest cells delaminate from the dorsal-most aspect of the neural tube by a process of epithelial–mesenchymal transition (EMT).

- The neural crest cells migrate and go onto form many specialized structures and tissues in the developing embryo by migration, proliferation and differentiation.
- Melanoblasts are derived from neural crest cells via a SOX10 positive melanoblasts/glial bipotent progenitor. The key step during melanocyte formation is the activation of microphthalmia transcription factor (MITF).
- Melanocytes express Melan A (A103), melanoma-associated antigen recognized by T cells 1 (MART-1), human melanoma black 45 (HMB-45) marker of immature melanocytes, S-100 protein, microphthalmia transcription factor (MITF), SOX100, PNL2, MUM1, tyrosinase and CD63.
- Immunomarkers for epithelioid melanoma are Melan A (A103), melanoma-associated antigen recognized by T cells 1 (MART-1), human melanoma black 45 (HMB-45), SOX100, PNL2 and MUM1. S-100 protein is expressed in epithelioid melanoma, spindle cell melanoma and desmoplastic melanoma and other tumors. Other markers for malignant melanoma are SOX100, tyrosinase, CD63, PNL2 and MUM1.

IMMUNOFLUORESCENCE MICROSCOPY

IMMUNOFLUORESCENCE MICROSCOPY: OVERVIEW

Immunofluorescence microscopy is a powerful technique that utilizes fluorescent labeled antibodies to detect specific target antigens. Fluorescein dye emits greenish fluorescence under UV light, which can be tagged to immunoglobulin molecules.

- Sometimes, immunofluorescence microscopy technique is used to make viral ocular plaque more readily visible to human eye. Immunofluorescent labeled tissue sections are studied using a fluorescent microscopy. It is very sensitive method.
- Disadvantages of immunofluorescence microscopy are expensive equipment and its reagents, cross reactivity and nonspecific immunofluorescence.

PRINCIPLE

Principle of immunofluorescence microscopy involves usage of fluorescein isothiocyanate labeled immunoglobulin to detect antigens or antibodies according to test systems under a fluorescent microscope.

The second layer antibody can be labeled with fluorescent dye such as fluorescein isothiocyanate (FITC), rhodamine or texas red in indirect immunofluorescence microscopy.

TYPES OF IMMUNOFLUORESCENCE MICROSCOPY

There are two types of immunofluorescence microscopy: direct and indirect (Table 14.68). Immunofluorescence microscopy by using probe is shown in Fig. 14.13.

DIRECT IMMUNOFLUORESCENCE MICROSCOPY

Direct immunofluorescence microscopy is simple and very common procedure. Antigen is fixed on a slide and fluorescein labeled antibodies are layered over it. Then the slides are washed to remove unattached antibodies and examined under UV light in fluorescent microscope. The site where the antibody attaches to its specific antigen shows apple green fluorescence. This technique is used for direct detection of pathogens or their antigens in tissue or in pathologic samples.

Table 14.68 Differences between direct and indirect immunofluorescence microscopy

Parameters	Direct Immunofluorescence Microscopy	Indirect Immunofluorescence Microscopy
Time	Shorter procedure, single labeling step	Longer procedure, double labeling step
Cross reactivity	Minimized	May cross react with other than the target
Sensitivity	Less	High due to amplification of signal
Cost	Expensive	Relatively inexpensive

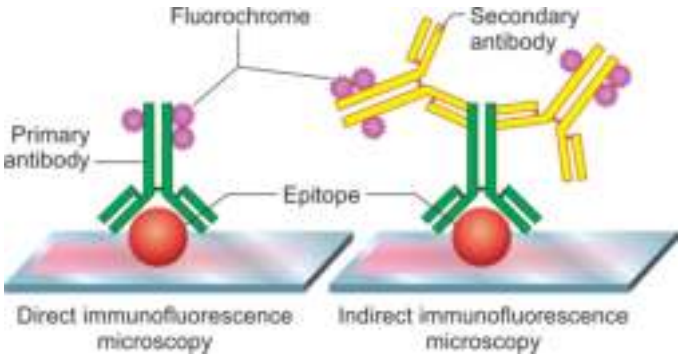


Fig. 14.13: Direct and indirect immunofluorescence microscopy.

INDIRECT IMMUNOFLUORESCENCE MICROSCOPY

Indirect immunofluorescence microscopy utilizes a two-step procedure to detect antigens on the slides. In the first step, a primary unlabeled antibody is used to bind and detect antigen. Then a second fluorescent labeled antibody binds the Fc portion of the primary antibody. This technique is more sensitive because multiple secondary antibodies can bind to a single primary antibody, thus amplifying the signal strength. Clinical applications of immunofluorescence microscopy are given in Table 14.69.

Table 14.69 Clinical applications of immunofluorescence microscopy

Type of Immunofluorescence	Sampling	Systems Involved	Applications in Diagnosing Disorders
Direct immunofluorescence microscopy	Tissue sample	▪ Skin and mucosa	▪ Bullous pemphigoid ▪ Pemphigus vulgaris ▪ Dermatitis herpetiformis ▪ Discoid lupus
		▪ Kidney (evaluation with fluorescein isothiocyanate—FITC conjugated method against IgG, IgM, IgA, C3, C1q, fibrinogen, albumin, κ and λ)	▪ Glomerulonephritis
		▪ Kidney (evaluation of C4D) ▪ Lung (evaluation of C4D)	▪ Humoral renal transplant rejection ▪ Humoral lung transplant rejection
Indirect immunofluorescence microscopy	Blood sample without anticoagulant (serum dilutions is removed and applied to an epithelial substrate together with fluorescein-labeled IgG)	Skin lesions	▪ Pemphigus vulgaris ▪ Paraneoplastic pemphigus ▪ Bullous and/or cicatricial pemphigoid

Other applications of immunofluorescence are to detect viruses (herpes, varicella-zoster, cytomegalovirus, influenza, HIV), bacteria (Mycobacterium tubercle bacilli and Treponema pallidum), and Entamoeba histolytica.

CHEMILUMINESCENCE VERSUS CHEMIFLUORESCENCE TECHNIQUES

Traditionally, radioisotopic protocols have been used in the laboratory. Keeping in view of high cost of isotopes and difficulty in their disposal, many scientists are turning to nonisotope methods as a substitute to isotopes. Currently, chemiluminescence and chemifluorescence techniques are used in the laboratories.

CHEMILUMINESCENCE TECHNIQUE

Chemiluminescence is nonisotope technique based on chemical reaction occurring between an enzyme horseradish peroxidase (HRP) and luminol resulting in light emission. The light emission is detected by changed couple device (CCD), X-ray film, photomultiplier tube (PMT) based detectors. This technique is more sensitive than calorimetric methods.

The antibody based systems provide target specificity. Enzyme component provides signal amplification. This has now become a protocol for substituting radioisotopes worldwide. Schematic diagram of chemiluminescence technique is shown in Fig. 14.14. Applications of chemiluminescence in clinical pratice are given in Table 14.70.

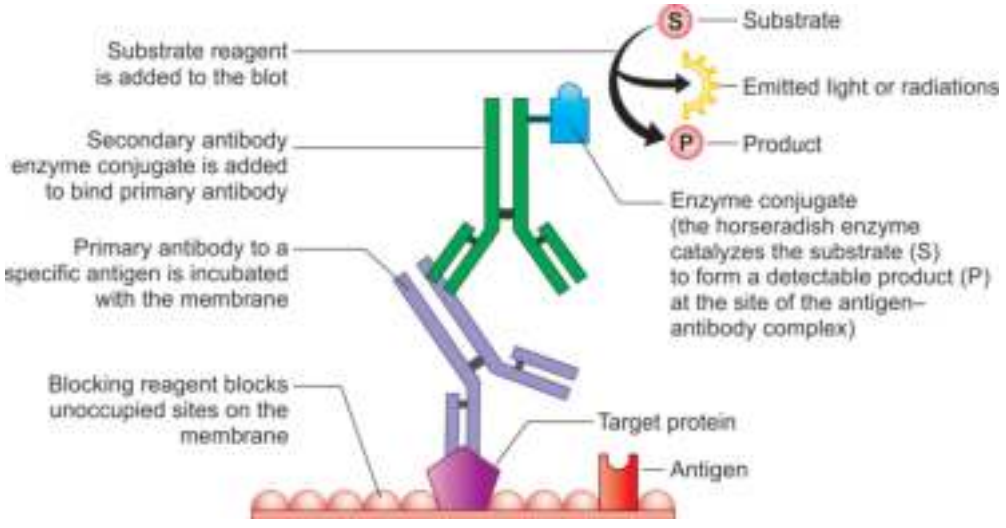


Fig. 14.14: Schematic diagram of chemiluminescence technique.

Table 14.70 Applications of chemiluminescence technique in clinical practice

Categories	Uses
Chemiluminescence immunoassay	Chemifluorescence is a sensitive technique used alternative to conventional colorimetric methodologies. Principle is same as ELISA technique and uses a chemiluminescent substrate for detection of the following: ▪ Hormones: Insulin, thyroxin, estradiol, testosterone, progesterone ▪ Vitamin: Vitamin B_{12} ▪ Tumor markers: Bone morphogenic protein 2, carcinoembryonic antigen (CEA), α-fetoprotein (AFP) and human β-chorionic gonadotropin (β-hCG) ▪ C-reactive protein and tumor necrosis factor: during inflammation
DNA hybridization detection	DNA hybridization technique uses specific chemiluminescent detecting reagents containing H_2O_2 and luminol for the following uses: ▪ Identifying DNA in crime case ▪ Paternal dispute ▪ Classify DNAs of various organisms
Western blotting	Western blotting (or protein immunoblotting) is a technique widely used to detect specific proteins in samples of tissue, cell lysates, cell culture supernatants or body fluids
Food analysis	Chemiluminescence technique is used to determine N-nitroso compounds, sugar, anabolic hormone, metabolites, food oxidations, metals and other interesting compounds
Forensic science	Chemiluminescence technique is used by criminalists to detect traces of blood at crime scene. Tiny amount of iron from hemoglobin in blood serves as catalyst for the chemiluminescence reaction that causes chemiluminescent reagent luminol to glow

CHEMIFLUORESCENCE TECHNIQUE

Chemifluorescence is an alternative technique used for molecular study, which combines fluorescence and chemiluminescence. Difference between chemiluminescence and chemifluorescence is based on detection mechanism. In chemiluminescence technique, light is generated based on enzymatic reaction, in which fluorescent molecule is attached to either secondary or tertiary antibody, which requires excitation via laser or some other high intensity light source. Excitation of fluorescent molecule generates a light emission collected by CCD or photomultiplier tube (PMT) based detector.

Chemifluorescence technique provides greater sensitivity, targeted specificity and amplification of signal as compared to calorimetric methods as well as chemiluminescence. Comparison between chemiluminescence and chemifluorescence technique is given in Table 14.71. Schematic diagram of chemifluorescence technique is shown in Fig. 14.15.

Table 14.71 Comparison between chemiluminescence and chemifluorescence technique

Parameters	Chemiluminescence Technique	Chemifluorescence Technique
Detection system	CCD, X-ray film, PMT based detectors	CCD, PMT based detector
Excitation	None	Required
Detection levels	Femtogram	Femtogram
Shelf life	Up to one year	<1 month
Cost	Inexpensive	Expensive

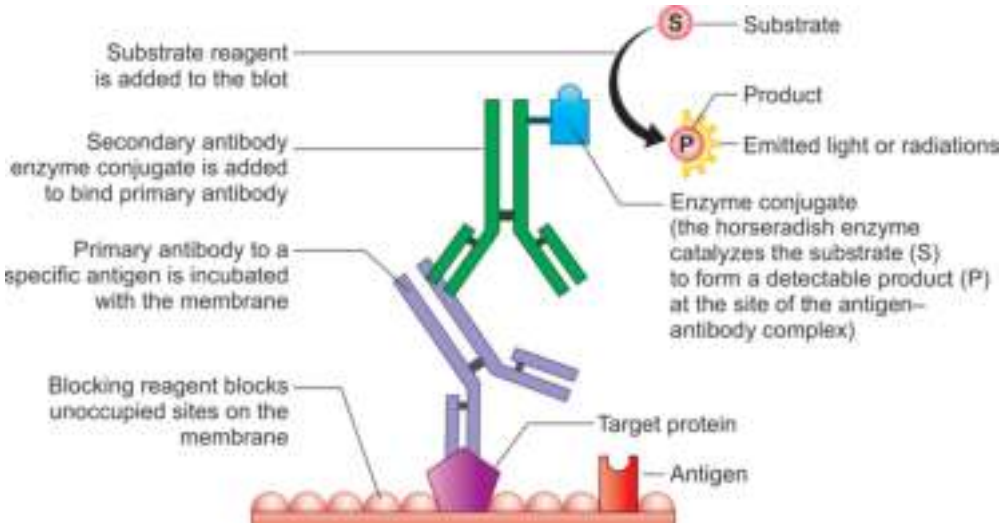


Fig. 14.15: Schematic diagram of chemifluorescence technique.

MOLECULAR DIAGNOSTICS TO ANALYZE CELL PROLIFERATION

There are three techniques to analyze proliferation of cells, which include Ki-67 (MB-1), mitotic cell count and flow cytometry technique/fluorescence activated cell sorting (FACS) analysis. FACS is a derivative of flow cytometry technique that adds an exceptional degree of functionality.

Ki-67 (MB-1) ANALYSIS

Ki-67 (MB-1) is applied on formalin-fixed paraffin-embedded tissue. It establishes prognostic marker for some malignant tumors. It captures all cells undergoing proliferation irrespective of the stage in the cell cycle. Therefore, Ki-67 (MB-1) is not a true analysis of mitosis.

MITOTIC CELL COUNT ANALYSIS

Mitotic cell count analysis is specific for mitotic cells. Technique can detect aberrant mitoses, aneuploidy in hematoxylin and eosin stained sections. It is unable to measure proliferating cells outside mitotic phase of cell cycle.

FLOW CYTOMETRY TECHNIQUE

By using a panel of selected monoclonal antibodies, flow cytometric analysis can determine cell lineage (i.e. B cell, T cell, natural killer cell or myelomonocytic cell), developmental stage (mature vs immature cells, thymic vs post-thymic cells) and clonality (monoclonal or polyclonal) of a given cell population. Flow cytometric analysis can also determine the heterogenous and aberrant morphologic features and percentage of malignant tumor cells.

- Flow cytometry can count cells in each phase of cell cycle and determine ploidy status, which can also be used as an ancillary technique in cytology. Cells are labeled with fluorochrome dyes, that can be attached to the antibodies for detection of specific proteins, e.g. cell lineage markers. Fluorochrome dyes can bind directly to cellular DNA or lipids.
- Fluorescence of the dyes is analyzed after exposure to an argon laser. Fluorescence occurs as the cells in liquid medium 'flow' past the laser. Cell size and number can also be determined by flow cytometry.

Table 14.72 Color of fluorochromes used in flow cytometry

Fluorochrome	Color
Propidium iodide	Red color
Peridinin chlorophyll	Red color
Texas red	Red color
Phycocyanin	Red color
Allophycocyanin	Red color
Cyanin 5	Red color
Fluorescein isothiocyanate	Green color
Phycoerythrin	Orange color
Rhodamine	Orange color

- A flow cytometer is versatile in its capability of measuring multiple parameters simultaneously, which analyzes multiple suspended cells ranging from 0.2 to 150 μm flowing in a fluid stream through a beam of light. Various parameters in flow cytometric analysis include cell size, cytoplasmic granularity, surface antigen, cytoplasmic antigen, nuclear antigen and DNA-RNA contents of individual cells in a cell suspension.
- Antigen expressed on cell surface, cytoplasm and nucleus are detected by means of fluorochrome conjugated antibodies. Color of fluorochromes used in flow cytometry is given in Table 14.72.
- Samples analyzed by flow cytometer include blood, bone marrow aspirate, fine needle aspirate, body fluids and fresh solid malignant tissue suspension.
- Cellular parameters are analyzed through an optical system. The light signal generated is registered in an electronic system. The computer system is responsible for data storage and graphic display on a screen.
- Samples are prevented clotting by addition of anti-coagulants such as disodium EDTA, sodium citrate or heparin. Concentration of white blood cells is enhanced by lysis of red blood cells by addition of ammonium chloride. Cells can also be obtained from solid malignant tissue suspension and converted into suspension for flow cytometric analysis suspensions. Comparison between immunohistochemistry and flow cytometry technique is given in Table 14.73.

COMPONENTS OF FLOW CYTOMETER

The flow cytometer is composed of flow system, optical system and electronic system. The flow system transports cells in a stream to the laser beam for analysis. The optical system consists of lasers to illuminate the cells in the sample stream and optical fibers to direct

Table 14.73 Comparison of immunohistochemistry and flow cytometry technique

Parameters	Immunohistochemistry Technique	Flow Cytometry Technique
Specimen required	Formalin fixed paraffin-embedded and frozen sections	Blood, bone marrow, body fluids, fine needle aspirates and fresh solid tissues
Equipment cost	Less expensive equipment	More expensive equipment
Morphologic correlation	Excellent visual correlation	No visual correlation
Available monoclonal antibodies	Limited monoclonal antibodies but gradually expanded	Abundant monoclonal antibodies
Dual stain applied on same cells	Limited	5–6 color staining
Quantitation	Estimation with imaging techniques	Accurate and reproducible
Distinction between surface and cytoplasmic staining	Difficult	Easy
Turn around time	4 hours with hematoxylin and eosin stained section	3 hours

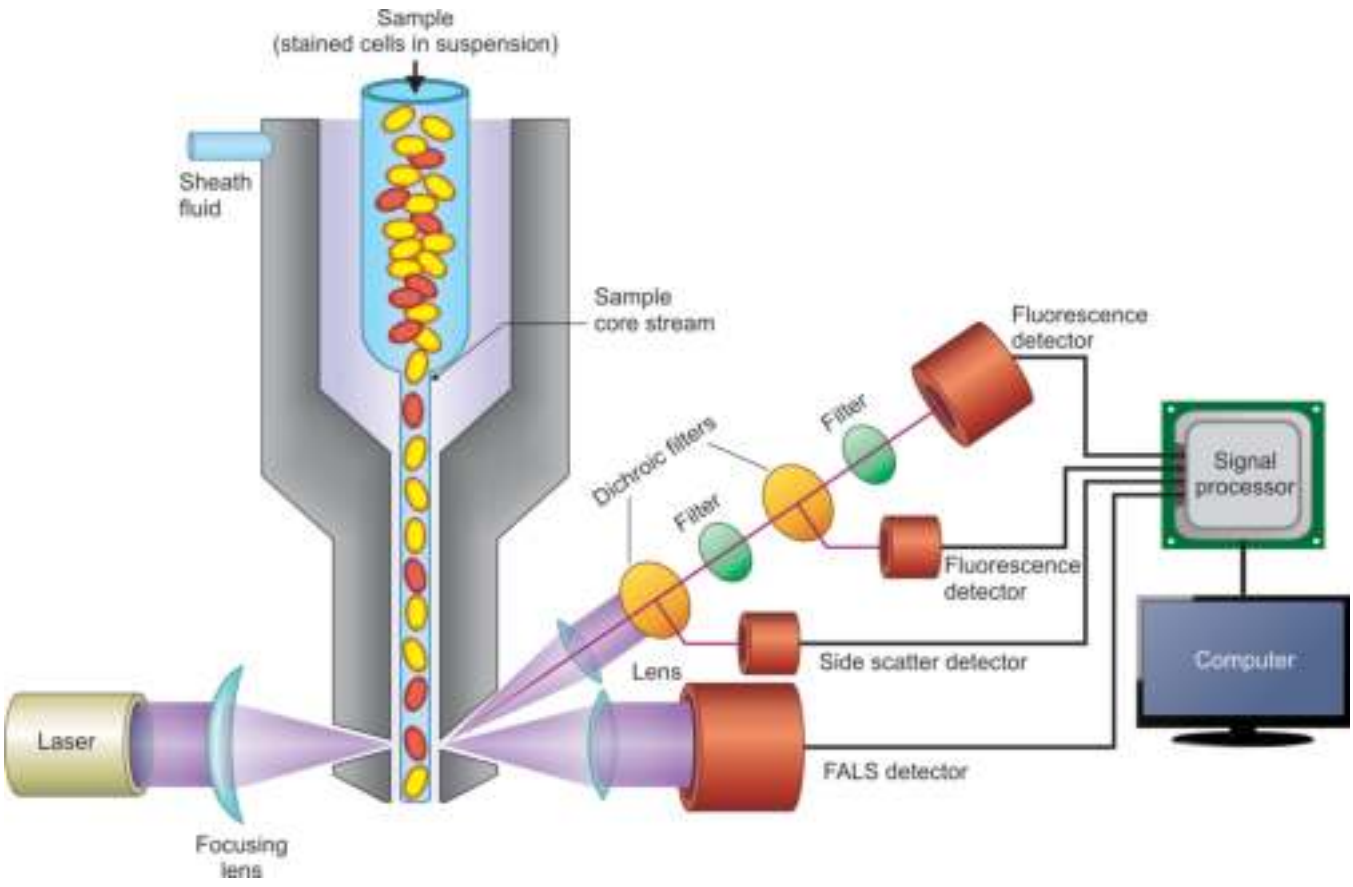


Fig. 14.16: The flow cytometer is composed of a flow system, optical system and electronic system.

the resulting light signals to the appropriate detectors. The electronic system converts light signals into electronic signals that are processed by the computer (Fig. 14.16). Flow cytometry is used in classification of leukemias and DNA analysis.

Flow System

Sample obtained from peripheral blood and bone marrow aspirate or solid malignant tissue suspension mixed with anticoagulant are injected into a stream of sheath. Cell particles are forced into center of the stream enabling transporting of single cell. Higher-flow rate is used for the immunophenotyping of cells. Lower flow rate is used for DNA analysis.

Optical System

Optical system consists of light scattering and fluorescence. Laser beam in flow cytometer participates in illumination of particles in the sample stream. Filters direct the generated light signals to the appropriate detectors. Most flow cytometers have argon ion laser as a source of light. The light signals are generated when laser beam strikes the cells. A system comprising optical mirrors and filters direct the specialized wavelength of light to the designated photodetectors.

Light Scattering

Light scattering occurs when cell deflects laser light. Leukocytes can be separated into different subpopulations of cells using forward scatter (FSC) and side scatter (SSC) are shown in Table 14.74. Neutrophils contain granular cytoplasm and complex nuclei, therefore, show both high forward light and side scatter. On the other hand, lymphocytes lack cytoplasmic granules and hence show both low forward light and low side scatter.

Conjugation of Fluorescent Dyes to Monoclonal Antibodies

Conjugation of fluorescent dyes to monoclonal antibodies directed toward antigens on a particular cell subset help in identification of cell subpopulations. There are two types of fluorescence staining procedures: direct and indirect (Table 14.75). Argon ion lasers are most common lasers used in most flow cytometers because 488 nm light emitted may be absorbed by more than one fluorochrome. Fluorescent dyes such as fluorescein isothiocyanate (FITC) and phycoerythrin (PE) are used to conjugate with monoclonal antibodies (Table 14.76).

Table 14.74 Type of light scattering

Type of Light Scattering	Characteristics
Forward scatter (FSC)	▪ Collected in the line with laser light beam ▪ Analyzes measurement of the cell surface size
Side scatter (SSC)	▪ Collected perpendicular to the laser light beam ▪ Analyzes internal complexity of a cell

Table 14.75 Type of fluorescence staining procedures

Steps	Direct Fluorescence Staining Procedure	Indirect Fluorescence Staining Procedure
First incubation	Incubation of cells with single fluorescence staining	Incubation of cells with a nonfluorescence staining directed towards specific antigen
Washing	Incubated cells are washed to remove non-specific bound antibodies	Incubated cells are washed remove nonspecific bound antibodies
Second incubation	Not required	Required with fluorescent dye directed against monoclonal antibody

Table 14.76 Comparison between fluorescein isothiocyanate (FITC) and phycoerythrin (PE)

Parameters	Fluorescein Isothiocyanate (FITC)	Phycoerythrin (PE)
Range of absorption of light and then fluorescence	460–510 nm	448–555 nm
Then fluorescence	510–60 nm giving green fluorescence	480–565 nm giving red fluorescence

Staining pattern of subpopulation of cells and percentage are defined.

Electronic System

Photodetectors convert the generated forward and side scattered cells, and fluorescent light signals into electrical impulses.

- There are two types of photodetectors in flow cytometer: photomultiplier tubes (PMTs) and photodiodes.
- Photomultiplier tubes (PMTs) amplify the electric current generated from light signals. Long amplifiers are used to negative from dim positive signals from cells stained with fluorochrome-labeled antibodies. Linear amplifiers are used to detect forward and side scatter signals.
- Photodiodes are used to detect strong forward scatter (FSC) signals. The intensity of the electronic impulses derived from the photodetectors is analyzed by digital converter.

APPLICATIONS OF FLOW CYTOMETER

Flow cytometry is an ancillary diagnostic technique to analyze cell marker/cell type in classification of leukemias/lymphomas (CD antigens), DNA ploidy analysis in molar pregnancy and cell cycle (G1, S, G2, M phases).

Classification of Leukemias

Flow cytometry is an ancillary method for classification of acute leukemias and lymphomas. Monoclonal antibodies specific to nuclear, cytoplasmic and surface antigens and characteristic of particular cell subsets are used in flow cytometer. These are organized as clusters of differentiation (CD) antigens, which differentiate hematopoietic malignancies and lymphomas.

- In cases of acute leukemias, it is essential to know the myeloid or lymphoid lineage required to know the prognosis and line of treatment in those cases in which routine cell morphology and cytochemistry fail to subtype or assign proper lineage in acute leukemias.
- Immunophenotyping using flow cytometry improves the diagnosis of acute lymphoblastic leukemia and differentiation of B cell ALL and T cell ALL (Table 14.77).
- Lineage markers of AML while analyzing on flow cytometric analysis include anti-MPO cytoplasmic, CD13, CD33 and CD117. Immunophenotyping of acute myelogenous leukemia (AML) is given in Table 14.78. Immunophenotyping of chronic lymphocytic leukemia (CLL) is given in Table 14.79.

Table 14.77 Immunophenotying of ALL (B cell) and ALL (T cell)

Characteristics	ALL (B Cell)	ALL (T Cell)
HLA-DR	Positive	Negative
cCD1	Negative	Positive
CD2	Negative	Positive
CD3	Negative	Positive
CD5	Negative	Positive
CD7	Negative	Positive
CD10 (CALLA)*	Positive	Negative
CD19	Positive	Negative
CD20	Positive	Negative

**CD10 (CALLA) is favorable prognostic marker and most amenable to therapy.*

Table 14.78 Lineage markers of acute myelogenous leukemia (AML) analyzing on flow cytometry technique

Parameters	Lineage Markers of AML
Myeloid lineage	▪ Anti-MPO is cytoplasmic lineage marker of AML ▪ CD13 ▪ CD33 ▪ CD117
Markers of immaturity	CD34
Monocytic differentiation	▪ CD14 ▪ CD64
Megakaryocytic differentiation	▪ CD41 ▪ CD61
Erythroid differentiation	▪ CD235A ▪ Glycophorin A

Table 14.79 Immunophenotyping of chronic lymphocytic leukemia (CLL)

Immunophenotype (Pan-B Cell Markers)	Expression
CD5*	Positive
CD23	Positive
CD19	Positive
CD20	Positive

**CD5 is positive in normal B cell and T cell.*

DNA Content Analysis

Flow cytometry technique can be used in analyzing DNA content of cell by using fluorescent stains, which target different DNA nucleotide base pairs within double helix.

- Fluorescent stains such as **HOECHST 33342** or **4', 6-diamino-2-phenylindole (DAPI)** are specific for the adenine/thymine nucleotide base pairs. Mithramycin and chromomycin A3 are target guanine/cytosine nucleotide base pairs.
- **Propidium iodide** stains all double-stranded nucleic acids, hence it is used in conventional flow cytometers with low power argon lasers because it absorbs light at 488 nm. DNA content analysis helps in differentiating normal cells from aneuploid malignant cells. The histograms from malignant tumors show abnormal peaks than normal cells.

Cell Cycle Analysis

Cell cycle shows G0 (resting phase), G1 (pre-synthetic phase), G2 (synthetic phase), S and M (mitotic) phases. Cells of G2 have finished DNA synthesis with double the amount of DNA. In M phase, two daughter cells are formed. G1/S and G2/M are checkpoints of cell cycle. The cell cycle can be plotted as a histogram with the number of cells per channel on the Y axis and fluorescence intensity of the cells stained for DNA content on the X axis.

CYTOGENETIC ANALYSIS

CYTOGENETIC ANALYSIS: OVERVIEW

The milestones in the history of clinical cytogenetics comprise preparation of chromosomes from blood cultures.

- Chromosomal spread is enhanced by development of hypotonic methods. Discovery of fluorescent quinacrine compounds helped in the demonstration of unique banding pattern for each human chromosome.
- Cytogenetic analysis is commonly used in the evaluation of congenital disorders, diagnosing syndrome associated with chromosomal abnormality, screening karyotypic abnormalities in patients with multiple birth defects and prenatal diagnosis. In surgical pathology, cytogenetic analysis recognizes cytogenetic abnormalities in patients with leukemias, lymphomas and soft tissue sarcomas.
- Cytogenetic analysis in cancers has aided targeted therapy, prognosis, and risk-based stratification of intensive therapy. Fresh tissue is required for cell culture and subsequent G-banding metaphase cell preparations or metaphase FISH procedures.

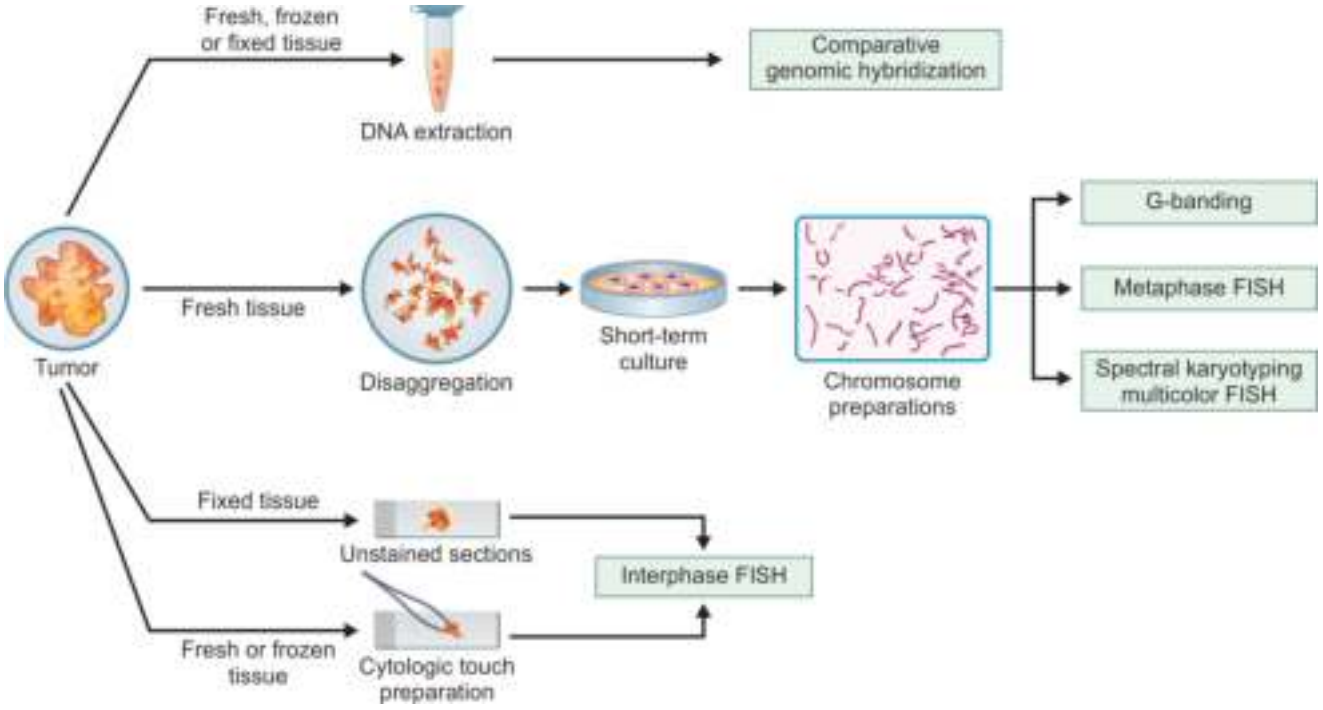


Fig. 14.17: Basic specimen requirements for cytogenetic analysis: Fresh tissue is required for cell culture and subsequent G-banded metaphase cell preparations or metaphase FISH procedures. DNA is extracted from fresh, frozen, or formalin-fixed paraffin-embedded tissue for comparative genomic hybridization platforms, and interphase FISH analysis can be conducted on cytologic touch preparations or formalin-fixed paraffin-embedded tissue sections.

- DNA is extracted from fresh, frozen, or formalin-fixed paraffin-embedded tissue for comparative genomic hybridization platforms, and interphase FISH analysis can be conducted on cytologic touch preparations or formalin-fixed, paraffin-embedded tissue sections (Fig. 14.17).
- Conventional cytogenetic analysis has ability to evaluate entire genome without any prior knowledge of the chromosomal regions involved in the disease process.
- Conventional cytogenetic analysis is performed on viable tissue specimens, which contain proliferating cells. It is only suitable for detection of numerical and structural chromosomal abnormalities. Cytogenetic technique lacks sensitivity to detect mutations such as deletions, amplifications, single nucleotide base substitutions and so on.

CYTOGENETIC ANALYSIS METHODOLOGY

Overall scheme for the production of metaphase chromosomes for conventional cytogenetic analysis is shown in Fig. 14.18.

Cell Culture Initiation

Blood sample is withdrawn and mixed with anticoagulant. Blood is kept in culture bottle with mitogen for 72 hours at 37°C. Different specimens are collected for traditional cytogenetic analysis as given in Table 14.80. Bone marrow and solid tumor consist of cells, which proliferate at slow rate. Therefore, mitogen is added to enhance proliferation (Table 14.81).

Cell Culture Maintenance

The length of *in vitro* culture depends on cell type is given in Table 14.82.

Cell Harvesting

Colcemid is added to the bottle to arrest cell division at metaphase. Colcemid, a synthetic analogue of colchicine (an alkaloid derived from the bulb of Mediterranean plant colchicine) prevents separation of sister chromatids and blocks proliferation of cells in metaphase stage.

- Cell cycle is asynchronous *in vitro* cells are obtained in acceptable mitotic index by addition of thymidine followed by deoxythymidine to obtain normal DNA replication. Alternately, 5-fluorodeoxyuridine may be used to stall cells at G1/S transition by addition of thymidine.
- The blood is now centrifuged in test tube. Then add hypotonic solution to the test tube to rupture red blood cells and swelling of white blood cells. Again centrifuge the test tube and then add methanol and acetic acid to the test tube to fix cells. Now centrifuge the test tube containing fixed white blood cells and place a few drops on glass slide. Dry the smear and

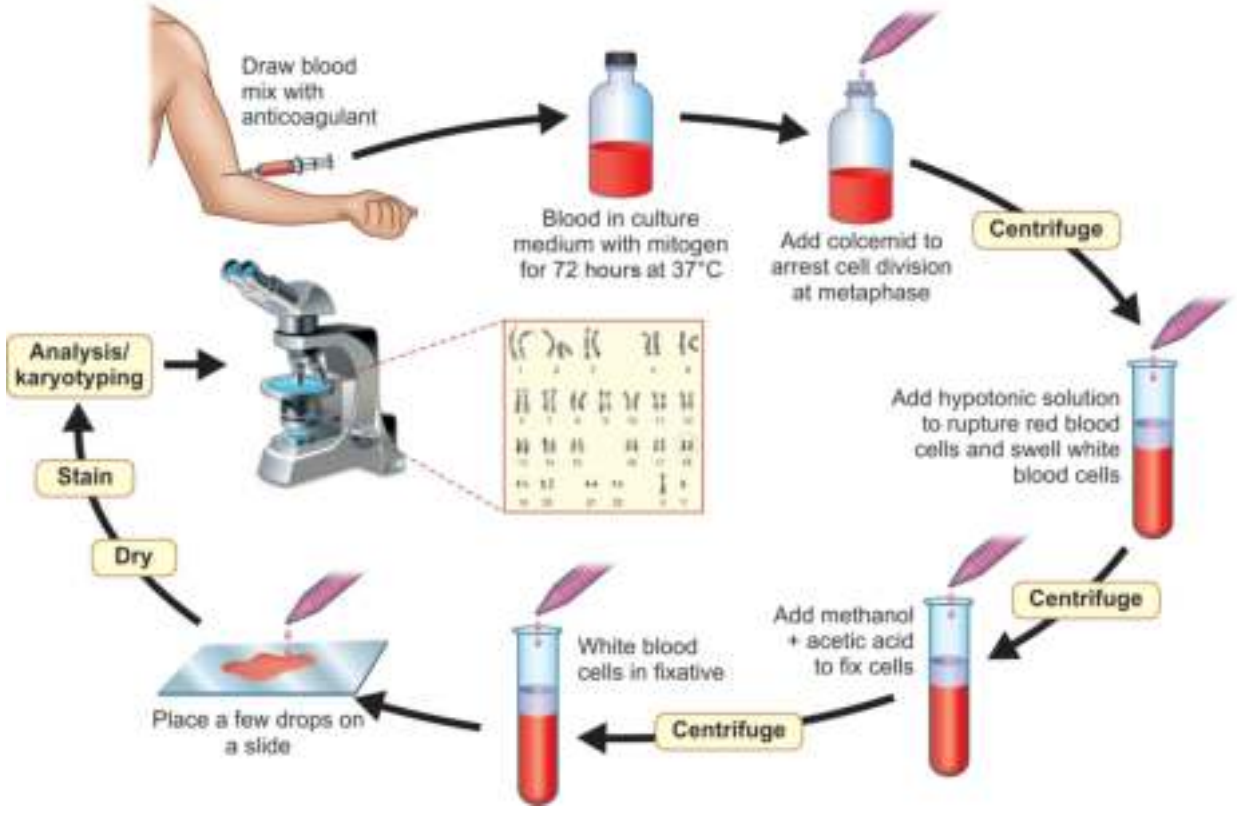


Fig. 14.18: Overall scheme for the production of metaphase chromosomes for conventional cytogenetic analysis.

Table 14.80 Specimen requirements for traditional cytogenetic analysis

Type of Sample	Sample Collection
Peripheral blood	Blood sample with anticoagulant kept in refrigerator or at room temperature
Bone marrow aspirate	Bone marrow aspirate with anticoagulant kept at room temperature
Solid tissue from tumor	▪ Viable tumor sample kept in sterile container containing broad spectrum antibiotics to prevent microbial overgrowth and autolysis ▪ Sterile container is transported on ice

Table 14.81 Mitogens used to enhance proliferation of cells of bone marrow and solid neoplasms

Mitogen	Cell Proliferation in Culture
Phytohemagglutinin (PHA)	T cells
Lipopolysaccharide (LPS)	B cells
Protein A	B cells
12-O-tetradecanoylphorbol-13-acetate (TPA)	B cells
Epstein-Barr virus	B cells
Synthetic oligonucleotides	B cells
Pokewood mitogen	B cells

then stain. The slide is analyzed for karyotyping. Quinacrine banding technique allows detection of the region containing repetitive DNA.

Table 14.82 Time required for proliferation of cells in culture medium

Cell Proliferation	Time Required
Peripheral blood cells	72 hours
Bone marrow cells	24 to 48 hours
Solid malignant neoplasms	≥2 weeks

CYTOGENETIC ANALYSIS TECHNIQUES

Bright field microscopy or fluorescence microscopy is used to visualize chromosomes. Electronic imaging system is used to capture the images. Assay failure occurs due to sampling from necrotic tumor, overgrowth of microbes, overgrowth of nonrepresentative clone of tumor cells, post-culture failure and wrong interpretation of results.

METAPHASE FLUORESCENCE *IN SITU* HYBRIDIZATION FOR CHROMOSOMAL KARYOTYPING TECHNIQUE

All metaphase chromosomes *in situ* hybridization analysis is performed by using probes labeled with fluorophores. Metaphase FISH technique is a modified Southern blot in which the target DNA comprises chromosomes rather than membrane bound DNA. Cytogenetic technique is used to stain metaphase chromosome as given in Table 14.83. Four steps of metaphase fluorescence *in situ* hybridization for chromosomal karyotyping are given in Table 14.84. Type of probes used in metaphase *in situ* hybridization is shown in Table 14.85.

MULTIPLEX METAPHASE FLUORESCENCE *IN SITU* HYBRIDIZATION FOR CHROMOSOMAL KARYOTYPING TECHNIQUE

Multiplex metaphase FISH technique is also known as **multicolor FISH**. Special karyotyping (SKY) is related technique. In both techniques, metaphase chromosome is hybridized with combination of probes labeled with different fluorophores. Five different fluorophores can produce different color combinations to uniquely labeled

Table 14.83 Cytogenetic technique is used to stain metaphase chromosome

Staining Technique	Staining Patterns
Technique producing specific alternating bands along each chromosome	
Giemsa banding (G-banding)	▪ Dark bands are AT-rich ▪ Light bands are CG-rich
Quinacrine banding (Q-banding)	Bright regions are AT-rich
Reverse banding (R-banding)	▪ AT-rich regions stain lightly (dull fluorescence) ▪ CG-rich regions staining darkly (bright fluorescence)
4,6-Diamidino-2-phenylindole (DAPI) staining	DAPI binds AT-rich regions produces a pattern similar to Q-banding (bright regions are AT-rich)
Technique staining selective chromosome regions	
Constitutive heterochromatin banding (C-banding)	▪ Stains heterochromatin (α-satellite DNA) around the centromere ▪ Used to demonstrate some inherited polymorphisms
Telomere banding (T-banding)	Technical variation of R-banding used to stain telomeres
Silver staining for nuclear organizer regions (NOR staining)	Stains the NORs (which contain rRNA genes) on the satellite stalks of acrocentric chromosome
Fluorescence *in situ* hybridization (FISH)	Staining pattern dependent on the probe

Table 14.84 Four steps of metaphase fluorescence *in situ* hybridization for chromosomal karyotyping

Steps	Procedure
Step 1	Denaturing of probe and metaphase target by high temperature and formamide
Step 2	Hybridization of probe to the chromosomal target
Step 3	Removal of unbound post-hybridization probe by washing
Step 4	Detection of bound probe by fluorescent microscopy

Table 14.85 Type of probes used in metaphase *in situ* hybridization

Characteristics	Repetitive Sequence Probes	Unique Sequence Probes	Whole Chromosome Probes (WCPs) also known as Painting Probes
Basis of development of probe	Differences in α-satellite sequences of centromeres	Derived from genomic clones or cDNA by using vectors such as bacterial artificial chromosomes (BACs) and yeast artificial chromosomes (YACs)	Probes isolated through flow sorting of specific chromosomes, microdissection of specific chromosomes accompanied by PCR amplification
Use	Most widely used to detect repetitive sequence	Used to detect sequences present on given chromosome	Used to identify chromosomal re-arrangements

WCPs to visualize 24 different human chromosomes, X and Y chromosomes in one hybridization.

- Multiplex FISH and special karyotyping techniques (SKY) are used to detect aneuploidy, interchromosomal rearrangements and marker chromosome (extrachromosomal material of unknown origin).
- Multiplex FISH and SKY do not provide direct information of the involved chromosomal bands. Both techniques do not detect intrachromosomal rearrangements such as inversions. These do not provide information on regions with repetitive DNA.
- Multiplex FISH and SKY techniques are modified by mixing with partial chromosome paints. These modified techniques detect translocation breakpoints and interchromosomal rearrangements.

COMPARATIVE GENOMIC HYBRIDIZATION TECHNIQUE

Comparative genomic hybridization (CGH) technique has higher sensitivity than conventional cytogenetic analysis. Different types of dyes are also used to label

Comparative genomic hybridization (CGH) technique in cancer

1. Comparative genomic hybridization technique allows a comprehensive analysis of multiple DNA gains and losses in entire genomes within a single analysis.
2. Equal amounts of test and reference DNA samples are fluorescently labeled respectively and mixed from the same individual.
3. Test DNA from tumor is fluorescently labeled as red and reference DNA from normal tissue as green. The mixture is applied to an array and allowed to hybridize *in situ* to normal metaphase chromosome. Microarray has 1,000,000 probes covering the human genome.
4. Red and green signal intensity is detected and mapped to genome location. Software clusters high and low intensity probes according to ratio of the concentrations of sequences in the two samples.
5. By comparing the fluorescence intensities of test DNA and reference DNA, changes in signal intensities caused by imbalances of the test tumor DNA can be identified.
6. The ratio of the binding intensities of test tumor DNA and control normal tissue DNA is proportional to the concentrations of sequences in the two samples.
7. Comparative genomic hybridization technique is performed to analyze alterations in DNA copy number which are involved in cancer associated with changes in the gene expression that occur in tumor development. Loss of DNA sequences contributes to the inactivation of tumor suppressor genes, while amplifications may activate oncogenes.
8. Comparative genomic hybridization technique application in Down syndrome is to demonstrate extra copy of DNA sequences from a portion of chromosome 21.

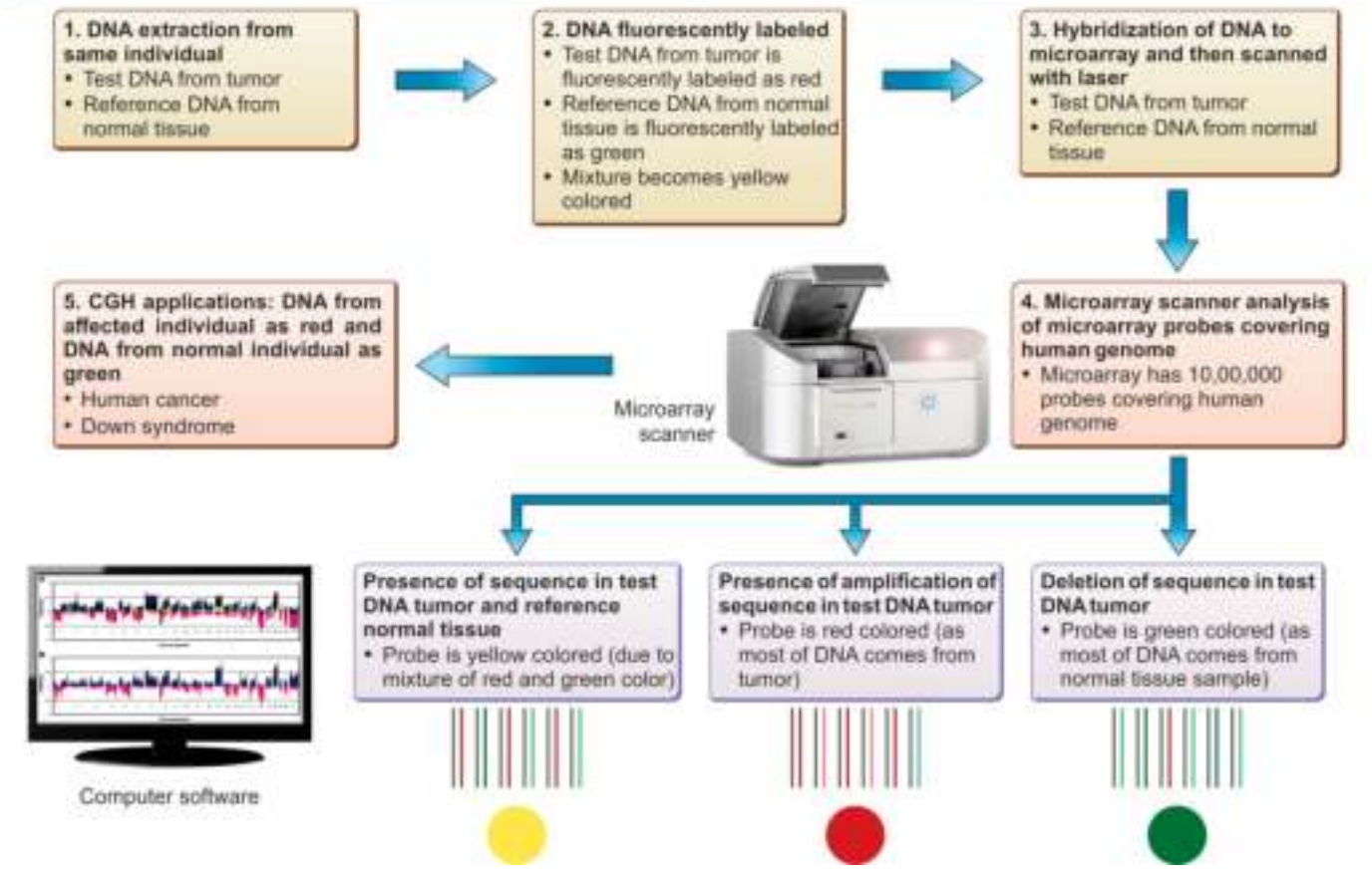


Fig. 14.19: Comparative genomic hybridization (CGH) technique

genomic DNA obtained from normal and diseased tissues. Microarray DNA gene probe is fixed to the slide. The major advantages of comparative genomic hybridization (CGH) technique is to detect copy number variations as small as several hundred nucleotide base pairs by DNA extraction from formalin fixed tissue. Level of fluorescence is compared with specific gene. CGH technique in cancer is shown in Fig. 14.19.

Metaphase Comparative Genomic Hybridization Technique

Metaphase comparative genomic hybridization (CGH) technique is a variation of metaphase FISH technique. Metaphase CGH technique is used to survey entire genome for chromosomal deletions and amplifications.

- For typical CGH test, genomic DNA extracted from tumor is labeled with red fluorophore, and genomic DNA from paired normal issue is labeled with green fluorophore. Both red and green probes are now mixed and used in single hybridization.
- The relative difference in DNA content between the normal and specimen DNA is represented by a difference in the green:red fluorescence ratio. Conventional chromosomal CGH is shown in Fig. 14.20.

Array Comparative Genomic Hybridization Technique

Array comparative genomic hybridization (CGH) technique utilizes a microarray comprising an orderly arrangement of DNA molecules linked to a solid matrix support. The labeled probe mixture is hybridized to the microarray. Ratio of green to red fluorescent signals is measured for each feature. Genomic microarrays are currently employed to detect genomic copy number changes, copy neutral changes and loss of heterozygosity. Comparative genomic hybridization *vs* conventional cytogenetic analysis is given in Table 14.86.

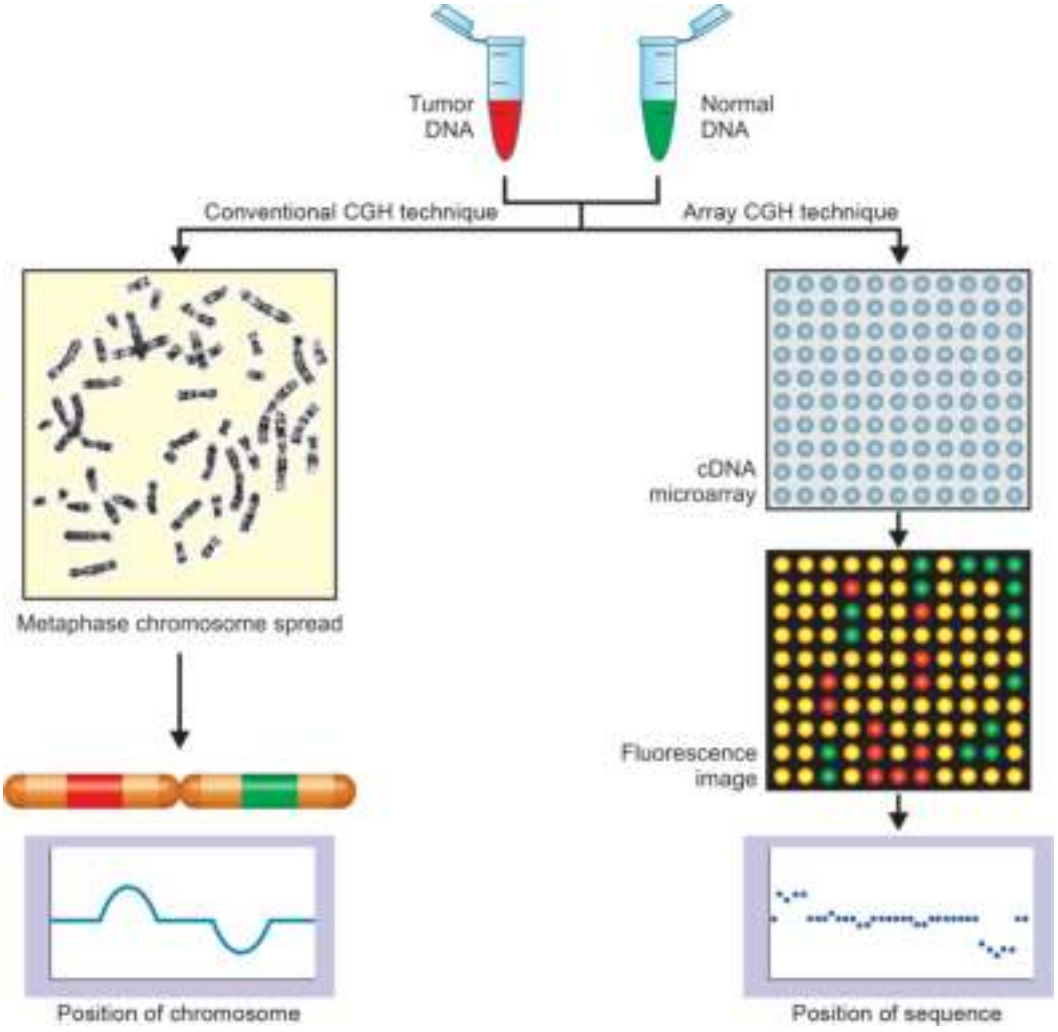


Fig. 14.20: Conventional chromosomal CGH: In conventional chromosomal CGH, the ratio of fluorescence intensities along each chromosome is analyzed. Increased DNA copy number in the tumor sample will be detected by increased red fluorescence, whereas decreased copy number will allow more binding of the normal DNA and increased green fluorescence. On the right, a similar hybridization to a cDNA array permits measurement of copy number at a higher resolution.

Table 14.86 Comparative genomic hybridization *versus* conventional cytogenetic analysis

Parameters	Comparative Genomic Hybridization (CGH)	Conventional Cytogenetic Analysis
Sensitivity	High sensitivity	Low sensitivity
Sample obtained	DNA extraction from fixed as well as fresh tumor	Peripheral blood, bone marrow aspirate and solid tumor
Utility	Genomic wide scan for structural alterations	Genomic wide scan for structural alterations is not possible

MICROARRAY ANALYSIS TECHNIQUE

Microarray analysis technique is used to detect the expression of thousands of genes at same time from experiments on DNA (gene chip analysis), RNA and protein microarrays. DNA microarrays are microscope slides that are printed with thousands of tiny spots in defined positions, with each spot containing a known DNA sequence (e.g. single-nucleotide polymorphisms, SNPs).

- Microarray analysis is used to evaluate patients with genomic imbalance especially in diagnostic testing for congenital anomalies, developmental delay and intellectual disabilities.
- However, this technique is not widely used in diagnosis of solid malignant tumors.
- Microarray analysis depends on the nucleic acid composition of the array (probe) and the test material (target) that is hybridized to it. Microarray application and technology are shown in Fig. 14.21A and B. SNP array procedures are shown in Fig. 14.22A and B.

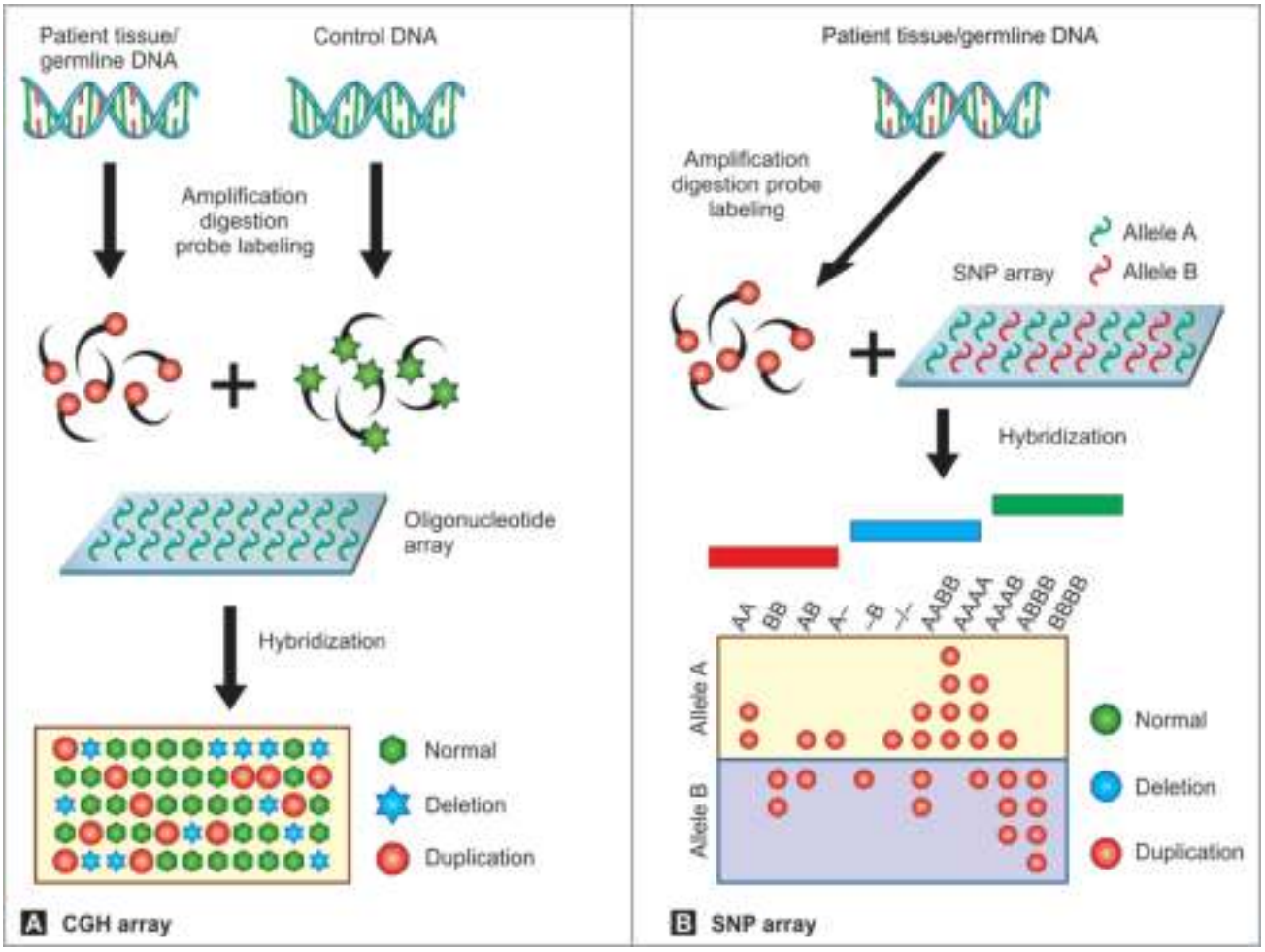


Fig. 14.21: Microarray application and technology. Microarray technology can be used in many different applications depending on the nucleic acid composition of the array (probe) and the test material (target) that is hybridized to it. (A) CGH array: in CGH array, fragments of genomic DNA are spotted as probes onto the microarray surface using robotics. Genomic DNA from the patient's tissue and germline are then co-hybridized to the array to identify regions of chromosomal gain or loss, (B) SNPs/re-sequencing: alternatively, genomic DNA sequence can be used to computationally design oligonucleotide probes that detect specific alterations in DNA samples (either germline or tissue) for either SNP genotyping or DNA sequencing studies.

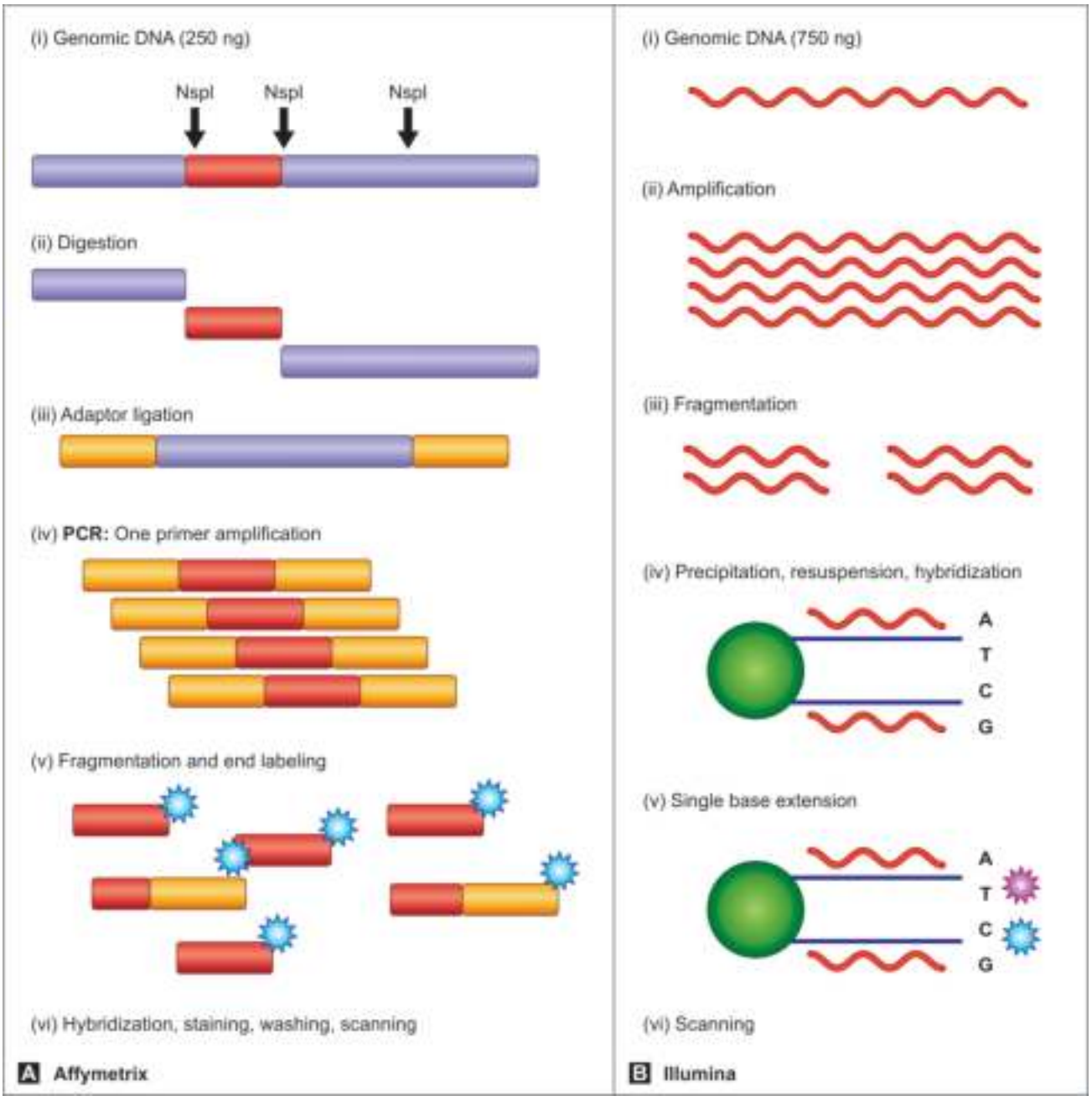


Fig. 14.22: SNP array procedures. (A) Affymetrix platform: genomic DNA is digested with NspI restriction enzyme. The resulted DNA fragments are ligated to adaptors and subsequently amplified. Amplification products are end-labeled and hybridized to the array. (B) Illumina platform: the entire genome is amplified and then hybridize to a bead array; allelic discrimination is achieved by single base extension reaction. In both platforms, the probe intensity is measured and compared with a silicoreference to evaluate DNA copy number.

Microarray Analysis Technique: Application and Technology

In microarray analysis technique, fragments of genomic DNA are spotted as probes onto the microarray surface using robotics. Genomic DNA from the patient's tissue is hybridized to the array to identify regions of chromosomal gain or loss.

Single-Nucleotide Polymorphism (SNP) Technique

Polymorphism is generic term that means 'many shapes'. Single-nucleotide polymorphism or SNP is most common type of gene variation among people. Each SNP represents a difference in single DNA building block, called a nucleotide. It is DNA sequence variation occurring when a single-nucleotide A, T, C

or G in the genome differs between members of species. For example, two sequenced DNA fragments from different individuals AAGCCTA to AAGCTTA contain a difference in a single-nucleotide base.

- In human beings, 99.9% nucleotide base pairs are same. Remaining 0.1% makes a person unique/ (different characteristics/traits). These variations can be harmless (change in phenotype) or harmful (diabetes mellitus, cancer, cardiovascular diseases, hemophilia).
- Single-nucleotide polymorphism (SNP) is present in coding (mostly) and noncoding regions of gene. In coding region, DNA/RNA sequences code for proteins. In noncoding regions, a segment of DNA

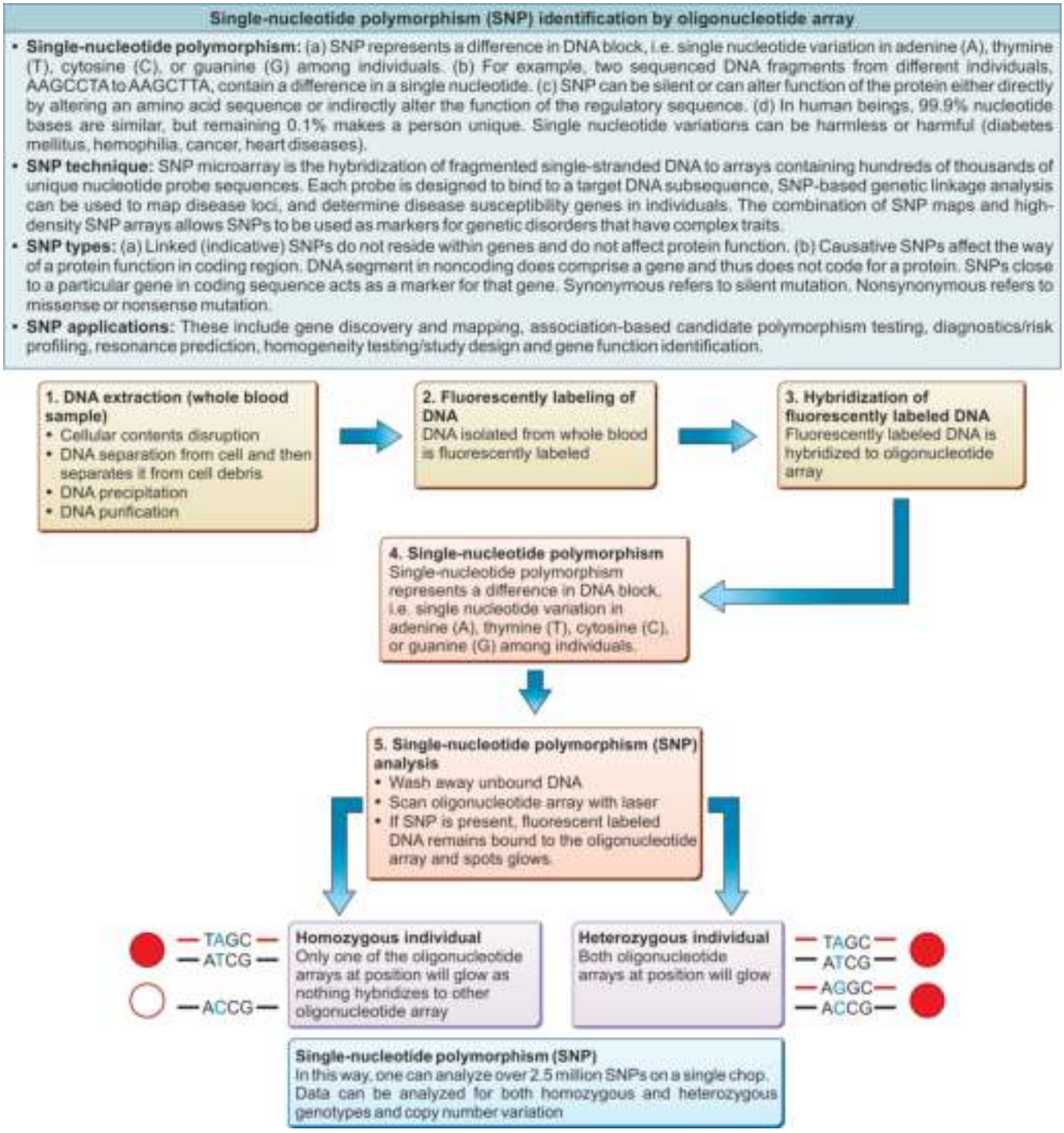


Fig. 14.23: Single-nucleotide polymorphism (SNP) identification by microarray.

does comprise a gene that does not code for a protein. Single-nucleotide polymorphism occurs with a very high frequency (1 in 100 nucleotide base pairs to 1 in 100–300 nucleotide base pairs).

- SNP close to a particular gene, acts as a marker for that gene. SNPs in coding regions may alter the protein structure made by that coding region. Single-nucleotide polymorphism (SNP) detection by microarray analysis and small format genotyping are shown in Figs 14.23 and 14.24. Comparison of single-nucleotide polymorphism and oligonucleotide arrays is given in Table 14.87.

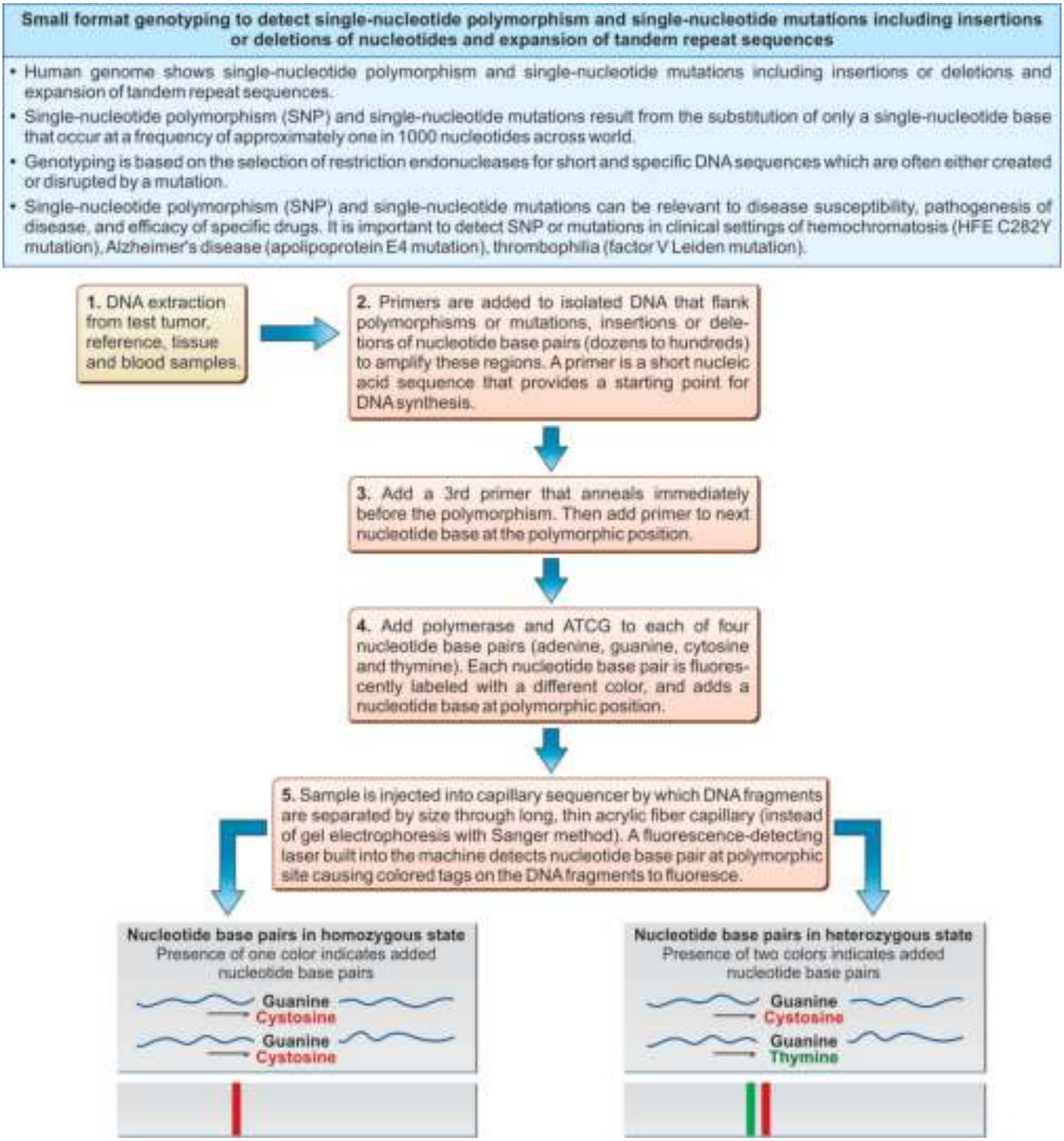


Fig. 14.24: Small format genotyping for detection of single-nucleotide polymorphism (SNP) and single-nucleotide mutations including insertions or deletions of nucleotide base pairs.

Table 14.87 Comparison between single-nucleotide polymorphism and oligonucleotide arrays

Array Attribute	Single-Nucleotide Polymorphism (SNP) Arrays	Oligonucleotide Arrays
Method of assessment of copy number imbalance	Probe signal intensities derived from patient's sample compared to a silicoreference mode	Patient's sample is directly hybridized against control DNA to detect relative gains or loss
Detection of balanced chromosomal rearrangements	Not detected	Not detected
Detects number of markers	1,000,000 markers	<2000,000 markers
DNA input requirement	200–500 ng	1–5 µg
Type of probe	SNP and oligo probes	Oligo probes
Limits of resolution	<10–20 kb	As low as 10–20 kb
Threshold for detection of mosaicism	As low as 5%	20–30%
Ability to detect copy of neutral LOH	Yes	No
Ability to detect uniparental disomy	Yes	No
Ability to detect consanguinity	Yes	No

Clinical Applications of Microarray Analysis Technique

Clinical applications of microarray analysis technique include molecular classification of breast cancer and lymphomas; defining origin of metastatic tumor, prediction of tumor behavior, response to therapy, prognosis and pathogenesis.

Advantages of Microarray Analysis Technique

Microarray analysis technique has markedly increased resolution of chromosomal analysis. It does not require cell viability since DNA serves as the starting material. The technique is employed on samples such as peripheral blood lymphocytes, bone marrow aspirates, lymph nodes, formalin-fixed paraffin-embedded tissue, aminocytes, products of conception and buccal mucosal cells.

Limitations of Microarray Analysis Technique

The technique can detect copy number changes. Microarray analysis has certain limitations. It cannot detect chromosomal rearrangements, balanced translocations, insertions and inversions. It also cannot analyze segmental duplication and repetitive sequences. Low level of mosaicism may not be detectable. Loss of heterozygosity (LOH) is demonstrated only by SNP-based platforms.

IN SITU HYBRIDIZATION, CHROMOGENIC *IN SITU* HYBRIDIZATION AND FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

IN SITU HYBRIDIZATION: OVERVIEW

In situ hybridization (ISH) is a gold standard technique for localizing specific nucleic acid targets within fixed tissues and cells to obtain information about gene expression and genetic loci.

- Basic principle of *in situ* hybridization is similar to that of blot hybridization, in which the nucleic acid probe is created by labeling DNA strand complimentary to a gene of interest with fluorescent radioactive tag.
- DNA specimen is denatured *in situ* or isolated and then transferred to a membrane slide. DNA specimen is now incubated with the labeled probe at a specific temperature determined by the type of probe used. Excess of probe is then washed away to produce an image by using an appropriate detection method.
- Clinical applications of DNA hybridization techniques are Southern blot DNA mutational analysis, comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH) for detection of gene amplification rearrangements and chromosomal translocations.

APPLICATIONS OF *IN SITU* HYBRIDIZATION

Prenatal Test during Pregnancy

Birth defects occur as a result of genetic disorders caused by mutations in one or more genes, chromosomal aneuploidy or environmental factors during

gestational period. Cytogenetic disorders occur in women older than 35 years.

- Women with high-risk pregnancies undergo prenatal diagnosis through amniocentesis for cytogenetic analysis of fetal cells for detection of chromosomal abnormalities in Down syndrome (trisomy 21), the number of chromosomes by karyotyping, a time-consuming diagnostic test.
- Currently, most laboratories perform rapid diagnostic tests for aneuploidy such as fluorescence *in situ* hybridization (FISH) as a part of a combination test with conventional cytogenetic analysis for prenatal diagnosis. In addition to FISH, other modern diagnostic techniques include multi-color primed *in situ* labeling (multi-PRINS), quantitative fluorescence polymerase chain reaction (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA).

Pharmacogenomics

Pharmacogenomics is the branch of genetics concerned with determining the likely response of an individual to therapeutic drug. For example, the enzyme CYP2C19 takes part in metabolism of several therapeutic drugs, such as the anti-clotting drug clopidogrel, into their active forms. Some patients possess polymorphisms in specific places on 2C19 gene that make poor metabolizers of those drugs. Clinicians can test for these polymorphisms by modern diagnostic techniques, and find out whether the drugs would be fully effective for that patient.

CHROMOGENIC *IN SITU* HYBRIDIZATION: OVERVIEW

In present scenario, there are two basic methods to visualize RNA and DNA targets fluorescence *in situ* hybridization and chromogenic *in situ* hybridization.

- Chromogenic *in situ* hybridization (CISH) is a cytogenetic diagnostic technique that combines the chromogenic signal detection method of immunohistochemistry (IHC) techniques with *in situ* hybridization. Both CISH and FISH are useful for very distinct applications. These are used to detect the presence or absence of specific regions of DNA.
- Both CISH and FISH use a labeled target specific probe that is hybridized with the sample. The instrumentation used in CISH and FISH to visualize the sample is different for each method.
- However, CISH is much more practical in diagnostic laboratories, because it uses bright-field microscopes rather than more expensive and complicated fluorescence microscopes used in FISH.

DNA PROBE DESIGN

Probe design for CISH as well as FISH is very similar with differences only in labeling and detection. FISH is generally labeled with a variety of different fluorescent tags analyzed under a fluorescence microscope. On the other hand, CISH probe is labeled with biotin or digoxigenin and analyzed by using a bright-field microscope after treatment steps have been applied. Differences and advantages of FISH and CISH are highlighted (Table 14.88).

FLUORESCENCE *IN SITU* HYBRIDIZATION: OVERVIEW

Fluorescence *in situ* hybridization (FISH) is a technique that hybridizes DNA nucleic acid probe to a target chromosomal-specific DNA sequence of interest contained within cell nucleus. FISH technique permits identification of both structural and numeric

Table 14.88 Comparison of fluorescence *in situ* hybridization (FISH) and chromogenic *in situ* hybridization (CISH)

Parameters	Fluorescence *in situ* Hybridization	Chromogenic *in situ* Hybridization
Instrument/visualization method	▪ Epifluorescence or confocal microscope ▪ Probe labeled with fluorescent tag	▪ Bright field microscope ▪ Probe labeled with biotin or digoxigenin
Primary advantage	▪ Visualization of multiple targets in the same sample ▪ FISH is not routinely used by pathologists	▪ Ability to view the CISH signal and tissue morphology simultaneously ▪ Pathologists are familiar with IHC signals
Primary application	Gene expression and cytogenetics	Molecular pathology diagnostics
FISH versus CISH signal	Fluorescence signals can fade within several weeks	Chromogenic reaction is permanent
Morphologic analysis	Morphology is not always easy to analyze	Morphology is easy to analyze
Recording of results	Results of FISH have to be recorded with an expensive CCD camera	Regular slide storage is done

aberrations characteristics of certain hematopoietic and nonhematopoietic cancers in many clinical settings (Table 14.89).

- FISH technique can be performed on nondividing (interphase) cells on air-dried or formalin-fixed specimens. It can detect molecular abnormalities in cancers with low proliferative rates such as multiple myeloma. This technique detects aneusomies/deletions, gene amplification and chromosomal translocations.
- On the other hand, conventional cytogenetic analysis has ability to evaluate entire genome for detection of numerical and structural chromosomal abnormalities. Conventional cytogenetic analysis lacks sensitivity to detect mutations such as deletions, amplifications, and single base substitutions.
- A variety of specimen types can be analyzed using FISH. The intact cells are attached to slide using standard cytogenetic methods. It allows one to look at multiple genomic changes within a single cell, without destruction of the cellular morphology.
- DNA probe is a nucleic acid that can be labeled with a marker which hybridizes to another nucleic acid on the basis of base complementarity allowing identification and quantitation. Various types of labeling are used—direct and indirect, radioactive (^{32}P, ^{35}S, ^{14}C, ^{3}H), fluorescent (FISH) and biotinylated (avidin-streptavidin).

Pathology Pearls: Methods of Fluorescence *in situ* Hybridization

- **Direct FISH method:** Fluorescent dye binds directly to DNA probe using polymerase enzymes and labeled nucleoside triphosphates.
- **Indirect FISH method:** DNA probe is tagged with a hapten (biotin/digoxigenin) and detected by antibody tagged with fluorescent dye, which gives easy amplification of signal.
- **Multicolor FISH method:** Multicolor FISH can provide colorized information relative to chromosome rearrangements, especially useful in specimens where chromosome preparations are less than optimal for standard cytogenetic banding analysis.

FISH TECHNIQUE METHODOLOGY

Sample is fixed in Carnoy's fixative (3:1 methanol and glacial acetic acid) on the slide. Denaturation is done by formamide at 42°C. Nucleic acid hybridization involves interaction of DNA probe and target DNA.

- Nucleic acid hybridization is the formation of a duplex between two complementary DNA sequences.
- Annealing is another term used to describe the hybridization of two complementary molecules. Post-hybridization washings are carried out.

Table 14.89 Diagnostic tests by FISH technique to detect various disorders

Diagnostic Tests	Disorders
Prenatal testing	▪ Trisomy 13, 18, 21 ▪ XY aneusomies
Microdeletion syndromes	▪ Cri du chat (5p) ▪ Prader-Willi/Angelman (15q) ▪ DiGeorge syndrome
Transplant pathology	▪ XY FISH on sex mismatched organ transplant ▪ Disease relapse using known genetic alterations in primary tumor
Oncology (diagnostic, predictive and prognostic markers of hematopoietic and nonhematopoietic cancers)	▪ Chromosome aneusomies ▪ Gene locus/locus deletions ▪ Gene amplifications ▪ Translocations

- DNA probe is labeled with a colored fluorescent molecule, which remains attached to the DNA during the hybridization process. The fluorescent molecule emits a particular color when viewed through a fluorescence microscope in the darkroom that is equipped with the appropriate filter sets. Fluorescence *in situ* hybridization (FISH) technique is shown in Figs 14.25 and 14.26.

Advantages and Limitations of FISH Technique

Specimen Type Applicable of FISH Technique

Fluorescence *in situ* hybridization (FISH) technique is applicable to various specimens including cytology smears, buccal smears, fresh/frozen specimens and formalin-fixed paraffin-embedded (FFPE) tissue specimens. Specimen analyzed by fluorescence *in situ* hybridization technique is given in Table 14.90.

FISH Versus Cytogenetic and Other Molecular Techniques

Fluorescence *in situ* hybridization (FISH) technique can be performed on nondividing (interphase) cells on air-dried or formalin-fixed specimens. It detects molecular abnormalities in malignant neoplasms with low proliferation rates such as multiple myeloma. FISH technique detects deletions, gains, chromosomal translocations and amplifications.

- FISH is more sensitive and quicker than conventional karyotyping, which provides targeted approach for molecular cytogenetic alterations, which have been identified by various global techniques such as classic cytogenetics, loss of heterozygosity (LOH), screening, comparative genomic hybridization (CGH), array CGH (aCGH), single-nucleotide polymorphism (SNP) array and gene expression profiling.

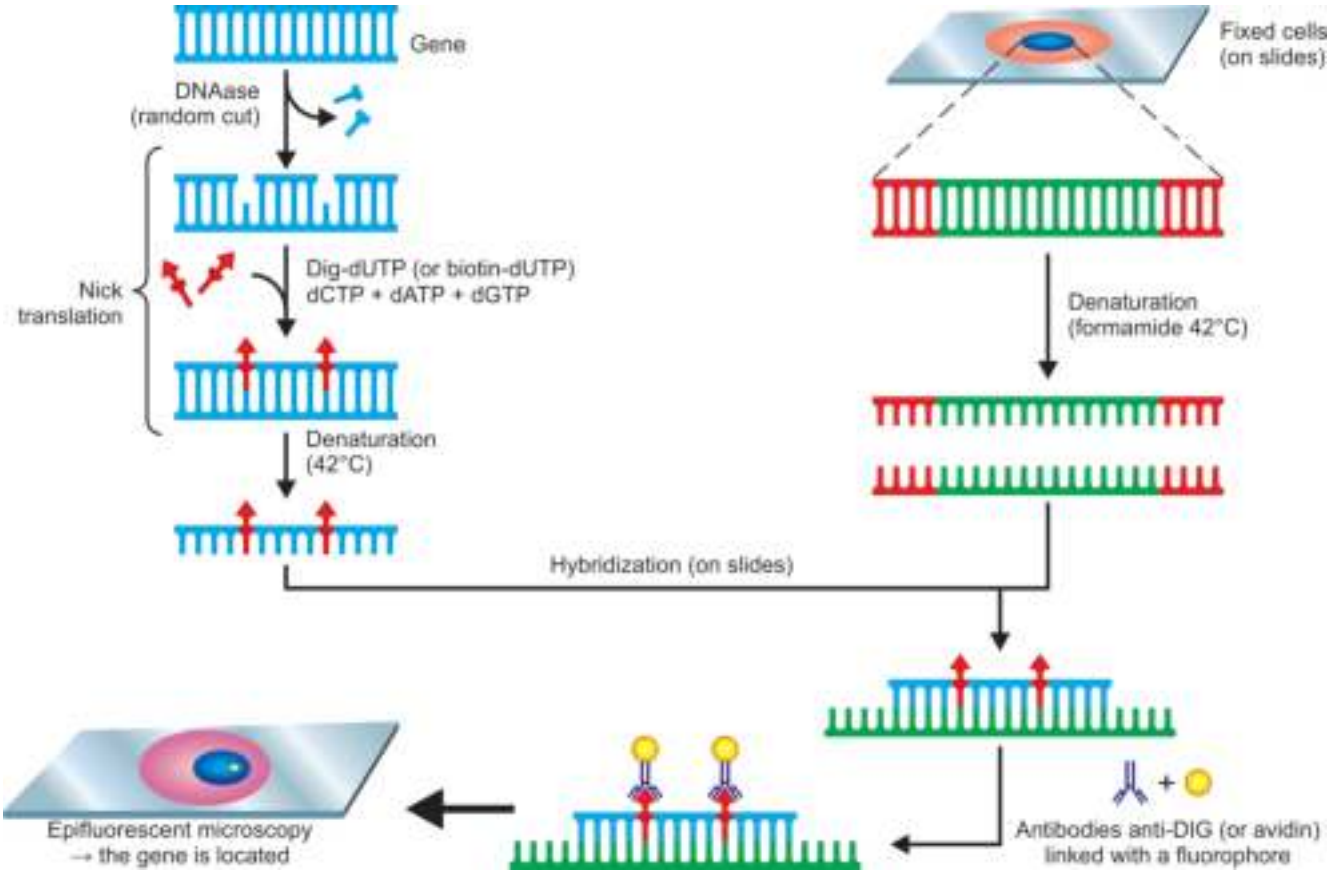


Fig. 14.25: Fluorescence *in situ* hybridization (FISH) technique.

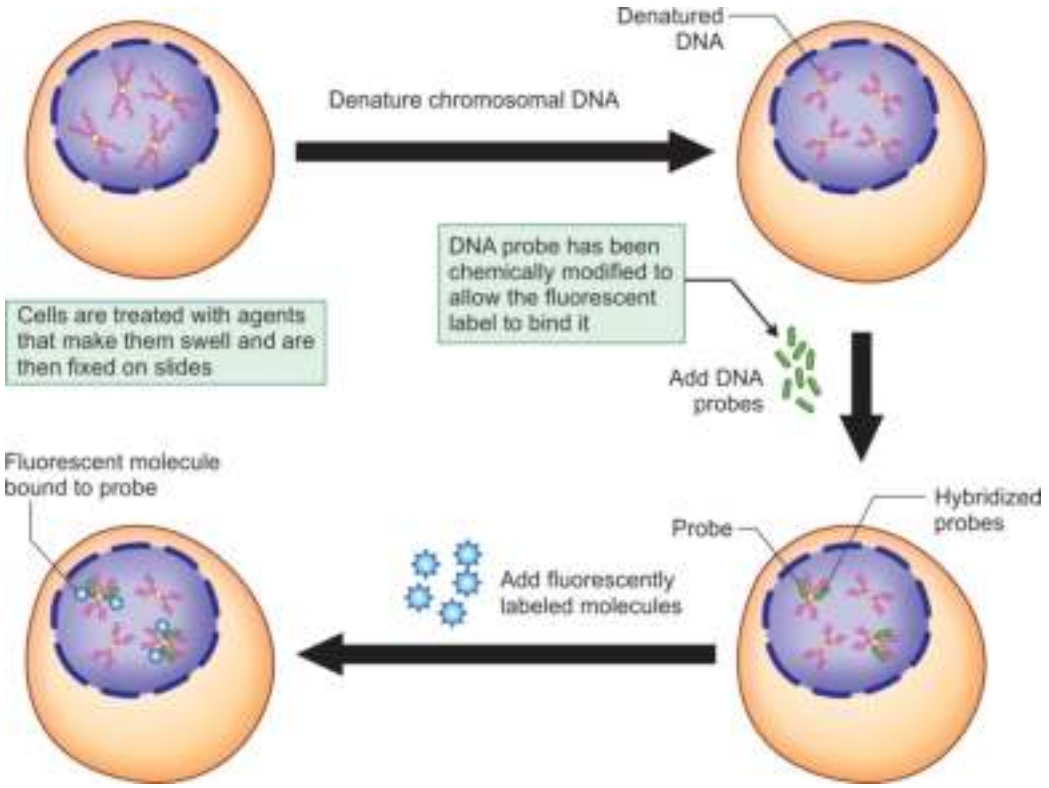


Fig. 14.26: Fluorescence *in situ* hybridization (FISH) technique.

Table 14.90 Specimens analyzed by fluorescence *in situ* hybridization technique

Categories	Characteristics
Peripheral blood and bone marrow aspirate	Rapid analysis required
Cytology specimens	▪ Body fluids, urine, pleural effusions ▪ Intraoperative cytology smears ▪ Cell culture preparations
Buccal smears	Used for sex determination
Fresh/frozen tissue	Fresh/frozen tissue analysis
Formalin fixed paraffin-embedded (FFPE) tissues	▪ Thin sections obtained (4–6 µm) ▪ Disaggregated nuclei ▪ Archived unstained sections ▪ Previously stained sections (e.g. negative immunohistochemistry control)

- Minimal residual disease or early recurrences are better detected by polymerase chain reaction (PCR).

Tissue Microarray—FISH Technique

Tissue microarray—FISH technology has advantage over multiple specimen paraffin blocks tissue microarrays, which is constructed from hundreds of 0.6–2.0 nm neoplastic, non-neoplastic and tissue cores of interest. The tissue microarray—FISH technique reduces data acquisition time, probe, reagents and storage space requirement space. Tissue microarray—FISH technique is excellent for validation of new DNA probe, proficiency testing and quality control.

Disadvantages and Pitfalls of FISH Technique

Artifacts due to underestimation of copy number, aneuploidy or hyperploidy, autofluorescence and partial hybridization failure make interpretation difficult. Signal fading is another disadvantage. Minimal residual disease or early recurrences are better detected by polymerase chain reaction (PCR). Permanent record is not possible unless chromogenic *in situ* hybridization (CISH) is used.

DEVELOPMENT OF DNA PROBES

Centromere Enumerating Probe (CEP)

Initially developed centromere enumerating DNA probes remain ideal for detection of gains and losses of whole chromosomes such as monosomy, trisomy and other polysomies.

A Whole Chromosome Paint Probe

A whole chromosome paint probe (WCP) consists of a cocktail of DNA fragments that targets all the nonrepetitive DNA sequences of an entire chromosome. As WCP covers large region, it produces a diffuse signal in interphase nuclei. WCP probe is not often used in interphase FISH technique, primarily utilized in advanced cytogenetic applications.

Locus Specific Identifier (LSI) Probe (Most Versatile)

Locus specific identifier (LSI) probe targets distinct chromosomal regions of interest, which utilizes single copy rather than repetitive DNA sequences. Assortment of locus specific is now commercially available. Various cloning vectors such as bacterial artificial chromosomes (BACs), P1-derived artificial chromosomes (PACs) and yeast artificial chromosomes (YACs) are important sources of development of probes used in FISH technique.

CLINICAL APPLICATIONS OF FISH TECHNIQUE

Clinical applications of FISH technique include detection of chromosomal microdeletion, gene arrangements, breakpoint mapping of chromosomes and interphase chromosome. Clinical applications of FISH technique in detection of cancer associated genetic alterations are given in Table 14.91.

- **Aneusomies and deletions:** Aneusomies represent gains or losses of whole chromosomes. Deletions refer to losses of distinct chromosomal regions, varying in size from loss of a specific gene or portion of a gene to entire chromosomal arm.
- **Gene amplifications:** Gene amplification occurs in one of two patterns demonstrated by FISH technique. Presence of amplified gene on extrachromosomal segments is known as double minutes and is demonstrated numerous individual signal by FISH technique.
  - Gene amplification consisting of numerous contiguously arranged gene copies within single chromosomal region gives abnormal globular signals by FISH technique. HER2/neu amplification is demonstrated in breast carcinoma in 20–35%. Herceptin administration is beneficial in breast carcinoma patients with HER2/neu positivity.

Table 14.91 Clinical applications of FISH technique in detection of cancer-associated genetic alterations

Systems Involved	Malignant Neoplasms	Genetic Alterations	Diagnostic/Predictive/Prognostic Significance
Aneusomes and deletions			
Nervous system	▪ Glioblastoma multiforme ▪ Oligodendroglioma ▪ Medulloblastoma ▪ Meningioma ▪ Malignant peripheral nerve sheath tumor	▪ + 7, –10 ▪ 1p– with 19q– ▪ 17q–, 17q+ (i17q) ▪ NF2 (22q–) ▪ NF1 (17q–)	▪ Diagnostic significance ▪ Diagnostic/predictive/prognostic significance ▪ Diagnostic significance ▪ Diagnostic significance ▪ Diagnostic significance
Hematologic system	▪ Chronic lymphocytic leukemia ▪ Leukemia/myelodysplastic syndrome ▪ Multiple myeloma	▪ 13q–, 11q–, 17q– ▪ + 8, + 12; –5, –7 ▪ RB1 (–13q), TP53 (17p–)	▪ Diagnostic/prognostic significance ▪ Diagnostic/prognostic significance ▪ Diagnostic significance
Respiratory system	Lung carcinoma	+ 17p, 7, or 17; 9q–	Diagnostic significance
Urogenital system	Urothelial carcinoma	+ 3 , 7 or 17; 9p–	Diagnostic significance
Soft tissue	▪ Malignant rhabdoid tumor ▪ Atypical rhabdoid tumor	INI1/Hsnf5 (22q–)	▪ Diagnostic significance ▪ Diagnostic significance
Gene amplifications			
Nervous system	▪ Glioblastoma multiforme ▪ Medulloblastoma ▪ Neuroblastoma	▪ EGFR ▪ c-Myc ▪ N-Myc	▪ Diagnostic significance ▪ Diagnostic/prognostic significance ▪ Diagnostic/prognostic significance
Breast	Breast carcinoma	HER2/neu	Diagnostic/predictive significance
Gastrointestinal tract	Gastric carcinoma	HER2/neu	Diagnostic/predictive significance
Chromosomal translocation			
Soft tissue	▪ Synovial sarcoma ▪ Alveolar rhabdomyosarcoma ▪ Desmoplastic round cell tumor ▪ Myxoid round cell liposarcoma ▪ Inflammatory myoblastic tumor (IMT)	▪ SYT-SSX, SYT-BA* ▪ PAX3-FKHR, FKHR-BA* ▪ EWS-WT1, EWS-BA* ▪ CHOP-BA* ▪ ALK-TPM3, ALK-TPM4, ALK-CARS, ALK-BA*	▪ Diagnostic significance ▪ Diagnostic significance ▪ Diagnostic significance ▪ Diagnostic significance ▪ Diagnostic/prognostic significance
Skeletal system	Ewing's sarcoma/primitive neuro-ectodermal tumors	EWS-FLI1, EWS-BA	Diagnostic significance
Hematologic lymphoid system	▪ Acute myelogenous leukemia ▪ Acute lymphoblastic leukemia ▪ Chronic myelogenous leukemia ▪ Myeloid and lymphoid neoplasms with eosinophilia ▪ Multiple myeloma ▪ Burkitt's lymphoma ▪ Follicular lymphoma ▪ Mantle cell lymphoma ▪ Anaplastic large cell lymphoma ▪ MALT lymphoma ▪ BCL, unclassified with features in between diffuse large B cell lymphoma and Burkitt's lymphoma	▪ AML1-ETO, CBFB-BA, PML-RARA, RARA-BA*, MLL-BA, BCR-ABL ▪ TEL-AML1, BCR-ABL, MLL-BA ▪ BCR-ABL ▪ PDGFRA, PGDFRB, FGFR1, FIP1L1-PDGFRA IGH-CCND1, IGH-FGFR3 ▪ IGH-BA* ▪ Myc-OGH, Myc-BA ▪ IGH-BCL-2 ▪ IGH-CCND1 ▪ ALK-BA* ▪ AP12-MALT1, IGH-MALT1, MALT1-BA* ▪ IGH-BCL-2, Myc-IGH, Myc-BA*	▪ Diagnostic/predictive/prognostic significance ▪ Diagnostic/predictive/prognostic significance ▪ Diagnostic/predictive significance ▪ Diagnostic minimal residual disease/predictive/prognostic significance ▪ Diagnostic significance ▪ Diagnostic significance ▪ Diagnostic significance ▪ Diagnostic significance ▪ Diagnostic/predictive/prognostic significance ▪ Diagnostic significance ▪ Diagnostic significance

**BA: Break apart*

- N-Myc and c-Myc amplifications have been demonstrated in neuroblastoma.

- **Chromosomal translocations:** Tumor-associated chromosomal translocations demonstrated by FISH technique are useful diagnostic aid in primitive appearing hematopoietic and soft tissue malignancies. Reliable DNA probes are commercially available for most common translocations. Three types of strategies are employed to demonstrate chromosomal translocations as given in Table 14.92.

DEVELOPMENT OF FISH TEST FOR CLINICAL USE

Development on the frequencies of various translocations and breakpoints, FISH-F, FISH-BA, FISH-ES, D-FISH or combination of strategies can be designated. Newly developed FISH technique is useful to demonstrate cytogenetic abnormalities such as chromosomal gain or loss, deletion of gene or locus, gene amplification and translocation, diagnostic, prognostic and predictive response or lack of response to patient therapy.

Table 14.92 Comparison among three types of strategies to demonstrate translocations

Parameters	FISH-F Technique	FISH-BA Technique	FISH-ES Technique
Probe used	Two loci-specific probes with different fluors (color tags) targeting two different partners (BCR on 22q and ABL on 9q)	Two probes localizing just proximal and distal to one of two breakpoints of interest (c-Myc at chromosome 8q24 can fuse with IGH at 14q32 in Burkitt's lymphoma)	One large probe that spans a breakpoint region demonstrated in minimal residual disease in patients with chronic myelogenous leukemia

ENZYME-LINKED IMMUNOSORBENT ASSAY TECHNIQUE

ENZYME-LINKED IMMUNOSORBENT ASSAY TECHNIQUE: OVERVIEW

Enzyme-linked immunosorbent assay (ELISA) technique is a sensitive microtiter plate-based immunoassay technique, also called solid-phase enzyme immunoassay as it employs an enzyme-linked antigen or antibody as a marker for the detection of specific protein (antigen or antibody).

- ELISA technique is used to assess the presence of specific protein (antigen or antibody) in the given sample and its quantification. Antigen–antibody reaction is immune-mediated reaction.
- Enzyme used in ELISA technique acts on colorless substrate and converts into colored (chromogenic) product. Color intensity of the colored product generated by enzyme is analysed.

PRINCIPLE

In ELISA technique, an enzyme conjugated with an immunoglobulin reacts with a colorless substrate to generate colored chromogenic product. A number of enzymes employed for ELISA include alkaline phosphatase, horseradish peroxidase, and β-galactosidase. Signals are developed by the action of hydrolyzing enzyme on chromogenic substrate and the optical density analyzed by microplate reader. General procedure of ELISA technique is shown in Fig. 14.27. Clinical applications of ELISA technique are given in Table 14.93.

ELISA TECHNIQUE DATA INTERPRETATION

ELISA technique yields three different types of data output.

- **ELISA technique quantitative data:** ELISA technique data can be interpreted in comparison to a standard curve (a serial dilution of a known, purified antigen) in order to precisely calculate the concentration of antigen in various samples.
- **ELISA technique qualitative data:** ELISA technique can also be used to achieve a yes or no answer indicating whether a particular antigen is present in a sample, as compared to a blank well containing no antigen or an unrelated control antigen.
- **ELISA technique semiquantitative data:** ELISA technique can be used to compare the relative levels of antigen in assay samples, since the intensity of signal will vary directly with antigen concentration.

TYPES OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) TECHNIQUE

There are various types of ELISA techniques such as direct, indirect, sandwich, competitive and noncompetitive are shown in Fig. 14.28.

Direct ELISA Technique

Direct ELISA technique uses directly labeling antibody itself. Sample of antigen is applied to the surface, often in the well of a microtiter plate. The antigen is fixed to the surface to render it immobile. The plate well and other surfaces are then coated with a blocking buffer.

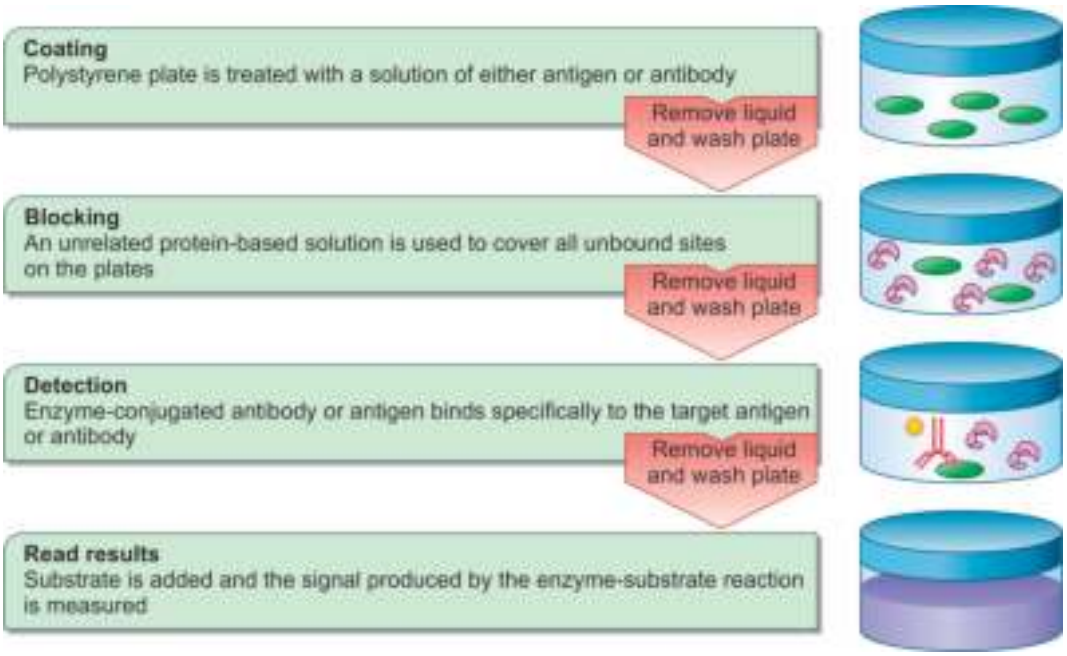


Fig. 14.27: General procedure of ELISA technique.

Table 14.93 Clinical applications of ELISA technique

Sample	Disease
Serum immunoglobulin concentrations	Clinical settings
Serum markers	Hepatitis B virus infection
Blood hormones	Pregnant women
Blood sample for antibody to infectious agents	▪ Human immunodeficiency virus ▪ Mycobacterium tubercle bacilli ▪ *Treponema pallidum* infection (syphilis) ▪ *Vibrio cholerae* (cholera) ▪ Bird flu ▪ Lyme disease ▪ Pernicious anemia ▪ Toxoplasmosis ▪ Varicella-zoster virus (chickenpox)
Feces	Rotavirus

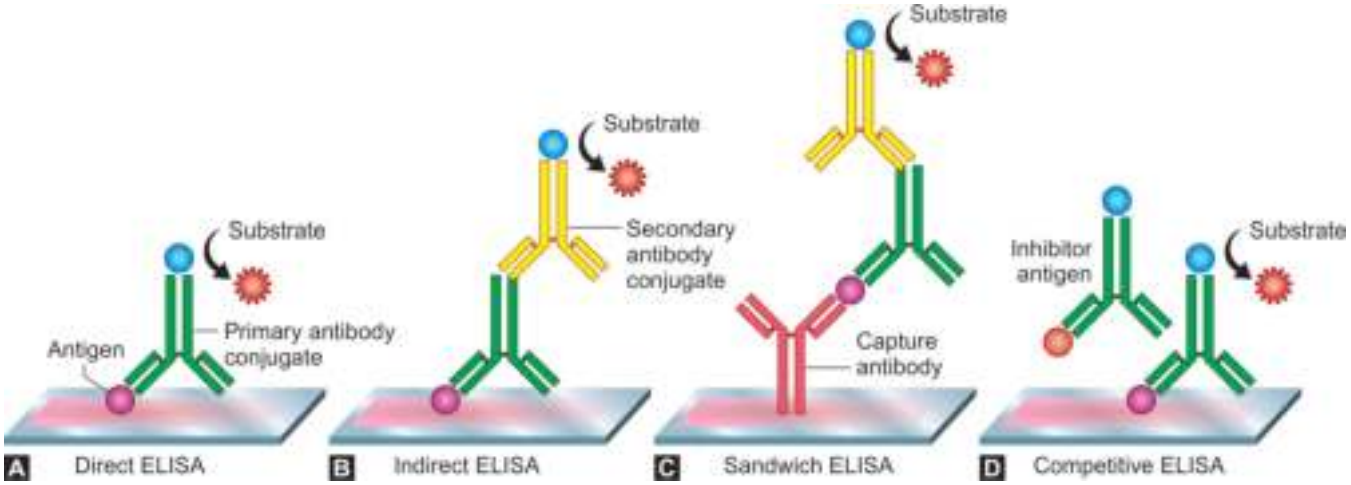


Fig. 14.28: Types of ELISA technique.

Antibody labeled by an enzyme (detecting antibody) is usually diluted in buffer and applied to the microtiter plate for binding to the antigen coated on plate.

Indirect ELISA Technique

Indirect ELISA technique detects the presence of antibody in a sample. The protein antigen to be tested is added to each well of ELISA microtiter plate; where it is given time to adhere to the plastic through charge interactions.

- Primary antibody present in the sample binds to the antigen after addition of sample.
- The solution is washed to remove unbound antibodies. Enzyme conjugated secondary antibodies are added to the solution. The substrate for enzyme is added to quantify the primary antibody through color change.
- The concentration of primary antibody present in the serum directly correlates with the intensity of the color. Procedure of indirect ELISA technique is shown in Fig. 14.29.
- A wide variety of labeled secondary antibodies is now available commercially. The indirect ELISA technique is versatile because many primary antibodies can be prepared in one species and the same labeled secondary antibody can be used for detection. Maximum immunoreactivity of the primary antibody is retained because it is not labeled. Sensitivity is increased because each primary antibody contains several epitopes that can bind with the labeled secondary antibody, permitting for signal amplification.
- Indirect ELISA technique, cross reactivity may occur with the secondary antibody resulting in nonspecific signal. An extra incubation step is required in the procedure. Comparison between direct ELISA and indirect ELISA technique is given in Table 14.94.

Sandwich ELISA Technique

In sandwich ELISA technique, microtiter plate is coated with antibody to detect specific antigen. Any nonspecific binding sites on the surface are blocked by using bovine serum albumin.

- Antigen-containing sample is applied to the microtiter plate. Unbound antigen is removed by washing.

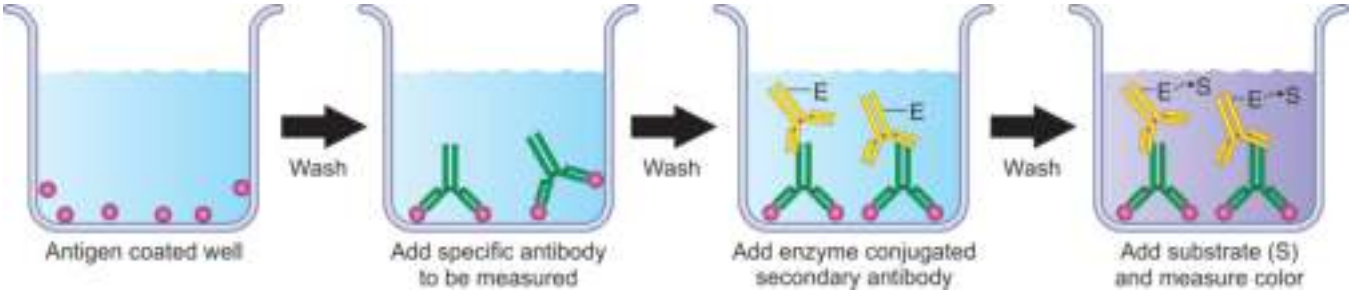


Fig. 14.29: Procedure of indirect ELISA.

Table 14.94 Comparison between direct ELISA and indirect ELISA technique

ELISA Technique	Direct ELISA Technique	Indirect ELISA Technique
Advantages	▪ Quick as only one antibody and fewer steps are used ▪ Cross reactivity of secondary antibody is eliminated	▪ A wide variety of labeled secondary antibodies is available commercially ▪ Maximum immunoreactivity of the primary antibody is retained because it is not labeled ▪ Increased sensitivity allowing signal amplification, as each primary antibody contains many epitopes bound by labeled secondary antibody ▪ Different visualization markers can be used with the same primary antibody
Disadvantages	▪ Immunoreactivity of primary antibody may be adversely affected by labeling with enzymes or tags ▪ Minimal signal amplification occurs ▪ Labeling primary antibody for each specific ELISA system is time-consuming and expensive	▪ Cross reactivity might occur with secondary antibody resulting in nonspecific signals ▪ Extra incubation step is required in the procedure

- Enzyme-linked primary antibodies are applied as detection antibodies, which also bind specifically to the antigen. Then the unbound antibody-enzyme conjugates are removed by washing.
- A chemical substance is added, which is converted by the enzyme into a colored product, absorbency of which is analyzed to determine the presence and quantity of antigen. Sandwich ELISA technique is shown in Fig. 14.30.

Advantages

Sandwich ELISA is highly-specific technique, in which antigen/analysate is specially captured and detected. It is suitable for complex (or crude/impure) samples as the antigen does not require purification prior to measurement. Sandwich ELISA technique is flexible and sensitive, by which both direct and indirect detection ELISA methods can be used.

Competitive ELISA Technique

Competitive ELISA technique is highly sensitive technique even when the specific detecting antibody is present in relatively small amounts.

- The technique depends on the competitive reaction between the sample antigen and antigen bound to the wells of microtiter plate with the primary antibody.
- Antigen and antibody are labeled with an enzyme and allowed to compete with unlabeled ones (in patient's serum) for binding to the same target.
- Hydrolysis signal from antigen–antibody complex (enzyme-labeled) is measured. Antigen or antibody in serum is then calculated.
- There is no need to remove the excess/unbound antigen or antibody from the reaction plate or tubes. Concentration of color is inversely proportional to the amount of antigen presents in the sample. Competitive enzyme-linked immuosorbent assay (ELISA) technique is shown in Fig. 14.31.

Noncompetitive ELISA Technique

In noncompetitive ELISA technique excess/unbound antigen or antibody must be removed before every step of reactions. It can be of various types—direct ELISA technique, indirect ELISA technique, sandwich ELISA technique and antibody capture ELISA technique (similar to sandwich ELISA technique but in first step, anti-IgM or IgG is coated on the plate and then antibodies in patient serum are allowed to capture in next step).

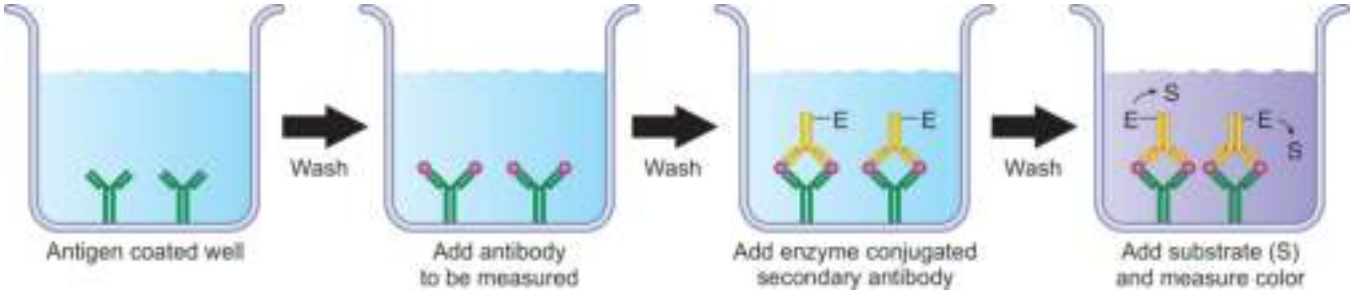


Fig. 14.30: Sandwich enzyme-linked immunosorbent assay (ELISA) technique.

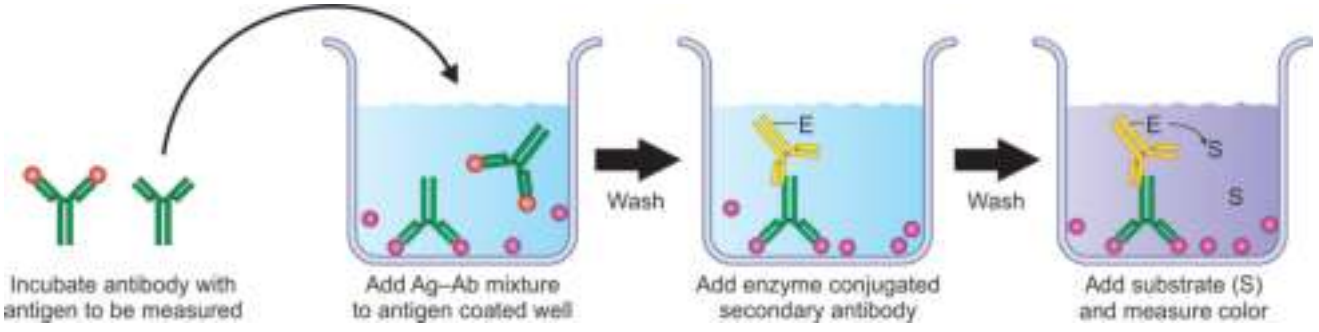


Fig. 14.31: Competitive enzyme-linked immunosorbent assay (ELISA) technique.

DNA SEQUENCING METHODS

DNA sequencing methods are used to determine diagnosis, prognosis, the detection of minimal residual disease, and the diagnosis of hereditary predisposition to cancer. Molecular profiling of malignant tumors by cDNA arrays can determine expression of large segments of the genome at once and can be useful in molecular stratification of otherwise identical tumors for the purpose of treatment. DNA sequencing methods are used for detection of gene mutations as given in Table 14.95. Techniques used to analyze DNA, RNA and proteins are given in Table 14.96. Next generation DNA sequencing is shown in Fig. 14.32.

POLYMERASE CHAIN REACTION TECHNIQUE

Most molecular tests performed in surgical pathology focus on somatic or acquired DNA variations in the cells of the disease process, which provide information of diagnostic, prognostic and predictive indicators. Polymerase chain reaction (PCR) is quick, reliable and sensitive technique, which has become a central diagnostic tool in clinical molecular genetic testing.

Pathology Pearls: Polymerase Chain Reaction Technique

Polymerase chain reaction (PCR) technique is used to amplify single or a few copies of a piece of DNA to generate thousands to millions of copies of a particular DNA sequence.

Methodology

Polymerase chain reaction (PCR) technique involves amplification of a target sequence of DNA using 3 and 5 DNA primers. After 25 cycles 10^7 copies of target DNA are produced.

Clinical Applications

- Polymerase chain reaction (PCR) is a diagnostic technique to detect genetic and acquired disorders.
- PCR technique detects direct DNA sequences in forensic medicine, circulating tumor or microorganisms, e.g. Myocobacterium tubercle bacilli, *Helicobacter pylori, Treponema pallidum*, hepatitis C virus, Epstein-Barr virus and *Toxoplasma gondii.*
- PCR technique detects paternal assay.
- PCR technique detects BCR-ABL translocation in chronic myelogenous leukemia, clonal assay in lymphomas, DNA mismatch repair (microsatellite sequence) in hereditary nonpolyposis colorectal cancer (HNPCC), and detection of EWS/FLI-1 fusion gene in Ewing sarcoma.
- RT-PCR, DNA hybridization *in situ* or Southern blot techniques are used to detect certain chromosomal translocations.

SPECIMENS REQUIRED

Nucleic acids are used for molecular testing by polymerase chain reaction (PCR). Specimen requirements are dictated by the disease process.

- Sources of nucleic acids are peripheral blood, bone marrow aspirate, fresh or frozen tissue stored at –200° to –700°C, formalin-fixed paraffin-embedded tissue (FFPE), cytology specimens, and enriched population of cells from flow cytometry.
- Transport of these specimens on ice reduces cell lysis, minimizes nuclease activity and reduces nucleic acid degradation. Peripheral blood and bone marrow aspirate are added anticoagulants. Samples obtained for DNA sequencing by polymerase chain reaction technique are given in Table 14.97.

Table 14.95 DNA sequencing methods are used for detection of gene mutations

Technique	Principle	Type of Gene Mutation Detection
Cytogenetic analysis	Unique visual appearance of various chromosomes	Numerical structural abnormalities in chromosomes
Fluorescence *in situ* hybridization (FISH) technique	Hybridization to chromosomes with fluorescent labeled probes	Numerical structural abnormalities in chromosomes
Southern blot technique	Hybridization with genomic probe or cDNA probe after digestion of high molecular DNA	Detection of large deletion, insertion, rearrangement
Polymerase chain reaction (PCR) technique	Amplification of DNA	Expansion of triplet repeats, variable number of tandem repeats (VNTR), gene rearrangement, translocation
Reverse transcriptase PCR (RT-PCR) technique	Reverse transcription, amplification of DNA segment, absence or reduction of mRNA transcription	To analyze the expression level of targeted genes
DNA sequencing technique	Direct sequencing of PCR products. Sequencing of DNA segment cloned into plasmid vectors	Point mutation, small deletions and insertions
Restriction fragment length polymorphism (RFLP) technique	Detection of altered restriction pattern of genome: DNA Southern blot or PCR products	Point mutation, small deletions and insertions

Table 14.96 Techniques used to analyze DNA, RNA and proteins

Technique	Sample Analyzed	Gel Used	Purpose
Southern blot technique	DNA	Yes	Detection of DNA changes
Northern blot technique	RNA	Yes	Analysis of mRNA amount and size
Western blot technique	Protein	Yes	Analysis of protein content
Allele specific oligonucleotides technique	DNA	No	Detection of DNA mutations
Microarray technique	RNA or cDNA	No	Measurement of many mRNA at one time
ELISA technique	Protein or antibodies	No	Detection of proteins (antigens) or antibodies

Next generation sequencing (NGS) of DNA by illumina genome analyzer sequencing process

- Next generation sequencing of DNA is a technology for determining the nucleic acid sequence in DNA (adenine, guanine, cytosine and thymine) and RNA (adenine, cytosine, uracil and guanine) to study genetic variation associated with genetic diseases and other diseases.
- Next generation sequencing of DNA has three steps: (a) library preparation (DNA fragmentation, adaptor ligation *in vitro*, sequencing), (b) clonal amplification (emulsion PCR and bridge PCR) and (c) cyclic array sequencing (sequencing by ligation and sequencing by synthesis).
- Methods for next generation sequencing of DNA include: massively parallel signature sequencing, Riche 454 pyrosequencing, reversible terminator sequencing, oligonucleotide ligation detection sequencing, Ion Torrent sequencing, illumina sequencing and single-molecule real-time sequencing.

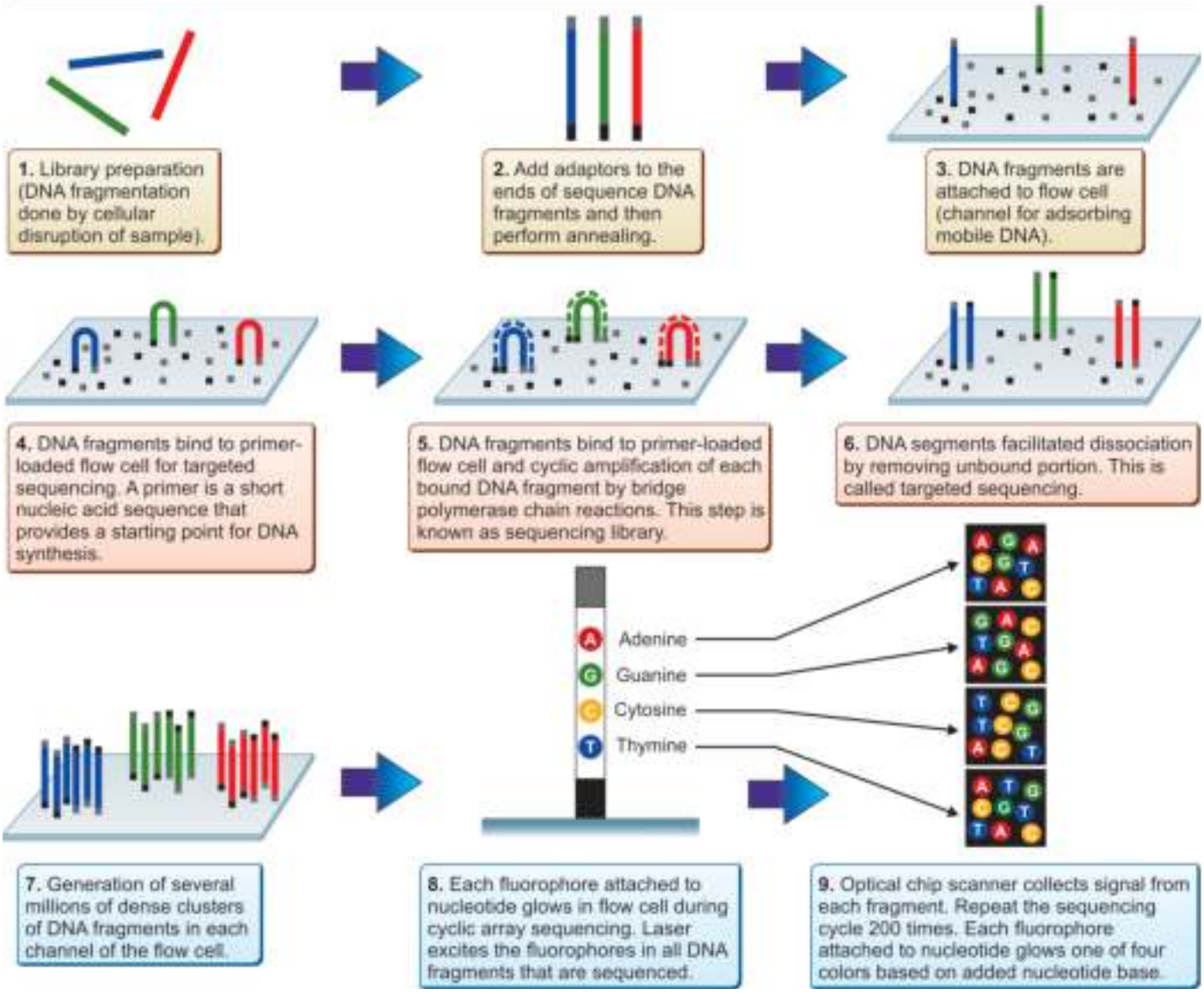


Fig. 14.32: Next-generation sequencing of DNA.

Table 14.97 Samples obtained for DNA sequencing by polymerase chain reaction technique

Prenatal/Postnatal Period and Cancer Genetic Sampling	Sample
Prenatal period	▪ Amniotic fluid ▪ Chorionic villus (fresh or formalin-fixed) ▪ Fetal cord blood
Postnatal period	▪ Peripheral blood ▪ Product of conception (fresh or formalin-fixed) ▪ Skin biopsy (fresh or formalin-fixed)
Cancer cytogenetic sampling	▪ Bone marrow ▪ Lymph node ▪ Peripheral blood ▪ Core biopsy ▪ Solid tumor ▪ Pleural effusion

Tissue Microdissection Method

Microdissection enables the targeted collection of cells or tissues from slide-mounted cytologic specimens or frozen or formalin-fixed paraffin-embedded (FFPE) tissue sections. After that, sample tissues may be treated for extraction of nucleic acids or protein. Microdissection method is carried out by dissecting microtome (simplest method) and laser capture microdissection (LCM) requiring a specialized microscopy. It is primarily a research application but also is useful in surgical pathology practice.

Nucleic Acid Extraction Method

Extraction of nucleic acids from pathologic samples involves cell lysis followed by selective DNA or RNA isolation and a quantitative or qualitative assessment. Pathologic samples used for molecular analysis include tissue samples (fresh or formalin-fixed paraffin-embedded, i.e. FFPE); body fluids (amniotic fluid, saliva, stools, urine); buccal and cervical scrapes; fine needle aspirates; peripheral blood; cell cultures and hair root.

DNA Extraction Methods

Classic DNA extraction methods are time-consuming (about 3 days) and require relatively large quantities of tissues (100 mg to 1 g).

- Numerous DNA extraction kits are now available that use glass-fiber filters that selectively bind DNA after tissue treatments with protease and chaotropic buffers (which disrupt protein and DNA secondary structures).
- With DNA extraction kits, pure DNA recovery from diverse pathologic samples is possible within several hours.
- Automated DNA extraction platforms are available for the processing of multiple patient samples.

RNA Extraction Methods

Classic RNA extraction methods cause rapid homogenization of large quantities of fresh tissues in protease and guanidinium thiocyanate solution to denature ubiquitous endogenous RNases. Current methods allow relatively rapid (1 day) recovery of RNA, again after tissue homogenization in chaotropic guanidinium salt solution that leaves RNA contained in an aqueous phase and protein and DNA in an organic phase.

DNA and RNA Extraction Preservation

DNA and RNA extraction from fresh tissue specimens gives high-integrity nucleic acids. Next best option is preservation of extracted nucleic acids from tissues in liquid nitrogen (commercially available storage reagents, e.g. RNA later, ambion).

- DNA and RNA extracts from FFPE block tissues tend to be degraded with block age. The concentration and purity of extracted nucleic acids are assessed spectrophotometrically (NanoDrop spectrophotometer).
- Nucleic acid integrity can be estimated by comparing nucleic acid fragment size against a molecular weight ladder after agarose gel electrophoresis.

Nucleic Acids Storage

Nucleic acids are generally stored at 4°C for assays performed within 1 week to 1 month of extraction and in aliquots at 20°C or 80°C for long-term storage. Repeated freeze-thawing may lead to DNA degradation. RNA is more labile than DNA and is susceptible to degradation by RNases. RNA is stored at 20°C for short-term use and at 80°C or under liquid nitrogen for long-term use.

PCR TECHNIQUE METHODOLOGY

Polymerase chain reaction technique generates thousands to millions of copies of a particular sequence. It is currently used in biomedical research laboratories for the diagnosis of hereditary and infectious diseases. Essential ingredients of polymerase chain reaction are given in Table 14.98. It is a method for the *in vitro* amplification of DNA involving automated cycles of denaturation, annealing, and extension or synthesis performed in a thermocycler.

Basic Steps of Polymerase Chain Reaction Technique

Basic steps of polymerase chain reaction (PCR) technique are denaturation, annealing and extension (Figs 14.33 and 14.34).

Table 14.98 Essential ingredients of polymerase chain reaction (PCR) technique

Essential Ingredients	Comments
Buffer	▪ pH is typically maintained using a Tris-HCl-based buffer ▪ Other ingredients include KCl (aid primer template annealing), bovine serum albumin (BSA) and nonionic detergents (aid Taq DNA polymerase enzyme stability)
DNase or RNase free pure water	Final PCR reaction volumes vary from 10 to 50 μl
Magnesium cations (Mg^{2++})	Stabilizes the interaction between the oligonucleotide primer, template DNA and Taq DNA polymerase enzyme
dNTP (deoxyribonucleoside triphosphate)	–
Oligonucleotide primers	18–25 bases in length
Template DNA	Sample amount in a reaction can range from 1 ng to 1 μg
Thermostable DNA polymerase enzymes	Taq DNA polymerase extracted from *Thermus aquaticus* isolated from a hot-springs dwelling bacterium of the Deinococcus–Thermus phylum

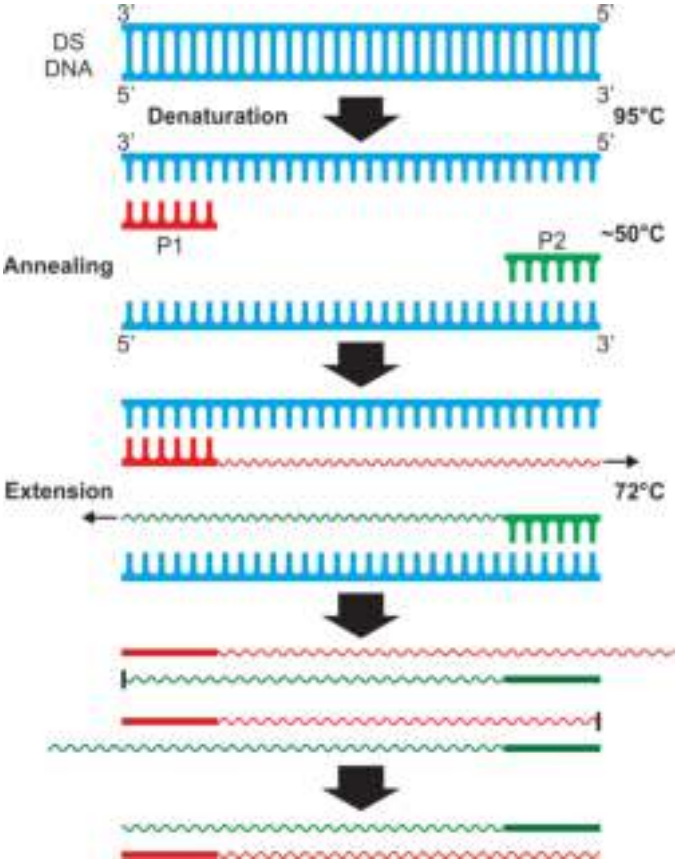


Fig. 14.33: Polymerase chain reaction shows denaturation, annealing and extension.

- **Denaturation step:** Sample specimen DNA of extracted is rendered single-stranded by heating to 94°–98°C.
- **Annealing step:** In annealing step, oligonucleotide primers hybridize with the target sequences. The annealing temperature depends on deoxyribonucleoside triphosphate (dNTP) composition of the primers and temperature in the range of 40°–60°C.
- **Extension step:** The extension step (72°C) leads to DNA synthesis (5′→3′) by thermostable DNA polymerase of new DNA strand. DNA amplification is accomplished by repetitive cycle (30–50 times) of the denaturation, annealing, and extension steps. The time period for each of the denaturation, annealing and extension steps can vary from 10 seconds to >1 minute, which depends on reaction volume size, amplicon base composition and length, thermostable DNA polymerase activity (about 1000 bp are extended per minute) and hardware specifications.

CLINICAL APPLICATIONS OF POLYMERASE CHAIN REACTION TECHNIQUE

Analysis of Molecular Genetic Alterations in Various Disorders

Polymerase chain reaction technique is performed to detect genetic disorders, BCR-ABL translocation in chronic myelogenous leukemia (CML), detection of EWS/FLI-1 fusion gene in Ewing sarcoma, hereditary nonpolyposis colorectal cancer (HNPCC) mutation analysis and detection of circulating tumor or pathogen. Molecular techniques used in nonhematologic and hematologic malignancies are shown in Figs 14.35 and 14.36. Polymerase chain reaction (PCR) technique variations are given in Table 14.99.

NUCLEIC ACID SIGNAL AMPLIFICATION TECHNOLOGY

Previously described techniques directly amplify target nucleic acid sequences to threshold of detection. Alternative strategy is to employ amplification technology at the level of detection, where a non-amplified nucleic acid is targeted using a probe and an amplified signal is generated from the probe.

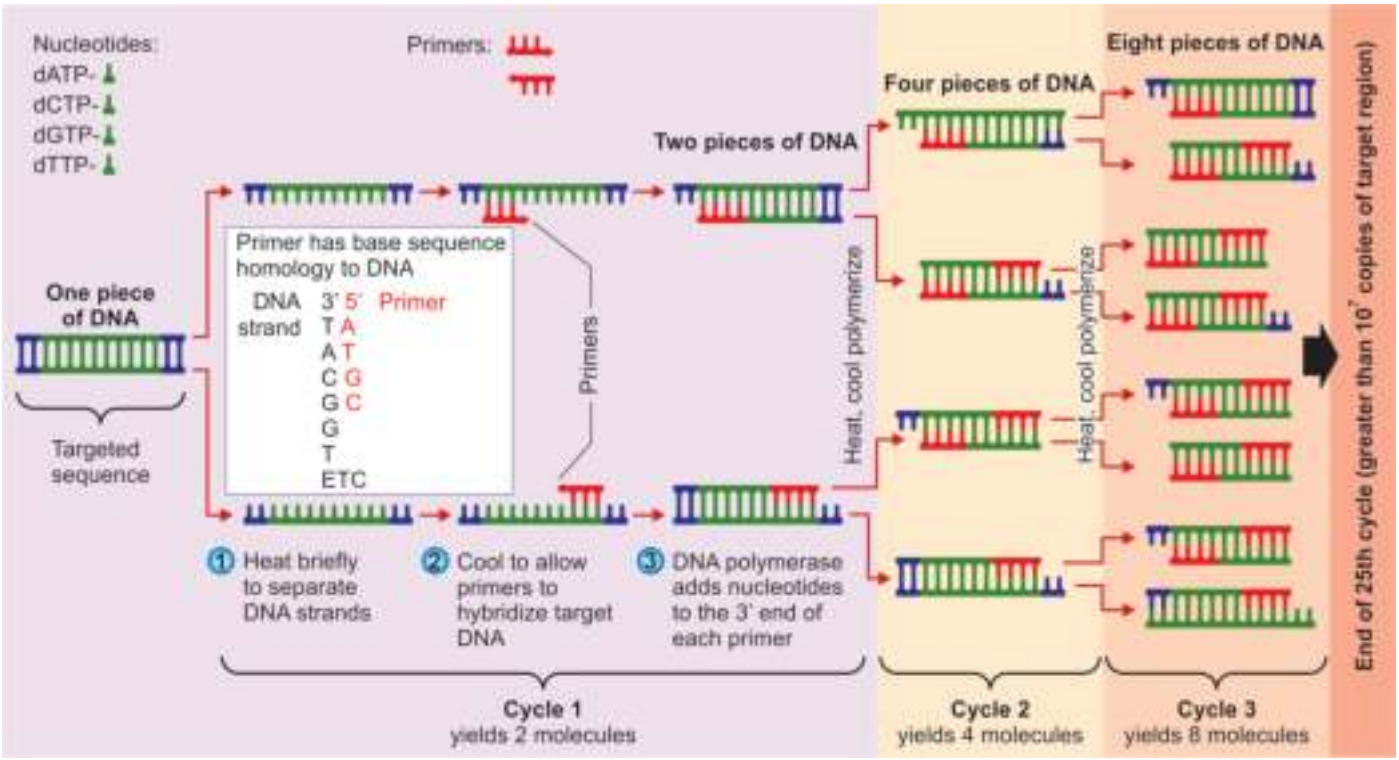


Fig. 14.34: Basic steps in polymerase chain reaction (PCR).

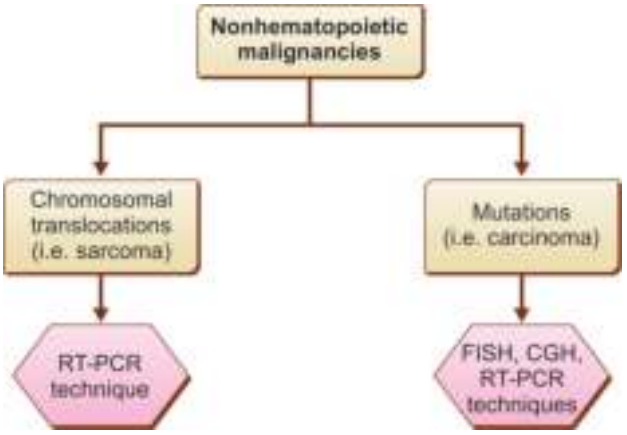


Fig. 14.35: Molecular techniques used in nonhematologic malignancies.

Nucleic acid amplification methods are given in Table 14.100.

Signal amplification techniques are less susceptible to false positive data and used in multiple ligation-dependent probe amplification (MLPA) and hybrid capture (Table 14.101).

WESTERN BLOTTING TECHNIQUE

Western blotting technique is widely used in molecular biology to identify specific antibodies in patient's blood and tissue samples by using a probe. A probe used is an antibody raised against that particular protein. Western blotting works on the principle of gel electrophoresis. Proteins are separated based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Gel electrophoresis is commonly used for separating proteins on the basis of size, shape and charge. In gel electrophoresis, protein of sample is separated according to molecular weight. Western blot technique is shown in Fig. 14.37.

SDS-PAGE Protocol

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDH-PAGE) is commonly used to obtain high resolution separation of complex mixture of proteins. Sample loading is done into the wells of gel electrophoresis equipment containing buffers.

Pathology Pearls: Blotting Techniques

- Blotting is a method of transferring proteins, DNA or RNA onto a carrier such as nitrocellulose membrane or polyvinylidene difluoride (PVDF) or nylon membrane.
- Western blotting technique is used to detect proteins.
- Southern blotting technique is used to detect RNA.
- Northern blotting technique is used to detect DNA.

SOUTHERN BLOT TECHNIQUE

Southern blot technique is used to detect specific DNA sequence in samples, which was developed by

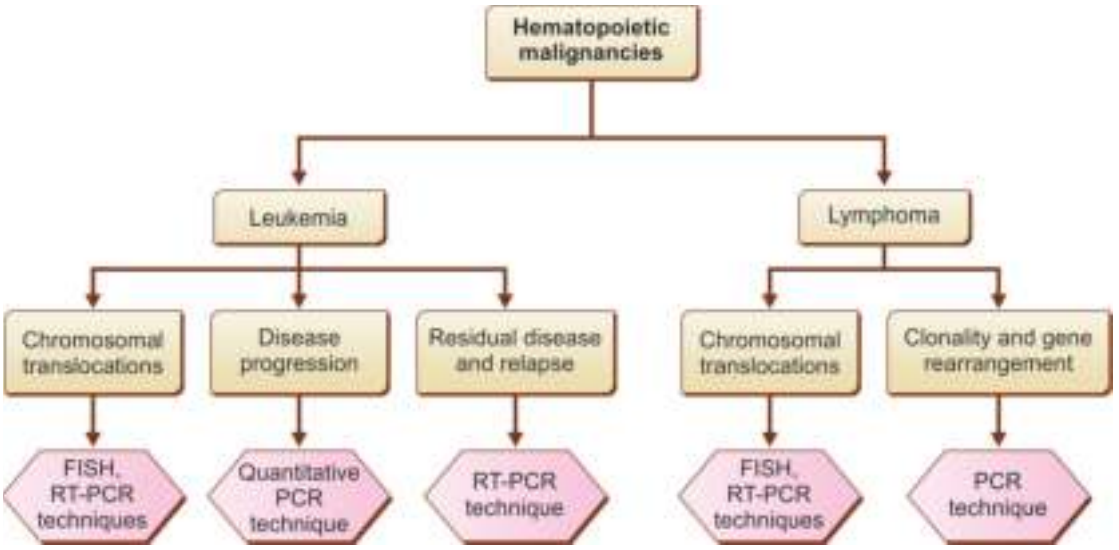


Fig. 14.36: Molecular techniques used in hematologic malignancies.

Table 14.99 Polymerase chain reaction (PCR) technique variations

PCR Technique Variations	Comments
Multiplex PCR technique	Simultaneous detection of more than one target by use of multiple primer pair sets
Consensus PCR technique	Used to amplify single target that has variable sequences or multiple targets that have similar (common) sequences
Degenerate PCR technique	Also used in the amplification of variable sequence target
Nested PCR technique	Used for improved PCR sensitivity and specificity
Reverse transcription PCR (RT-PCR) technique	For investigation of RNA expression through PCR, first step is conversion of total RNA or mRNA into single-stranded complementary DNA (cDNA) as thermostable DNA polymerases require DNA as a substrate
Real-time quantitative PCR (qPCR) technique	Used for the accurate quantification of DNA or RNA and cDNA target in a sample. Note: In standard PCR/end-point PCR, final product is obtained after 30–50 cycles. Although end-point PCR can be semiquantitative, it is essentially a qualitative assay
Allele-specific PCR (AS-PCR) technique	Allows direct detection of any point mutation in human DNA by analyzing PCR products in an ethidium bromide stained agarose or polyacrylamide gel
PCR amplification of specific alleles (PASA) technique	Generally applicable technique for detection of known point mutations, small deletions, insertions and polymorphisms
Long and accurate PCR (LA-PCR) technique	Allows the amplification of sequences 5–>20 kb in length
Amplification refractory mutation system (ARMS) technique	Simple method for detecting any mutation involving single base change, uses sequence-specific primers that allow amplification of test DNA only when the target allele is contained within the sample

Table 14.100 Nucleic acids amplification methods

Amplification Methods	Description	Applications
Transcription-mediated amplification (TMA) technique	Amplification of RNA targets, including species-specific rRNA sequences	Detection of *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycobacterium tuberculosis* and hepatitis C virus (HCV)
Nucleic acid sequence-based amplification (NASBA) technique	Isothermal amplification of a DNA or RNA target, identical to TMA but uses a separate RNase H enzyme and fluorescence resonance energy transfer (FRET) based detection technology	Detection of CMV and human immunodeficiency virus (HIV) RNA
Strand displacement amplification (SDA) technique	Requires a DNA polymerase that has strand displacement activity	Detection of *Chlamydia trachomatis, Neisseria gonorrhoeae*, and *Legionella pneumophila*
Ligase chain reaction (LCR) technique	Involves cycles of DNA denaturation and annealing and uses a thermostable DNA ligase	Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Table 14.101 Signal amplification techniques

Techniques	Description	Applications
Multiple ligation-dependent probe amplification (MLPA)	Involves ligation of two oligonucleotides hybridized immediately adjacent to each other at the target of interest. The ligation product is then amplified by PCR	Detection of familial cancers (BRCA1 and BRCA2 testing in breast cancer, colon polyposis (APC), retinoblastoma, Wilms' tumor); prenatal and postnatal screening; syndromes (Turner and Klinefelter syndromes)
Hybrid capture	Involves an *in vitro* solution hybridization of a target DNA sequence with an RNA probe, followed by a signal amplification step	Screening of 13 high-risk HPV genotypes (16, 18, 31, 33, etc.); detection and quantitation of CMV, *Chlamydia trachomatis, Neisseria gonorrhoeae,* HBV and herpes simplex virus (HSV)

Dr EM Southern in 1975 as a method for transferring DNA out of an agarose slab gel onto solid support like nitrocellulose or nylon membrane.

- Cellular DNA is digested by using restriction endonucleases to cut genomic DNA into different sized fragments.
- Gel electrophoresis is now used to separate DNA fragments. Alkaline buffer is used for denaturing DNA to ssDNA. Digested DNA is then transferred to nitrocellulose membrane.
- Complementary probe is now hybridized to the nitrocellulose membrane to detect specific gene of interest by labeling with radioactive or fluorescent dye.
- Southern blot technique requires relatively large quantity of high molecular weight DNA. Southern blotting technique is shown in Fig. 14.38A to F.

APPLICATIONS OF SOUTHERN BLOT TECHNIQUE

Southern blot technique can be used to detect chromosomal rearrangements, DNA amplifications, deletions and loss of heterozygosity. It can be used in combination with polymerase chain reaction (PCR) technique. Southern blotting technique is mostly used in restriction fragment length polymorphism (RFLP) analysis.

Pathology Pearls: Clinical Applications of Southern Blot Technique

- Autosomal dominant cerebellar ataxia evaluation
- Myotonic dystrophy evaluation
- Epstein-Barr virus clonality assay
- Beckwith-Wiedemann syndrome
- Fragile X syndrome evaluation
- Hemophilia A analysis for inversion, deletion, and carrier
- Ig gene rearrangement detection in B cell lymphoma and minimal residual disease
- T cell receptor gene rearrangement in T cell lymphoma
- MLH1, MSH2, MSH6 deletion/duplication screening
- Partial Duchenne muscular dystrophy (DMD) deletion/duplication assay
- Huntington disease (trinucleotide repeat)
- Sickle cell disease (noncoding repeats or satellite DNA sequence variations).

- Southern blot technique has advantages over polymerase chain reaction (PCR) technique when available sequence data are insufficient to design PCR primers specific to the site of a chromosomal rearrangement. Comparison between PCR and Southern blot technique is given in Table 14.102.
- Southern blot technique requires large quantities of DNA. It is a time-consuming process.

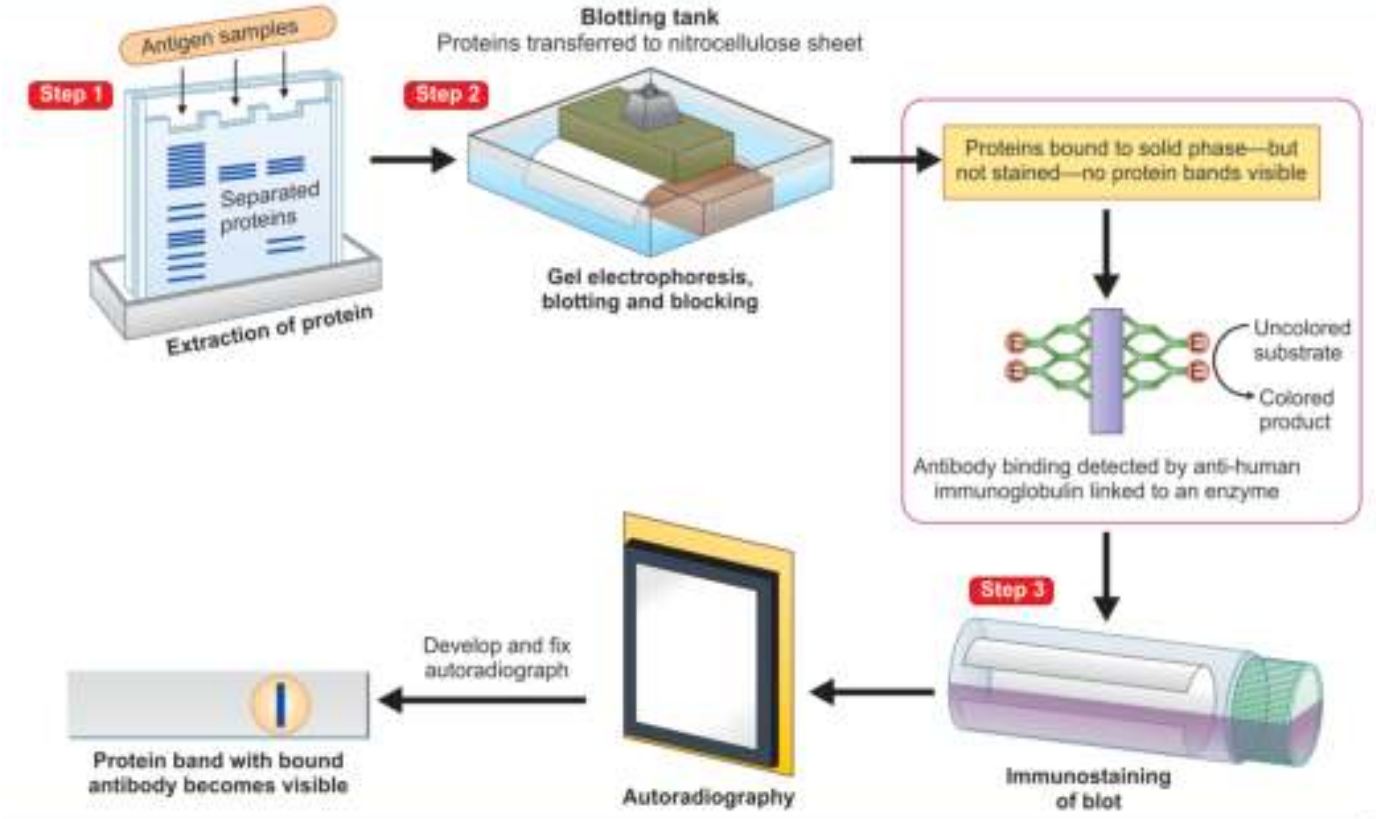
NORTHERN BLOT TECHNIQUE

Northern blot technique is used to analyze special sequences of messenger RNA (mRNA). Messenger RNA (mRNA) constitutes up to 5% of the total cellular RNA. RNA molecules are separated by electrophoresis and transferred to nitrocellulose membrane. RNA molecules are now identified by specific hybridization probe.

- **Northern blot technique methodology:** Extracted mRNA is denatured with formaldehyde or glyoxal to prevent the formation of secondary RNA structures.
  - RNA digestion into smaller fragments is not required as native mRNA fragment sizes range from about 300–12,000 nucleotide base pairs (average size is 1,000–3,000 nucleotide base pairs).
  - After agarose gel electrophoresis, RNA is transferred to membrane by capillary, vacuum, or electrotransfer process, and then the membrane is hybridized with a labeled probe to the gene target.
  - The resulting data indicates whether a gene is overexpressed or underexpressed or if an abnormally sized transcript is expressed.
- **Disadvantages of Northern blot technique:** Northern blotting is time-consuming technique, which requires relatively large amounts of high-integrity RNA and requires a high level of laboratory skill. Differences between Southern blotting and Northern blotting techniques are given in Table 14.103.
- **Clinical applications of Northern blot technique:** Northern blot technique is used to compare the expression of normal tissue versus malignant

Western blot (immunoblotting) technique: Basic goal

- Western blot technique is used to separate the blood proteins and detect the specific proteins (HIV antibodies that indicate HIV infection).
- Labeled antibody against particular protein is used to identify the desired protein.
- Western blot technique is used to confirm a positive ELISA test, and the combined tests are 99.9% accurate.
- A positive Western blot test confirms an HIV infection. A negative Western blot test indicates ELISA test as false positive.
- Calorimetric, chemiluminescence and fluorescence techniques are used to visualize a Western blot.
- As the antibodies only bind to the protein of interest, only one band should be visible.
- Thickness of the band corresponds to the amount of protein present.



Step 1. Extraction of protein from cell (called tissue separation)

- Cell lysate is most common sample used in Western blot test.
- Protein is extracted from mechanical or chemical induced lysis of cell.
- Protease inhibitor is added to prevent denaturing of protein.
- Protein concentration is determined by spectroscopy.

Step 2. Gel electrophoresis, blotting and blocking

- **Gel electrophoresis:** The sample is loaded in well of SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins are negatively charged, so they move toward positive (anode) pole as the electric current is applied.
- **Blotting:** The nitrocellular membrane is placed on the gel. Proteins are transferred to nitrocellular paper by capillary action.
- **Blocking:** Blocking is very important step in Western blot test, as it prevents antibodies from binding to the membrane before adding the primary antibody. Blocking is often made with 5% bovine serum albumin (BSA) or casein to reduce the background.

Step 3. Treatment with primary and secondary antibodies; and suitable substrate

- Proteins bound to solid phase are not stained, hence no protein band is visible.
- The primary antibody is specific to the desired protein so it forms antigen–antibody complex.
- The secondary antibody is enzyme labeled (e.g. alkaline phosphatase or horseradish) and against primary antibody so it can bind with antigen–antibody complex.
- The mixture is incubated with specific substrate to visualize the enzyme action. The enzyme converts the substrate to give visible colored product, so band of color can be visualized in the membrane.
- Western blotting is also a quantitative test to determine the amount of protein in the sample.

Fig. 14.37: The Western blot (sometimes called the protein immunoblot), or Western blotting is a widely used analytic technique in molecular biology and immunogenetics to detect specific proteins in a sample of tissue extract or homogenate. The sample undergoes protein denaturation, followed by gel electrophoresis.

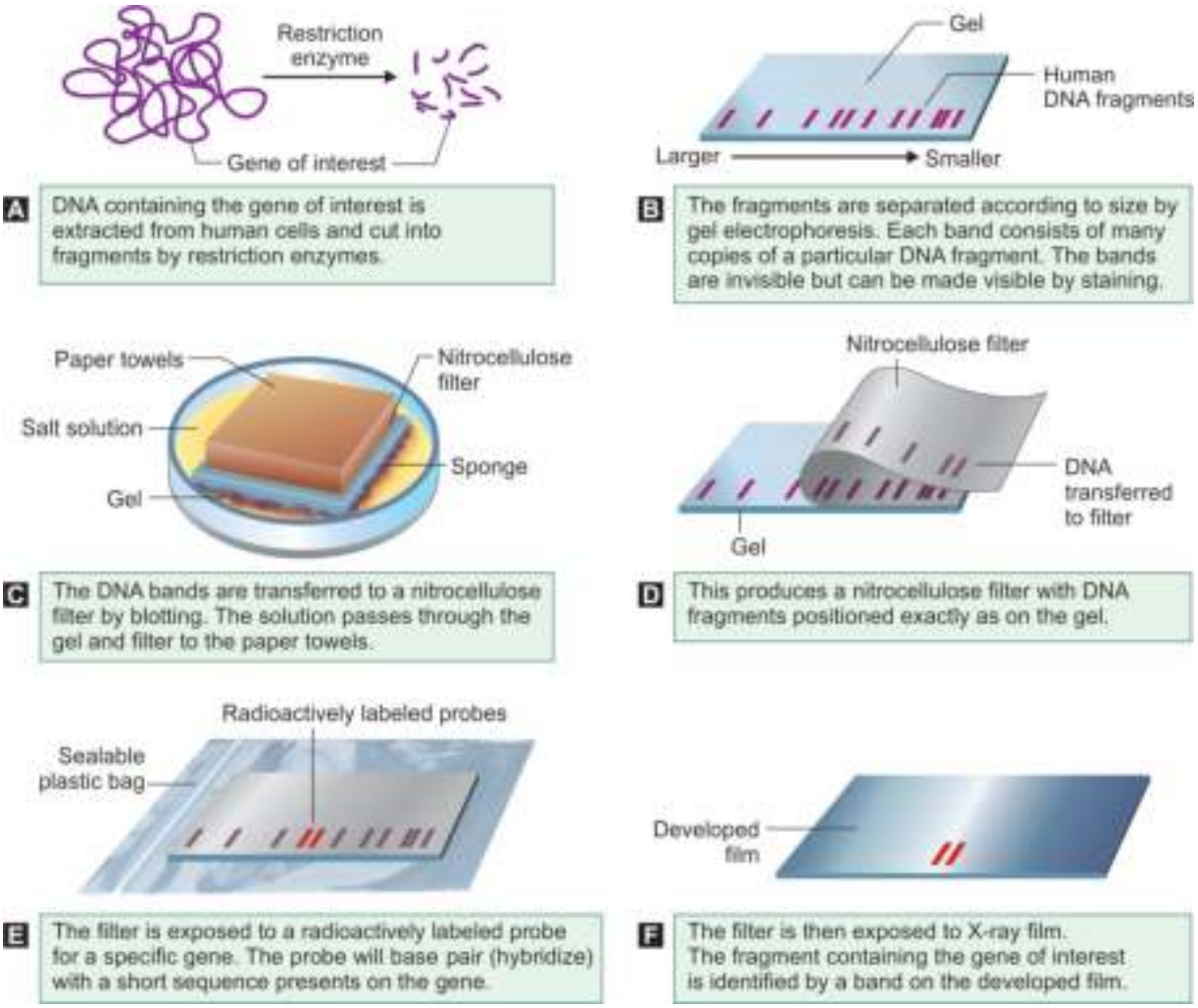


Fig. 14.38A to F: Southern blotting technique.

Table 14.102 Comparison between polymerase chain reaction (PCR) technique and Southern blot technique

Features	Polymerase Chain Reaction (PCR) Technique	Southern Blot Technique
DNA amount	≤1 µg	At least 30 µg per probe
Restriction enzyme digestion	Not needed	Required
DNA quality and size	Can be severely degraded and 100–300 bp DNA	High quality DNA and at least 20 kb
Gel electrophoresis	Polyacrylamide gel required	Agarose gel required
Detection methods	Fluorescence dye, silver stain, chemiluminescence radioactivity	Usually radioactivity, less often chemiluminescence
Time	1–2 days	1–2 weeks
False negative results	Common for B cell lymphomas, but uncommon in T cell lymphomas	Rare
Sensitivity	1 cell per 1000 cells	1–5% of total DNA

tumors and identification of potential oncogenes. This technique provides prognostic information via expression of profiling.

Northern blot technique is also used to study pathogenesis of disease by analyzing specific messenger RNA (mRNA) levels in tissues.

Table 14.103 Differences between Southern blotting and Northern blotting techniques

Features	Southern Blotting Technique	Northern Blotting Technique
Naming of blotting	Named after inventor Dr EM Southern	It is a misnomer
Separation	DNA	RNA
Denaturation step	Required	Not required
Membrane used	Nitrocellulose filter membrane	Aminobenzyloxymethyl filter paper
Hybridization	DNA-DNA	RNA-DNA

TISSUE MICROARRAY (TMA) TECHNIQUE

TISSUE MICROARRAY TECHNIQUE: OVERVIEW

Tissue microarray (TMA) technique involves cylindrical tissue core needle biopsies of multiple tissues constructed in the same paraffin-embedded tissue block by inserting tissue core from the donor's block and inserted into recipient's block.

- Tissue microarray is a new technique used to analyze several hundred tissues, especially tumor samples on single slide by *in situ* technology.
- Difficulty with paraffin-embedded tissue relates to antigenic changes in proteins and mRNA degradation induced by the fixation and embedding processes.
- Tissue microarray (TMA) technique allows rapid visualization of morphology and molecular targets (DNA/RNA/protein) in thousand's of tissue specimens at a time, which correlates gene and protein expression patterns in intact tissues with clinical outcomes. Tissue microarray technique is shown in Fig. 14.39.

TISSUE MICROARRAY METHODOLOGY

Selection of Donor's Block

Primary donor paraffin-embedded cylindrical tissue block may be obtained from surgical pathology, autopsy or research material. Ideally donor block should contain 2–3 mm thickness of target tissue to permit large number of histologic sections (usually 100–300 sections) from it. A morphologically representative area of interest within the donor block is identified under light microscope using hematoxylin and eosin stained section on a glass slide as a guide.

Creation of New Paraffin-embedded Tissue Block

Cylindrical tissue cores are removed from the donor and inserted into a recipient paraffin-embedded tissue block. Using a precise spacing pattern, tissues can be inserted at high density, with up to 1,000 tissue cores in single paraffin-embedded tissue block.

Cutting Section from Tissue Block

Sections from tissue block that are cut with a microtome are placed onto standard glass slides that can be used or *in situ* analysis. Depending on the overall depth of tissue remaining in the donor blocks, tissue arrays can generate between 100 and 500 sections.

Recording of Source Tissue Data

Recording of source tissue data comprises clinical history, metastatic disease, staging, treatment history, tumor gross description such as size, shape, infiltration of margins and core tissue.

Tissue Processing

Marked slide and paraffin wax block are transferred to the person who will make the array. Then the core biopsies from selected tissue are taken either manually or by automated arrays. In tissue microarray (TMA) technique, cylindrical tissue cores are taken with intercore space of 0.8 mm. This TMA core size just fills the field of 40X objective. The core thickness should be 2–3 mm uniformly, if this is variable some sections of the block face will have empty spaces.

TISSUE MICROARRAY DESIGN

The cylindrical tissue cores are placed in X-Y axis positioning. While scoring TMA microscopically, it is easy to loose place when >10 cores in either axis. In such cases, sub-arrays of 5 rows and 5 columns can be made with empty spaces between arrays. Histologic sections/blocks can be stored for 70 years at room temperature as antigenicity of most tissues stored in paraffin wax is retained. However, RNA *in situ* hybridization (ISH) sections can be stored up to 1 year are best.

Cutting Sections from Tissue Block

Most tissue microarray (TMA) technique, cylindrical tissue cores are slip fit into recipient block holes without any bonding material between tissue cores and wax.

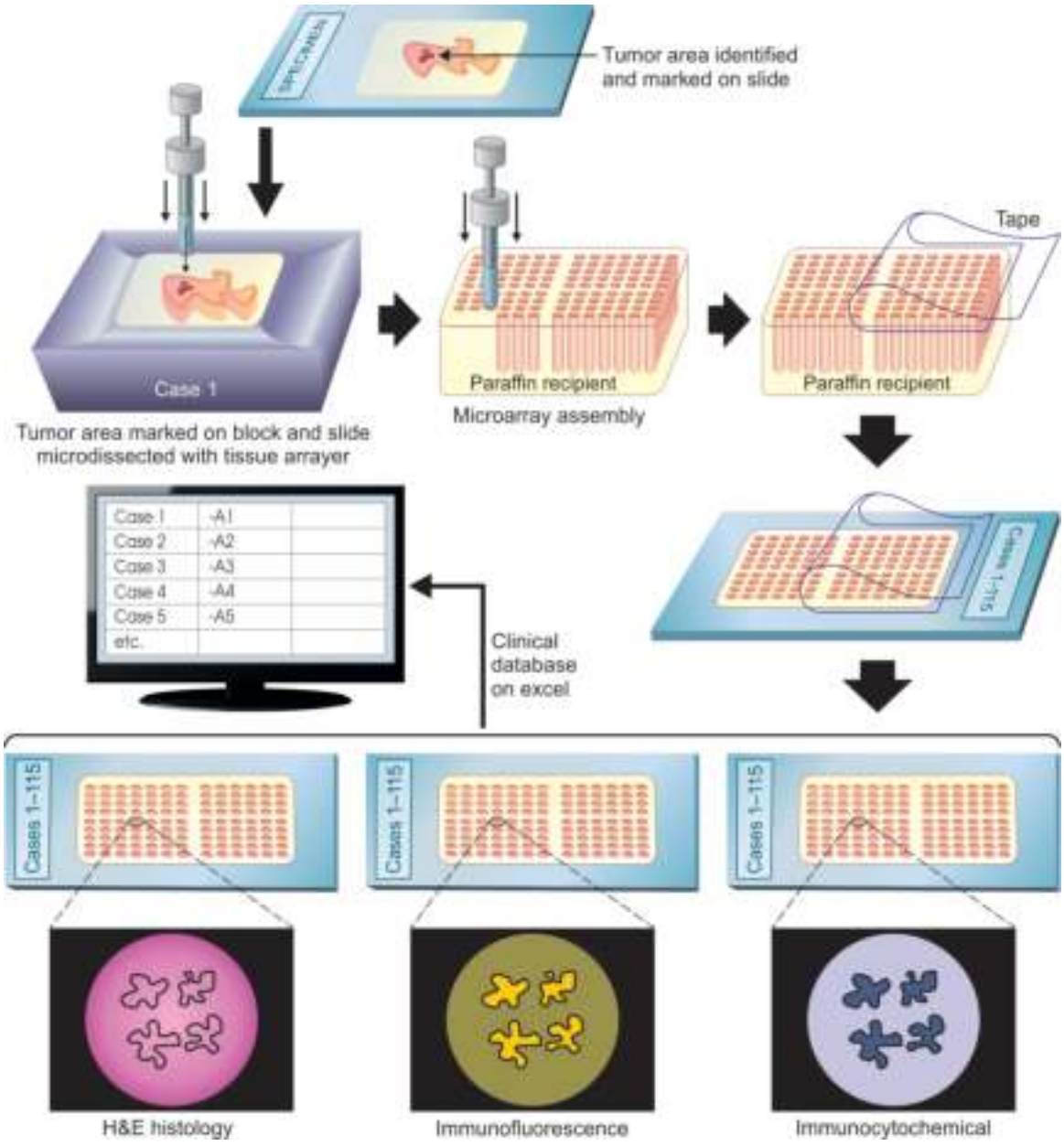


Fig. 14.39: Tissue microarray technique.

Thus, sections cut from block face have nothing to hold tissue cores in place. Ideally melting temperature and hardness of donor and recipient block's paraffin wax should match.

Hematoxylin and Eosin Staining

Hematoxylin and eosin stain permits visualization of morphology of area of interest from different donor blocks in one slide. Also since morphology of tissues may change with more sections from the TMA; 1st and every 50th section of TMA block may be stained with hematoxylin and eosin (H&E) to monitor morphology and representability of the specimens.

Analysis of Tissue Sections

Once constructed, tissue microarrays can be used with a wide range of techniques including histochemical staining, immunohistochemical/immunofluorescent staining or *in situ* hybridization for either DNA or mRNA.

Image Capturing

Regular bright field microscope can be used for analysis and scoring TMA slides, which can image 8–10 slides on single run in each of bright field, dark field and 3-color fluorescent mode if needed and view them simultaneously. It can also do morphometric analysis of these images.

APPLICATIONS OF TISSUE MICROARRAY TECHNIQUE

Tissue microarray (TMA) is an emerging diagnostic technique tool for research which has high throughput, uniform reaction conditions and experimental uniformity. TMA does not destroy the original block for diagnosis and economizes the use of reagents.

- TMA also facilitates data recording and linking to clinical data. Main application of TMA is understanding of tumor biology.
- There are different types of TMAs: multi tumor TMA, progression TMA and prognostic TMA.
  - In multi-tumor TMA, samples obtained from multiple tumors are analyzed for role of newly identified genes in normal and neoplastic tissues.
  - Progression TMA is used to study molecular changes in different stages of one particular tumor type.
  - Prognostic TMA comprises samples from tumors with available clinical follow-up data.

Surgical Pathology: Molecular Diagnostic Techniques in Clinical Practice
Immunohistochemistry Technique
Immunohistochemistry technique measures the detection of protein antigens. One IHC experiment can give quantitative (fluorescence intensity value) and semiquantitative (pathologist's score) data.
Fluorescence *in situ* Hybridization (FISH) Technique
FISH is used for analysis of genetic alterations. A single hybridization provides visualization of specific gene change in up to 1,000 tissues.
mRNA *in situ* Hybridization Technique
This detects mRNA transcripts in multiple different tissues.
DNA Microarray Technique
DNA microarray technique can be used along with TMA to generate information regarding DNA in different solid tissue cancers.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE

Main function of high-performance liquid chromatography (HPLC) is to separate, identify, quantify and purify nonvolatile and thermally stable components in the target sample.

PRINCIPLE OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The principle of separation in normal phase mode and reverse phase mode is adsorption in chromatography column of high-performance liquid chromatography (HPLC). The component, which has less affinity towards the stationary phase travels faster. The component which has same affinity towards the stationary phase, the components are separated.

COMPONENTS OF HPLC

HPLC has various components: mobile phase solvent reservoir, degasser, high-pressure pump, injector system, reversed-phase column compartment, detector, data processor and waste collection.

- Mobile phase solvent reservoir is delivery system that introduces the sample under pressure with the help of high-pressure pump from one or several reservoirs and flows through the column at a constant rate.
- A degasser is required to remove dissolved air and other gases from the solvent in the mobile phase.
- Column compartment controls the temperature of the column, if required.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows identification and quantification of the different substances. Waste is collected from the liquid.

TYPES OF HPLC

High-performance liquid chromatography (HPLC) types are based on: (a) modes of chromatography (normal mode HPLC or reverse mode HPLC), (b) principle of separation technique (adsorption HPLC, ion exchange HPLC, size exclusion, i.e. gel permeation HPLC and affinity HPLC), (c) elution technique (isocratic separation HPLC and gradient separation HPLC), and (d) purpose of separation technique (analytic HPLC and preparative HPLC).

- Ion exchange high-performance liquid chromatography is based on an exchange of ions between charged stationary surface and ions of the opposite charge in the mobile phase.

- Schematic representation of HPLC functioning, separation, identification, and quantification of substances in biological fluids, newborn screening by high-performance liquid chromatography–mass spectroscopy to analyze blood sample are shown in Figs 14.40 to 14.43.

SEPARATION OF COMPONENTS IN TARGET SAMPLE IN HPLC

Modes of separation in high-performance liquid chromatography (HPLC) include normal phase chromatography, reverse phase chromatography, reverse phase ion exchange chromatography and size exclusion chromatography.

- Normal phase chromatography is generally used for separation of nonpolar compounds. On the other hand, reverse phase chromatography is used for separation of polar compounds.
- In ion exchange chromatography, the stationary phase contains ionic groups, which interacts with the ionic groups of the sample molecules. This method is suitable for the separation of charged molecules only. Ion pair chromatography may be used for the separation of ionic compounds. Strong acids and basic compounds may be separated by reverse phase chromatography by forming ion pairs with suitable counter ions.

PRESSURE PUMP COMPONENT IN HPLC

The pressure pump is one of the most important components of HPLC, since its performance directly affects retention time, reproducibility and detector sensitivity.

- Various types of pressure pumps can be used in HPLC: displacement pump, reciprocating pump and pneumatic or constant pressure pump. Displacement pressure pump produces a flow that tends to be independent of viscosity and back pressure and also is pulse free out but possesses limited capacity (250 ml).
- Reciprocating pressure pump has small internal volume and its high output pressure at constant flow rates but it produces pulsed flow.
- Pneumatic or constant pressure pump has small internal limited capacity, and flow rate depends on viscosity and column back pressure.

SAMPLE INJECTION SYSTEM COMPONENT IN HPLC

Sample injection system component includes loop injection, valve injection and column injection. A fixed

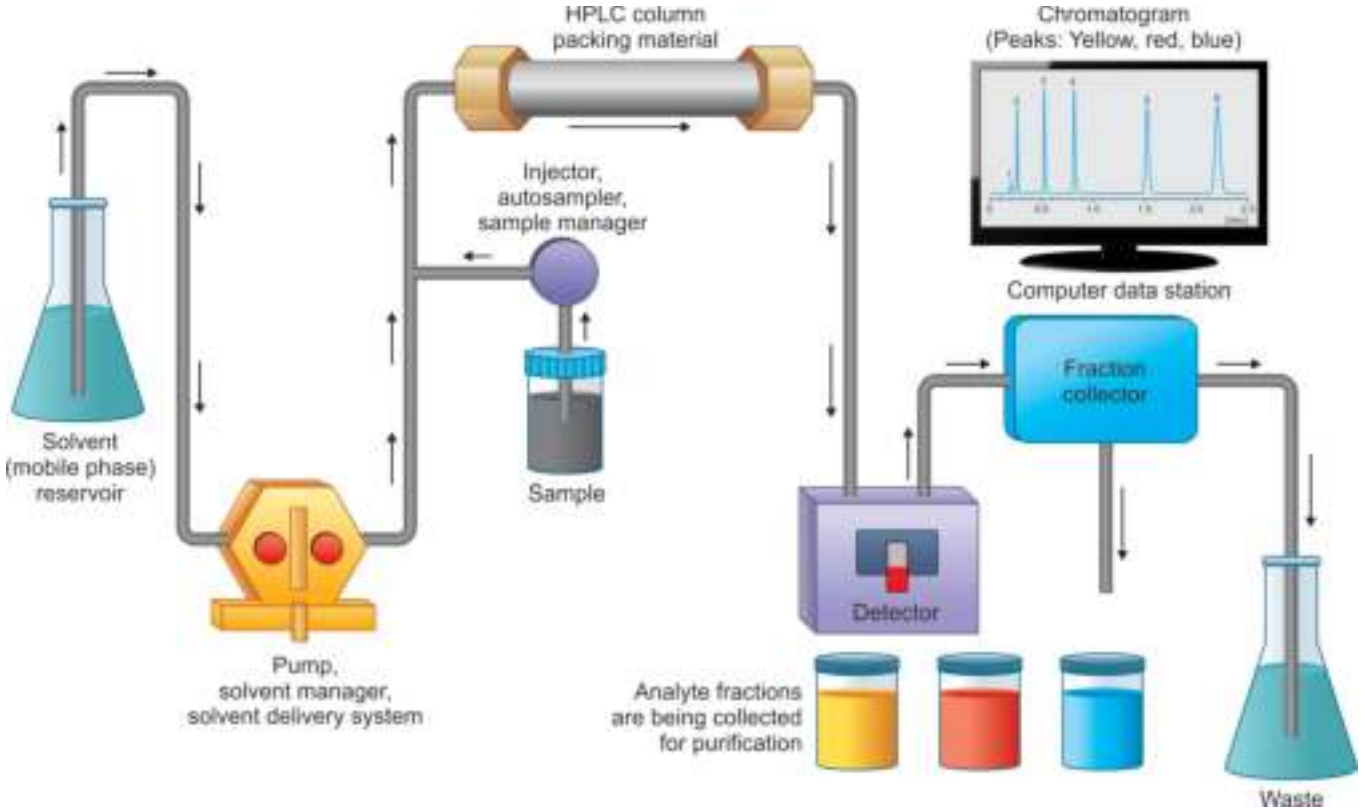


Fig. 14.40: Schematic representation of high-performance liquid chromatography functioning.

Chromatography technique for separation, identification, and quantitation of substances in biologic fluids

- Chromatography is an analytic laboratory technique commonly used for separating a mixture of chemical substances into individual components so that individual components such as proteins, peptides, nucleic acids and polynucleotides can be thoroughly analyzed in biologic fluids. Biologic fluid sample is extracted to remove extraneous molecules.
- Chromatography techniques include gas chromatography, high-performance liquid chromatography (ion exchange, paper, thin layer and gel chromatography), and affinity chromatography.
- Biologic fluid sample is injected into instrument with column chromatography.
- Low-molecular weight compounds in biologic fluid analysis require chemical derivation to increase volatility in gas phase by gas chromatography.
- High-molecular weight compounds in biologic fluid analysis is done by high-performance liquid chromatography (HPLC).

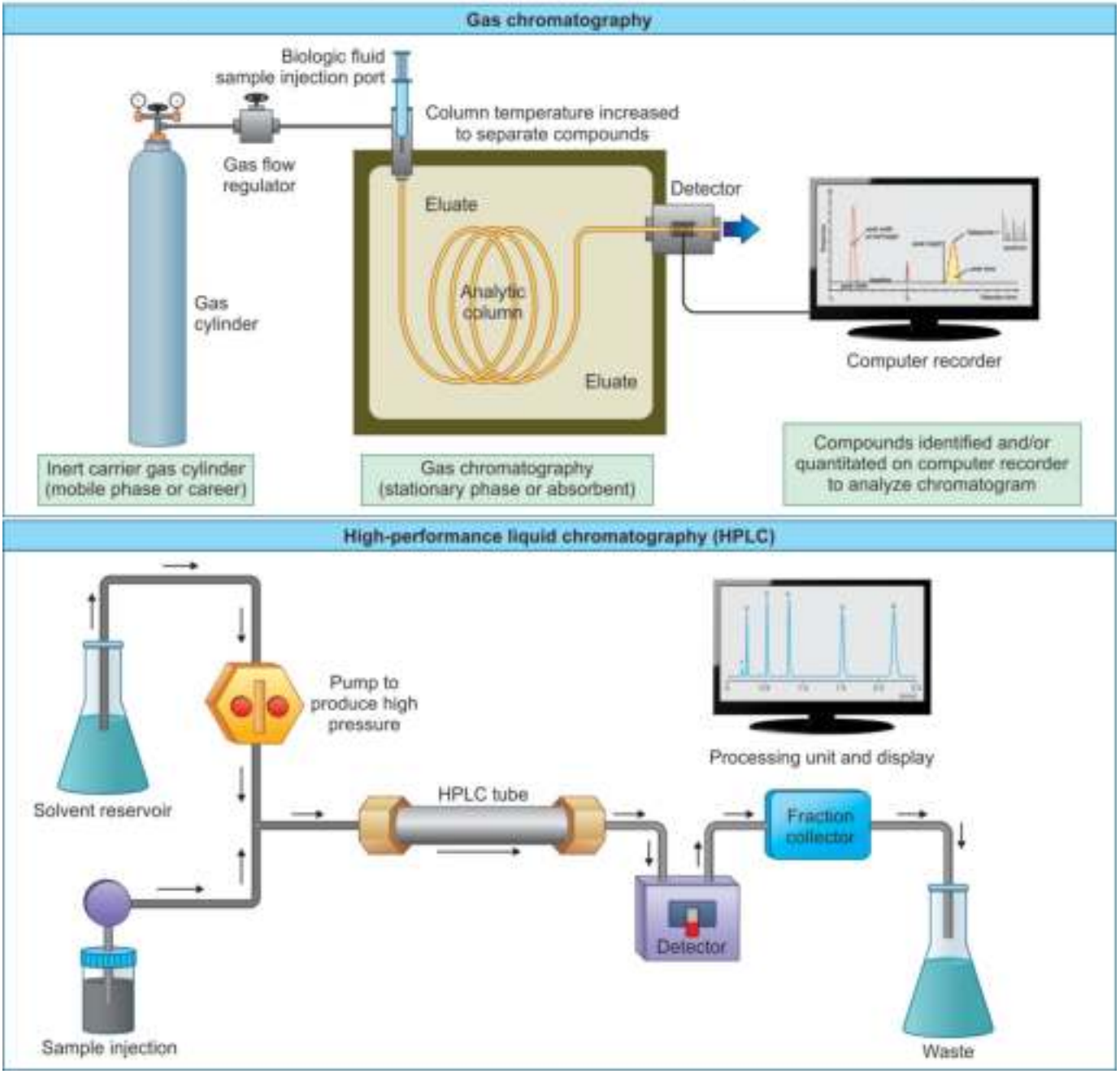


Fig. 14.41: Schematic representation of high-performance liquid chromatography (HPLC) for separation, identification, and quantification of substances in biological fluids.

Rapid analysis of newborn screening to detect early disease using liquid chromatography–mass spectrometry (LC–MS) technique

- **Principle:** Liquid chromatography–mass spectrometry (LC–MS) is a powerful analytic technique used for separation, identification and quantification of both unknown compounds as well as to elucidate the structure and chemical properties of different molecules. After 24 hours of life, heal of newborn is punctured to obtain whole blood which is spotted onto a special card and processed in the central laboratory. Extraction of molecular compounds of interest from a portion of the blood spot and separated by liquid chromatography and then analyzed by mass spectrometry.
- **Purpose:** LC–MS is performed for screening newborn babies blood samples and analyzing and detecting early disease and prevention with respect to disorders of amino acid metabolism and other congenital disorders.
- **LC–MS in clinical practice:** LC–MS is performed to diagnose early diseases: (a) hemoglobin disorders (sickle cell, hemoglobin sickle cell disease, β-thalassemia), (b) metabolic [phenylketonuria, methylmalonic acidemia, maple syrup urine disease (MSUD), tyrosinemia, citrullinemia, medium chain acyl-CoA dehydrogenase (MCAD) deficiency], (c) endocrine disorders (congenital hypothyroidism, congenital adrenal hyperplasia), and (d) rare disorders (galactosemia, cystic fibrosis, severe combined immunodeficiency, Pompe disease, mucopolysaccharidosis type 1, spinal muscle atrophy, X-linked adrenoleukodystrophy, biotinidase deficiency).

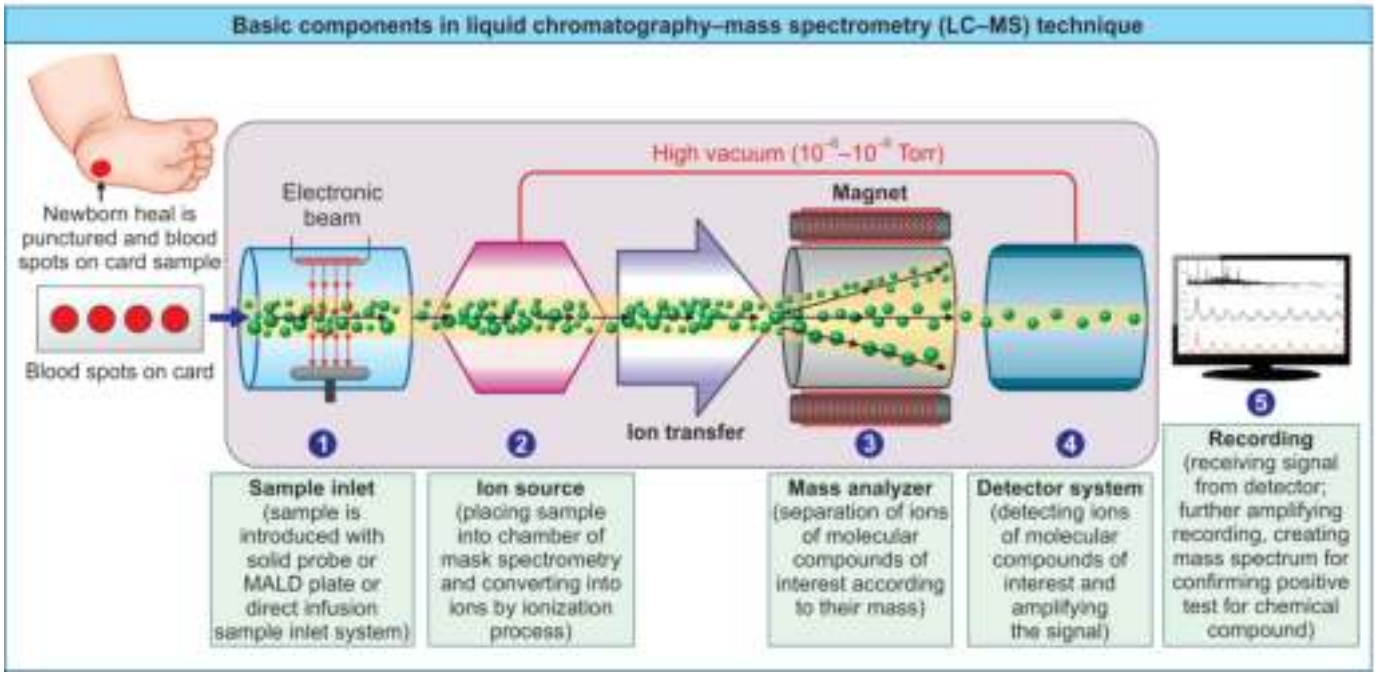


Fig. 14.42: Schematic representation of newborn screening by high-performance liquid chromatography (HPLC)–mass spectroscopy to analyze blood sample.

amount of volume is introduced by making use of fixed volume loop injection. Injection valve and column valve can introduce variable volume of sample.

COLUMNS COMPONENT IN HPLC

Columns are the main component of high-performance liquid chromatography (HPLC), because the columns are responsible for the separation of the sample components. The sample passes through the columns with the mobile phase and separates its components when sample comes out from the columns. The material filled in the HPLC columns is known as stationary phase. There are different types of chromatography columns on the basis of their composition and method of separation, which include: normal phase columns, reverse phase columns, ion exchange columns and size exclusion columns.

DETECTOR COMPONENT IN HPLC

The function of detector component in high-performance liquid chromatography (HPLC) is to monitor the mobile phase as it merges from the columns. Solute property detector responds to a physical property of the solute which is not exhibited by pure mobile phase, e.g. ultraviolet absorbance, fluorescence diffusion current.

- Bulky property detector compares overall changes in physically property of the mobile phase with and without an eluting solute, e.g. refractive index, dielectric constant or density.
- Types of detectors in high-performance liquid chromatography (HPLC) include: ultraviolet/visible detector, photodiode array detector, fluorescence detector, electrochemical detector, refractive index detector, evaporative light scattering detector and mass spectroscopy.

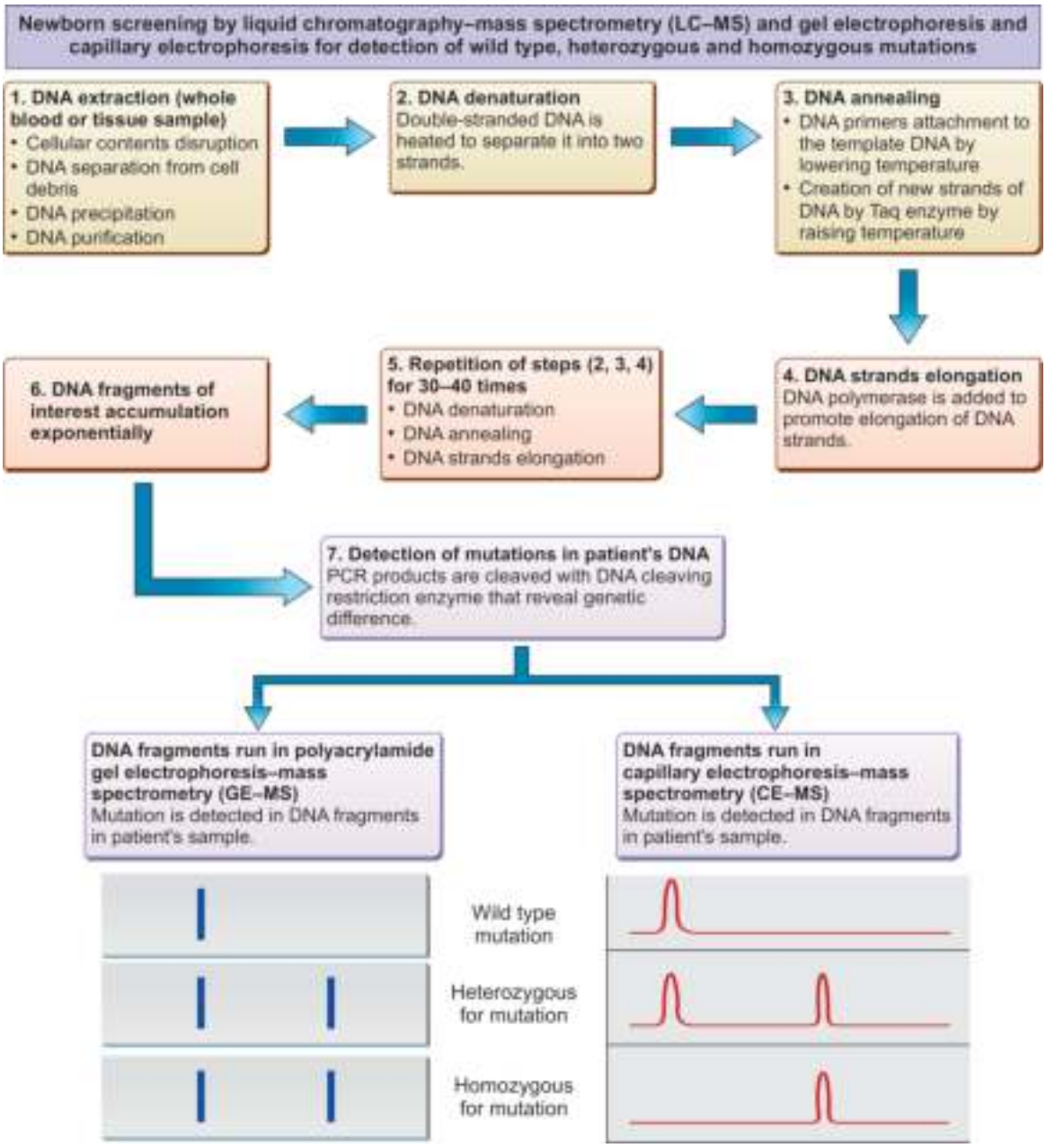


Fig. 14.43: Schematic representation of high-performance liquid chromatography–mass spectroscopy for detection of mutations in DNA by using polyacrylamide gel and capillary electrophoresis.

VALIDATION OF HPLC

Validation is the procedure, which proves that high-performance liquid chromatography technique yields the expected results with reliability, adequate precision and accuracy. Validity parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantitation and robustness.

ADVANTAGES OF HPLC

High-performance liquid chromatography is ideally suited for separation and identification of proteins, amino acids, nucleic acids, hydrocarbons, carbohydrates, pharmaceuticals, environmental (monitoring of water pollution), forensic science (drug in biological samples), pesticides, pigments, antibiotics, steroids and other organic substances.

DISADVANTAGES OF HPLC

Cost of high-performance liquid chromatography (HPLC) equipment and reagents is more. Overall HPLC has more advantages than electrophoresis technique and routinely performed in many laboratories.

APPLICATIONS OF HPLC

High-performance liquid chromatography (HPLC) can be performed on very small venous sample to estimate quantity of normal and abnormal hemoglobins.

- Different hemoglobins have different affinity for the stationary phase. Hemoglobins possess strong positive charge and have higher affinity for stationary phase and appear in the elute later than other hemoglobins. Retention time is the time taken by normal and variant hemoglobins to appear in the eluate.
- Cation exchange high-performance liquid chromatography (CE-HPLC) technique achieves good separation and quantitation of HbF and HbA_2 in addition to screening for variant hemoglobins along thalassemia. A cut-off level of HbA_2 >4% predicts the majority of carriers of classic β-thalassemia.
- Thalassemia and thalassemic hemoglobinopathies pose serious health problem leading to severe morbidity and mortality in Indian population. Detection of asymptomatic carriers of thalassemia major by reliable laboratory methods is the cornerstone of prevention of this serious health problems.
- Genetic studies are indicated to confirm borderline thalassemia cases and to detect silent carriers of β-thalassemia, α-thalassemia, and area and novel variants in routine clinical practice.
- High-performance liquid chromatography coupled with electrospray mass spectroscopy (HPL-MS) is widely used for neonatal screening for metabolic disorders, quantitative determination of therapeutic immunosuppressive drugs in biological fluids, and toxicological investigations.
- High-performance liquid chromatography is used to test water samples to analyze pollution in lakes and rivers. Ion exchange chromatography is more effective in the separation of macromolecules such as nucleic acids and proteins.
- High-performance liquid chromatography (HPLC) is widely used in industrial and scientific applications such as pharmaceutical, environmental, forensic science and chemicals agents.

HEMOGLOBIN ELECTROPHORESIS AND GEL ELECTROPHORESIS TECHNIQUES

HEMOGLOBIN ELECTROPHORESIS TECHNIQUE

Hemoglobin electrophoresis is a diagnostic tool to measure different types of hemoglobin in the blood in suspected disorder associated with abnormal hemoglobin sickle cell anemia and thalassemia. Hemoglobin electrophoresis technique is used to analyze HbA, HbA_2, HbF, HbS, HbC and HbE.

PRINCIPLE

The principle of hemoglobin electrophoresis technique is that when proteins are exposed to charge gradient, they separate from each other by forming bands that separate toward one end or the other end in the electric field. The hemoglobins are separated on variety of supporting media on the basis of electric charge differences.

- The commonly used mediums in electrophoresis technique are cellulose acetate membrane, starch gel, agarose gel, citrate agar gel and filter paper.
- Cellulose acetate hemoglobin electrophoresis is performed at alkaline pH of 8.2–8.6.
- Citrate agar or agarose gel hemoglobin electrophoresis is done in acidic pH of 6.0–6.2. HPLC is rapidly taking its place in many laboratories. Principle of hemoglobin electrophoresis technique is shown in Fig. 14.44.

BLOOD SAMPLING

Blood transfusions within the previous 12 weeks may alter test results. Hemoglobin electrophoresis is done on venous blood samples taken in anticoagulant EDTA container. Red blood cell hemolysate is prepared using a lysing reagent (Na_4 EDTA and KCN).

TYPES OF HEMOGLOBIN ELECTROPHORESIS TECHNIQUES

Cellulose acetate hemoglobin electrophoresis at pH 6.8 is the method of choice in most clinical laboratories. In this technique, there is overlapping of bands of hemoglobins S, D, G and Lepore. Agar gel hemoglobin electrophoresis using citrate buffer at pH 6.0 is useful in supplementing technique that helps in getting separate hemoglobin S band (Fig. 14.45A and B).

Cellulose Acetate Hemoglobin Electrophoresis Technique (pH 8.6)

Cellulose acetate hemoglobin electrophoresis is the primary screening technique used to detect variant of abnormal hemoglobins. The major portion of normal adult Hb is HbA. In addition, up to 3.5% HbA_2 is normally present, along with <2% HbF. The more common mutant Hbs are S, C, E, D, G, and Lepore.

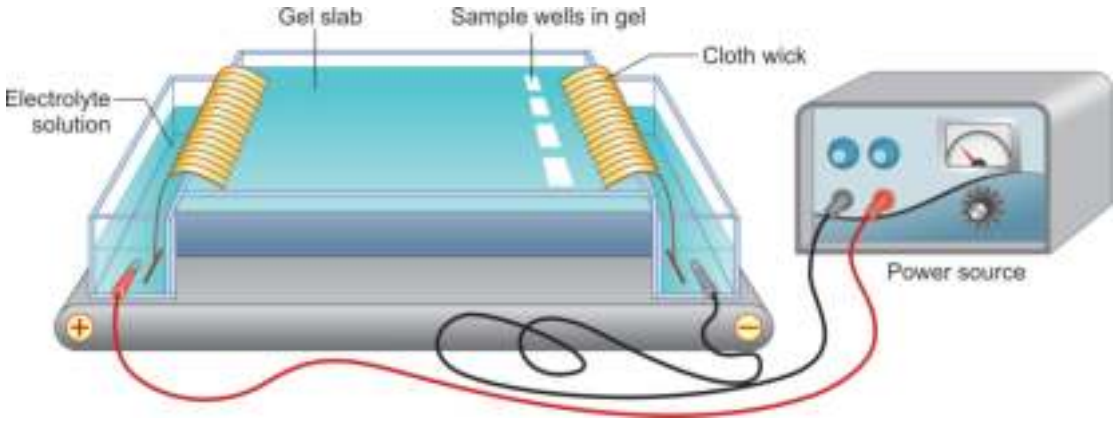


Fig. 14.44: Principle of hemoglobin electrophoresis technique.

Principle

In an alkaline pH (8.2–8.6), Hb is a negatively charged molecule and will migrate toward the anode (+). The various Hbs move at different rates depending on their net negative charge in cellulose acetate hemoglobin electrophoresis.

Methodology

The red blood cell hemolysate (red blood cell membranes are destroyed to free the Hb molecules for testing) is placed in a cellulose acetate membrane, which is positioned in an electrophoresis tray with the inoculated red blood cell hemolysate near the cathode (–).

- One end of the cellulose acetate strip is immersed in the buffer (pH 8.2–8.6) on the cathode side and the other end is placed in the buffer on the anode (+) side. An electric current of specific voltage is allowed to run for timed period. During electrophoresis, the hemoglobin molecules migrate toward the anode because of their negative charge.
- The difference in the net charge of the hemoglobin molecule determines its mobility and manifests its self by the speed with which it migrates to the anode (+) pole.
- The cellulose acetate membrane is then stained in order to color of the proteins (Hbs). By noting the distance, each Hb has migrated and comparing this distance with the migration distance of known controls, the types of hemoglobins may be identified. Example of the fast moving hemoglobin is Hb Bart and the two fastest hemoglobin variants include HbH and I, while HbC is the slowest common Hb. Cellulose acetate hemoglobin electrophoresis at pH 8.6 is shown in Fig. 14.46.

Citrate Agar Hemoglobin Electrophoresis Technique (pH 6)

Citrate agar hemoglobin electrophoresis technique separates hemoglobin fractions that migrate together

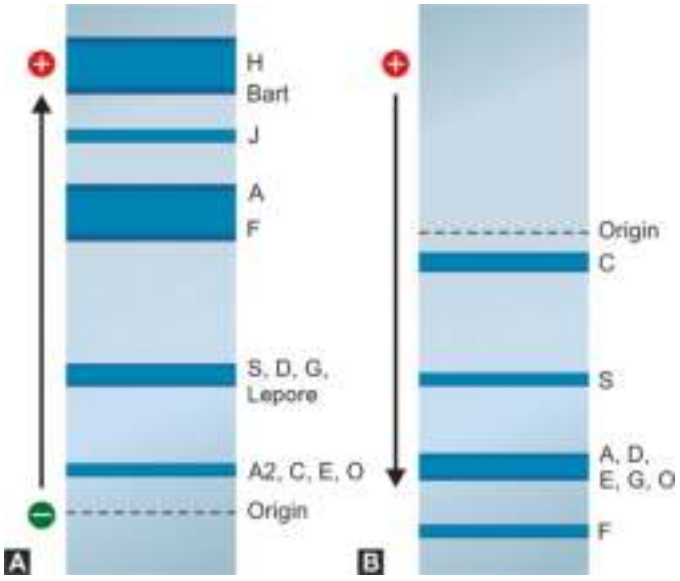


Fig. 14.45: (A) Schematic representation of electrophoretic mobility of normal and abnormal hemoglobins on cellulose acetate at pH 8.6, (B) agar gel at pH 6.

on cellulose acetate agar. All hemoglobin specimens that show an abnormal electrophoretic pattern in alkaline media (cellulose acetate agar) should undergo electrophoresis on an acid citrate agar. Citrate agar hemoglobin electrophoresis technique is used to confirm variant Hbs and further differentiates HbS from HbD and G and HbC from HbE, O_{Arab}, and C_{Harlem}. The procedure should not be used as a screening procedure because many abnormal hemoglobins migrate with HbA.

INTERPRETATION OF HEMOGLOBIN ELECTROPHORESIS

Each of the major hemoglobin types has an electrical charge of a different degree. Hemoglobin electrophoresis separates and measures normal and abnormal

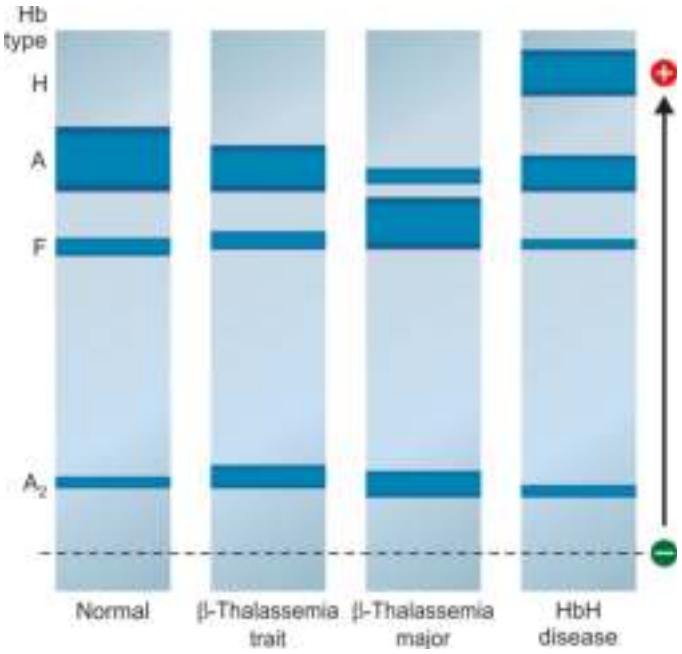


Fig. 14.46: Cellulose acetate hemoglobin electrophoresis at pH 8.6.

hemoglobins. The components then move away from each other at different rates, and when separated from a series of distinctly pigmented bands, which are then compared with those of a normal sample.

- Each pigmented band is further assessed as a percentage of total hemoglobin, thus indicating the severity of any hemoglobin abnormality.
- Hemoglobin electrophoresis technique detects many different types, the most common being HbA_1, HbA_2, HbF, HbS, and HbC.
- Normal reference values of hemoglobins can vary by laboratory, but are generally within the following ranges.

Hemoglobin Electrophoresis Patterns

In normal adults, HbA_1 is the major component of hemoglobin in the normal red blood cell. HbA_2 is a minor component of normal hemoglobin, comprising approximately 2–3% of the total. In children, HbF is the major hemoglobin component in the fetus, but usually exists only in minimal quantities in the normal adult. Levels of HbF >2% in patients over three years of age are considered abnormal. Hemoglobin electrophoresis pattern in normal persons and hemoglobin disorders is given in Tables 14.104 and 14.105.

Table 14.104 Hemoglobin electrophoresis pattern in normal persons

Hemoglobin Type	Hemoglobin Percentage
Adults	
HbA_1	95–98%
HbA_2	2–3%
HbF	0.8–2.0%
HbS	0%
HbC	0%
Children	
HbF (newborn)	50–80%
HbF (6 months age)	8%
HbF (>6 months age)	1–2%

Table 14.105 Hemoglobin electrophoresis pattern in hemoglobin disorders

Hemoglobin	Hemoglobin Disorder	Hemoglobin Percentage
HbA_2	▪ β-Thalassemia ▪ HbH disease	▪ 4.00–5.8% ▪ <2%
HbF	▪ β-Thalassemia major ▪ β-Thalassemia minor ▪ Homozygous hereditary persistence of fetal hemoglobin ▪ Heterozygous hereditary persistence of fetal hemoglobin ▪ Heterozygous HbS	▪ 10–90% ▪ 2–5% ▪ 100% ▪ 5–35% ▪ 15%
HbS	Sickle cell disease (homozygous)	70–98%
HbC	Hemoglobin C disease (homozygous)	90–98%

GEL ELECTROPHORESIS TECHNIQUE

Gel electrophoresis is a technique used to separate DNA fragments or other macromolecules, such as RNA and proteins depending on their size.

- DNA samples are loaded into the pocket like wells at one end of gel, and an electric current is passed to pull them through the gel. Gel is made out of polysaccharide called agarose. Based on their size and charge, the molecules travel through gel in different directions at different speeds, allowing them to be separated from one another.
- DNA fragments are negatively charged; therefore, they move towards the positive electrode. Small fragments of DNA move through the gel faster than large ones.
- Because DNA and RNA are negatively charged molecules, they move towards the positive electrode.
- Proteins however, are not negatively charged, thus when one desires to separate proteins using gel electrophoresis, they must first mix the proteins with a detergent called sodium dodecyl sulphate,

Table 14.106 Various types of gel electrophoresis

Electrophoresis Types	Comments
Agarose gel electrophoresis	▪ Agarose composed of multiple linked repeat units of disaccharide agarobiose (d-galactose and 3, 6-anhydro-l-galactose) is manufactured from seaweed such as Rhodophyta ▪ *Applications:* Analysis of end-point PCR or RT-PCR assays; analysis of restriction fragment length polymorphism (RFLP) assays; routinely used in molecular biology for the analysis of recombinant DNA experiments and can also be used for the purification of probes for ISH
Polyacrylamide gel electrophoresis (PAGE)	▪ Polyacrylamide is produced from monomers of acrylamide in a reaction initiated by free radicals generated by reduction of ammonium persulfate; higher the concentration of acrylamide, the finer the resolution of DNA fragments ▪ The advantage of polyacrylamide over agarose is that size differences at the base-pair level can be distinguished ▪ *Applications:* End-point PCR fragment analysis where fragment size differences are slight; for sequencing assays and for microsatellite marker-based assays using autoradiography or fluorescence-labeled fragments
Pulsed-field gel electrophoresis (PFGE)	▪ Used for improved resolution (accomplished by alternating the direction of the electrical field) of high molecular weight DNA ▪ *Applications:* Identification of microorganism strains such as *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Listeria*, or *Campylobacter* species; evaluation of autosomal dominant ataxia
Capillary gel electrophoresis	▪ It supports automated DNA sequencing and fragment analyses ▪ *Applications:* Widely used for DNA sequencing and microsatellite assay

which makes the proteins unfold into linear shape and coats them with a negative charge, thus allows them to migrate toward the positive end of the gel and be separated.

- Finally, after the DNA, RNA, or protein macromolecules have been separated using gel electrophoresis, gel is stained with dyes and observed as bands representing molecules of different sizes can be observed.
- Each band contains a large number of DNA fragments of the same size that have travelled as a group to the same position. A single DNA fragment will not be visible by itself on gel electrophoresis.
- By comparing the bands in a sample to the DNA ladder, one can determine their approximate sizes. For example, the bright band on the gel above will be roughly 700 nucleotide base pairs in size. Various types of gel electrophoresis are given in Table 14.106.

Hematoxylin and Eosin (H&E) Stain in Diagnostic Surgical Pathology

Hematoxylin and eosin (H&E) is the standard stain for histologic examination of human tissues in surgical pathology. Hematoxylin is a positively charged (cation) basic dye, which stains nucleus giving basophilic (blue color) appearance. Eosin is a negatively charged (anionic) acidic dye, which stains cytoplasm giving eosinophilic (red/pink) appearance. Cells may have affinity for both basic dye and acidic dye, which exhibit amphophilic (purple) appearance. Hematoxylin itself has no staining properties not until it has been oxidized and combined with mordant such as aluminum alum. Sequential application of hematoxylin and eosin dyes to the histologic sections results in nuclei being stained blue, and cytoplasm and extracellular matrix pink. Hematoxylin and eosin-stained tissue sections are examined under light microscope. Combined H&E stains are vital for distinguishing many tissues and for making a tentative diagnosis of malignancy.

- **True nuclear inclusions and pseudoinclusions:** Abnormal substances in the nuclei that can be observed by light microscopy are referred to nuclear inclusions and pseudoinclusions. Their presence can aid in the diagnosis of some disease entities and tumor types.
  - **True nuclear inclusions:** True nuclear inclusions consist of material that is foreign to the nucleus and completely contained within the nucleus. True nuclear inclusions are continuous with the cytoplasm and consist of cytoplasmic organelles, such as the endoplasmic reticulum, Golgi apparatus and secretory granules. Immunoreactivity of true nuclear inclusions are same as that of the cytoplasm. True nuclear inclusions are classically caused by viral infections. Basophilic nuclear inclusions are diagnostic of acinic cell carcinoma and malakoplakia (Michaelis-Gutmann bodies).

- **Nuclear pseudoinclusions:** Nuclear pseudoinclusions are formed by invagination of cytoplasm into nucleus and delimited by the nuclear membrane in cytologic and histologic tissue sections. Tumors with pseudoinclusions include papillary thyroid carcinoma, hyalinizing trabecular adenoma of thyroid, pituitary adenoma, meningioma, hepatocellular carcinoma, paraganglioma, pheochromocytoma, melanoma, ductal hyperplasia of breast and inspissated surfactant in lung adenocarcinoma. Nuclear pseudoinclusions can be observed in Arias Stella reaction. Nuclear pseudoinclusions are found in benign and malignant melanocytic tumors. The presence of nuclear pseudoinclusions is not a reliable criterion for differentiating melanoma from benign melanocytic lesions, although it is useful in distinguishing melanocytic from non-melanocytic tumors.

- **Inclusion bodies:** Inclusion bodies are tiny aggregates of specific type of protein within the cytoplasm or nucleus of neurons observed. Amyotrophic lateral sclerosis adversely affects motor neurons. Inclusion body myositis adversely affect skeletal muscle cells. Dementia with Lewy bodies adversely affect central nervous system.
  - **Inclusion bodies in skeletal muscles:** Inclusion bodies of aggregations of multiple proteins are also observed in skeletal muscle cells affected by inclusion body skeletal myositis and hereditary inclusion body skeletal myopathy.
  - **Inclusion bodies in neurons:** Inclusion bodies in neurons in the form of misfolded proteins may be accumulated in the cytoplasm or nucleus in the setting of neurodegenerative diseases including Lewy bodies in Lewy body dementias and Parkinson's disease, neuroserpin inclusion bodies called 'Collins bodies' in familial encephalopathy and Huntington's disease, and Papp-Lantos bodies in multiple system atrophy.
  - **Viral inclusion bodies:** Viral inclusion bodies can be cytoplasmic and nuclear eosinophilic (acidophilic), nuclear basophilic. Examples of intracytoplasmic eosinophilic (acidophilic) viral inclusion bodies are Negri bodies in rabies, Henderson-Patterson bodies in molluscum contagiosum, and Guarnieri bodies in variola (smallpox). Examples of intranuclear eosinophilic (acidophilic) viral inclusion bodies are Cowdry bodies type A in herpes simplex viral infection, Cowdry bodies type B in adenovirus viral infection, Torres bodies in yellow fever, and Warthin-Finkeldey bodies in measles and HIV/AIDS. Intranuclear basophilic viral inclusion bodies in cytomegalovirus infection giving 'Owl eye appearance'.
- **Intracellular inclusions not bound by membranes:** Intracellular inclusions are stored nutrients (glycogen in liver, lipids, and proteins), secretory products and pigment granules. Crystalline inclusions are found in Sertoli cells and Leydig cells of human tissues. Intracellular yellow or brown material may include bile, hemosiderin, lipofuscin, melanin pigment chromoblastomycosis.
- **Extracellular matrix inclusions:** Amyloid, monoclonal immunoglobulin deposits, collagen, basement-like material, fibrin, tubular myelin figures (lamellar bodies in lungs), and crystals (such as Charcot-Leydon crystals and crystalloids within the lumens of neoplastic acinic of prostatic carcinoma) and are present in extracellular compartment of tissue.

CHAPTER 15

Blood Vessels

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

ANATOMY AND PHYSIOLOGY OF BLOOD VESSELS

ARTERIOSCLEROSIS: HISTOLOGIC VARIANTS

ATHEROSCLEROSIS
- Locations of atherosclerosis
- Major and minor risk factors
- Pathogenesis of atherosclerosis
- Complications of atherosclerosis
- Consequences and clinical manifestations of atherosclerosis
- Therapeutics for atherosclerosis

ANEURYSMS
- Aneurysms: overview
- Aneurysms: causes
  - Atherosclerotic aneurysms
  - Syphilitic (luteic) aneurysms
  - Aortic dissecting hematoma
  - Berry aneurysm
  - Mycotic aneurysm
  - Aneurysm of sinus of Valsalva
  - Arteriovenous fistula
  - Capillary microaneurysm

VASCULITIS
- Vasculitis: overview
- Large vessel vasculitides
- Medium vessel vasculitides
- Small vessel vasculitides

VENOUS SYSTEM
- Varicose veins
- Deep vein thrombosis (thrombophlebitis)
- Thoracic vena cava syndrome

LYMPHATIC SYSTEM
- Acute lymphangitis
- Lymphedema
- Chylous effusion

NEOPLASTIC DISORDERS OF BLOOD VESSELS
- Reactive vascular proliferation
- Capillary hemangioma
- Cavernous hemangioma
- Kaposi sarcoma
- Angiosarcoma

HYPERTENSION
- Hypertension: overview
  - Determinants of hypertension
- Essential (primary) hypertension
- Secondary hypertension
- Vascular changes in hypertension
  - Vascular changes in benign hypertension
  - Vascular changes in malignant hypertension
- Complications of hypertension

ANATOMY AND PHYSIOLOGY OF BLOOD VESSELS

Arteries are blood vessels responsible for carrying oxygen-rich blood from the heart to the body. Veins are blood vessels that carry blood low in oxygen from the body back to the heart for oxygenation.

- Large or elastic arteries, e.g. aorta, innominate arteries, subclavian arteries, origin of common carotid arteries and origin of pulmonary arteries.
- Medium-sized/muscular arteries, e.g. radial artery, renal artery and mesenteric artery.
- Small-sized arterioles <2 mm are present within substance of tissues and organs (peripheral resistance vessels). Arterioles consist of three layers: tunica intima, tunica media and tunica adventitia.
- Arterioles transport blood from heart, maintain blood pressure and regulate peripheral resistance through vasoconstriction and vasodilation.
- Capillaries participate in exchange of gases and nutrients between blood and interstitial tissue.
- Venules transport blood from capillary beds toward the heart.
- Vein is composed of tunica intima, thinner tunica media and tunica adventitia. Its lumen is larger than arteries. Veins participate in transport of blood from venules to the heart (Table 15.1).
- Lymphatic system consists of lymphatic vessels, lymphoid organs and lymphoid tissues.

Table 15.1 The structure and function of blood vessels

Blood Vessel	Structure	Function
Artery	▪ Tunica intima, thick tunica media and tunica adventitia ▪ Contractility and elasticity	▪ Transports blood from heart ▪ Maintenance of blood pressure
Arteriole	▪ Tunica intima, tunica media and tunica adventitia ▪ Lumen smaller than artery	▪ Transports blood from heart and maintenance of blood pressure ▪ Regulation of peripheral resistance through vasoconstriction and vasodilation
Capillary	Microscopic size vessel with single-layered endothelium	Exchange of gases and nutrients between blood and interstitial tissue
Venule	Tunica intima, tunica media and tunica adventitia and gradually enlarge as reaching near the heart	Transports blood from capillary beds toward the heart
Vein	▪ Tunica intima, tunica media and tunica adventitia with larger lumen than artery ▪ Presence of valve aids in the unidirectional blood flow towards the heart	Transports blood from venules to the heart
Lymphatic system	Consists of lymphatics, lymph nodes and lymphoid tissues	▪ Maintains fluid levels in our body tissues ▪ Functions as immune system

ARTERIOSCLEROSIS: HISTOLOGIC VARIANTS

Arteriosclerosis is defined as thickening and loss of elasticity (hardening) of arterial walls. It is responsible for morbidity and mortality due to narrowing and weakening of arteries. There are three histologic variants of arteriosclerosis (e.g. Mönckeberg's arteriosclerosis, arteriolosclerosis and atheromatous plaque) described as under.

MÖNCKEBERG'S ARTERIOSCLEROSIS

Mönckeberg's arteriosclerosis is age-related clinically insignificant entity seen in >50 years of age. Calcium is deposited in the media of arteries in a ring-like fashion resulting in stiff pipe stem arteries. Blood vessels are often palpable.

- Calcium deposition in arteries can be demonstrated on radiological examination. This form of Mönckeberg's arteriosclerosis may coexist with atheromatous plaque, but it is distinct and unrelated to it (Fig. 15.1).
- Mönckeberg's arteriosclerosis involves the media of medium-sized muscular arteries, i.e. radial, ulnar, femoral and uterine arteries.
- These calcific deposits do not encroach the lumen of blood vessels; hence blood supply to tissues is not impaired.

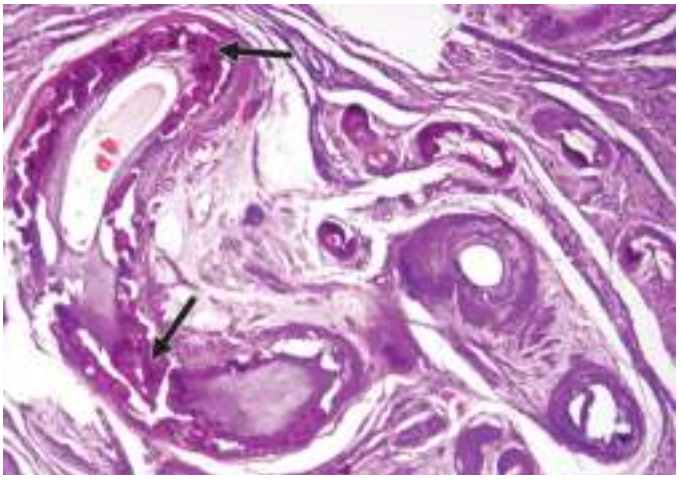


Fig. 15.1: Mönckeberg's arteriosclerosis shows calcium deposition in the media of blood vessels (arrows).

ARTERIOLOSCLEROSIS

Arteriolosclerosis is characterized by thickening of the walls of small arteries and arterioles that narrows the lumen and can lead to ischemia or insufficient blood flow to organs. Hyaline arteriolosclerosis is usually associated with benign hypertension, normotensive elderly persons and diabetes mellitus. Renal arterioles involvement in benign hypertension and malignant

hypertension are known as benign nephrosclerosis malignant hypertension respectively. Renal arterioles in malignant hypertension exhibit two histologic patterns: hyperplastic arteriolosclerosis and fibrinoid necrosis (necrotizing arteriolitis).

HYALINE ARTERIOLOSCLEROSIS

Hyaline arteriolosclerosis is characterized by thickening of the arterioles due to the accumulation of homogenous hyaline material in their walls in the kidneys of patients who have diabetes mellitus or benign arterial hypertension. Hyaline material in arterioles stains eosinophilic pink in hematoxylin and eosin-stained tissue sections. The increased intraluminal pressure in benign hypertension imposed on the arteriolar walls drives proteins and get deposited into the subintimal region of arterioles and occlude the lumen leading to progressive organ atrophy (Fig. 15.2).

HYPERPLASTIC ARTERIOLOSCLEROSIS

Hyperplastic arteriolosclerosis is characterized by thickening of the wall of arterioles due to proliferation of smooth cells arranged in concentric laminated pattern exhibiting an '**onion-skin pattern**'. There is subsequent marked narrowing of the lumen of arterioles. These changes represent an adaptive response of arterioles to severe (malignant) hypertension and progressive systemic sclerosis. Hyperplastic arteriolosclerosis may be accompanied by damage to arterioles known as fibrinoid necrosis (Fig. 15.3).

FIBRINOID NECROSIS OF ARTERIOLES

Fibrinoid necrosis of arterioles is most often associated with malignant hypertension with diastolic blood pressure >120 mm Hg and immune complex vasculitis that can lead to bleeding and organ damage throughout the body. Histologic examination reveals arterioles exhibiting intraluminal deposition of fibrinoid material, vascular necrosis, inflammation, focal hemorrhage and narrow and fixed lumen (Fig. 15.4).

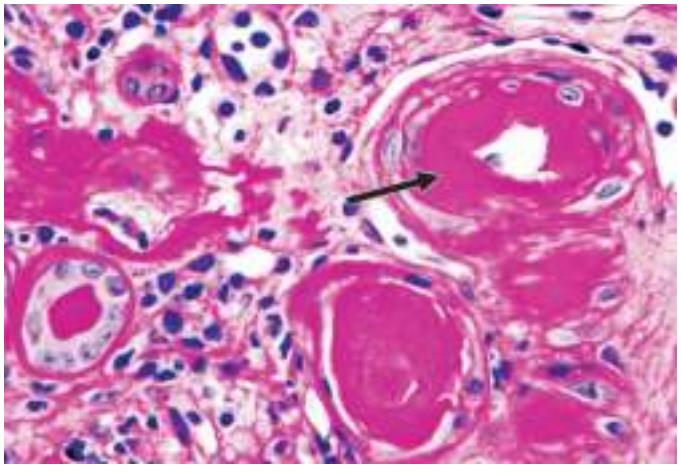


Fig. 15.2: Hyaline arteriolosclerosis (arrow) (400X).

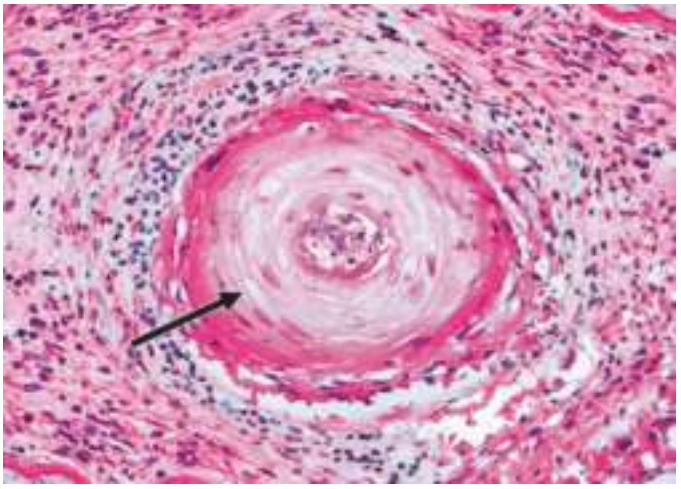


Fig. 15.3: Hyperplastic arteriolosclerosis—blood vessel shows proliferation of intimal and smooth muscle cells arranged in concentric laminated pattern exhibiting an onion-skin pattern (arrow) (400X).

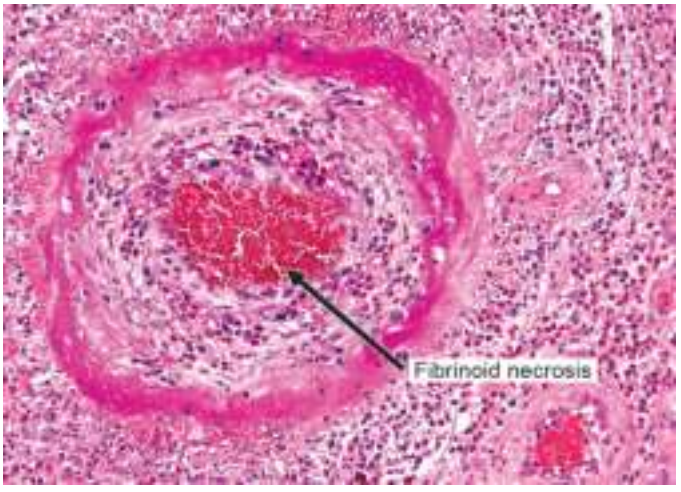


Fig. 15.4: Fibrinoid necrosis. Malignant hypertension leads to fibrinoid necrosis of small arteries as shown here. The damage to the arteries leads to formation of pink fibrin, hence the term fibrinoid (arrow) (400X).

ATHEROMATOUS PLAQUE

Atherosclerosis is a progressive thickening and hardening of medium and large elastic arteries as a result of lipid deposition on their intima. Atheromatous plaques are most often eccentrically located in the arterial lumen as a result of vascular hemodynamic stress at branching points and posterior wall of the abdominal aorta.

ATHEROSCLEROSIS

Atherosclerosis is hardening and narrowing of arteries caused by the deposition of lipids, cholesterol, calcium, and other substances in the innermost layer of endothelium of the large- and medium-sized arteries. Risk factors include hyperlipidemia, hypertension, diabetes mellitus, tobacco smoking, obesity, lack of exercise and diet high in saturated fat. Atheromatous lesions are frequently found in the aorta and in large aortic branches, which may cause partially or total blockage of blood flow through the arteries supplying vital body organs (i.e. heart, brain, kidneys) and lower extremities. Patient may develop angina pectoris, myocardial infarct, cerebral stroke, renal infarct and peripheral vascular disease.

Clinical Pearls: Atheromatous Plaque

- Atherosclerosis occurs due to focal accumulation of lipid in the intima of the large elastic and medium size arteries with inflammation and fibrosis forming atheromatous plaques. It causes luminal narrowing, associated with ischemia in the heart, brain, intestine, lower extremities and kidneys. Atheromatous plaque rupture with superimposed thrombosis causes acute tissue ischemia and infarction.
- Acute myocardial infarction, cerebral stroke and peripheral vascular disease are the main causes of morbidity and mortality in developed countries.
- Overtime, atheroma undergoes changes resulting in complicated plaque. Occlusive atheroma occludes most of the artery's lumen leading to tissue/organ ischemia. On the other hand, in ruptured atheroma, fissures of the fibrous cap and defects of the endothelial lining cause thrombosis and embolism. The formation of an occlusive thrombus over a ruptured atheromatous plaque in coronary arteries is the most important cause of myocardial infarction. Removal of the thrombus by coronary angioplasty or administration of tissue plasminogen activator (tPA) may save the patient's life.
- Clinical management of atherosclerosis is based on minimizing risk factors. Hypertension is controlled by antihypertensive medication (e.g. diuretics, β-blockers), HMG-CoA reductase inhibitors (statins-lipid lowering agents), anticoagulants, i.e. 75 mg aspirin daily to minimize thrombotic risk; and lifestyle advice (e.g. stopping tobacco smoking, healthier diet and increasing exercise).

LOCATIONS OF ATHEROSCLEROSIS

Atheromatous plaques formed in the large and medium size arteries include abdominal aorta, proximal region of coronary arteries, carotid arteries, vertebral arteries (circle of Willis), iliac arteries, popliteal artery, anterior tibial artery, posterior tibial artery, and renal artery. Arteries of the upper extremities and internal mammary artery are always spared. This is the reason that internal mammary artery is used as graft in coronary artery bypass surgery. Depending on the duration of ischemia to tissues/organs, main clinical syndromes caused by atheromatous plaque are given in Table 15.2. Locations of atheromatous plaques in order of frequency and clinical manifestations are shown in Fig. 15.5.

Table 15.2 Main clinical syndromes caused by atheromatous plaque

Atheromatous Plaque Location and Clinical Manifestations	
Ischemic Heart Disease (Coronary Arteries Involved)	
▪ Angina pectoris (intermittent ischemia)	▪ Heart failure
▪ Myocardial infarction (acute ischemia)	▪ Chronic ischemic heart disease
▪ Sudden cardiac death (SCD)	
Cerebrovascular Disorders (Internal Carotid and Middle Cerebral Artery Involved)	
▪ Transient cerebral ischemic attacks (transient ischemia)	▪ Dementia (arteriopathic)
▪ Cerebral stroke/infarction (acute ischemia)	
Mesenteric Vascular Disease (Celiac, Superior and Inferior Mesenteric Artery Involved)	
▪ Intestinal infarction/gangrene intestine (acute ischemia)	▪ Mesenteric claudication also known as abdominal angina (intermittent ischemia)
Peripheral Vascular Disease (Popliteal, Anterior Tibial and Posterior Tibial Artery Involved)	
▪ Intermittent claudication (intermittent ischemia)	▪ Gangrene foot (acute ischemia)
Renovascular Diseases (Extrarenal or Intrarenal, Renal Artery Involved)	
▪ Hypertension	▪ Renal failure
Aneurysm (Abdominal Aorta)	
Abdominal aortic aneurysm associated with rupture	

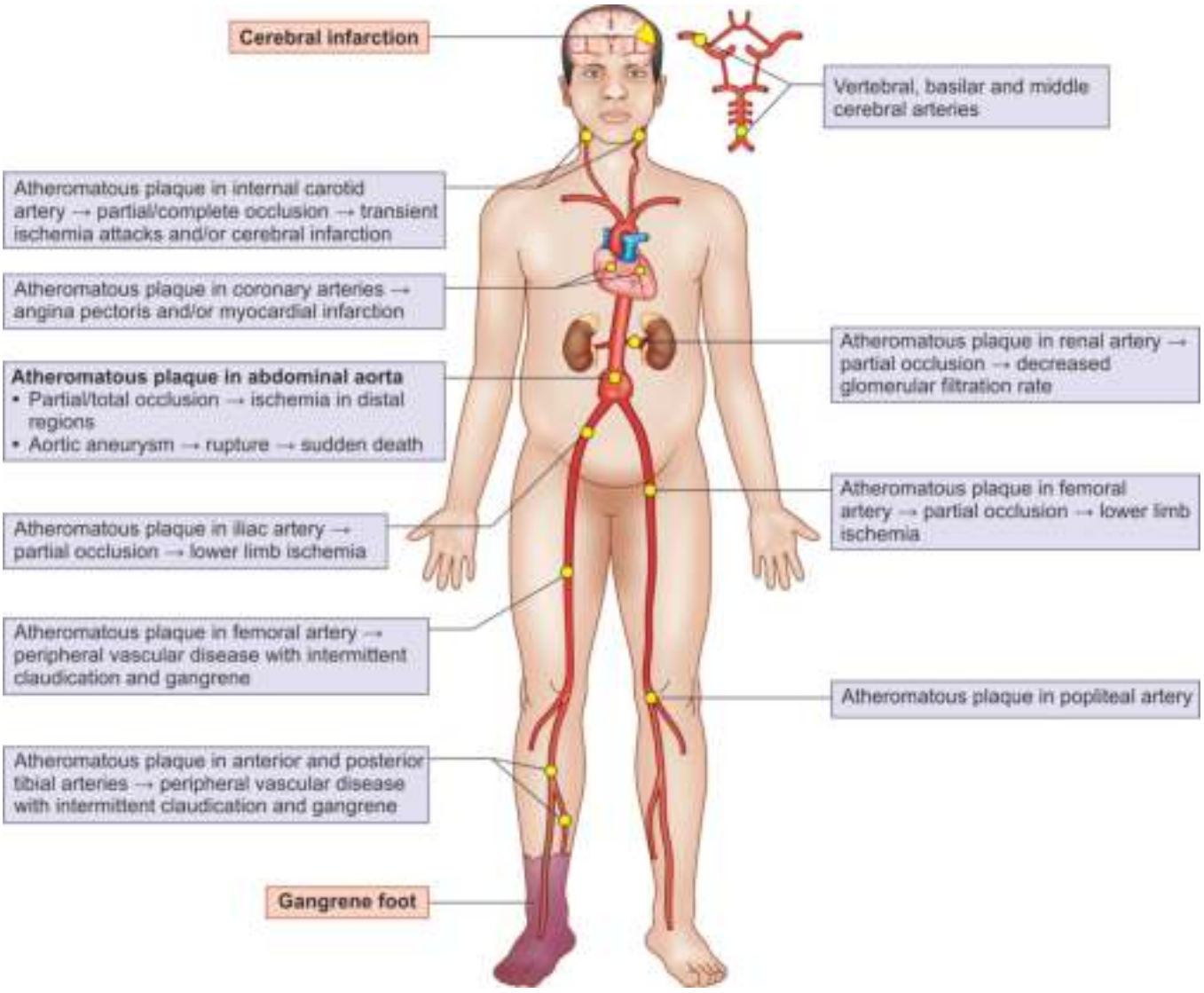


Fig. 15.5: Locations of atheromatous plaques in order of frequency and clinical manifestations.

MAJOR AND MINOR RISK FACTORS

Major modifiable risk factors for atherosclerosis include hyperlipidemia, hypertension, tobacco smoking and diabetes mellitus; and nonmodifiable factors are increasing age, male gender, family history of atherosclerosis and genetic abnormalities (ApoA1, ApoE, hepatic lipase and LDL receptor). Minor risk factors include obesity, stress type A personality, thrombophilia, high intake of carbohydrates, homocysteinemia, racial factors and infectious agents. Major and minor risk factors for atherosclerosis are given in Table 15.3.

HYPERLIPIDEMIA

Disorders of the lipid metabolism play major role in the pathogenesis of atherosclerosis and coronary artery disease. A clear-cut relationship is evident between elevated serum cholesterol and myocardial infarction. At the tissue level, cholesterol deposits occur in areas of endothelial cell damage in atheromatous plaques. Normally, cholesterol is a vital structural component of cell membranes and a source of steroid hormone. Cholesterol and triglycerides are the most important lipids in the study and management of coronary artery disease. Increased LDLs and IDLs are directly related to the risk for atherosclerosis. On the other hand, increased HDL exerts protective effect of risk for atherosclerosis by removing cholesterol from the tissues.

Major Lipoprotein Classes

Lipids are soluble in nonpolar solvents, such as ether and chloroform; but relatively insoluble in polar solvents such as water. Thus, triglycerides and cholesterol circulate in plasma as a part of watersoluble lipoproteins. The lipoproteins act as lipid shuttle, which transport cholesterol through the blood stream. Apolipoproteins

Table 15.3 Major and minor risk factors for atherosclerosis

Risk Factors	Comments
Major modifiable risk factors	
Hyperlipidemia	Diet high in saturated fats, diabetes mellitus, hypothyroidism, hepatic metabolic syndrome and nephrotic syndrome (hyperlipidemia controlled by diet and lipid lowering drugs)
Hypertension	Persistent high blood pressure
Diabetes mellitus	Fasting sugar >126 mg/dl, random blood sugar >200 mg/dl (advanced glycosylated end products atherogenic)
Tobacco smoking	Tobacco smoking injures vascular endothelium, causes deranged lipid profile (increased LDL, decreased HDL) and promotes aggregation of platelets in the intima of atheromatous plaque
Sedentary lifestyle	Accelerates atherosclerosis (prevented by regular exercise)
Major nonmodifiable risk factors	
Increasing age	Aging process results in loss of elasticity of blood vessels (peak incidence at 60 years in men and >70 years in women)
Male gender	Men (>55 years) are more affected than women (>45 years), high estrogen and HDL are antiatherogenic influence in premenopausal women
Family history of premature atherosclerosis	Premature atherosclerosis incidence in children
Genetic abnormalities	▪ ApoA1 mutation: Severe atherosclerosis ▪ ApoE mutation: Premature atherosclerosis ▪ Hepatic lipase deficiency: Premature atherosclerosis ▪ LDL receptor mutation: Premature atherosclerosis by inhibiting cholesterol uptake by liver
Minor risk factors	
Obesity	Obesity is most important risk factor for atherosclerosis
Stress type A personality	Incidence is more in these persons
Factors affecting thrombosis	Factor V Leiden, prothrombin G20210A mutation, antithrombin III deficiency, protein C deficiency, protein S deficiency, activated protein C resistance
High intake of carbohydrates	Accelerates atherosclerosis
Homocysteinemia	>100 µmol/L
Racial factors	White population is more affected than Black persons
Infectious agents	*Chlamydia pneumoniae*, *Helicobacter pylori*, herpesvirus, cytomegalovirus promote atherosclerosis

(Apo) are the protein moiety of lipoproteins. Four major lipoprotein classes have been identified based on the buoyant density of the lipid particles: chylomicron (**CM**), very lowdensity lipoprotein (**VLDL**), lowdensity lipoprotein (**LDL**) and highdensity lipoprotein (**HDL**). Several minor lipoproteins have been identified such as intermediatedensity lipoproteins and lipoprotein-a, i.e. Lp-a. LDL and intermediate-density lipoproteins (**IDLs**) are bad cholesterol and directly related to the risk of atherosclerosis. Major classes of human plasma lipoproteins are given in Table 15.4 and Fig.15.6. Relative concentration of lipoprotein fractions is analyzed as clinical predictors for development of atherosclerosis. Ideally, the ratio between LDL cholesterol to HDL cholesterol should be 4:1. Desirable lipid profile is given in Table 15.5.

Table 15.4 Major classes of human plasma lipoproteins

Lipid Particle	Major Apolipoproteins
Chylomicrons	ApoA-1, ApoA-IV, ApoC-1, ApoC-II, ApoC-IIIE
VLDL	ApoB-100, ApoC-I, ApoC-II, ApoC-IIIE
IDL	ApoB, ApoE1.006–1.019
LDL	ApoB-100
HDL2	ApoA-II, ApoA-I, ApoE
HDL3	ApoA-II, ApoA-I, ApoE
La(a)	ApoB-100, Apo(a)

Exogenous and Endogenous Transport Pathways

Exogenous and endogenous pathways for triglyceride and cholesterol transport are shown in Fig. 15.7.

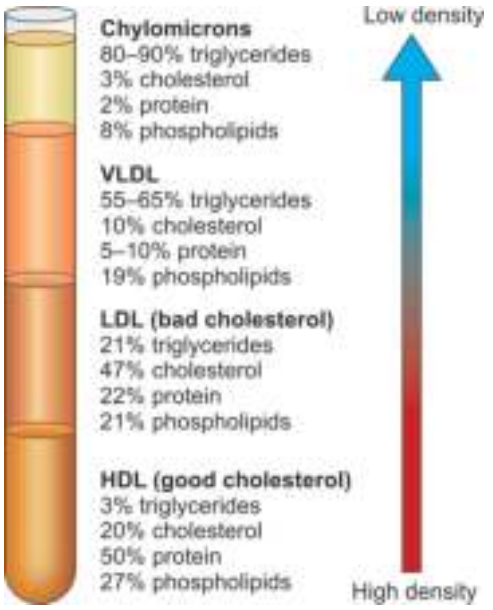


Fig. 15.6: Major classes of human plasma lipoproteins. Lipoproteins are named on their protein content, which is measured in density. These include chylomicrons, VLDL, LDL and HDL. Lipids are less dense than proteins. As the proportion of triglyceride decreases, the density increases.

Table 15.5 Desirable lipid profile

Parameters	Lipid Fraction Range
▪ Cholesterol	<200 mg/dl
▪ Triglyceride	<150 mg/dl
▪ LDL	<100 mg/dl
▪ HDL	>60 mg/dl
▪ Ratio of LDL cholesterol to HDL cholesterol	4:1

- **Exogenous pathway for triglyceride and cholesterol transport:** In the exogenous pathway, cholesterol and fatty acids from food are absorbed through intestinal mucosa. Fatty acid chains are linked to glycerol to form triglycerides. Cholesterol and triglycerides are packaged into chylomicrons and returned via the lymph into the blood circulation. The lipids are coupled to proteins by enzymes. The ester bonds holding the fatty acids in glycerides are split by lipoprotein lipase in the capillaries of adipose tissue and skeletal muscles. Now the fatty acids are removed, leaving cholesterol-rich lipoprotein remnants. These lipoprotein remnants bind to special remnant receptors and are taken up by hepatocytes.
- **Endogenous pathway for triglyceride and cholesterol transport:** The cholesterol of the lipoprotein remnant is either secreted into the intestine, largely as bile

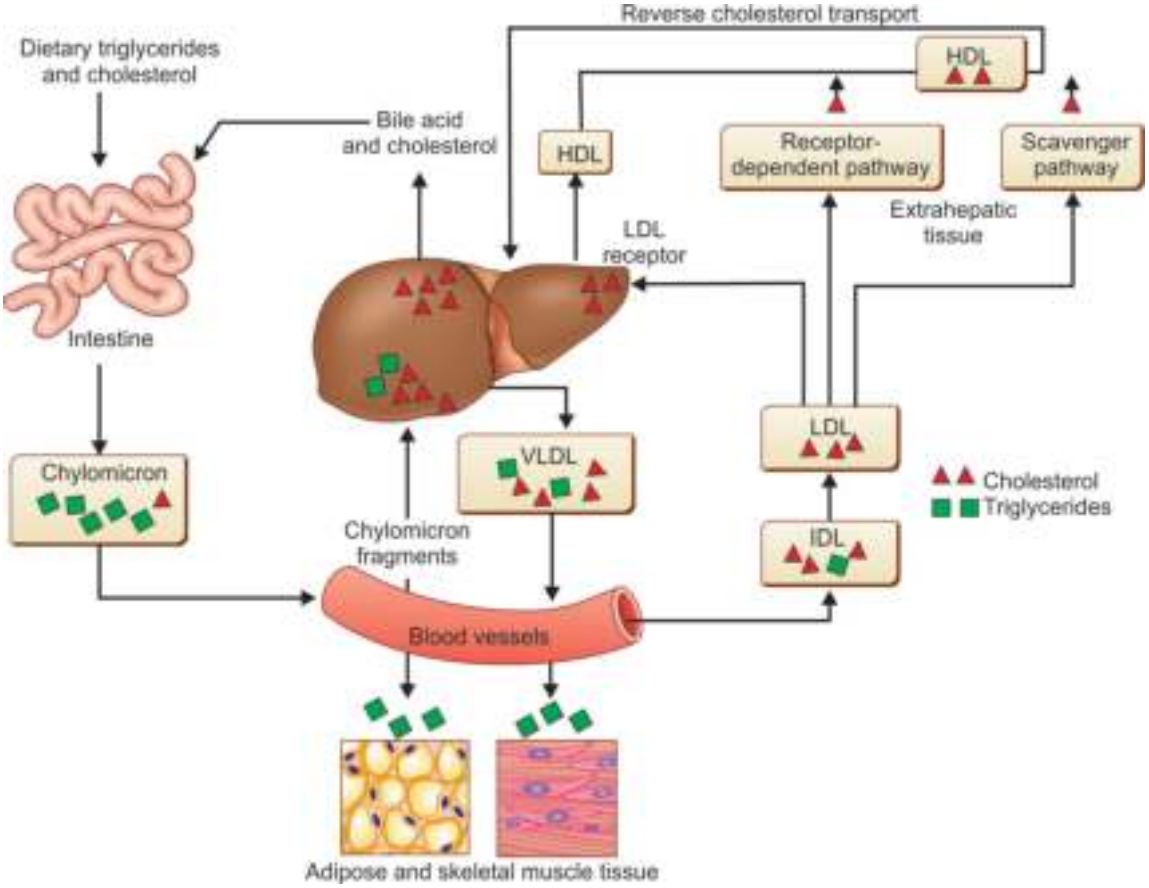


Fig. 15.7: Exogenous and endogenous pathways for triglyceride and cholesterol transport.

acids, or packaged as very low-density lipoprotein (VLDL) particles, which are then secreted into the blood circulation. This is the first step in the endogenous pathway for lipid transport. In adipose tissue or skeletal muscles, the triglyceride is removed from VLDLs with the help of lipoprotein lipase. The intermediate density lipoprotein (IDL) particles remain in the blood circulation. Some IDLs are immediately taken up by hepatocytes via the mediation of LDL receptor for ApoB/ApoE. The remaining IDLs in the blood circulation bind to the hepatocytes and other cells, and are removed from the blood circulation. HDLs take up cholesterol from the cells. The cholesterol is now esterified by the enzyme lecithin cholesterol acyltransferase, after which the esters are transferred to LDLs and then taken up by the cells. Classification of total LDL cholesterol in children with targeting fasting screening in children over age two years with family history of dyslipidemia or premature coronary artery disease is given in Table 15.6.

Apolipoproteins (Apo) and Dyslipoproteinemias

Apolipoproteins (Apo) constitute the major protein moiety of lipoproteins. These are commonly referred by using the nomenclature introduced in 1971. Apolipoproteins, their major sites of synthesis and functions are given in Table 15.7.

Familial Hypercholesterolemia

Familial hypercholesterolemia is inherited autosomal disorder, that causes LDL (bad) cholesterol level to be

Table 15.6 Classification of total LDL cholesterol in children with targeting fasting screening in children over age two years with family history of dyslipidemia and premature coronary artery disease

Category	Total Cholesterol	LDL Cholesterol
Acceptable	>170 mg/dl	<110 mg/dl
Borderline	171–199 mg/dl	111–129 mg/dl
High	≥200 mg/dl	≥130 mg/dl

Table 15.7 Apolipoproteins, their major sites of synthesis and functions

Apolipoprotein	Major Density Class	Chromosome	Major Sites of Synthesis	Functions in Lipoprotein Metabolism
ApoAI	HDL	11	Liver, intestine	Activation of lecithin: Cholesterol acyltransferase
ApoAII	HDL	1	Liver, intestine	Unknown
ApoAIV	Chylomicrons	11	Intestine	Unknown
ApoB-100	VLDL, IDL, LDL	2	Liver	Binds to LDL receptor
ApoB-48	Chylomicrons, VLDL, IDL	2	Intestine	Unknown
ApoCI	Chylomicrons, VLDL, HDL	19	Liver	Activation of lecithin: Cholesterol acyltransferase
ApoCII	Chylomicrons, VLDL, HDL	19	Liver	Activation of lipoprotein lipase
ApoCIII	Chylomicrons	11	Liver	Inhibition of lipoprotein uptake by the liver
ApoD	HDL	3	Unknown	Cholesteryl ester exchange protein
ApoE	Chylomicrons, VLDL, HDL	19	Liver, macrophage	Binds to E receptor
ApoF	VLDL	12	–	Transport and/or esterification of cholesterol
ApoH	VLDL	17	–	Encoded by APOH gene and function is to bind cardiolipin
ApoJ	HDL	8	–	Encoded by CLU gene on chromosome 8, regulates cholesteryl ester transfer protein (CETP)
ApoL	HDL	22	–	May be linked to reverse cholesterol transport
ApoM	Chylomicrons, VLDL, LDL, HDL	6	–	May be linked to reverse cholesterol transport
ApoO	HDL, LDL, VLDL	X	–	Homologue to plasminogen, may be prothrombotic, bound to ApoB-100 by disulfide bond
Apo(a)	Lp(a)	6	–	Carries cholesterol load has role in wound healing

LDL: Low density lipoprotein; IDL: Intermediate density lipoprotein; HDL: High density lipoprotein.

very high. The condition begins at birth and can cause myocardial infarction at an early age. The defect makes the body unable to remove LDL from the blood. It results in a high level of LDL in the blood circulation, which is deposited in the large elastic and medium size arteries. These blood vessels show atheromatous plaques in the intima encroaching lumen.

- Mutations of ApoA1, ApoE and LDL receptor; and hepatic lipase deficiency leads to hypercholesterolemia. Cholesterol gets deposited in the intima of large elastic and medium size arteries.
- Normally, LDL receptors are cell surface proteins, which regulate plasma cholesterol by mediating endocytosis and recycling of apolipoprotein E. Capillary lipoprotein lipase participates in degradation of lipids.
- Gene mutations of apolipoproteins lead to defective assembly of lipid with apolipoprotein. Defective LDL receptors lead to defective internalization of lipids. Defective capillary lipoprotein lipase leads to defective degradation of lipids. Net result of defective degradation of lipids is hypercholesterolemia.
- High level of cholesterol in the blood is endocytosed by tissue macrophages in the large elastic and medium size arteries; and under skin resulting in atherosclerosis and xanthomas respectively. Molecular defects of dyslipoproteinemias and pathologic features are given in Table 15.8. Serum lipids and dyslipoproteinemias are given in Table 15.9.

Table 15.8 Molecular defects of dyslipoproteinemias and pathologic features

Defects in Apolipoprotein, Enzymes and Receptors	Genetic Defect	Pathologic Features
Apolipoprotein defects		
ApoAI deficiency	ApoAI cutting or rearrangements (11q23)	▪ HDL absence ▪ Severe atherosclerosis
ApoAI variant deficiency	ApoAI point mutation (11q23)	▪ Reduced HDL level ▪ Severe atherosclerosis
ApoB-100 and ApoB-48 deficiency	Microsomal triglyceride protein mutation (4q22–24)	▪ Hemolytic anemia ▪ Mild ataxia, malabsorption ▪ Visual disturbance ▪ Absence of atherosclerosis
ApoB-100 deficiency	Mutation (2p24)	▪ Mild ataxia ▪ Malabsorption ▪ Absence of atherosclerosis
ApoCII deficiency	ApoCII mutation (19q13.2)	▪ Type I hyperlipidemia ▪ Severe hypertriglyceridemia ▪ Variable atherosclerosis
ApoE variant deficiency	ApoE mutation (19q13.2)	▪ Type III hyperlipidemia ▪ Elevated hypertriglyceridemia ▪ Premature atherosclerosis
Enzyme defects		
Lipoprotein lipase deficiency	Lipoprotein lipase mutation (8p22)	▪ Type I hyperlipidemia ▪ Hypertriglyceridemia ▪ Minimal atherosclerosis
Hepatic lipase deficiency	Hepatic lipase mutation (8p21–23)	▪ Elevated IHD and HDL levels ▪ Severe atherosclerosis
Lecithin: Cholesterol acyltransferase (LACT) deficiency	LCAT mutation (16q22.1)	▪ Mild hypertriglyceridemia ▪ Reduced HDL ▪ Variable atherosclerosis ▪ Corneal opacities
Receptor defect		
Familial hypercholesterolemia	LDL receptor mutation (19p13.2)	▪ Type II hyperlipidemia with severe elevation of LDL ▪ Premature atherosclerosis

LDL: Low density lipoprotein; IDL: Intermediate density lipoprotein; HDL: High density lipoprotein.

Table 15.9 Serum lipids and dyslipoproteinemias

Most useful lipid and lipoprotein testing are done by enzymatic analysis
Low-density lipoprotein cholesterol can be analyzed after fasting. It is currently considered the most important value in assessing risk for ischemic heart disease and directing therapy
Lipid profile currently recommended for initial screening in persons includes total cholesterol, low density lipoproteins, high density lipoproteins and triglycerides. Testing should be repeated at least once every five years
Other tests, including apolipoprotein levels and lipoprotein subclasses, may prove valuable in fine-tuning risk assessment and evaluating response to therapy
New guidelines enhance the perceived atherosclerotic plaque risk due to diabetes mellitus and support aggressive intervention in patients suffering from diabetes mellitus and metabolic syndrome

Acquired Hypercholesterolemia

Foods rich in cholesterol, saturated fat and *trans*-fats can raise blood cholesterol. Foods rich in fat include cheese, egg yolk, fried and processed food, pastries and red meat. High intake of these dietary products is associated with atherosclerosis.

- On the other hand, unsaturated fatty acids in ground nut consist of double bond with low melting point, hence less stable and not deposited in the arteries and under skin. Hypercholesterolemia can occur in the settings of diabetes mellitus, hypothyroidism and nephrotic syndrome. Progesterone therapy increases plasma levels of LDL, cholesterol and triglyceride and decreases HDL level.

Clinical Pearls: Lipid Lowering Drugs (Statins) in Hypercholesterolemia

- Diet regulation and administration of lipid lowering drugs have beneficial effect on reducing the risk for development of atherosclerosis in large elastic and medium size arteries supplying organs/tissues. Intake of omega-3 fatty acid derived from fish oil is beneficial to lower plasma cholesterol levels.
- Statins are lipid lowering drugs, which inhibit HMG-CoA reductase, which is rate-limiting enzyme in hepatic biosynthesis of cholesterol.
- Regular exercise, weight loss, intake of ethanol and estrogen intake increase HDL and decrease LDL cholesterol.

HYPERTENSION

Uncontrolled blood pressure is a major risk factor for atheromatous plaque. Persistent high systolic blood pressure over 160 mm Hg increases the risk for myocardial infarction and cerebral stroke. The use of antihypertensive drugs has significantly decreased the risk for myocardial infarction and cerebral stroke.

- Hypertension enhances the severity of atherosclerotic plaques further supports a role for hemodynamic disturbances inducing several factors in the development of atherosclerotic plaques.
- Hemodynamic forces induce gene expression patterns in the endothelial cells that promote atherosclerosis, e.g. endothelin, platelet endothelial cell adhesion molecule 1 (PECAM-1), FGF2, tissue factor and plasminogen activator.

DIABETES MELLITUS

Both type 1 and type 2 diabetes mellitus are powerful and independent risk factors for coronary artery disease, cerebral strokes and peripheral vascular disease. Atherosclerosis accounts for virtually 80% of all deaths among diabetic patients. Microvascular complications include retinopathy, nephropathy and neuropathy.

- Prolonged exposure to hyperglycemia induces a large number of alterations at cellular levels of vascular tissue that potentially accelerates atherosclerotic process.
- Increased plasma glucose combines with apolipoproteins and other proteins to form Schiff base (reversible reaction by controlling plasma glucose). Schiff base is converted to early glycosylated end products and then to advanced glycosylated end products. Glycosylation is a process affecting circulating lipoproteins and protein component of the vasculature. Advanced glycosylated end products play important role in the pathogenesis of atherosclerosis. Glycosylated LDL is less susceptible to oxidation and thus delivers cholesterol to vasculature.
- Human studies have elucidated major mechanisms that encompass most of the pathologic alterations in diabetic vasculature. Nonenzymatic glycosylation of proteins and lipids can interfere with normal function by disrupting molecular conformation, alter enzymatic activity and interfere with receptor recognition. In addition, glycosylated proteins interact with specific receptors present on all the cells in atherosclerotic plaque, including endothelial cells, monocyte-derived macrophages and smooth muscle cells. The interaction of glycosylated proteins with their receptors leads to induction of oxidative stress and proinflammatory responses. Protein kinase C (PKC) activation leads to subsequent alterations in expression of growth factors.

Clinical Pearls: Glycosylated Hemoglobin (HbA1C) Analysis

- Glycosylated hemoglobin (HbA1C) is a form of hemoglobin that is covalently bound to glucose. Hemoglobin is exposed to glucose in the blood and both are bound together through the glycosylation process.

- HbA1C is a measure of the β-N-1-deoxy fructosyl component of hemoglobin.
- Glycosylated hemoglobin is tested to monitor the long-term diabetes mellitus to assess average blood sugar over the past 2–4 months.
- Normal range for HbA1C is <6%; and 6–6.4% in prediabetics. An HbA1C of 6.5 is recommended as the cut off point for diagnosing diabetes mellitus.

TOBACCO SMOKING

Tobacco smoking is an important major risk factor for atherosclerosis. Tobacco smoke contains tar, polycyclic aromatic hydrocarbons, nicotine, phenol, nitrosamines, nitrogen oxide and carbon monoxide. Prolonged tobacco smoking doubles the death rate from ischemic heart disease. Tobacco smoke causes endothelial dysfunction, deranged lipids (increased LDL and decreased HDL), increase adhesiveness and activation of platelets.

INFECTIONS AND ATHEROSCLEROSIS

Serological and epidemiological studies suggest that some infectious agents such as *Chlamydia pneumoniae*, cytomegalovirus, *Helicobacter pylori*, herpesvirus may participate in the pathogenesis of atherosclerosis. DNA obtained from these infectious agents has been demonstrated in atheromatous plaque, but the nature of their association is obscure.

HOMOCYSTEINEMIA

Homocysteinemia is rare autosomal recessive disorder caused by mutation in gene encoding homocysteine synthase. The disorder enhances formation of premature and severe atheromatous plaque. Raised plasma homocysteine level is an independent risk factor for atheromatous plaque.

- Homocysteine has toxic effects on endothelial cells resulting in impairment of several antithrombotic mechanisms in endothelial cells.
- Homocysteine inhibits thrombomodulin on the surface of endothelial cells, antithrombin III binding activity of heparan sulphate proteoglycans and binding of tissue plasminogen activator, which accelerates atherosclerosis.

FAMILIAL GENETIC ALTERATIONS

Family history is the most important independent factor for atherosclerosis. Familial hypercholesterolemia is well established risk factor for atherosclerosis and ischemic heart disease. Familial clustering of diabetes mellitus and hypertension may predispose to premature atherosclerosis.

GENDER PREDILECTION

Premenopausal women are relatively protected against atherosclerosis due to increased estrogens compared with age-matched men. Estrogens have antiatherogenic properties. Postmenopausal women are more prone to develop atherosclerosis as a result of decreased circulating estrogens.

INFLAMMATION DURING ATHEROGENESIS

Inflammation is present during all stages of atherogenesis. It is linked with atheromatous plaque formation and its disruption. C-reactive protein is increased in disrupted atheromatous plaque. It is an acute phase reactant synthesized in the liver. Its expression is enhanced by IL-6 cytokine. Increased plasma level of C-reactive protein is an independent risk factor for coronary artery disease, sudden cardiac death, cerebral stroke and peripheral arterial disease.

Clinical Pearls: C-Reactive Protein Analysis

- Estimation of C-reactive protein synthesized by liver is an important marker in inflammation and disrupted atheromatous plaque with superimposed thrombosis.
- C-reactive protein level is increased in patients with disrupted atheromatous plaque with superimposed thrombosis in acute myocardial infarction, cerebral stroke and peripheral vascular disease.

PATHOGENESIS OF ATHEROSCLEROSIS

Over the years, many theories have been proposed to explain the pathogenesis of atherosclerosis. With the new knowledge from experimental and clinical observations, a comprehensive description of the pathogenesis of atheroma formation, evolution and clinical features of the atheromatous plaques vary from patient to patient.

- Virchow in 1952 stated that atherosclerosis occurs due to low-grade inflammation of blood vessels. Imbibing of lipids from the blood causes proliferation of the intimal cells. Rokitansky in 1852 stated that incorporation of small thrombus results in formation of atheromatous plaque.
- According to monoclonal hypothesis, atherosclerosis was suggested as a neoplastic lesion due to monoclonal proliferation of smooth muscle cells as a result of alterations in lipoproteins. Response to injury hypothesis is most widely accepted. Injury to intima results in dysfunctional endothelium. Lipids and monocytes from blood reach subintimal region through dysfunctional endothelium. Lipid accumulation in the macrophages, synthesis of cytokines and migration of smooth muscle cells lead to formation of atheromatous plaque.

- Atherosclerosis progresses through endothelial injury and dysfunction, accumulation of lipoproteins, monocyte adhesion to endothelium and transmigration into intima, platelet adhesion and activation, growth factors synthesized by endothelium, macrophages, smooth muscle cells and T cells; smooth muscle proliferation and synthesis of extracellular matrix (ECM) and lipid accumulation. Pathogenesis of atheromatous plaque and its complications is shown in Fig. 15.8.

CHRONIC INJURY TO VASCULAR ENDOTHELIUM

Hyperlipidemia, sustained hypertension, tobacco smoking, diabetes mellitus, homocysteinemia, hemodynamic disturbances at ostia, branching points, reactive oxygen species and infectious agents cause chronic endothelial injury of large elastic and medium size arteries resulting in dysfunctional endothelium.

- The dysfunctional endothelium is more permeable to lipoproteins and monocytes. Hyperlipidemia can

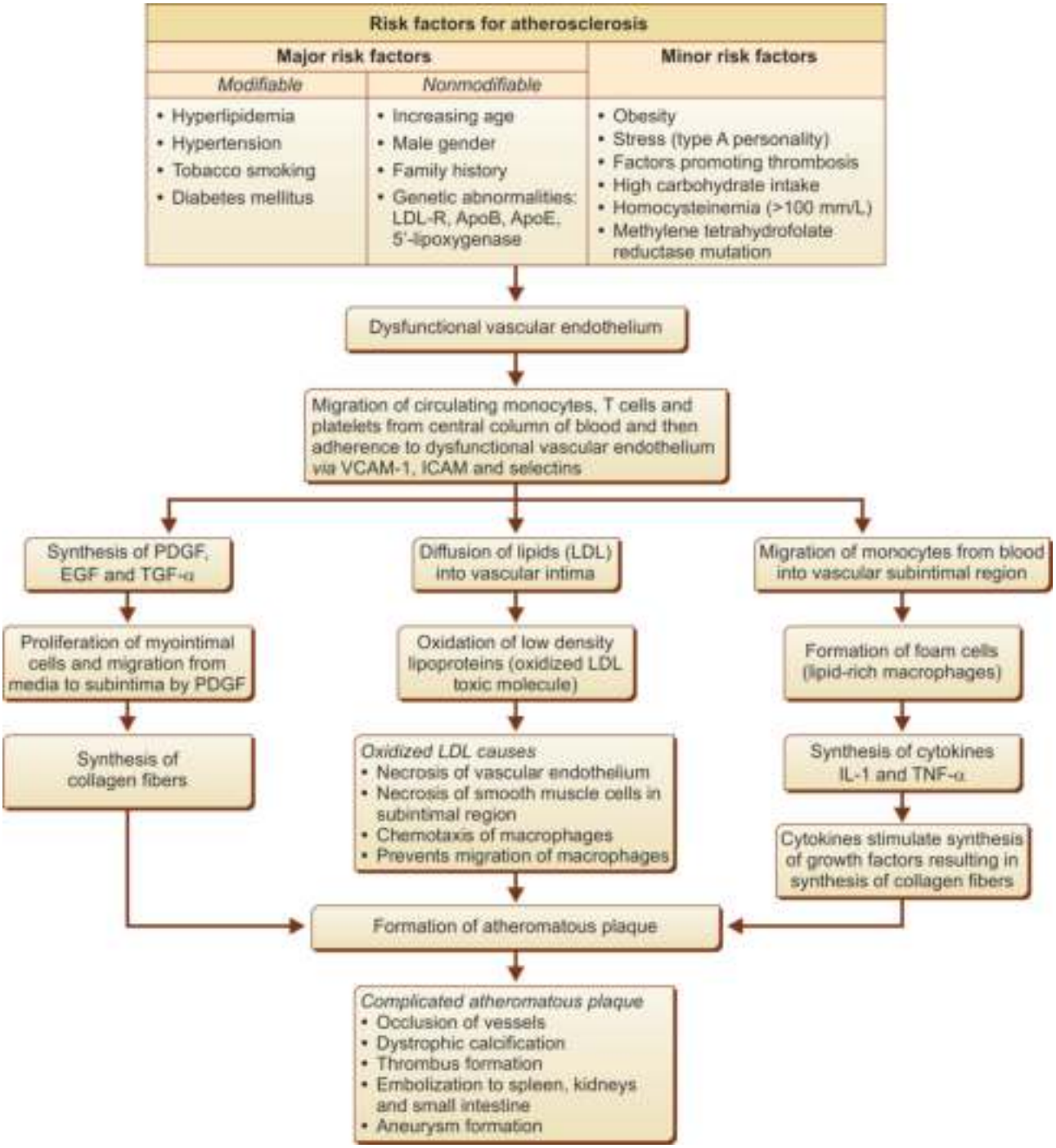


Fig. 15.8: Pathogenesis of atheromatous plaque and its complications.

directly impair endothelial function. Lipids increase production of reactive oxygen species, which decay nitrous oxide (vasodilator). Lipids also increase local shear and stress.

- However, hemodynamic disturbances and hypercholesterolemia are the most important causes of dysfunctional endothelium.
- Hemodynamic disturbances occur at the ostia of exiting blood vessels, branching points and along the posterior wall of the abdominal aorta. Laminar blood flow induces superoxide dismutase antioxidant, which gives protection against atherosclerosis.

ADHERENCE OF BLOOD CELLS TO DYSFUNCTIONAL VASCULAR ENDOTHELIUM

At the sites susceptible to development of atheromatous plaque, expression of proinflammatory genes leads to synthesis of cell adhesion molecules such as vascular cell surface adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), which recruit monocytes into the vessel wall. However, stress and shear also induce gene expression and synthesis of antiatherogenic agents such as nitrous oxide (vasodilator) and plasminogen activator inhibitor 1 (PAI-1).

HEMODYNAMIC FORCES

Hypertension enhances the severity of atherosclerotic plaques further supports a role for hemodynamic forces induce several factors in the development of atherosclerotic plaques. Hemodynamic forces induce gene expression and synthesis of endothelin, fibroblast growth factor 2, tissue factor and plasminogen activator in the endothelial cells, which promote atherosclerosis.

INFLUX OF MONOCYTES

Monocyte-macrophage recruitment is one of the early events in the development of atheromatous plaque. It is orchestrated through a multistep process involving adhesion, transmigration and chemotaxis. Adhesion of monocytes–macrophages is regulated by cell surface adhesion molecules including VCAM-1, ICAM-1, E-selectin, P-selectin and several chemokines such as monocyte chemotactic protein 1 (MCP-1) and macrophage colony stimulating factor (M-CSF). Transmigration involves platelet endothelial cell adhesion molecule 1 (PECAM-1). Chemokines (MCP-1 and M-CSF) participate in recruitment of additional monocytes in blood vessel wall.

INFLUX OF LIPIDS AND OXIDIZED LDL MOLECULE

Lipid accumulation depends on disruption of integrity of the endothelial barrier through inter-endothelial gaps and endothelial dysfunction. Dysfunctional endothelium is caused by hypercholesterolemia, abnormal laminar flow, reactive oxygen species, cytokine-induced inflammation, advanced glycosylated end products in diabetes mellitus and homocysteinemia.

- Platelet synthesizes malondialdehyde enzyme that acts on the LDL molecule, which gets converted to modified LDL molecule. LDL carries lipids into the intima. Modified LDL molecule enters the subintimal region. Oxygen reactive species synthesized by macrophages and dysfunctional endothelium participate in the oxidation of LDL molecule to form oxidized LDL molecule.
- Oxidized LDL activates cell surface adhesion molecules. Macrophages can adhere to activated endothelial cells and transmigrate between endothelial cells into the intima, carrying lipids with them.

Uptake of Oxidized LDL Molecule by Macrophages

Monocyte-derived macrophages express receptors such as β-VLDL receptors and scavenger receptors, which bind modified LDL molecule. Lipid laden macrophages are known as foam cells. Smooth muscle cells also imbibe LDL molecules. Some of foamy macrophages laden with lipid undergo necrosis and release lipids in extracellular compartment. Alterations in extracellular matrix proteoglycans synthesized by smooth muscle cells in the intima also render these sites prone to lipid accumulation. Reduced egression of lipids out of artery also promotes accumulation of lipid. Accumulation of cholesterol in foam cells leads to mitochondrial dysfunction, apoptosis, necrosis and release of cellular proteases, inflammatory cytokines and prothrombotic molecules.

Toxic Effects of Oxidized LDL Molecules

Oxidized LDL molecules imbibed by macrophages and smooth muscle cells play important role in the pathogenesis of atheromatous plaque.

- Oxidized LDL molecules cause necrosis of foamy macrophages, smooth muscle cells and endothelium. Oxidized LDL molecules participate in the recruitment of additional monocyte-derived macrophages with the help of monocyte-binding protein synthesized by endothelium; and also inhibit egression of macrophages.
- LDL molecules also participate in migration of smooth muscle cells from media to subintimal region. Role of oxidized low-density lipoproteins in the pathogenesis of atheromatous plaque is shown in Fig. 15.9.
- The nuclear factor-κB (NF-κB) pathway is an important signal transduction system that participates in

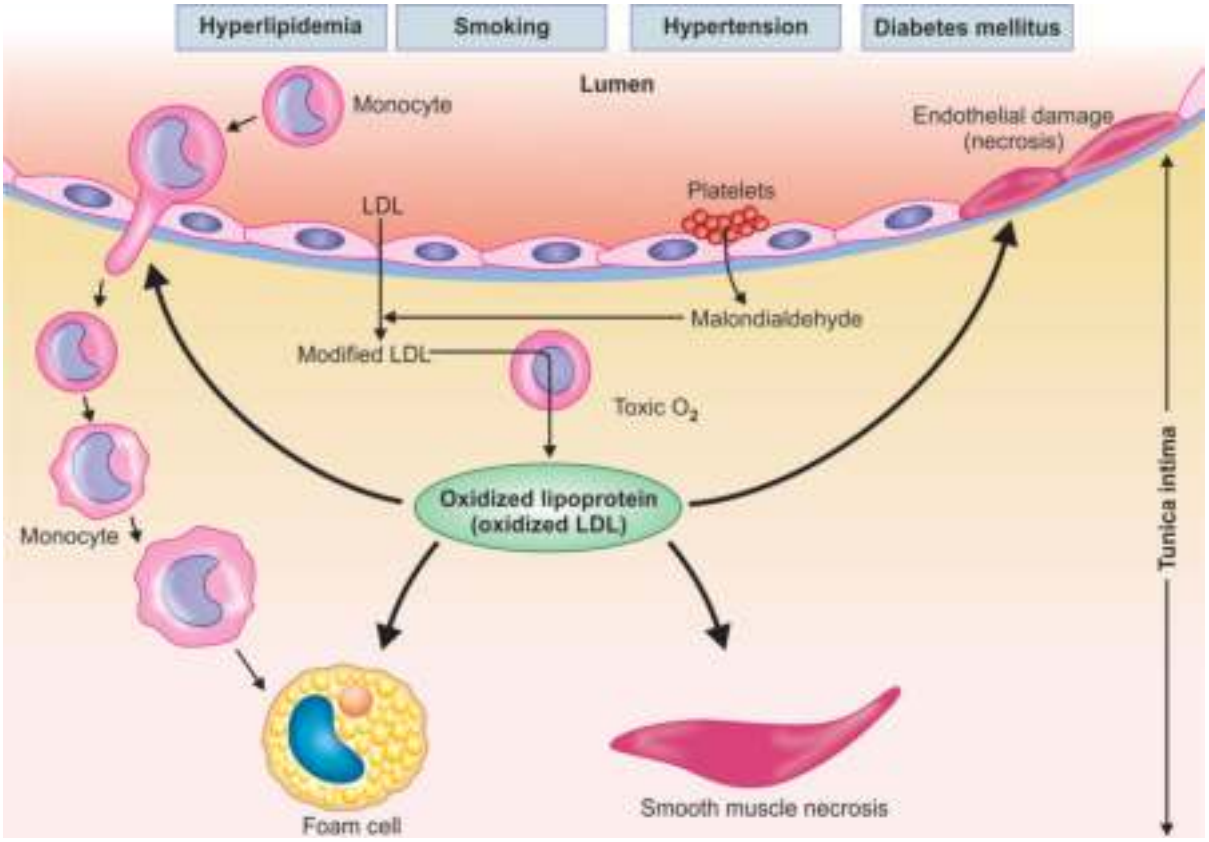


Fig. 15.9: Role of oxidized low-density lipoproteins in the pathogenesis of atheromatous plaque. Oxidized LDL causes necrosis of the endothelial and smooth muscle cells; migration and proliferation of smooth muscle cells; chemotaxis of monocyte-derived macrophages, and inhibition of egress of monocyte derived macrophages.

atheromatous plaque growth and progression. It also enhances recruitment of leukocytes, synthesis of cytokines and remodeling of extracellular matrix in atheromatous plaque.

ROLE OF GROWTH FACTORS AND CYTOKINES

There are cellular interactions in the progression of the atheromatous plaque. Endothelium, platelets, macrophages, T cells and smooth muscle cells synthesize

Table 15.10 Cytokines, growth factors and other molecules synthesized by endothelial cells, platelets, macrophages, T cells and smooth muscle cells

Cells	Growth Factors
Endothelial cells	PDGF, FGF-β, IL-1, TGF-β, PGI_2, IGF-1,NO, OxLDL, TF, TFPI
Platelet	PDGF, EGF, TGF-β, TxA_2, IGF-1, thrombin
Macrophages	PDGF, FGF, HB-EGF, TGF-β,TNF-α, IL-1, PGF, oxidized LDL, MMP/TMP, TF, TFPI
T cells	IFN-γ, TGF-β, TNF-α, IL-1
Smooth muscle cells	▪ Growth factors and cytokines ▪ PDGF, FGF, IGF-1, IL-1, M-CSF, TGF-β, TNF-α, PGE, HB-EGF, MCP-1, GM-CSF, TF, TFPI
Synthesis of other products	Collagen fibers, proteoglycans, MMP/TIMP

PDGF: Platelet derived growth factor; FGF-β: Fibroblastic growth factor β; FGF: Fibroblastic growth factor; IL-1: Interleukin 1; PGI_2: Prostaglandin I_2; PGE: Prostaglandin E; IGF-1: Insulin growth factor 1; NO: Nitric oxide; TFPI: Tissue factor pathway inhibitor; EGF/TGF-β: Epidermal growth factor/transforming growth factor β; TXA_2: Thromboxane A_2; TNF-α: Tumor necrosis factor-α; HB-EGF: Heparin binding epidermal growth factor; TF: Tissue factor; IFN-γ: Interferon γ; MMP/TMP: Matrix metalloproteinase/tissue metalloproteinase; MCP-1: Monocyte chemotactic protein 1; GM-CSF: Granulocyte/monocyte-colony stimulating factors; M-CSF: Macrophage colony stimulating factor; PGF: Placental growth factor; Oxidized LDL: Oxidized low density lipoproteins.

a variety of cytokines, growth factors and other molecules, which act on smooth muscle cells. The cellular interactions promote the proliferation of smooth muscle cells. Cytokines, growth factors and other molecules synthesized by endothelial cells, platelets, macrophages, T cells and smooth muscle cells are given in Table 15.10.

Source of Growth Factors

- **Macrophages:** Activated macrophages synthesize various growth factors such as PDGF, FGF, HB-EGF, TGF-β, TNF-α, IL-1, PGF, oxidized LDL, MMP/TMP, TF and TFPI. These also synthesize collagen fibers, proteoglycans, MMP/TIMP. PDGF, EGF and TGF-β induce migration and proliferation of smooth muscle cells in the subintimal region; and synthesis of extracellular matrix proteins such as collagen, elastin and proteoglycans.
- **Smooth muscle cells:** Activated smooth muscle cells synthesize PDGF, FGF, IGF-1, IL-1, M-CSF, TGF-β, TNF-α, PGE, HB-EGF, MCP-1, GM-CSF, TF and TFPI. PDGF has autocrine action.
- **Platelets:** Platelets synthesize PDGF, EGF, TGF-β, TXA_2, IGF-1 and thrombin. Thrombin stimulates protease activated receptors, which send signal for migration and proliferation of smooth muscle cells; and synthesis of extracellular matrix. PDGF, EGF, TGF-β induce migration and proliferation of smooth muscle cells in the subintimal region; and synthesis of extracellular matrix proteins such as collagen, elastin and proteoglycans.
- **T cells:** T cells synthesize IFN-γ, TGF-β, TNF-α and IL-1. TNF-α and IL-1 cytokines increase the expression of cell surface adhesion molecules on dysfunctional endothelium. These cytokines also participate in migration and proliferation of smooth muscle cells in subintimal region; and formation of new blood vessels in the atheromatous plaque. Cytokines IFN-γ, TGF-β and IL-1 activate macrophages, which synthesize matrix metalloproteinases (MMPs). Metalloproteinases cause degradation of collagen fibers in the atheromatous plaque.
- **Endothelial cells:** Activated endothelium PDGF, FGF-β, IL-1, TGF-β, PGI_2, IGF-1, NO, OxLDL, TF and TFPI. PDGF and TGF-β participate in migration and proliferation of smooth muscle cells beneath intima.

ROLE OF SMOOTH MUSCLE CELLS (SMCs)

Smooth muscle cells are quiescent (qSMCs) in the normal arterial wall. The qSMCs exhibit differentiated contractile phenotype. These are derived embryonically from the progenitor mesenchymal cells and neural crest cells. Progenitor smooth muscle cells (pSMCs) are derived from the hematopoietic stem cells in the bone marrow.

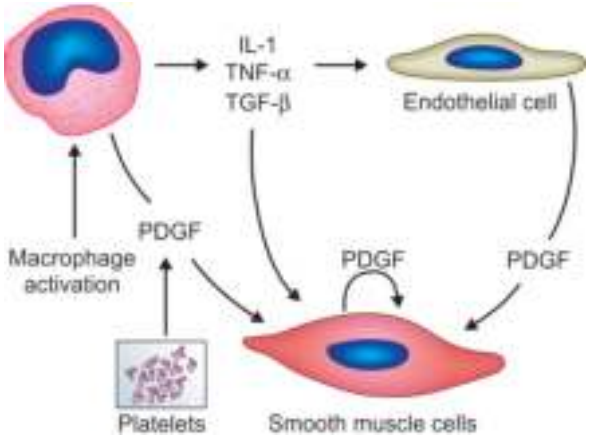


Fig. 15.10: Cellular interactions in the progression of atheromatous plaque that promote proliferation of smooth muscle cells.

- The qSMCs are activated by lipids, cytokines, chemokines and other chemical mediators synthesized by macrophages and endothelial cells through paracrine pathways in the atheromatous plaque. Activated smooth muscle cells (aSMCs) participate in repair and remodeling of the atherosclerotic lesion. Activated smooth muscle cells migrate, proliferate and synthesize extracellular matrix, and can accumulate lipids and become foam cells in the atheromatous lesion.
- Endothelial progenitor cells (EPCs) derived from mesenchymal stem cells in the bone marrow may replenish SMCs in the vasculature, especially during a response to endothelial injury. The aSMCs interact with endothelial cells (ECs) and macrophages directly or indirectly to regulate atheromatous plaque growth. Cellular interactions in the progression of atheromatous plaque that promote proliferation of smooth muscle cells are shown in Fig. 15.10.

SYNTHESIS OF EXTRACELLULAR MATRIX

The nuclear factor-κB (NF-κB) pathway is an important signal transduction system that participates in growth and progression and remodeling of extracellular matrix in atheromatous plaque. Smooth muscle cells participate in synthesis of extracellular matrix, i.e. collagen fibers, proteoglycans, metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). Extracellular matrix subsequently produces fibrotic atherosclerotic plaque. Synthesis of connective tissue determines the stiffness of the blood vessels and further progression of atheromatous plaque.

THROMBUS FORMATION

Thrombus formation in atheromatous plaque induces fibrin generation. Fibrin stimulates protease activated receptors, which transmit signal for migration and proliferation of smooth muscles, and synthesis of extracellular matrix.

MORPHOLOGY OF ATHEROSCLEROSIS

Grossly recognizable atherosclerosis begins as a fatty streak and progresses to an atheroma and then fibroatheroma. Histology of atherosclerosis shows three components of fibroatheroma: fibrous cap, cellular layer and necrotic care.

COMPLICATIONS OF ATHEROSCLEROSIS

Complications of atherosclerosis include acute occlusion of vessel lumen, chronic narrowing of vessel lumen, bleeding in atheromatous plaque, mural thrombus formation, embolism, aneurysm, dystrophic calcification and restenosis of blood vessel involved.

Pathology Pearls: Gross Morphology and Histologic Features of Atheromatous Plaque

Morphologic Features of Fibroatheroma

- **Fatty streak:** Fatty streak is characterized by intimal accumulation of foam cells (lipid filled macrophages). It may represent as the initial precursor of atherosclerosis. However, all fatty streaks are not precursor of atherosclerosis. Virtually, these are present in thoracic aorta of children by the age of 10 years. These lesions are not sufficiently raised to cause any significant blood flow obstruction. Fatty streaks are multiple, minute, yellow elongated slightly raised streaks measuring ≥1.0 cm in length and 0.1 cm in diameter in thoracic aorta of infants and children. Fatty streaks are also demonstrated in the coronary arteries.
- **Atheroma:** Atheroma is characterized by intimal collection of foam cells and extracellular lipid resulting in restriction of the circulation and risk for thrombosis. Atheromatous plaques are white yellow, raised usually eccentric lesion, measuring 0.3–1.5 cm or more in size encroaching vascular lumen.
- **Fibroatheroma:** Atheroma with development of a fibrous cap is called fibroatheroma. Lesion is yellow to white elevated above intima measuring 0.3–1.5 cm or more in size protruding toward the vascular lumen (Figs 15.11 and 15.12).

Histologic Features of Fibroatheroma

There are three main components of a fibroatheroma: Fibrous cap, atheroma core and shoulder. Components of atheromatous plaque is shown in Fig. 15.13A to C and Table 15.11. Comparison of fatty streak and atheromatous plaque is given in Table 15.12. Pathophysiologic events and consequences of atherosclerotic are given in Table 15.13. Complications of atheromatous plaque are shown in Fig. 15.14.

- **Fibrous cap:** The thick fibrous separates the vessel lumen from the central core of fibroatheroma. It is more stable and less likely to develop complications such as hemorrhage and rupture. The fibrous cap is composed of smooth muscle cells and extracellular matrix (e.g. collagen). Fibrous is composed of smooth muscle cells, foam cells, fibrin, and extracellular matrix such as collagen, elastin, glycosaminoglycans and proteoglycans. The endothelium over the fibrous cap appears intact.
- **Central core and shoulder of atheromatous plaque:** Central core of the atheroma contains cholesterol and cholesterol esters, lipid-filled macrophages (foam cells), necrotic smooth muscle cell debris and calcium. The shoulder of the atheromatous plaque is located at the junction of normal intima near the fibroatheroma lesion. It contains macrophages, leukocytes including T cells and newly formed blood vessels.



Fig. 15.11: Autopsy finding in a long-standing diabetes mellitus. Abdominal aorta demonstrates severe atherosclerosis and a mural thrombus. (*Courtesy*: Dr. Sujata Kanetkar, Professor and Head of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

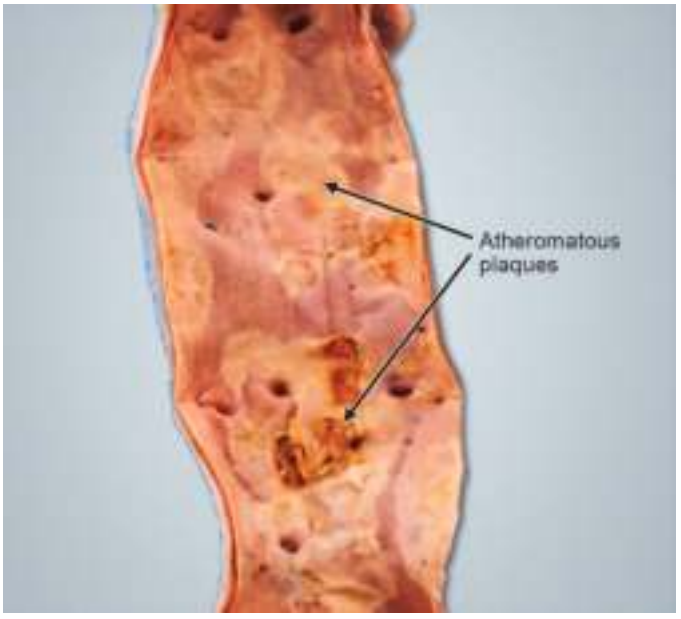


Fig. 15.12: Atheromatous plaques in abdominal aorta. Multiple atheromatous plaques of various sizes are demonstrated in the abdominal aorta. Ostia of the small branches arising from the abdominal aorta show narrowing due to deposition of lipid.

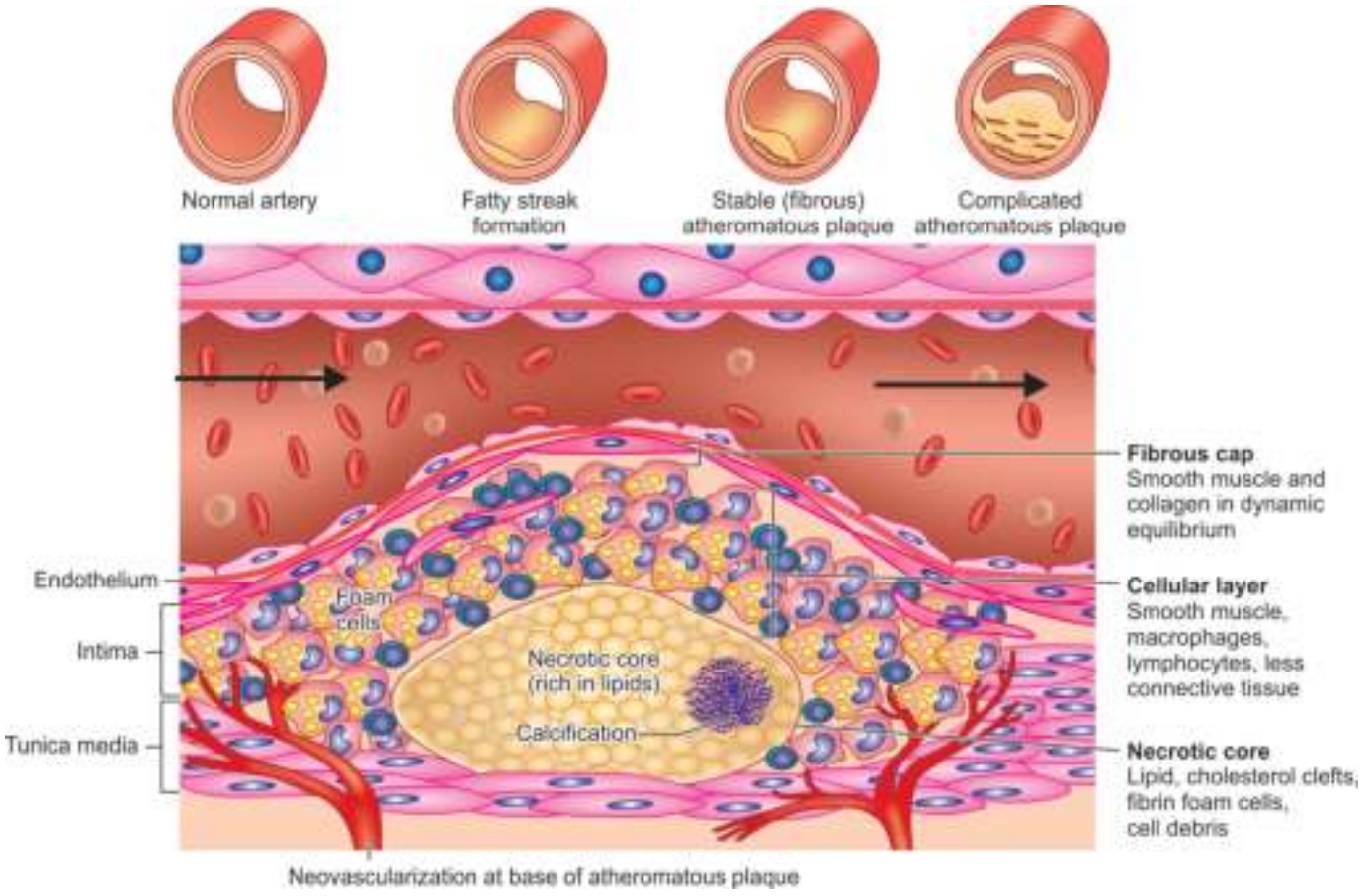


Fig. 15.13A: Evolution of the atheromatous plaque. It shows normal blood vessel, development of fatty streaks, stable fibrous plaque and complicated atheromatous plaque. Atheromatous plaque consists of fibrous cap, central core and shoulder. The thick fibrous cap is more stable and less likely to develop complications such as hemorrhage and rupture. The fibrous cap is composed of smooth muscle cells and extracellular matrix (e.g. collagen). Central core of the lesion contains cholesterol and cholesterol esters, lipid-filled macrophages (foam cells), necrotic smooth muscle cell debris and calcium.



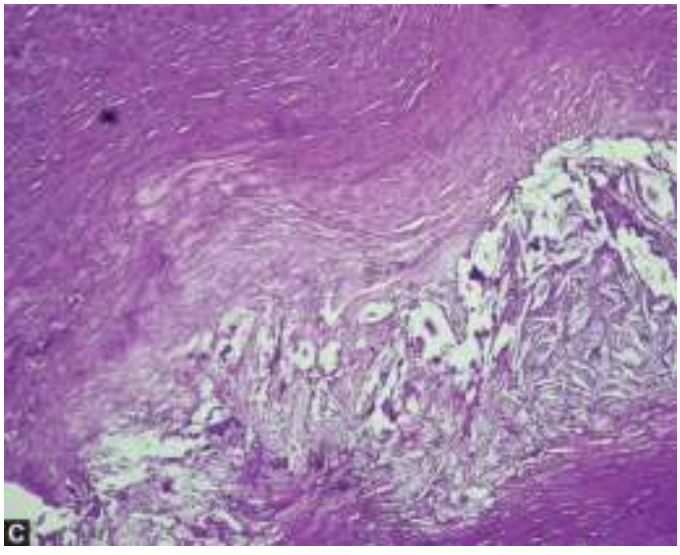


Fig. 15.13B and C: Evolution of the atheromatous plaque. (B) Gross morphology of abdominal aorta shows atheromatous plaque, (C) atheromatous plaque is initially covered by endothelium. Area between the blood vessel lumen and necrotic central core, termed the fibrous cap, contains smooth muscle cells, macrophages, T cells, lipid laden foam cells and connective tissue components. Cholesterol crystals are present within the fibrous tissue and the necrotic areas. It is worth mentioning that newly formed blood vessels are fragile, which may rupture and resulting in acute expansion of the atheromatous plaque due to intraplaque hemorrhage (40X).

Table 15.11 Components of atheromatous plaque

Cellular Component	Extracellular Matrix Component	Lipid Component
▪ Endothelial cells ▪ Smooth muscle cells ▪ Macrophages ▪ Foam cells ▪ Paltelets ▪ Mast cells ▪ T cells ▪ B cells ▪ Neutrophils	▪ Collagen fibers ▪ Elastin ▪ Proteoglycans ▪ Glycoproteins ▪ Proteolytic enzymes (MMPs)* ▪ Tissue inhibitors of MMPs	▪ Lipids (cholesterol and cholesterol esters) ▪ Lipoproteins

**MMPs: Matrix metalloproteinases*

Table 15.12 Comparison of fatty streak and atheromatous plaque

Features	Fatty Streak	Atheromatous Plaque
Age group	Children	Older age group
Composition	Fatty streak is characterized by intimal accumulation of foam cells (lipid filled macrophages), lipid accumulation mainly intracellular, small amount in extracellular compartment	Central core of the atheromatous plaque contains lipid accumulation mainly in the extracellular region
Gross morphology	Fatty streaks are multiple, minute, yellow elongated slightly raised streaks measuring 1.0 cm in length and 0.1 cm in diameter in thoracic aorta of infants and children, and not encroaching lumen	Atheromatous plaques are white yellow, raised usually eccentric lesion, measuring 0.3–1.5 cm or more in size encroaching lumen
Vasculature involved	Thoracic aorta and coronary arteries	Large- and medium-sized blood vessels such as abdominal aorta, coronary arteries, internal carotid artery, middle cerebral artery, celiac artery, superior mesenteric artery, inferior mesenteric artery, popliteal artery, anterior tibial artery, posterior artery, renal artery
Geographic distribution	May be demonstrated in clinical normal persons	Developing and developed countries
Complications	No complication	Atheromatous plaque may occlude lumen, bleeding in plaque, mural thrombus, embolism, aneurysm, calcification
Clinical consequences	Not affecting blood flow in vessels	Patient may develop clinical manifestations related to heart (angina, myocardial infarction, sudden cardiac death, heart failure), brain (transient ischemic attacks, cerebral infarction), intestine (gangrene intestine), lower leg (gangrene foot)

Table 15.13 Pathophysiologic events and consequences of atherosclerotic plaque

Pathophysiologic Events	Consequences
Acute occlusion of artery secondary to atheromatous plaque rupture/thrombosis	End organ/tissue infarction such as myocardial infarction, cerebral stroke, gangrene intestine, gangrene foot
Progressive lumen narrowing of vessel in atherosclerosis	Chronic ischemic heart disease
Erosion/replacement of media by atheromatous plaque with loss of tensile strength	Aneurysm especially abdominal aorta
Dislodgment of thrombus in atheromatous plaque and or release of the highly thrombogenic components of the central core of atheroma	Thromboembolism, atheroembolism

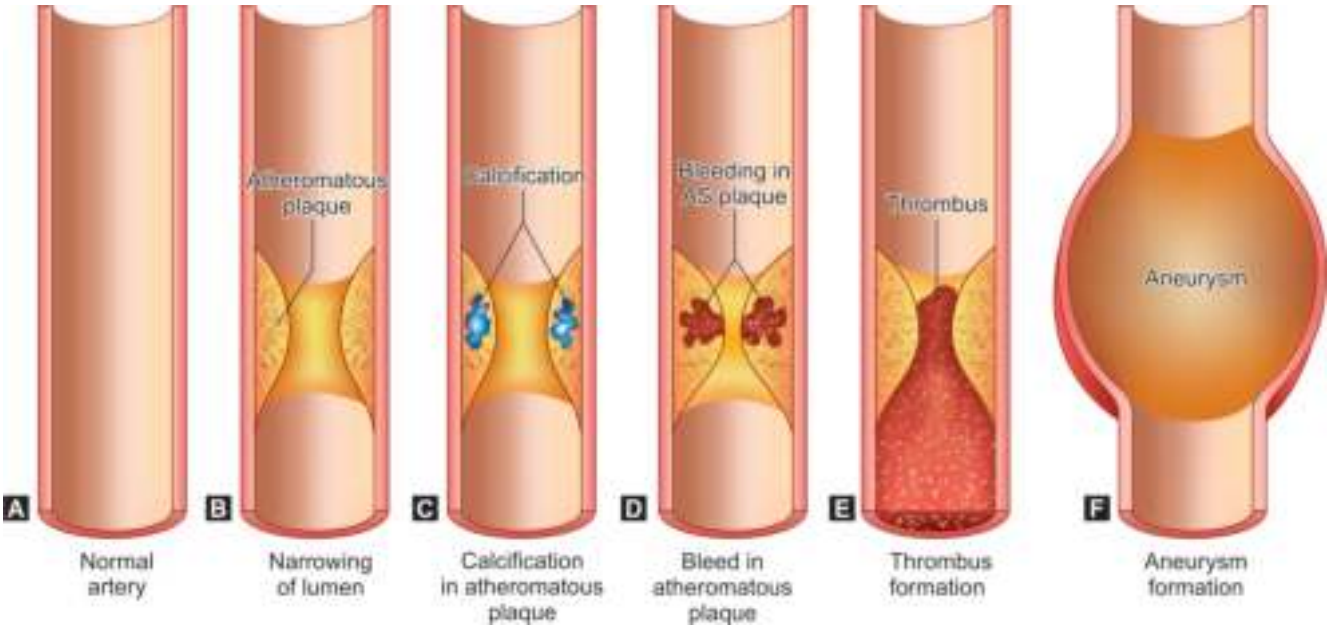


Fig. 15.14: Complications of atheromatous plaque. It shows a normal blood vessel, narrowing of blood vessel as a result of atheromatous plaque and its complications such as dystrophic calcification, bleed into the atheromatous plaque, thrombus formation and aneurysm.

ACUTE OCCLUSION OF THE VESSEL LUMEN

Thrombosis on an atheromatous plaque can abruptly occlude medium size artery resulting in ischemic necrosis (infarction) of the tissue supplied by that blood vessel. Patient may present with myocardial infarction, cerebral stroke or gangrene of the intestine or gangrene of lower extremities. Some occlusive thrombi can be dissolved therapeutically by thrombolytic agents (streptokinase and tissue plasminogen activator) that can activate fibrinolytic activity of blood.

CHRONIC NARROWING OF THE VESSEL LUMEN

As an atheromatous plaque grows, it may cause narrowing of the vascular lumen, progressively reducing the blood flow to the tissue/organ supplied by that artery. Chronic ischemia of the affected tissue leads to atrophy of the organ. For example, unilateral renal artery stenosis results in renal atrophy. Stenosis of the mesenteric artery causes intestinal gangrene. Stenosed blood vessel in a case of diabetes mellitus may result in peripheral vascular disease.

HEMORRHAGE INTO ATHEROMATOUS PLAQUE

The initial stage is associated with formation of new vasa vasorum in areas of subsequent atheromatous plaque. The overall appearance of these vasa vasorum newly formed blood vessels is fairly disorganized. In the progression stage, there is extension of adventitial new blood vessels to the media and eventually into the enlarging atheromatous plaque. Eventually, most advanced atheromatous plaques are fairly neovascularized. Moreover, neovascularization seems to characterize the active atheromatous plaque rather than calcified inactive atheromatous plaque. Newly formed small blood vessels within atheromatous plaque may rupture resulting in sudden enlargement of the atheromatous plaque and impairment of the blood supply to the tissue or organ.

ATHEROMATOUS PLAQUE DISRUPTION

Vasoconstriction can potentiate disruption of atheromatous plaque. Vasoconstriction at the site of atheromatous plaque is stimulated by circulating adrenergic agonists, platelet adhesions, activation and secretion; endothelial dysfunction with impaired synthesis of endothelial derived vasodilator nitric oxide and endothelin; and the synthesis of inflammatory mediators from perivascular inflammatory cells.

MURAL THROMBUS FORMATION

Mural thrombus results from turbulent blood flow around atheromatous plaque, where it protrudes into the vessel lumen and creates reduced luminal blood flow or stasis. The disturbance in blood flow also causes damage to the endothelial lining, which may become dysfunctional or locally ulcerated, and hence patient no longer has thromboresistant endothelial surface.

- Thrombi most often form at the sites of erosion and fissuring on the surface of the fibrous cap of atheromatous plaque. Mural thrombi located in

the more proximal region of a coronary artery may embolize to more distal sites in the blood vessel.

- Thrombotic vascular occlusion may manifest as myocardial infarction (coronary arteries), cerebral stroke (internal carotid and middle cerebral artery), gangrene intestinal loop (celiac trunk, superior mesentery and inferior mesenteric artery) and gangrene limb (popliteal, anterior tibial and posterior tibial arteries).

THROMBOEMBOLISM

A thrombus formed over an atheromatous plaque may detach and lodge as an embolus in the distal blood vessel. Thus, embolization from a thrombus in an abdominal aortic atherosclerotic aneurysm may cause sudden occlusion of blood vessels supplying distal tissues/organs. Ulceration of an atheromatous plaque may become dislodged and produce so-called cholesterol crystal emboli, which look like needle-shaped cholesterol clefts in the affected tissues/organs, most often in the kidney.

ANEURYSM FORMATION

Complicated atheromatous plaque may extend into the media of large elastic arteries and weaken their walls resulting in aneurysm formation in abdominal aorta. The reduced elastin of the large elastic artery promotes thinning and ballooning of the vessel wall, while matrix metalloproteinases (MMPs) synthesized by macrophages and smooth muscle cells cause of breakdown collagen fibers.

- Such aneurysms contain mural thrombi, which may embolize to distant organs. Sudden rupture of the abdominal aneurysm has fatal outcome.
- The abdominal aortic aneurysms may be saccular, fusiform or cylindrical in shape, which may be demonstrated by ultrasonography and MRI. Patient presents with pulsatile mass abdomen, pressure exerted by aneurysm related symptoms, rupture into the cavities and thromboembolism.

DYSTROPHIC CALCIFICATION

Dystrophic calcification involving osteochondrocytic differentiation occurs in the central areas of necrosis in the atheromatous plaque. Oxidized lipids and inflammatory cytokines enhance vascular calcification.

- Dystrophic calcification in the vasculature depends on mineral deposition and resorption, which are regulated by osteoblast-like cells and osteoclast-like cells present in the vessel wall. Several transcriptional factors including RUNX2, MSX2, osterix and SOX9 promote development of osteoblast-like cells.
- Dystrophic calcification of atheromatous plaque may undergo vasospasm, which results in rupture of the atheromatous plaque.

RESTENOSIS OF CORONARY ARTERIES

Percutaneous transluminal coronary angioplasty (PTCA) is an important treatment in stenosed epicardial coronary artery disease as a result of atheromatous plaque. With a catheter-based approach, coronary arteries are inflated by a balloon catheter to dilate stenotic coronary artery, so that blood flow to the heart may be restored.

- Ballooning of coronary arteries causes endothelial damage and tear in the atheromatous plaque and media. Restenosis of coronary arteries may occur within 3–6 months in 30–40% of cases.
- Restenosis of coronary arteries occurs as a result of intimal hyperplasia, smooth muscle cell proliferation, extracelluar matrix deposition with or without organized thrombus on the luminal surface.
- Restenosis of coronary arteries is treated by using stents coated with biocompatibility polymers and biologically active agents. Drug-eluting stents with antiproliferative agents inhibit cell cycle progression and thus inhibit over proliferation of smooth muscle cells in the blood vessel.

CONSEQUENCES AND CLINICAL MANIFESTATIONS OF ATHEROSCLEROSIS

Consequences and clinical manifestations of atherosclerosis include cardiac manifestations (angina pectoris, myocardial infarction, sudden cardiac death, heart failure and chronic ischemic disease), brain manifestations (transient ischemic attacks, cerebral infarction), gangrene intestine, peripheral vascular disease and hypertension.

CARDIOVASCULAR MANIFESTATIONS

Angina pectoris is the most important manifestation of myocardial ischemia due to coronary artery disease. Coronary angiography revolutionized the diagnosis and management of coronary artery disease. Some persons present with chest pain, nausea, fatigue, shortness of breath, sweating and dizziness.

- Complicated atheromatous plaque involving >75% of vessel lumen can cause myocardial infarction and sudden cardiac death.
- Survivors may develop heart failure and chronic ischemic heart disease. Abdominal aorta below the origin of renal arteries is the most common site for atherosclerosis. Atheromatous plaque can undergo aneurysm formation in abdominal aorta that may rupture with fatal outcome.

CEREBROVASCULAR DISEASES

Internal carotid artery and circle of Willis are the most important sites of atheromatous plaque. Patient presents with transient ischemic attacks as a result of partial blockage of these vessels by atheromatous plaques. Complete occlusion of blood vessels supplying brain results in cerebral stroke/infarction. Clinical manifestations depend on the deprivation of blood supply to brain.

PERIPHERAL VASCULAR DISEASE

Popliteal, anterior tibial and posterior tibial arteries are sites of atheromatous plaque. Depending on the encroachment of lumen of the arteries, there may be partial or complete blockage. Partial blockage in the supplying blood vessels presents with intermittent claudication, i.e. cramps and pain in the leg. Complete occlusion of peripheral blood vessels presents with gangrene foot.

BOWEL ISCHEMIC NECROSIS (GANGRENE)

Celiac trunk, superior and inferior arteries are most sites for atherosclerosis. Complete occlusion of blood supply results in necrosis in the watershed regions of intestine. It is serious condition that can have fatal outcome. Patient presents with pain, tenderness and cramp in abdomen, passage of bright red or maroon blood in stool, nausea and diarrhea.

RENAL HYPERTENSION

Renal artery is also site for atherosclerosis. Decreased in blood supply to kidney activates renin-angiotensin-aldosterone system. Angiotensinogen synthesized by liver is converted to angiotensin II in lungs. Angiotensin II causes vasoconstriction and thus increases the peripheral resistance. Angiotensin II activates adrenal cortex to synthesize increased aldosterone production, that helps in the absorption of sodium and water in the kidney. Increased peripheral resistance and expansion of blood volume lead to renal hypertension.

THERAPEUTICS FOR ATHEROSCLEROSIS

HMG-CoA reductase inhibitors (statins lipid lowering agents) administration reduces cholesterol level by inhibiting cholesterol synthesis by liver. It is essential to treat the underlying major risk factors for atherosclerosis such as hyperlipidemia, hypertension, and diabetes mellitus. Daily intake of 75 mg aspirin minimizes thrombotic risk. Change of lifestyle advice, i.e. stopping tobacco smoking, healthier diet and increasing exercise also minimizes risk for atherosclerosis.

Clinical Pearls: Management of Atheromatous Plaque

- Major risk factors for atheromatous plaque include hyperlipidemia, hypertension, diabetes mellitus and tobacco smoking. It is essential to treat the underlying major risk factor. Patient is put on antihypertensive drugs, e.g. β-blockers and diuretics to control hypertension. Diabetes mellitus should be treated to maintain normal blood sugar level.
- Clinical management of atherosclerosis is based on minimizing its risk factors: hypertension is controlled by antihypertensive medication (e.g. diuretics, β-blockers), HMG-CoA reductase inhibitors, i.e. statins as lipid lowering agents, anticoagulants, i.e. 75 mg aspirin daily to minimize thrombotic risk; and lifestyle advice (e.g. stopping tobacco smoking, healthier diet and increasing exercise).
- Regular exercise reduces the risk of development of atheromatous plaque by maintaining elasticity of arteries and increasing high-density lipoproteins also reduces risk of development of diabetes mellitus and hypertension.
- HMG-CoA reductase inhibitors (statins-lipid lowering agents), i.e. administration reduces cholesterol level by inhibiting cholesterol synthesis by liver. HMG-CoA reductase (or 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase abbreviated as HMGCR) is the rate-limiting enzyme for cholesterol biosynthesis by liver. Normally, this enzyme is suppressed by cholesterol derived from the internalization and degradation of low density lipoproteins (LDL) via the LDL receptors. Competitive inhibitors of the HMG-CoA reductase induce the expression of LDL receptors in the liver, which in turn increases the catabolism of plasma LDL and lowers the plasma concentration of cholesterol, an important determinant of atherosclerosis.

ANEURYSMS

ANEURYSMS: OVERVIEW

Aneurysms are localized, abnormal permanent dilatation of cardiovascular tree that results from weakening of the wall of large elastic and medium size artery. Aneurysm can erode adjacent structures and cause pressure symptoms, pain, edema, rupture resulting in hypotensive shock and gangrene. The most important cause of aneurysm is an atheromatous plaque in the abdominal aorta. However, any abnormality that weakens the vessel media may predispose to an aneurysm.

Most common sites of aortic aneurysms and their etiology are given in Table 15.14.

Table 15.14 Most common sites of aortic aneurysms and their etiology

Site of Aneurysm	Etiology
Ascending aorta	Hypertension, syphilis
Descending aorta especially below the origin of renal arteries	Atherosclerosis
Aortic arch of aorta	Atherosclerosis
Popliteal artery	Atherosclerosis

Aneurysm is more common in splenic artery than hepatic artery.

Aneurysms may be congenital or acquired. These may be true or false. False aneurysm occurs due to trauma to intima and media. Aneurysms may take various shapes, i.e. saccular, fusiform, cylindrical, berry aneurysms, racemose/cirsoid (scalp), serpentine (torturous), arteriovenous fistula, and dissecting hematoma. Various shapes of aneurysm are described as under:

- **Saccular aneurysm:** It most often occurs in abdominal or thoracic aorta. The aortic wall bulges like a rounded sac-like structure. It throbs and pushes against surrounding structures. Mural thrombus is most often found within aneurysmal sac of saccular aneurysm.
- **Fusiform aneurysm:** It is most common type of aortic aneurysm assuming round and more tubular shape. The aorta is widened like a sausage for a few inches.
- **Cylindrical aneurysm:** Aortic aneurysm assumes cylindrical shape due to atherosclerosis (Figs 15.15 and 15.16).
- **Berry aneurysm:** It is small aneurysm located in the arteries of brain.
- **Dissecting hematoma:** It is not a true aneurysm and least common, but most painful type of aortic damage. Increased blood pressure and weakened wall split the layers of the aorta between the tunica intima (inner-most) and the tunica media (muscular layer). It is possible to have a dissecting hematoma of aorta without aneurysm.
- **Racemose/cirsoid aneurysm:** It is most often seen in scalp.
- **Serpentine aneurysm:** Aneurysm may assume serpentine (torturous) shape.
- **Arteriovenous fistula:** Arteriovenous fistula is formed secondary to trauma. Arteriovenous (AV) fistulas are abnormal communications (direct shunt) between arteries and veins.

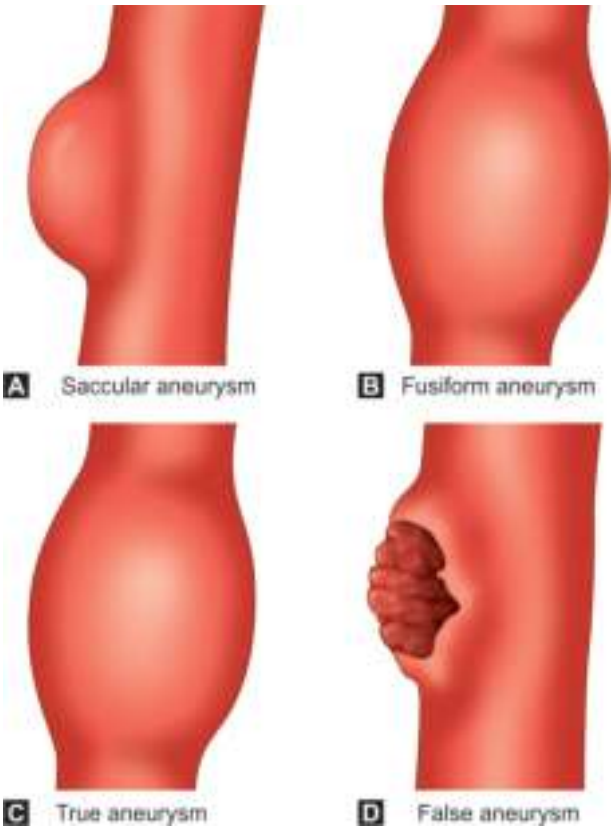


Fig. 15.15A to D: Shape of aneurysms.

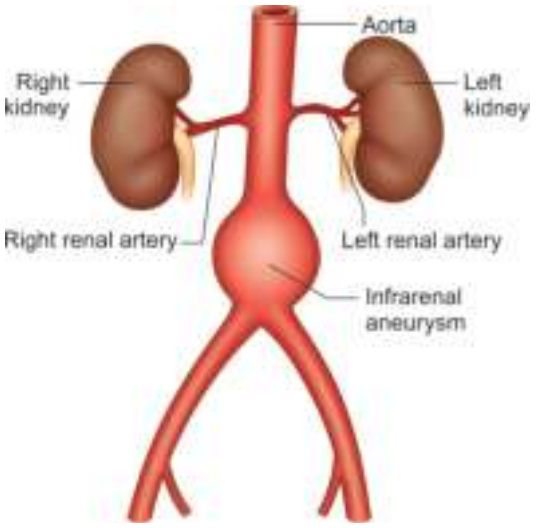


Fig. 15.16: Aneurysm in abdominal aorta due to atheromatous plaque.

ANEURYSMS: CAUSES

Several risk factors participate in the development of aneurysms, which include atheromatous plaques, smoking, congenital weakness of arterial wall (Marfan's syndrome or Ehlers-Danlos syndrome), inflammation (bacterial endocarditis), untreated syphilis and trauma.

ATHEROSCLEROTIC ANEURYSMS

Most important cause of aneurysm is an atheromatous plaque in the abdominal aorta. Major risk factors include hyperlipidemia, hypertension, diabetes mellitus and tobacco smoking. Males are more affected than females above 50 years of age.

Locations and Morphology

Atherosclerotic aneurysms occur in descending order, i.e. abdominal aorta below the origin of renal arteries (most common site), common iliac arteries and rarely aortic arch, which may take any shape, i.e. fusiform, cylindrical or saccular. Most abdominal aortic atherosclerotic aneurysms are fusiform in shape extending to involve common iliac arteries. Femoral or popliteal aneurysms are relatively uncommon.

Clinical Features

Patient presents with lower abdominal pain and dull backache, pulsatile abdominal mass, pressure symptoms, massive retroperitoneal hemorrhage and thromboembolism.

Imaging Technique

Atherosclerotic aneurysms are diagnosed by imaging techniques such as ultrasonography and MRI.

Blood Biochemistry

Serum C-reactive protein (CRP) is increased in patients with disrupted atheromatous plaques.

SYPHILITIC (LUTEIC) ANEURYSMS

Syphilitic (luteic) aneurysm is associated with tertiary syphilis. It typically affects the ascending arch of thoracic aorta resulting in dilatation and aortic valve insufficiency (Fig. 15.17).



Fig. 15.17: Syphilitic aneurysm of the arch of aorta. The aortic wall shows thickening and numerous wrinkled whitish plaques on the intimal surface. Patient may die due to rupture of the aneurysm (arrow).

Pathophysiology

Syphilitic aortitis shows obliterative endarteritis and periarteritis of the vasa vasorum.

- Vasa vasorum penetrate the outer- and middle-third of the aorta. In syphilitic aortitis, the vasa vasorum are surrounded by lymphocytes, plasma cells, and macrophages.
- Obliteration of the vasa vasorum causes focal necrosis, scarring of the media, and calcification, which results in disruption and disorganization of the elastic lamellae of aortic arch. Differences between atherosclerotic aneurysm and sphilitic aortitis are given in Table 15.15.

Table 15.15 Differences between atherosclerotic aneurysm and syphilitic aortitis

Parameters	Atherosclerotic Aneurysm	Syphilitic Aortitis
Etiology	▪ Hyperlipidemia ▪ Diabetes mellitus ▪ Hypertension ▪ Tobacco smoking	*Treponema pallidum* (tertiary syphilis)
Age group	More common >50 years	Young >20 years
Sex predilection	Males > females	Males and females equally affected
Site of blood vessel involved	Abdominal aorta below origin of renal arteries	Aortic arch of thoracic aorta
Morphology	Fibrofatty plaques measuring 0.3–1.5 cm in blood vessel affecting lumen	Obliterative endarteritis of vasa vasorum leads to necrosis of aortic media. Healing lesion in aorta gives tree bark appearance in intima due to fibrosis
Consequences	▪ Ulceration of atheromatous plaque ▪ Thrombus formation ▪ Embolism ▪ Dystrophic calcification ▪ Pressure symptoms on surrounding structures ▪ Atherosclerotic aneurysm rupture leads to fatal outcome	▪ Superior mediastinal compression ▪ Bronchial obstruction ▪ Aortic valve incompetence ▪ Massive cardiomegaly occurs known as cor bovinum ▪ Vertebral compression ▪ Recurrent laryngeal nerve paralysis of left site

Surgical Pathology: Syphilitic Aortitis

Gross Morphology

- The inner surface of the affected thoracic aorta shows a typical tree bark appearance as a result of fibrosis.
- Intima shows gray white, and gelatinous lesion.

Light Microscopy

- In cardiovascular syphilis, the vasa vasorum of the aortic arch show dense chronic inflammatory infiltrate mainly confined to the adventitia and the media.
- Obliterative endarteritis leads to infiltration by chronic inflammatory cells and replacement of the elastic layer muscle fibers by fibrous tissue causing the aortic wall to undergo weakening and dilatation due to inflammation of the wall of the ascending thoracic aorta, the coronary artery orifices narrowing and aortic valve incompetence.
- Aortic valve incompetence and coronary artery stenosis occur due to mesoarteritis of the proximal portion of the aorta and ischemic fibrosis of myocardium.
- Aortic arch is the most common site for syphilitic aneurysm.
- Tertiary syphilis may also involve other large elastic arteries and cerebral arteries.

Clinical Manifestations

- **Blood vessels involved:** *Treponema pallidum* involves aortic arch (most common) and abdominal aorta (less common).
- **Bronchial obstruction:** Syphilitic aneurysm may cause superior mediastinal compression and bronchial obstruction.
- **Hoarseness of voice:** Syphilitic aneurysm can cause paralysis of left recurrent laryngeal nerve, which is a branch of vagus nerve and found inferior to the aortic arch.
- **Vertebral erosion:** Syphilitic aneurysm may lead to painful vertebral erosion.
- **Cor bovinum:** Syphilitic aneurysm may cause aortic valve incompetence resulting in massive cardiomegaly known as '**cor bovinum**' (most common). Auscultation reveals '**Austin Flint murmur**' due to excessive blood dropping back from incompetent aortic valve onto the anterior mitral valve leaflet during diastole. This finding indicates the need for aortic valve replacement (Fig. 15.18).

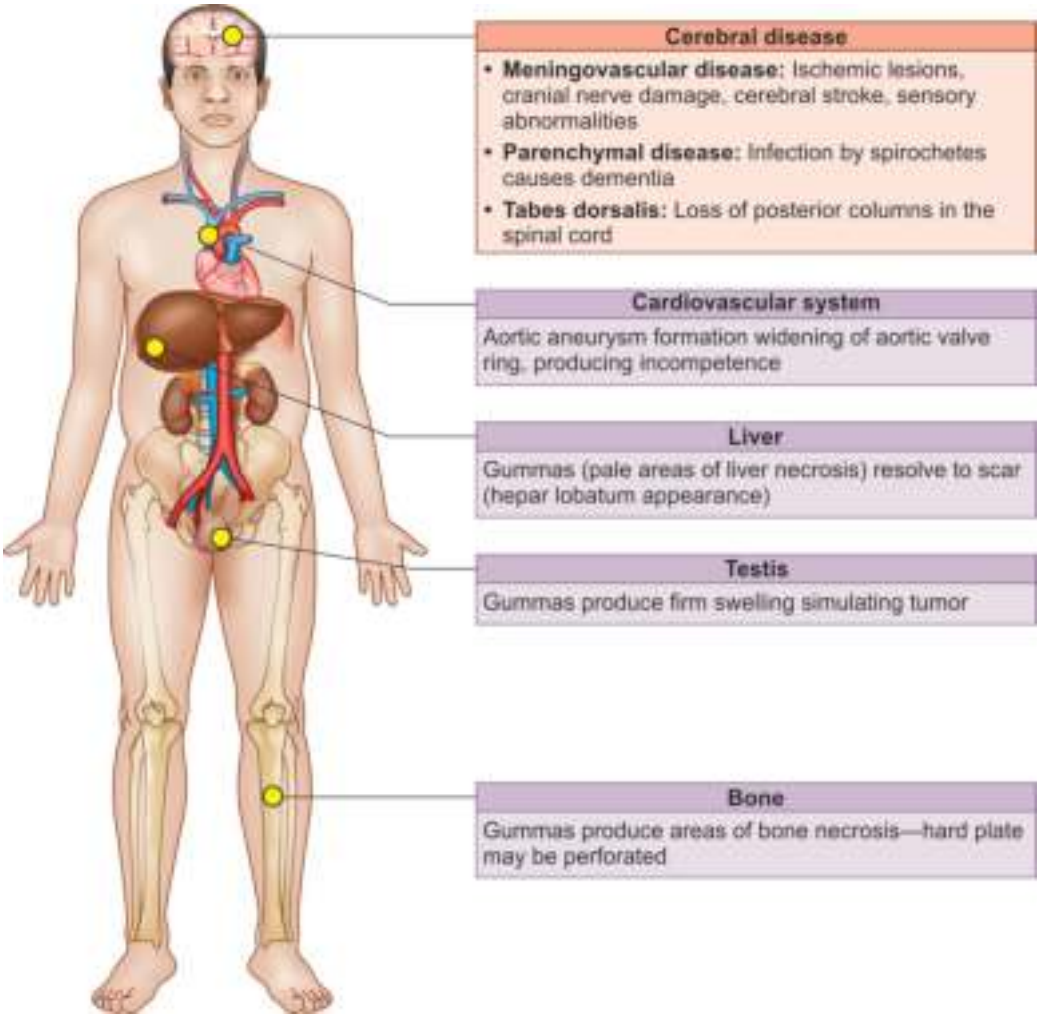


Fig. 15.18: Organs involved in tertiary syphilis.

AORTIC DISSECTING HEMATOMA

Aortic dissecting hematoma is associated with hypertension or diseases affecting the vascular media, most notably Marfan's syndrome (Figs 15.19 to 15.21).

Locations

Aortic dissecting hematoma usually occurs due to longitudinal tear in thoracic aorta and major branches; within 10 cm of the aortic valve.

- According to Stanford classification of aortic dissecting hematoma, type A involves either ascending thoracic aorta only or both ascending and descending thoracic aorta (most common and worst type); and type B affects descending thoracic aorta only usually distal to origin of left subclavian artery.
- According to DeBakey classification of aortic dissecting hematoma, type 1 involves ascending thoracic aorta and rest of aorta, type 2 affects ascending thoracic aorta only; and type 3 arises after ascending thoracic aorta. Blood dissects aorta under arterial pressure through the weakness of its wall.

Risk Factors

- **Hypertension:** It is the single most important factor responsible for aortic dissecting hematoma.
- **Atheromatous plaques:** Severe atheromatous plaque in abdominal aorta weakens aortic wall and produces aortic dissecting hematoma.
- **Traumatic injury:** Trauma to chest may cause tear in aorta.
- **Pregnancy:** Accumulation of proteoglycans rich extracellular matrix in aorta and increased blood volume weaken aortic wall in pregnant women.

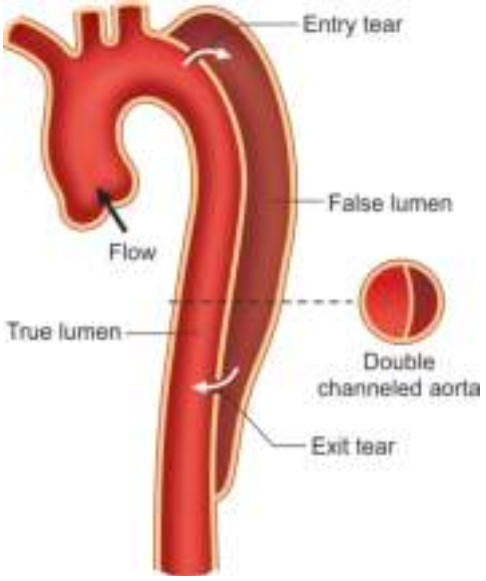


Fig. 15.19: Dissecting hematoma. Illustration shows splitting of aortic intima, entry of blood in false lumen and forming double barrel aorta.

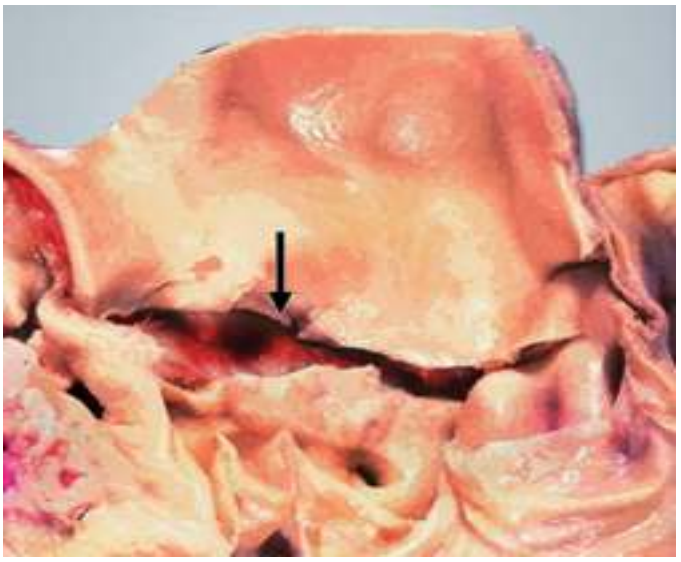


Fig. 15.20: This aortic dissection occurred just above the aortic root. The tear extends across the aorta. Hemopericardium with tamponade occurred within minutes of this event (arrow).

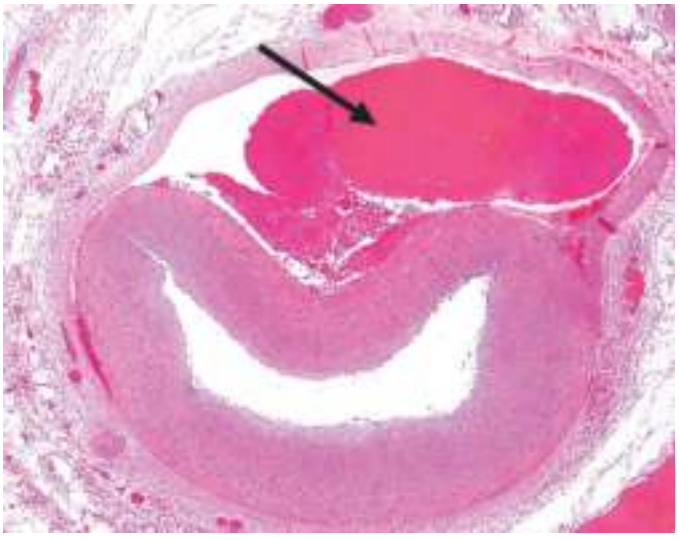


Fig. 15.21: Aortic dissection. It shows dissection extending into the muscular wall. Blood dissecting up around the great vessels can close off the carotids. Blood can dissect down to the coronaries and shut them off (arrow).

- **Marfan's syndrome:** It is an autosomal dominant disorder caused by missense mutations of the fibrillin gene 1 (FGN 1) located on chromosome 15. Fibrillin is a family of connective tissue proteins analogous to the collagen fibers. Defective cross-linkage collagen fibers weaken aortic wall. Mucin stain of the wall of the aorta demonstrates cystic medial necrosis.
- **Ehlers-Danlos syndrome:** Genetic defect in the synthesis of procollagen fibers in Ehlers-Danlos syndrome weakens the aortic wall. Mutation of lysyl hydroxylase gene is demonstrated in patient with Ehlers-Danlos syndrome.

- **Advancing age:** Blood vessels may undergo myxoid degeneration in advancing age.
- **Copper deficiency:** Copper is cofactor in lysyl oxidase, which takes part in collagen synthesis. Its deficiency leads to defective collagen synthesis which weakens the blood vessel.
- **Coarctation of aorta (wall stress):** It may result in aortic tear.

Pathologic Effects

Aortic dissecting hematoma may egress proximally and distally. It can cause vascular occlusion of branches arising from thoracic arch (i.e. loss of peripheral pulses), hemopericardium (i.e. retrograde spread), formation of second arterial lumen within the media (double-barreled aorta) and external rupture (retroperitoneal and mediastinal hemorrhage).

Clinical Features

- **Retrosternal pain:** Patient presents with severe tearing retrosternal chest pain often radiating to the back, which may be clinically confused with acute myocardial infarction. Abnormal electrocardiogram, increased serum troponin I and serum myocardial enzymes such as CK-MB and LDH indicate acute myocardial infarction (AMI).
- **Absence of pulse in subclavian artery:** There is absence of pulse due to compression of the subclavian artery.
- **Cerebral stroke:** It occurs due to carotid artery dissection.
- **Cardiac tamponade:** Aortic dissection resulting in cardiac tamponade has fatal outcome.

Imaging Techniques

The mediastinum is often widened and demonstrated by radiographic examination. Retrograde arteriography is a gold standard diagnostic tool.

BERRY ANEURYSM

The most common site of berry aneurysm is between the anterior communicating and the anterior cerebral arteries in the circle of Willis. These are not present at birth.

Shape of Aneurysm

Berry aneurysm develops as saccular lesions (1.0–1.5 cm) at site of congenital defects in smooth muscle distribution at a branch point of the arterial wall. It occurs in 10–15% of patients with adult polycystic kidney disease.

Risk Factors

Risk factors for berry aneurysm include hemodynamic stress, hypertension and coarctation of aorta.

Clinical Features

Rupture of berry aneurysm is the most common cause of subarachnoid hemorrhage. Patient presents with history of severe headache, nuchal rigidity from irritation of the meninges.

MYCOTIC ANEURYSM

Mycotic aneurysm occurs in the root of aortic arch and its descending branches, due to direct extension from aortic valve endocarditis.

Clinical Features

Emboli from infective endocarditis may involve cerebral blood vessels resulting in hemorrhage into the basal ganglia and extending into the subarachnoid space.

ANEURYSM OF SINUS OF VALSALVA

Aneurysm of sinus of Valsalva occurs due to lack of continuity between aortic media and fibrous ring of aortic valve resulting in thin paper-like aneurysm. It may be congenital or acquired.

Clinical Features

Patient presents with chest pain, heart failure, coarse cardiac murmur and thrill bounding pulse with fatal outcome. Arteriography establishes the diagnosis of aneurysm of sinus of Valsalva.

ARTERIOVENOUS FISTULA

Arteriovenous fistulas are often secondary to trauma. Arteriovenous (AV) fistulas are abnormal communications (direct shunt) between arteries and veins. Arteriography with thorostat dye establishes the diagnosis.

Etiology and Locations

Penetrating knife injury is the most common cause of arteriovenous fistula. Most common sites are scalp, arm and leg vessels. Veins are arterialized.

Clinical Features

Patient develops increased circumference of the limbs. There is presence of bruits and thrills. Large arteriovenous fistulas reduce systemic resistance and bypass the microcirculation, hence increasing venous return to the heart and producing high output cardiac failure.

CAPILLARY MICROANEURYSM

Capillary microaneurysm is also known as Charcot-Bouchard aneurysm. It is formed in intracerebral capillaries due to hypertension and diabetes mellitus.

Clinical Features

Patient may present with cerebral hemorrhage due to hypertension or retinal hemorrhage due to diabetic retinopathy. Table 15.16 shows clinical effects of aneurysms.

Table 15.16 Clinical effects of aneurysms

Types	Site and Mechanism	Clinical Effects
Atherosclerotic aneurysm	Thinning and fibrous replacement of media affecting lower abdominal aorta and iliac arteries	▪ Pulsatile abdominal mass ▪ Lower limb ischemia ▪ Rupture with massive retroperitoneal hemorrhage
Aortic dissection	Aortic and major branches	▪ Loss of peripheral pulses (e.g. radial artery) ▪ Hemopericardium ▪ External rupture (retroperitoneal hemorrhage)
Berry aneurysm	▪ Congenital defect(s) in elastic lamina/media ▪ Circle of Willis	Subarachnoid hemorrhage
Microaneurysms (Charcot-Bouchard)	Intracerebral capillaries	Intracerebral hemorrhage associated with hypertension
Syphilitic aneurysm	Inflammatory destruction of media and fibrous replacement affecting ascending arch of aorta	Aortic valve incompetence
Mycotic (infective) aneurysm	▪ Destruction of wall by root of aorta (direct extension from aortic valve endocarditis) ▪ Any vessel	Thrombus or rupture causing cerebral infarction or hemorrhage

VASCULITIS

VASCULITIS: OVERVIEW

Vasculitis is usually immune-mediated disorder, which affects arteries, arterioles, capillaries or venules that involves multi-systems with a predilection for highly vascular tissue such as skin, renal glomerulus, synovium and eye. Classification of the vasculitides is described in Table 15.17.

ETIOLOGICAL CLASSIFICATION

- **Immune-mediated vasculitis:** It is mediated by immune complex formation and complement

Table 15.17 Classification of the vasculitides

Examples	Diagnostic Features	Light Microscopy	Clinical Features
Large vessel vasculitides			
Giant cell (temporal) arteritis	**Three of five diagnostic criteria:** Age >50 years, recent localized headache, temporal artery tenderness, ESR 50 mm/hour and biopsy temporal artery shows vasculitis	Transmural granulomatous inflammation and giant cells	Recent localized headache, temporal artery tenderness
Takayasu arteritis (pulseless disease)	Aortic arch, carotid, subclavian arteries (most common), pulmonary arteries and abdominal aorta involved in men (50%), and in women under 30 years (>90%)	Granulomatous inflammation	Absence of pulse in carotid, radial or ulnar arteries, and visual disturbances

Contd...

Table 15.17 Classification of the vasculitides (*Contd...*)

Examples	Diagnostic Features	Light Microscopy	Clinical Features
Medium vessel vasculitides			
Polyarteritis nodosa	Necrotizing vasculitis weakens wall, aneurysm formation resulting in rupture associated with HBV (30–40%), intravenous amphetamines, sulfonamides and penicillin	Necrotizing vasculitis of medium- or small-sized arteries, segmental involvement with partial circumferential not associated with glomerulonephritis	Renal artery (85%), coronaries (75%), hepatic artery (75%), mesenteric arteries (65%), skeletal muscles arteries (40%), cerebral arteries, arteries supplying nerves, pancreas, and eye
Kawasaki disease	Large-, medium-to-small-sized arteries (especially coronaries)	Acute necrotizing vasculitis similar to polyarteritis nodosa	Febrile illness of childhood, self-limited acute vasculitis syndrome associated with mucocutaneous lymph node syndrome
Thromboangiitis obliterans	Tibial and radial arteries in heavy tobacco smokers. Segmental involvement with thrombus formation, sometimes involving nerves and veins of extremities	Vessel shows acute and chronic inflammation	Pain on claudication
Small vessel vasculitides (ANCA positive)			
Wegener's granulomatosis (ANCA positive)	Necrotizing vasculitis of arteries, capillaries and venules due to autoantibodies PR3-antineutrophilic cytoplasmic antibody (c-ANCA) against proteinase 3, MPO-ANCA and perinuclear ANCA (p-ANCA) against myeloperoxidase	Destructive nasal, lung and renal lesions	Clinical features related to organs involved
Microscopic polyangiitis (ANCA positive)	▪ Necrotizing vasculitis of capillaries, venules and arterioles involving glomeruli and lungs ▪ Immune-complex deposits (a few or absent)	Necrotizing vasculitis of glomeruli and lungs	Lung (most common) and renal manifestations
Churg-Strauss syndrome (ANCA positive)	Bronchial asthma, granuloma and eosinophilia	Granulomatous inflammation	Asthmatic attacks as a result of involvement of respiratory tract
Drug-induced small vessel vasculitides involving skin (ANCA positive)	Dermal post-capillary venules inflammation due to administration of penicillin or phenylbutazone	Acute or chronic inflammation	Skin rash 1–3 weeks after the drug therapy
Henoch-Schönlein purpura (ANCA negative)	IgA deposition occurs in vessel of skin often similar to IgA nephropathy. Other antigens may include drugs or foods	Vasculitis	Nephritic syndrome and skin rashes on extensor surfaces of the arms, legs and buttocks, painful joints, gastrointestinal involvement (colicky abdominal tract pain with melena)
Cryoglobulinemia vasculitis (ANCA negative)	Necrotizing vasculitis involving arterioles, capillaries, and venules. Glomeruli and small vessels of skin are involved in elderly population infected with hepatitis C infection. Serum cryoglobulin (IgM) is increased	Necrotizing vasculitis	Purpura of skin, arthralgia, and glomerulonephritis. Renal involvement can be life-threatening

Contd...

Table 15.17 Classification of the vasculitides (*Contd...*)

Examples	Diagnostic Features	Light Microscopy	Clinical Features
Diabetic vascular disease (ANCA negative)	▪ Microangiopathy in uncontrolled diabetes mellitus ▪ Hyperglycemia produce premature atherosclerosis, and microangiopathy resulting to kidneys, nerves and retina	Atheromatous plaque in large- and medium-sized arteries	Gangrene foot, renal failure and blindness
Raynaud's phenomenon (ANCA negative)	Arterial insufficiency of the digital vessels secondary to systemic lupus erythematosus and progressive systemic sclerosis. Raynaud's phenomenon may also occur in thromboangiitis obliterans, Takayasu's arteritis, cryoglobulinemia, ergot poisoning, thoracic outlet syndrome, and cold agglutinin diseases (IgM)	Vasculitis	Extreme cold temperatures and emotional stress may trigger color changes of the fingers from white to blue to red

activation. Examples of vasculitis are polyarteritis nodosa, Wegener's granulomatosis, systemic lupus erythematosus and rheumatoid arthritis. Vasculitis and antineutrophil cytoplasmic antibodies are given in Table 15.18.

- **Infective vasculitis:** It occurs due to bacteria (pyogenic bacteria, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, fungi (Candida, Aspergillus, and Mucormycosis), viruses (HBV, HCV, rubella, herpes zoster), and *Rickettsia rickettsii*.
- **Metabolic disorder:** Diabetic vascular disease occurs in uncontrolled diabetes mellitus.
- **Unknown etiology:** Vasculitis of unknown etiology includes cranial or giant cell (temporal) arteritis, Takayasu's arteritis (pulseless disease), thromboangiitis obliterans (Buerger's disease).

LARGE VESSEL VASCULITIDES

TEMPORAL (CRANIAL/GIANT CELL) ARTERITIS

Temporal (cranial/giant cell) arteritis is the most frequent form of granulomatous vasculitis, which primarily involves branches of the carotid artery, particularly the temporal artery. Involvement of the ophthalmic branch of the external carotid can lead to blindness. Aorta, its major branches and coronaries may also be affected.

Diagnostic Criteria

Three of five diagnostic criteria of temporal arteritis include: Age >50 years, recent localized headache, temporal artery tenderness, ESR 50 mm/hour and biopsy temporal artery showing vasculitis.

Table 15.18 Vasculitis and antineutrophil cytoplasmic antibodies

ANCA (Antineutrophil Cytoplasmic Antibody) Positive	ANCA (Antineutrophil Cytoplasmic Antibody) Negative
Wegener granulomatosis	Temporal arteritis
Microscopic polyarteritis	Takayasu arteritis
Polyarteritis nodosa	Glomerulonephritis
Systemic lupus erythematosus	Henoch-Schönlein purpura*
Idiopathic crescentic arteritis	Connective tissue diseases*
Kawasaki disease	Drug-induced vasculitis*
Churg-Strauss syndrome	

*ANCA (antineutrophil cytoplasmic antibody) rarely positive: *Henoch-Schönlein purpura, connective tissue diseases and drug-induced vasculitis.*

Pathogenesis

Exactly etiology is unknown, but type 4 cell-mediated hypersensitivity mediates this form of vasculitis.

Clinical Features

Patient presents with unilateral headache (most common) or claudication of the jaw, painful, palpable enlarged, tender temporal artery, morning stiffness in the neck and fever. Temporary or permanent impaired vision occurs due to involvement of ophthalmic artery.

Surgical Pathology: Temporal Arteritis
Light Microscopy
▪ Histologic examination of temporal arteritis shows transmural granulomatous inflammation of blood vessel, giant cells, thrombus formation, organization and intimal fibrosis, disrupted elastic lamellae, and marked intimal edema. ▪ Neovascularization in media is a useful indicator of previous inflammation. It can be quite focal, requiring serial sections of a small biopsy.

Laboratory Diagnosis

Elevated erythrocyte sedimentation rate (ESR) (>100 mm/hour) in conjunction with tender temporal artery is highly suggestive of cranial or giant cell arteritis (temporal arteritis).

Treatment

Treatment of patient with temporal arteritis is immediate institution of corticosteroid therapy to prevent blindness.

TAKAYASU ARTERITIS (PULSELESS DISEASE)

Takayasu arteritis involves branches of the aortic arch, i.e. carotid and subclavian arteries, pulmonary arteries and abdominal aorta (50% cases). Disorder is characterized by chronic progressive granulomatous inflammation and stenosis of aortic arch and its branches (carotid, subclavian arteries), resulting in aortic arch syndrome (Fig. 15.22).

Age and Sex Predilection

More than 90% of patients are women under 30 years of age. Takayasu arteritis may affect middle-aged women of Asian descent.

Pathogenesis

Type 4 hypersensitivity reaction is mainly responsible for Takayasu arteritis.

Clinical Features

Patient presents with absence of pulse in carotid, radial and ulnar arteries, visual disturbances, myocardial infarction due to involvement of coronary arteries and hypertension due to involvement of renal artery. Constitutional symptoms include fever, night sweats, malaise, myalgia, arthritis and arthralgia, ocular problems, and painful skin nodules.



Fig. 15.22: Takayasu's arteritis. It is common in Japan with unknown etiology. The aortic wall is thickened. Intima shows thick plaques. The walls of the innominate and common carotid arteries show thickening and narrowing. Consequences of occlusions lead to pulseless disease. Gross morphology and histopathological appearances are identical to syphilitic aortitis (arrow).

Surgical Pathology: Takayasu Arteritis
Gross Morphology
On gross examination, the aorta is thickened, and the intima exhibits focal, raised plaques.
Light Microscopy
Blood vessel shows granulomatous inflammation.

Diagnosis

Arteriography is a diagnostic tool for Takayasu arteritis.

Therapeutic Correlation

Patient with Takayasu arteritis is treated by bypass surgery.

MEDIUM VESSEL VASCULITIDES

POLYARTERITIS NODOSA (PAN)

Polyarteritis nodosa is systemic necrotizing vasculitis of medium- or small-sized arteries with segmental involvement or partial circumferential lesions and associated with glomerulonephritis, which leads to marked destruction of media and internal elastic lamella of arteries resulting in aneurysm formation.

- Small and medium size arteries undergo thrombosis, ulceration and infarction of organs. Affected blood vessel is firm and nodular that gives beaded appearance.
- Pathologic changes at different stages of disease process may show acute, chronic or healing stages.

Pathogenesis

Polyarteritis nodosa is mediated via type 3 immune complex mediated hypersensitivity through antigen–antibody complexes, in which antineutrophilic cytoplasmic autoantibodies are formed resulting in liberation of hydrolytic enzymes, which damage to mediam and small size arteries.

- Hepatitis B virus (30–40%) and intravenous amphetamines play an important role in its pathogenesis.
- Drugs, such as sulfonamides and penicillin, may form immunogenic hapten–protein complexes, which play key role in its pathogenesis.

Clinical Features

Patient presents with clinical manifestations due to involvement of renal arteries (85%), coronary arteries (75%), hepatic artery (75%), mesenteric arteries (65%), and arteries supplying skeletal muscles (40%), brain, nerves, pancreas and eyes (Fig. 15.23 and Table 15.19).

Laboratory Findings

Laboratory findings in polyarteritis nodosa include peripheral neutrophilic leukocytosis and eosinophilia, positive antineutrophil cytoplasmic antibodies with perinuclear staining (p-ANCA), and renal abnormalities (hematuria with RBC casts).

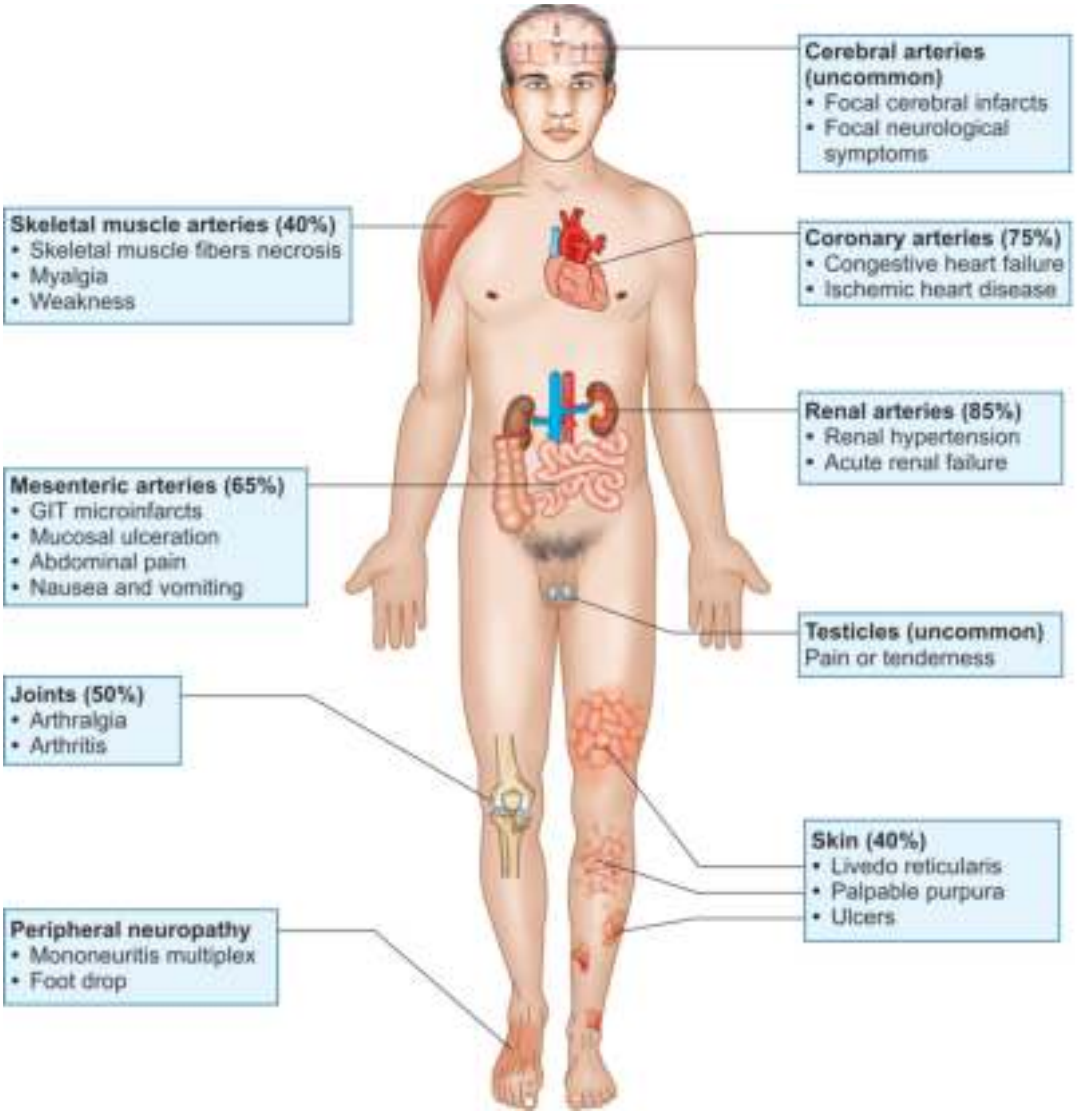


Fig. 15.23: Clinical manifestations of polyarteritis nodosa.

Table 15.19 Clinical manifestations of polyarteritis nodosa

Blood Vessels Affected	Percentage of Cases	Clinical Manifestations
Renal arterioles and glomeruli	85%	▪ Renal hypertension ▪ Acute renal failure due to microinfarcts
Coronary arteries	75%	▪ Ischemic heart disease ▪ Congestive heart failure
Mesenteric arteries	65%	Gut microinfarcts and mucosal ulceration, nausea, vomiting or abdominal pain
Skeletal muscle arteries	40%	Skeletal muscle fibers necrosis resulting in myalgia, weakness, arthralgia or arthritis
Cerebral arteries	Few cases	Focal infarcts with focal neurological symptoms

Diagnosis

Arteriography and biopsy of palpable nodular lesions in the skin and organ are analyzed to establish diagnosis of polyartertis nodosa.

Therapeutic Significance

Untreated polyarteritis nodosa disease is progressive, but most patients improve with corticosteroids and/or cytotoxic drug therapy.

Surgical Pathology: Polyarteritis Nodosa

Light Microscopy

- Lesion shows similar morphology as seen in polyarteritis nodosa, i.e. acute, chronic and fibrosis stages.
- Early lesion shows fibrinoid necrosis with thickening and infiltration of the vessel wall by neutrophils, eosinophils and lymphocytes.
- In mature lesion, vessel occlusion occurs secondary to intimal and mural fibrosis.

KAWASAKI DISEASE

Kawasaki disease is also known as mucocutaneous lymph node syndrome, which involves the coronary arteries. It is an acute febrile self-limited illness of children under the age of 10 years in Japan. Disorder is characterized by acute necrotizing vasculitis of small- and medium-sized vessels.

Clinical Features

Patient presents with fever, desquamating skin rash, mucosal inflammation (conjunctivae, lips, and oral mucosa) and cervical lymphadenopathy. Coronary artery vasculitis results in coronary thrombosis and aneurysm formation associated with fatal outcome.

THROMBOANGIITIS OBLITERANS

Thromboangiitis obliterans also known as Buerger's disease is acute disorder seen in young men especially in Jewish populations.

Blood Vessels Involved

Thromboangiitis obliterans involves arteries of extremities such as tibial, popliteal, and radial, extending to adjacent veins and nerves, i.e. neurovascular compartment.

Pathology

The arteries of the extremities in thromboangiitis obliterans undergo thrombosis resulting in painful gangrenous toes and fingers due to ischemia. Disorder is precipitated by heavy cigarette smoking. Blood vessels show thrombosis and microabscesses formation. Distal gangrene often requires amputation below knee joint.

SMALL VESSEL VASCULITIDES

Immunologic mechanism is responsible for most cases of small vessel vasculitides. Identification of autoantibodies in serum is used in diagnostic evaluation of patients with possible vasculitis.

- **Antineutrophil cytoplasmic antibody (ANCA):** ANCA IgG is formed against antigens, i.e. myeloperoxidase and proteinase 3 present in cytoplasm of neutrophils. Autoantibodies attack on small arteries, arterioles, capillaries and venules resulting in vasculitis. ANCA, i.e. p-ANCA (MPO-ANCA) and c-ANCA (PR3-ANCA) can be demonstrated by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence microscopy.
- **Antineutrophil cytoplasmic autoantibodies (c-ANCA):** Anti-proteinase 3 autoantibodies are formed against proteinase 3 antigen of cytoplasmic component of polymorphonuclear cells leading to subsequent release of hydrolytic enzymes and toxic oxygen-derived free radicals. Immunofluorescence study reveals cytoplasmic pattern, which correlates with anti-proteinase 3 (anti-PR3) antibody. It is more indicative of Wegener's granulomatosis.
- **Perinuclear antineutrophil cytoplasmic autoantibodies (p-ANCA):** Immunofluorescence study reveals perinuclear pattern of staining, which correlates

antimyeloperoxidase antibody (anti-MPO antibody). It strongly suggests microscopic polyarteritis. This pattern may also appear in other vasculitis as well as crescentic glomerulonephritis. Antinuclear antibody test is helpful in diagnosis of autoimmune disorder, i.e. systemic lupus erythematosus.

WEGENER'S GRANULOMATOSIS (ANCA POSITIVE)

Wegener's granulomatosis is a systemic necrotizing granulomatous vasculitis of unknown etiology, which involves respiratory tract such as nose, sinuses and lungs in 90–95% of cases. Patient develops destructive lesions in nasal cavity, lung and kidneys. It mainly affects 50–60 years of age group. Males are more affected than females. Disorder is usually positive for ANCA (Fig. 15.24A—histology and Fig. 15.24B—clinical features).

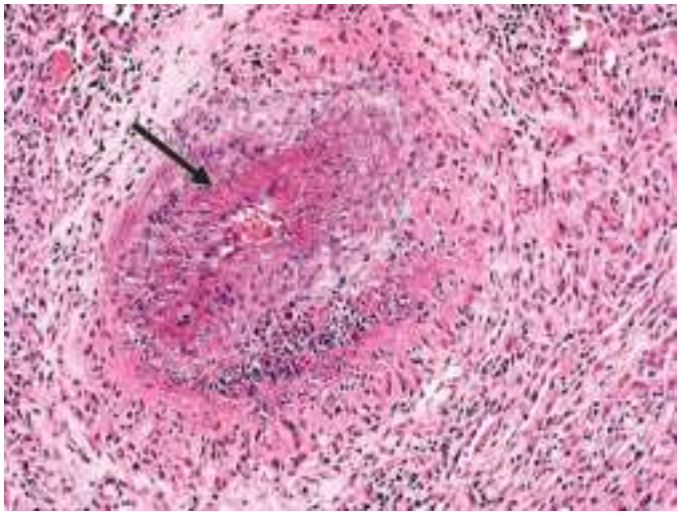


Fig. 15.24A: Wegener's granulomatosis. Blood vessel shows granulomatous inflammation (arrow) (100X).

Etiopathogenesis

Wegener's granulomatosis occurs due to inhalation of some infectious agents. PR3-antineutrophil cytoplasmic antibody (c-ANCA) against proteinase 3, MPO-ANCA and perinuclear ANCA (p-ANCA) autoantibodies against myeloperoxidase are formed. Immune complexes formed are deposited in small vessels of upper and lower respiratory tract, kidneys and other organs resulting in necrotizing vasculitis.

Clinical Features

Many organ systems are involved in Wegener's granulomatosis usually upper and lower respiratory tract (90–95%) and kidneys. Patient develops chronic

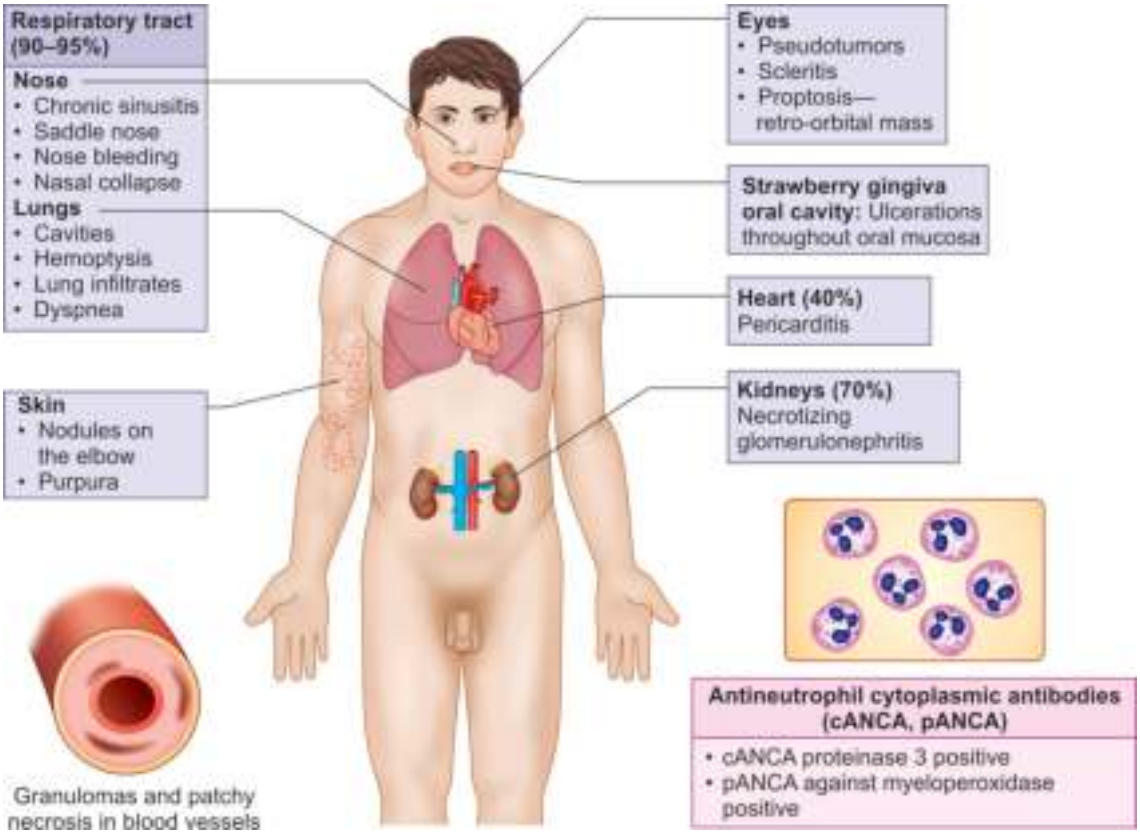


Fig. 15.24B: Clinical features of Wegener's granulomatosis.

Table 15.20 Clinical manifestations of Wegener's granulomatosis

Location	Lesions
Upper respiratory tract	Ulcerative lesions of nose, sinuses and pharynx; chronic sinusitis
Kidney	Chronic necrotizing glomerulonephritis
Lower respiratory tract	Necrotic areas and cavitation in lungs, cough, dyspnea, hemoptysis and chest pain

sinusitis (95%), saddle nose deformity, nasopharyngeal ulcers or pneumonias. Patient may also develop necrotizing glomerulonephritis (70%) resulting in renal failure, arthralgia due to joints involvement (60%), heart (40%), skin, eyes, and ears (Table 15.20).

Chest Radiograph

Chest radiograph may demonstrate bilateral nodular infiltrates or cavitary pulmonary lesions in patients with Wegener's granulomatosis.

Surgical Pathology: Wegener's Granulomatosis

Light Microscopy

- Histologic examination of involved tissue in Wegener's granulomatosis shows fibrinoid necrosis of small arteries and veins, early infiltration by neutrophils, subsequent macrophage cell infiltration, and fibrosis.
- Fibrosis occurs with chronic lesions.
- Granuloma formation with giant cells is prominent.

Treatment

Cyclophosphamide plus corticosteroids are useful in treating these patients. In untreated patients with Wegener's granulomatosis outcome is fatal within one year.

MICROSCOPIC POLYANGIITIS (ANCA POSITIVE)

Microscopic polyangiitis is necrotizing vasculitis involving glomeruli and pulmonary capillaries, which occurs most often in old age. Immune complex deposits may be few or absent.

Surgical Pathology: Microscopic Angiitis

Light Microscopy

- Histologic features are similar to polyarteritis nodosa.
- Early lesion shows fibrinoid necrosis with thickening and infiltration of the vessel wall by acute and chronic inflammatory cells.
- Immune complex deposits in the involved vessels are not common, but may be demonstrated only in a few cases. The p-ANCA is often positive.

CHURG-STRAUSS SYNDROME (ANCA POSITIVE)

Churg-Strauss syndrome is necrotizing vasculitis also known as allergic granulomatous angiitis, which involves pulmonary artery, coronary artery and skin vessels.

Pathogenesis

Types 1, 3, and 4 hypersensitivity reactions contribute to the pathogenesis of this form of vasculitis.

Clinical Features

Patient develops bronchial asthma and peripheral eosinophilia.

DRUG-INDUCED VASCULITIDES INVOLVING SKIN (ANCA POSITIVE)

Administration of penicillin or phenylbutazone causes vasculitis of post-capillary venules of skin.

Pathology

Dermal post-capillary venules show infiltration by polymorphonuclear cells, nuclear dusts due to karyorrhexis of neutrophils and leakage of RBCs leading to purpuric skin rashes. In some cases, blood vessels are mainly infiltrated by lymphocytes.

Clinical Features

Patient develops purpuric skin rash 1–3 weeks after the drug penicillin or phenylbutazone therapy.

HENOCH-SCHÖNLEIN PURPURA (ANCA NEGATIVE)

Henoch-Schönlein purpura (HSP) may be precipitated by exogenous antigens such as drugs, foods, or infectious organisms. HSP can develop after respiratory tract infections with streptococci (β-hemolytic, Lancefield group A) and viruses.

Pathology

IgA deposition occurs in small blood vessels of skin often similar to IgA nephropathy. Various drugs or foods may play role in the pathogenesis of Henoch-Schönlein purpura.

Clinical Features

Patient presents with nephritic syndrome and skin rashes on extensor surfaces of the arms, legs, and buttocks, painful joints, gastrointestinal tract involvement (colicky abdominal pain with melena).

CRYOGLOBULINEMIA VASCULITIS (ANCA NEGATIVE)

Cryoglobulinemia vasculitis is a necrotizing vasculitis, which involves arterioles, capillaries, and venules. Glomeruli and small blood vessels of skin are involved in elderly population infected with hepatitis C.

Clinical Features

Patient presents with purpuric skin rashes, arthralgia, and glomerulonephritis. Renal involvement can be life-threatening.

Laboratory Findings

Serum cryoglobulin (IgM) concentration is increased, that precipitates at 4°C and redissolves at 37°C. Complement C4 is decreased, while C3 is normal.

DIABETIC VASCULAR DISEASE (ANCA NEGATIVE)

In untreated patients with diabetes mellitus, hyperglycemia produces premature atherosclerosis, and microangiopathy involving vessels of kidneys and retina.

Complications

Complications of diabetic vascular disease include gangrene, renal failure and blindness.

Management

Effective control of diabetes reduces the incidence of renal and retinal disease.

RAYNAUD'S PHENOMENON (ANCA NEGATIVE)

Raynaud's phenomenon (ANCA negative) is arterial insufficiency of the digital vessels, which most often affects young healthy women.

Pathogenesis

Raynaud's phenomenon is most often secondary to systemic lupus erythematosus, progressive systemic sclerosis. It may also occur in thromboangiitis obliterans, Takayasu's arteritis, cryoglobulinemia, ergot poisoning, thoracic outlet syndrome, and cold agglutinin disease (IgM).

- Extreme cold temperatures and emotional stress may trigger color changes of the fingers from white to blue to red.
- CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) is a limited variant of progressive systemic sclerosis. It is associated with calcinosis of the fingertips. Patient develops Raynaud's phenomenon, esophageal motility disorder, sclerodactyly, telangiectasia, and a positive anti-centromere antibody.

Clinical Features

Patient presents with recurrent vasospasm of small arteries and arterioles leading to pallor or cyanosis, most often in the fingers and toes.

LYMPHOMATOID GRANULOMATOSIS

Lymphomatoid granulomatosis is a rare granulomatous vasculitis characterized by infiltration by atypical lymphocytoid and plasmacytoid cells.

Blood Vessels Involved

Lymphomatoid granulomatosis most often involves pulmonary vasculature but also has a predilection for vessels of the central nervous system, skin, liver, and kidneys.

Predisposing Factors

Lymphomatoid granulomatosis usually arises in the setting of immunosuppression and infection with Epstein-Barr virus. At present, it is thought to represent B cell neoplasm with an exuberant T cell response.

VENOUS SYSTEM

VARICOSE VEINS

Varicose veins and venous thrombosis together account for 90% of clinical venous disorders. A varicose vein is an enlarged and tortuous blood vessel. Varicose veins may occur in leg, esophagus (esophageal varices), anorectal region (hemorrhoids) and spermatic cord (varicocele).

VARICOSE VEINS IN LEGS

Varicose veins most often affect superficial veins of the lower extremities due to prolonged increased intraluminal pressure and loss of vessel wall support. Superficial varicosities of the leg veins, usually in the saphenous system, are among the most common ailments of humans due to upright posture.

Physiologic State

Superficial saphenous veins drain into deep saphenous veins) via communicating branches.

- Valves prevent blood flow from the deep into the superficial venous system except around the ankles, where blood flow is normally in that direction.

- Muscle contraction in the legs reduces hydrostatic pressure in the veins below the resting pressure, hence increasing the return of blood to the heart.

Pathologic State

- **Predisposing factors:** Prolonged standing in certain occupations, pregnancy, obesity, and inflamed deep vein thrombosis increase the venous pressure up to 10 times of normal resulting in varicose veins. Defective venous wall development is responsible for superficial varicosity in approximately 20% of general population. Individuals with normal veins may develop pedal edema (simple orthostatic edema) during prolonged standing or during journey.
- **Morphology:** Varicose veins are dilated and tortuous. Cut opened affected varicose veins reveal thrombus in lumen. Histologic examination shows variations in thickness as a result of hypertrophy of smooth muscle and subintimal fibrosis. Spotty dystrophic calcification is also demonstrated in varicose veins.
- **Clinical features:** Patient develops venous stasis, congestion, edema, pain, venous thrombosis, stasis dermatitis, ulceration, rupture, poorly healing wounds and superadded infections.

ESOPHAGEAL VARICES

Esophageal varices are dilated and tortuous veins in the submucosa of the lower esophagus in patients with cirrhosis developing portal hypertension. Esophageal varices commonly cause massive upper gastrointestinal hemorrhage (hematemesis—vomiting of blood), requiring emergency care to control hemorrhage and prevent hypovolemic shock.

HEMORRHOIDS

Hemorrhoids are dilatation of veins of the rectum and anal canal, which may occur inside or outside the anal sphincter.

- Hemorrhoids are aggravated by repeated pregnancy, straining at defecation and venous obstruction by rectal tumors.
- Thrombosis of hemorrhoids is exquisitely painful, may result in ulceration. Internal hemorrhoids typically bleed without pain, whereas external hemorrhoids typically cause pain but do not bleed.

VARICOCELE

A varicocele is a varicose dilatation of the veins draining the testis. Normally, veins draining testis and epididymis form the pampiniform plexus resulting in drainage into testicular veins. Left testicular vein directly opens into left renal vein, and the right into the inferior vena cava. A varicocele may develop as a result of poorly functioning valves that are normally found in veins.

Clinical Features

Most patients develop varicocele on the left side due to obstruction of testicular venous drainage due to spread of left-sided renal cell carcinoma into left renal vein. Patient presents with dragging discomfort. On palpation, with patient standing, the varicose plexus feels like a bag of worms.

DEEP VEIN THROMBOSIS (THROMBOPHLEBITIS)

Venous thrombosis occurs most often in the deep veins of the lower extremities due to stasis of venous blood especially in immobilized patients. Inflammation of veins with thrombus formation is known as thrombophlebitis.

PREDISPOSING FACTORS AND LOCATIONS

Cardiac failure, pregnancy, prolonged bed rest, or varicose veins cause venous circulatory stasis, which results in venous thrombus formation in the deep veins of lower extremities.

- Patient with cancers of pancreas, colon, or lung may develop venous thrombosis at one site and then disappear to be followed by thrombosis in other veins, is known as migratory thrombophlebitis (Trousseau sign).
- Pregnant women develop thrombosis in iliofemoral vein (painful white leg). Gravid uterus and hypercoagulable state during pregnancy initiate inflammation of thrombus with painful swelling. It is also known as plegmasia alba dolens.
- Venous thrombi are present in deep veins in the lower extremities below knee joint (most common in 90%); superior saphenous, periprostatic, pelvic (ovarian or uterine) veins.
- Less common sites of venous thrombosis include hepatic veins, renal veins, and dural sinuses.

CLINICAL FEATURES

- Patient presents with pain, tenderness and distal edema. In some cases, forced dorsiflexion of the foot resulting in pain is known as **Homan sign**.
- Deep vein thrombosis is entirely asymptomatic in 50% of affected patients. It is recognized only after thrombus is embolized to lung resulting in pulmonary hemorrhagic infarcts.

THERAPEUTIC CORRELATION

Anticoagulant therapy with heparin and warfarin prevents formation of venous thrombi.

THORACIC VENA CAVA SYNDROME

Superior vena cava syndrome is a collection of clinical signs and symptoms resulting from either partial or complete obstruction of blood flow through the superior vena cava. Thoracic vena cava syndrome occurs due to compression of superior vena cava or inferior vena cava. Depending on the involvement of vena cava, these are categorized into superior or inferior vena cava syndrome.

SUPERIOR VENA CAVA SYNDROME

Superior vena cava obstruction is caused by bronchogenic carcinoma in 90% of cases. Mediastinal lymphoma and cervical rib are less common causes.

Clinical Features

- Patient presents with dusky cyanosis, marked dilatation of veins of the face, arms, and shoulders giving purple discoloration.
- Compression of pulmonary veins may result in respiratory distress.
- Involvement of nerves results in neurologic manifestations.
- Visual disturbances occur due to congestion of retinal veins.
- The jugular veins are visibly distended resulting from raised jugular venous pressure.

INFERIOR VENA CAVA SYNDROME

Inferior vena cava syndrome may be caused by compression of inferior vena cava by a tumor, or propagation of thrombus from the iliofemoral vein, or growing of renal cell carcinoma as well as hepatocellular carcinoma into inferior vena cava, and occasionally into the right atrium.

Clinical Features

Due to obstruction of inferior vena cava, patient presents with marked pedal edema, distension of the superficial collateral veins of the lower abdomen. Involvement of renal vein may result in massive proteinuria.

LYMPHATIC SYSTEM

Lymphatic vessels have an incomplete basement membrane, hence predisposing them to infection and tumor invasion. Drainage of harmful pathogens to regional lymph nodes leads to reactive hyperplasia with enlarged, tender nodes. Lymphatic drainage of tumor emboli first occurs in the subcapsular sinus of draining lymph nodes.

ACUTE LYMPHANGITIS

Acute lymphangitis is inflammation of the lymphatic channels (red streak). It is most commonly secondary to group A β-hemolytic streptococci in majority of cases and *Staphylococcus aureus* to a lesser degree.

- Affected lymphatic channels are dilated and filled with inflammatory exudate, which extends through the wall and produce cellulites or focal abscess.
- Patient presents with painful red streaks in subcutaneous region along the course of inflamed lymphatic channels; and painful lymphadenopathy. Septicemia occurs due to spillage of bacteria into venous system.

LYMPHEDEMA

Lymphedema is an abnormal interstitial collection of lymphatic fluid due to obstruction to lymphatic flow. Increased concentration of proteins in lymphedema gives fibrogenic stimulus resulting in dermal fibrosis in chronic lymphedema. Lymphedema usually occurs due to secondary causes.

LYMPHEDEMA VARIANTS

Lymphedema is a chronic condition that occurs when lymph (also known as lymphatic fluid) pools in a person's legs, arms, hands, feet, or other body parts. Lymphedema is classified into primary and secondary forms.

- **Primary lymphedema:** It occurs due to congenital defect of lymphatic channels. It is known as **Milroy disease** or **heredofamilial lymphedema**. Other form of primary lymphedema is known as **lymphedema praecox** affects females of 10–25 years. Females develop slow onset edema feet extending to trunk resulting in superimposed infection or ulcerations.

- **Secondary lymphedema:** Lymphatic channels can be obstructed by (a) malignant neoplasms, (b) fibrosis resulting in inflammation, filariasis (elephantiasis) or irradiation, and (c) surgical ablation (axillary dissection of radical mastectomy). Breast cancer cells obstructing lymphatic channels produce peau d'orange of the breast in inflammatory carcinoma.

CHYLOUS EFFUSION

Chylous effusion is collection of lymphatic fluid (chylomicrons plus lymphocytes) in a body cavity. It is caused by rupture of obstructed lymphatic channels due to invasion by an infiltrating malignant lymphoma (most common) or trauma.

NEOPLASTIC DISORDERS OF BLOOD VESSELS

Vascular tumors of soft tissues occur in the deep subfascial soft tissue and/or subcutis. Some vascular tumors occurring in the dermis may involve deep soft tissue. Vascular tumors according to recent WHO classification of soft tissue are given in Table 15.21.

Table 15.21 Vascular tumors according to recent WHO classification of soft tissue

Reactive Vascular Endothelial Hyperplasia
▪ Papillary endothelial hyperplasia ▪ Pyogenic granuloma (lobular capillary hemangioma) ▪ Bacillary angiomatosis ▪ Glomeruloid hemangioma
Benign Tumors
▪ Capillary hemangioma ▪ Cavernous hemangioma ▪ Hemangioma of subcutaneous/deep soft tissue ▪ Angiomatosis ▪ Epithelioid hemangioma (angiolymphoid hyperplasia with eosinophilia) ▪ Intramuscular hemangioma ▪ Spindle cell hemangioma ▪ Lymphangioma
Intermediate (Borderline) Malignant Tumors
▪ Kaposiform hemangioendothelioma ▪ Dabska type (papillary intralymphatic angioendothelioma) and retiform hemangioendothelioma ▪ Kaposi sarcoma
Malignant Tumors
▪ Angiosarcoma ▪ Epithelioid hemangioendothelioma

REACTIVE VASCULAR PROLIFERATION

Reactive proliferation of vascular endothelial cells occurs due to infectious or paraneoplastic disorders. Reactive proliferation of vascular endothelial cells occurs in the cutaneous and mucosal regions. Variants of reactive vascular proliferations include papillary vascular endothelial hyperplasia, pyogenic granuloma, bacillary angiomatosis and glomeruloid hemangioma (Table 15.22).

PROGNOSIS AND TREATMENT

Prognosis is excellent in pyogenic granuloma treated by simple surgical excision. Bacillary angiomatosis is treated by antibiotics and correction of immunosuppressive state. Glomeruloid hemangioma is treated by simple surgical excision. It is difficult to treat polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes (**POEMS syndrome**).

Table 15.22 Variants of reactive vascular proliferations

Variants	Features
Papillary vascular endothelial hyperplasia	▪ Most common sites are superficial soft tissue of extremities especially thumb, head and neck region ▪ Patient presents with vascular endothelial hyperplasia in the superficial soft tissue of extremities especially thumb, head and neck region
Pyogenic granuloma	▪ Pyogenic granuloma is also known as lobular capillary hemangioma ▪ Most common sites are buccal mucosa and skin. It may affect any age group ▪ Patient presents with ulcerated polypoidal mass on the mucosal surface and skin
Bacillary angiomatosis	▪ Bacillary angiomatosis occurs in HIV positive persons and other immunosuppressive state ▪ Most common sites are mucosal surface and skin in the immunocompromised persons
Glomeruloid hemangioma	▪ Glomeruloid hemangioma occurs in patient with POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome. It occurs in elderly patients without sex predominance ▪ Patient presents with small, red to violaceous papules on proximal extremities and trunk

Surgical Pathology: Reactive Vascular Proliferations
Gross Morphology
Mucosal lesion shows polypoidal small red to blue mass. Skin biopsy shows erythematous lesions.
Light Microscopy
▪ **Papillary vascular endothelial hyperplasia:** Early lesion is composed of ingrowth of vascular endothelial cells into the thrombus with formation of pseudopapillae lined by plump endothelial cells. In the fully developed lesion, proliferation of vascular endothelial cells may create inter-anastomosing vascular channels simulating angiosarcoma. ▪ **Pyogenic granuloma:** It is well circumscribed and lobular lesion inolving large vessel in the central region and peripheral aggregates of well-formed capillaries. Lesion is infiltrated by acute and chronic inflammatory cells. ▪ **Bacillary angiomatosis:** It shows a vaguely lobular pattern. It is composed of capillary-sized vessels lined by endothelial cells. Stroma shows numerous neutrophils, fibrin and Bartonella bacilli demonstrated by Warthin-Starry stain. ▪ **Glomeruloid hemangioma:** It is composed of ectatic small vessels containing intraluminal papillae resembling glomeruli. Endothelial cells are vacuolated and containing eosinophilic proteinaceous material.
Differential Diagnosis
▪ **Pyogenic granuloma:** It should be differentiated from angiosarcoma, Kaposi sarcoma and vascular proliferation seen in other disorders. ▪ **Bacillary angiomatosis:** It should be differentiated from granulation tissue and Kaposi sarcoma. ▪ **Glomeruloid hemangioma:** It should be differentiated from papillary endothelial hyperplasia and Dabska tumor (low grade angiosarcoma).
Immunohistochemistry Technique
Vascular endothelial cells show positivity for CD31, CD34, Fli-1 protein and vWF.

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ Fli-1 protein	▪ Positive
▪ vWF	▪ Positive

CAPILLARY HEMANGIOMA

Capillary hemangioma is a regressing most common benign neoplasm of vascular endothelial cells either present at birth or during infancy in 15–20% of cases and children. Initially, it grows rapidly followed by stabilization and involution. Familial capillary hemangioma may show abnormality of chromosome 5.

LOCATIONS, AGE AND SEX PREDILECTION

Most common sites of capillary hemangioma are skin and mucosal surface followed by viscera. Large capillary hemangioma in infants on the head and neck region occurs as a part of **PHACES syndrome** (posterior fossa refers to possible abnormal structure in the brain especially cerebellum, hemangiomas of the cervicofacial region, abnormalities of arteries, cardiac and ocular regions). Lumbosacral capillary hemangioma occurs in infants with occult spinal malformations. Capillary hemangioma affects infants and children without sex predominance.

CLINICAL FEATURES

Patient with small superficial capillary hemangioma presents with red macules, usually level with the surface of the skin or slightly elevated (Fig. 15.25). Large capillary hemangioma appears as firm, blue-violet tortuous mass. Some capillary hemangiomas are associated with Sturge-Weber syndrome and von Hippel-Lindau syndrome.

- **Port-wine stain:** It is flat, red or purple benign lesion demonstrated on face of infants. It is composed of large vascular channels frequently interspersed with small capillaries.
- **Sturge-Weber syndrome:** It is characterized by port-wine stain of face, ipsilateral glaucoma, vascular lesions of choroidal tissue and extensive hemangiomatous involvement of meninges. Patient presents with convulsions, mental retardation and retinal detachment.
- **von Hippel-Lindau syndrome:** It is an autosomal dominant disorder characterized by multiple vascular tumors, other tumors and cysts widely scattered in many organ systems.

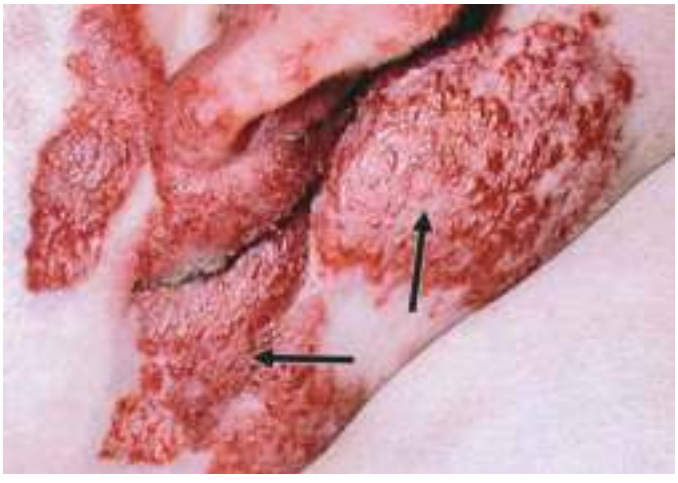


Fig. 15.25: Cutaneous hemangioma. Patient with small superficial hemangioma presents with red macules, usually level with the surface of the skin or slightly elevated (arrows).

PROGNOSIS AND TREATMENT

Treatment of capillary hemangioma depends on the age of patient, size of lesion and location. Large capillary hemangioma is treated by administration of interferon-α or glucocorticoids or both. Unresectable capillary hemangioma is treated by anti-angiogenesis agents.

Surgical Pathology: Capillary Hemangioma

Gross Morphology

- Capillary hemangioma is well circumscribed but lacking capsule. Gross morphology of capillary hemangioma varies depending on its size and location.
- Small-sized capillary hemangioma in the superficial region appears as red macules. Capillary hemangioma located in deep tissues appears as firm and blue violet mass.

Light Microscopy

- Capillary hemangioma is composed of closely packed aggregates of thin-walled capillaries usually filled with blood lined by single layer of bland endothelial cells. Each capillary is surrounded by scant connective tissue stroma.
- The lumen of capillaries may be partially or completely thrombosed and organized (Fig. 25.26).
- Juvenile capillary hemangioma in young children may be highly cellular and appearing solid with lumen formation due to protrusion of plump endothelial cells into the lumen and admixed with large number of pericytic cells. Mitoses may be demonstrated.
- In adults, lumen of capillaries is more obvious due to flattening of endothelial cells. Each capillary is surrounded by scant connective tissue stroma. Old lesions are usually fibrotic.

Differential Diagnosis

Capillary hemangioma should be differentiated from kaposiform hemangioendothelioma (KHE) and pediatric round cell tumors.

Immunohistochemistry Technique

Capillary hemangioma shows positivity for CD31, CD34, Fli-1, vWF and GLUT-1 (glucose transporter protein 1).

Markers	Expression
CD31	Positive
CD34	Positive
Fli-1	Positive
vWF	Positive
GLUT-1	Negative

CAVERNOUS HEMANGIOMA

Cavernous hemangioma is a benign tumor of endothelial origin, which tends to be larger than capillary hemangioma. It never undergoes regression and is less common than capillary hemangioma. Most common site of cavernous hemangioma is deep structures such as liver (most common), pancreas, spleen, placenta and brain. It affects young children with sex predominance.

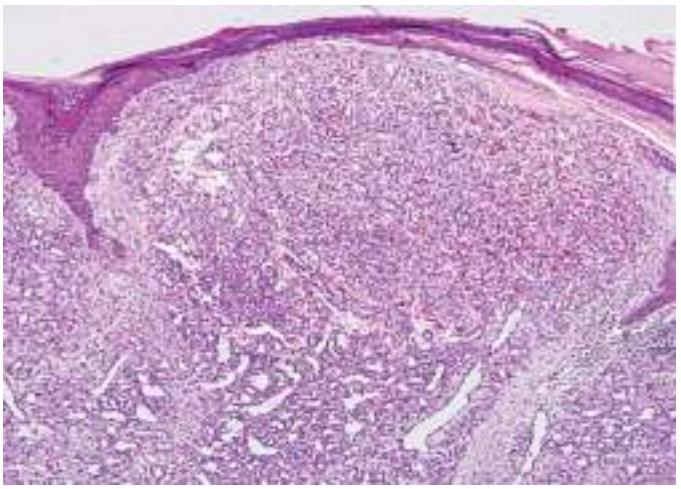


Fig. 15.26: Capillary hemangioma. Capillary hemangioma is composed of closely packed aggregates of thin-walled capillaries usually filled with blood lined by single layer of bland endothelial cells. Each capillary is surrounded by scant connective tissue stroma. The lumen of capillaries may be partially or completely thrombosed and organized (100X).

CLINICAL FEATURES

Cavernous hemangioma located in cutaneous region presents with irregular reddish tumor. Kasabach-Merritt syndrome is characterized by cavernous hemangioma and coagulopathy. Cavernous hemangioma located in skin and gastrointestinal tract has been as 'Blue bleb nevus syndrome'.

TREATMENT

Cavernous hemangioma never undergoes regression. It may produce destruction and compression of surrounding tissue. It does not respond to medicines. Surgical excision of tumor is curative.

Surgical Pathology: Cavernous Hemangioma

Gross Morphology

- Cavernous hemangioma located in skin shows irregular reddish lesion. Tumor located in the deep soft tissue especially in liver shows blue and nonpigmented.
- Cut surface shows sponge-like appearance.

Light Microscopy

- Cavernous hemangioma is composed of large dilated blood-filled inter-anastomosing vascular spaces lined by flattened endothelial cells resembling a sponge. Walls of the vessels are usually fibrotic.
- Thrombosis and calcification are most often demonstrated in the tumor. Thrombosis and recanalization in cavernous hemangioma may produce a sinusoidal pattern.

Differential Diagnosis

Cavernous hemangioma should be differentiated from angiosarcoma, spindle cell hemangioma and capillary hemangioma.

Immunohistochemistry Technique	
Capillary hemangioma shows positivity for CD31, CD34, Fli-1 and vWF. It is negative for GLUT-1 (glucose transporter protein 1).	
Markers	**Expression**
▪ CD31 ▪ CD34 ▪ Fli-1 ▪ vWF ▪ GLUT-1	▪ Positive ▪ Positive ▪ Positive ▪ Positive ▪ Negative

LYMPHANGIOMA

Lymphangiomas are malformations of lymphatic system characterized by formation of thin walled cysts. These can occur anywhere on the skin and mucous membrane.

- Lymphangiomas can be categorized as deep or superficial based on the size and depth of the abnormal lymphatic vessels or congenital or acquired.
- Superficial form of lymphangioma is lymphangioma circumscriptum also known as **lymphangiectasia**.
- Deep forms of lymphangioma include two specific congenital entities: cavernous lymphangioma and cystic hygroma.

LOCATIONS

Lymphangioma in skin is also called **lymphangioma circumscriptum**. Most common sites of cystic lymphangioma are neck, axilla and groin regions. Cavernous lymphangioma most often occurs in the oral cavity, upper trunk region, vulva and conjunctiva. Lymphangioma affects pediatric age group especially during first year of life. It may affect any age group without sex predominance.

PATHOPHYSIOLOGY

Congenital lymphangioma is formed due to the blockage of the lymphatic system during fetal development, though its cause remains unknown.

- Cystic hygromas are associated with genetic disorders including trisomies 13, 18 and 21; Noonan syndrome, Turner syndrome and Down syndrome.
- Acquired lymphangioma circumscriptum occurs in association with chronic lymphedema that leads to disruption of previously normal lymphatic drainage such as surgery, trauma, malignancy and radiation therapy.

CLINICAL FEATURES

Clinically, lymphangioma circumscriptum appears as multiple, grouped or scattered, translucent or hemorrhagic vascular papules.

- Cystic hygromas are lymphatic malformations that are clinically more circumscribed than cavernous lymphangioma and typically occur as cystic lump in the neck, axilla and groin regions. It may extend into the mediastinum in children compress lungs, heart and nerves.
- MRI can be useful in determining the extent of anatomical involvement of cystic hygroma or cavernous lymphangioma.

PROGNOSIS AND TREATMENT

Lymphangioma is not encapsulated tumors. It is treated by surgical excision. Recurrence may occur due to incomplete surgical excision.

Surgical Pathology: Lymphangioma	
Gross Morphology	
Multicystic spongy mass of lymphangioma is not encapsulated and containing watery/milky fluid.	
Light Microscopy	
▪ Lymphangioma is composed of large irregular vascular spaces lined by flattened bland endothelial cells with fibroblastic or collagenous stroma. Surrounding stroma may contain lymphoid aggregates. ▪ Abdominal lymphangioma may show marked inflammatory changes that obscure its lymphatic origin. Histology of cavernous lymphangioma is shown in Fig. 15.27.	
Immunohistochemistry Technique	
Lymphangioma shows positivity for CD31, CD34, D2–40 (podoplanin), PROX1, Fli-1 and CD141.	
Markers	**Expression**
▪ CD31 ▪ CD34 ▪ D2–40 (podoplanin) ▪ PROX1 ▪ Fli-1 ▪ CD141	▪ Positive ▪ Positive ▪ Positive ▪ Positive ▪ Positive ▪ Positive

SPINDLE CELL HEMANGIOMA

Spindle cell hemangioma (SCH) was originally described as spindle cell hemangioendothelioma. Later, it was believed to be low-grade angiosarcoma. Now, it has been reclassified by World Health Organization as spindle cell hemangioma.

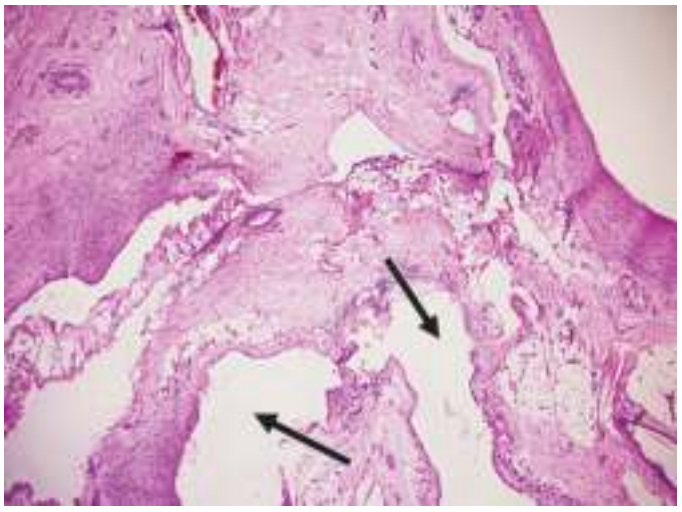


Fig. 15.27: Cavernous lymphangioma. It is composed of large irregular vascular spaces lined by flattened bland endothelial cells with fibroblastic or collagenous stroma (arrows). Surrounding stroma may contain lymphoid aggregates. Abdominal lymphangioma may show marked inflammatory changes that obscure its lymphatic origin (400X).

LOCATIONS

Spindle cell hemangioma is a benign possibly reactive endothelial tumor associated with Maffucci syndrome in 5% of cases. Most common site of spindle cell hemangioma is dermis or subcutis of distal extremities. It affects young adults without sex predominance.

CLINICAL FEATURES

Patient presents with violaceous nodule in dermis or subcutis of distal extremities. Maffucci syndrome is characterized by hemangiomas and endocrinopathies in 5% of cases. Spindle cell hemangioma is treated by surgical excision. It may recur through propagation of along blood vessel.

Surgical Pathology: Spindle Cell Hemangioma

Gross Morphology

Spindle cell hemangioma is small solitary mass in the dermis or cutis.

Light Microscopy

- Spindle cell hemangioma most often shows organizing thrombi, calcification and phleboliths. It is composed of two zones diagnostic of spindle cell hemangioma.
  - First zone shows thin-walled dilated blood vessels, reminiscent of cavernous hemangioma.
  - Second zone shows proliferation of spindle cells with normochromatic nuclei and eosinophilic cytoplasm, creating slit-like vascular spaces and vacuolated epithelioid cells indicating of spindle cell tumor.
- Mitoses and necrosis are not demonstrated in the tumor. Absence of chronic inflammatory cells helps in distinguishing spindle cell hemangioma from Kaposi sarcoma.

Differential Diagnosis

Spindle cell hemangioma should be differentiated from Kaposi sarcoma, kaposiform hemangioendothelioma and angiosarcoma.

Immunohistochemistry Technique

Spindle cell hemangioma shows positivity for CD31, CD34, desmin and smooth muscle actin (SMA).

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ Desmin	▪ Positive
▪ SMA	▪ Positive

DEEPLY LOCATED HEMANGIOMA INCLUDING ANGIOMATOSIS

Deeply located hemangioma is a benign progressive vascular tumor. Angiomatosis must involve multiple tissue planes or skeletal muscles.

LOCATIONS

Most common sites of deeply located hemangioma are synovium and nerves. Deeply located hemangioma affects any age group without sex predominance. On the other hand, angiomatosis can involve multiple planes (e.g. skin, subcutis, muscle, bone) or multiple skeletal muscles in a contiguous fashion.

CLINICAL FEATURES

Patient presents with large nonspecific lump simulating sarcoma. Deeply located hemangioma and angiomatosis are treated by surgical excision.

Surgical Pathology: Deeply Located Hemangioma including Angiomatosis

Gross Morphology

Gross morphology of deeply located hemangioma depends on the relative proportion of fat, capillary hemangioma and cavernous-like appearance.

Light Microscopy

- Deeply located hemangioma may show histologic features of both capillary and cavernous hemangiomas; and large malformed blood vessels resembling both arteries and veins.
- Deeply located hemangioma in the skeletal muscle most often splays apart the skeletal muscle in a checkerboard pattern and contain adipose tissue. Presence of adipose tissue poses difficulty in determination of surgical margin status.

Differential Diagnosis

Deeply located hemangioma and angiomatosis should be differentiated from epithelioid hemangioendothelioma and epithelioid angiosarcoma.

Immunohistochemistry Technique

Deeply located hemangioma shows positivity for CD31, CD34, Fli-1 and vWF. It is negative for GLUT-1 (glucose transporter protein 1).

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ Fli-1	▪ Positive
▪ vWF	▪ Positive
▪ GLUT-1	▪ Negative

KAPOSIFORM HEMANGIOENDOTHELIOMA

Kaposiform hemangioendothelioma is an intermediate (borderline) malignant vascular tumor. Kasabach-Merritt syndrome is characterized by kaposiform hemangioendothelioma, thrombocytopenia and hemorrhage. Most common sites are extremities in dermis and cutis; and retroperitoneum. It usually affects in the first year of life.

CLINICAL FEATURES

Patient presents with violaceous plaque in the dermis or subcutis of extremities; and large vascular tumor in the retroperitoneal region.

TREATMENT

Kaposiform hemangioendothelioma is a locally aggressive borderline malignant tumor. Complete excision of the tumor is difficult. There is little risk of metastases to distant organs. **Kasabach-Merritt syndrome** is characterized by kaposiform hemangioendothelioma, thrombocytopenia and hemorrhage resolve with complete excision of the tumor.

Surgical Pathology: Kaposiform Hemangioendothelioma

Gross Morphology

Kaposiform hemangioendothelioma located in the skin appears as large violaceous plaques; and large nodular infiltrative vascular tumor in retroperitoneal region associated with fibrosis.

Light Microscopy

- Tumor is composed of dilated lymphatic channels at the periphery of tumor resembling lymphangioma. It shows crescentic vascular spaces identical to angioma.
- Tumor shows admixture of well-formed capillary-sized vessels resembling angioma; and solid appearing foci with spindled and slit-like vascular spaces simulating Kaposi sarcoma. Glomeruloid foci contain small thrombi.

Differential Diagnosis

Kaposiform hemangioendothelioma should be differentiated from capillary hemangioma and Kaposi sarcoma.

Immunohistochemistry Technique

Kaposiform hemangioendothelioma shows positivity for CD31, CD34, Fli-1 and vWF. It is negative for GLUT-1 (glucose transporter protein 1).

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ Fli-1	▪ Positive
▪ vWF	▪ Positive
▪ GLUT-1	▪ Negative

DABSKA-TYPE PAPILLARY INTRALYMPHATIC ANGIOENDOTHELIOMA AND RETIFORM HEMANGIOENDOTHELIOMA

Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma are vascular tumors of intermediate malignancy defined by the presence of distinctive 'hobnailed' endothelial cells, most likely exhibiting lymphatic endothelial differentiation. Both entities show overlapping histologic features.

- Most common sites are extremities in the dermis and cutis regions; and head and neck regions. Both entities affect infants and young children without sex predominance.
- Patient presents with ill-defined and sometimes violaceous and plaque-like lesions in the extremities; and head and neck regions. Both Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma are locally aggressive intermediate malignant vascular tumors. Wide surgical excision of tumor is curative.

Surgical Pathology: Dabska-type Papillary Intralymphatic Angioendothelioma and Retiform Hemangioendothelioma

Gross Morphology

Both Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma are ill-defined violaceous lesions.

Light Microscopy

Both Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma are composed of distinctive 'hobnailed' endothelial cells with scant cytoplasm and irregular

hyperchromatic nuclei. There is presence of lymphocytic infiltrate and dense fibrosis in the lesions.

- **Dabska-type papillary intralymphatic angioendothelioma:** It is composed of relatively well-formed blood vessels with intraluminal papillae. The intraluminal papillae are composed of hyaline core and 'hobnailed' endothelial cells. There is presence of dilated lymphatic channels near lesion. Few mitoses are demonstrated.
- **Retiform hemangioendothelioma:** It shows infiltrative proliferation of elongated branching blood vessels lined by 'hobnailed' endothelial cells. The intraluminal papillae are composed of hyaline core and 'hobnailed' endothelial cells identical to Dabska-type papillary intralymphatic angioendothelioma. Few mitoses are also demonstrated.

Differential Diagnosis

Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma should be differentiated from tumors showing hobnailed endothelial cells and intraluminal papilla, e.g. hemangioma and angiosarcoma.

Immunohistochemistry Technique

Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma show positivity for CD31, CD34, Fli-1, D2–40 and VEGFR3. It is negative for vWF.

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ Fli-1	▪ Positive
▪ D2–40	▪ Positive
▪ VEGFR3	▪ Positive
▪ vWF	▪ Negative

EPITHELIOID HEMANGIOENDOTHELIOMA

Epithelioid hemangioendothelioma is a low-grade malignant vascular tumor of adult life, which is characterized by an 'epithelioid' or histiocytoid' endothelial cells. Tumor arises from small blood vessel in skin, deep soft tissue, bone, lung and liver. It may rarely present as an intravascular neoplasm. It affects children and adults.

- Patient presents with vascular thrombosis and painful lump in superficial or deep soft tissue. Patient with bone lesion presents with asymptomatic osteolytic lesion. Involvement of lung and liver by tumor may be asymptomatic.
- Epithelioid hemangioendothelioma is treated by wide surgical excision. Adjuvant radiotherapy may be in selected cases. Hepatic epithelioid hemangioendothelioma should be treated by liver transplantation.
- Tumor may metastasize to distant sites in 17% of cases with poor prognosis. Classic low-grade and high-grade epithelioid hemangioendotheliomas have risk of local recurrence and distant metastasis.

Surgical Pathology: Epithelioid Hemangioendothelioma

Gross Morphology

Epithelioid hemangioendothelioma neoplasm excised from superficial and deep soft tissue is white, firm and sometimes calcified. Lesion from lung may show thrombosed blood vessel.

Light Microscopy

- Epithelioid hemangioendothelioma is composed of small uniform rounded or slightly spindled eosinophilic endothelial cells with rounded nuclei and prominent cytoplasmic vacuolization. The latter feature probably represents primitive lumen formation by single cell.
- The tumor cells are arranged in small nests or cords or single file-array and only line well-formed vascular channels embedded in dense myxochondroid or myxohyaline matrix. Necrotic debris, hyaline collagen and thrombosed material may be demonstrated within the blood vessel (Fig. 15.28).
- Approximately 30% of epithelioid hemangioendothelioma shows anaplastic features, i.e. high nuclear grade, numerous atypical mitoses and necrosis with aggressive clinical course referred as malignant or high grade epithelioid hemangioendothelioma.

Differential Diagnosis

Epithelioid hemangioendothelioma should be differentiated from epithelioid hemangioma, epithelioid angiosarcoma, and metastatic carcinoma especially lobular carcinoma of breast.

Immunohistochemistry Technique

Epithelioid hemangioendothelioma shows positivity for CD31, CD34, Fli-1, vWF and low molecular weight cytokeratins (low molecular weight) (25%). CD34 positivity is shown in Figs 15.29 and 15.30.

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ Fli-1	▪ Positive
▪ vWF	▪ Positive
▪ Cytokeratins (LMW)	▪ Positive

KAPOSI SARCOMA

Kaposi sarcoma is a locally aggressive vascular neoplasm. It occurs in the four clinicopathological settings (Table 15.23).

- **Classic Kaposi sarcoma:** It affects elderly males of East European and Mediterranean. Most common site is lower extremities in the subcutaneous region. Disease has indolent course.

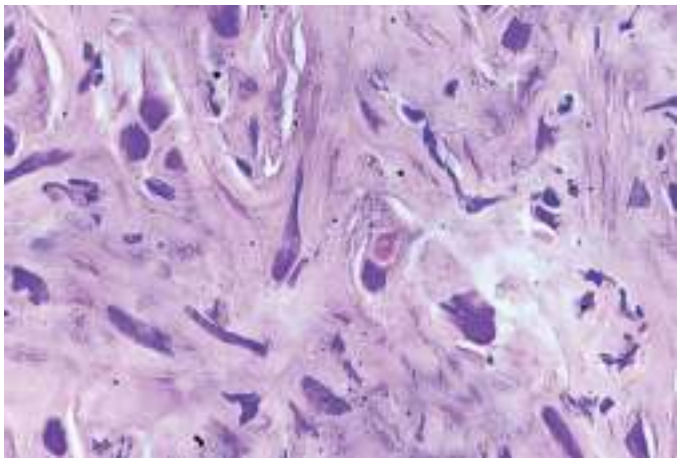


Fig. 15.28: Epithelioid hemangioendothelioma of liver. Tumor is composed of small uniform round and spindled endothelial cells with round nucleus, vacuolated cytoplasm and well-formed vascular channels, arranged in small nests or single file pattern embedded in dense myxochondroid or myxohyaline matrix (400X).

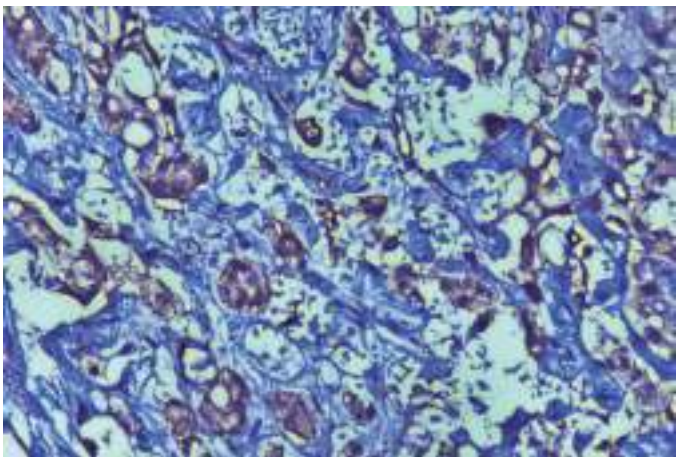


Fig. 15.30: Epithelioid hemangioendothelioma of liver. Vascular endothelial cells of epithelioid hemangioendothelioma of liver demonstrate immunohistochemical marker CD34 positivity (400X). It is a multifocal vascular tumor in liver of childhood most often first year of life.

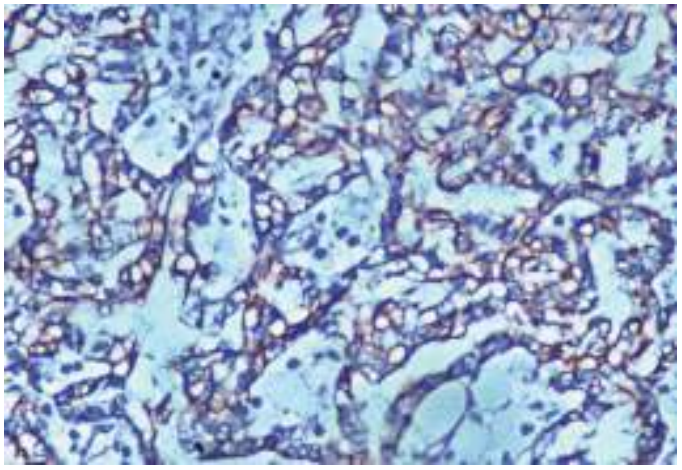


Fig. 15.29: Epithelioid hemangioendothelioma of liver. Vascular endothelial cells of the neoplasm demonstrate immunohistochemical marker CD34 positivity (400X).

- **Endemic (African) Kaposi sarcoma:** It affects young African males. Patient presents with bulky lymphadenopathy and soft tissue tumor. Disease has indolent clinical course in adults; and aggressive clinical course in African children with bulky lymphadenopathy.
- **Iatrogenic Kaposi sarcoma:** It occurs in renal transplantation cases, who develop cutaneous or visceral lesions. Disease has unpredictable clinical course. Lesions resolve on withdrawal of immunosuppressive therapy.
- **Epidemic AIDS-related Kaposi sarcoma:** Most common sites of epidemic AIDS-related Kaposi sarcoma are mucosal surfaces, skin, face, lower extremities, visceral and cutaneous lesions in HIV infected homosexual or bisexual persons.

Table 15.23 Clinicopathological types of Kaposi sarcoma

Kaposi Sarcoma Type	Clinical Groups	Location of Lesion	Clinical Course
Classic Kaposi sarcoma	Elderly males of East European and Mediterranean descent	Skin of lower extremities in the subcutaneous region	Indolent clinical course
Endemic (African) Kaposi sarcoma	Children and middle-aged males from equatorial Africa	Skin of lower extremities; and possibly viscera (bulky lymph nodes in children) and soft tissue tumor	Indolent clinical course in adults; and aggressive clinical course in African children with bulky lymphadenopathy
Iatrogenic Kaposi sarcoma	Immunocompromised persons (e.g. post-renal transplantation patients)	Skin of lower legs; and possibly viscera	Unpredictable clinical course; lesions resolve on withdrawal of immunosuppressive therapy
Epidemic (AIDS related) Kaposi sarcoma	Homosexual or bisexual males with HIV infection	Widespread involvement of skin, face, lower extremities, genitalia; and widespread involvement of viscera (e.g. lungs, lymph nodes, gastrointestinal tract, oral mucosa)	Aggressive clinical course

CLINICAL COURSE

Patient with Kaposi sarcoma presents with cutaneous violaceous patch, plaque, papule, macule or tumor mass. Lymphedema is demonstrated in occasional patient. Involvement of viscera and lymph nodes presents with hemorrhagic mass.

- Kaposi sarcoma is treated by cryotherapy and radiotherapy. Prognosis is good in immunocompetent patients with cutaneous lesions.
- Prognosis is poor in immunocompromised patients without high active antiretroviral therapy (HAART). Prognosis can be improved by administration of antiretroviral HAART and chemotherapy.

Surgical Pathology: Kaposi Sarcoma

Gross Morphology

- Cutaneous lesions in Kaposi sarcoma are variable, i.e. violaceous patch, plaque, papule, macule or tumor mass.
- Visceral and lymph nodes involved in Kaposi sarcoma are large hemorrhagic mass.

Light Microscopy

Histologic features of Kaposi sarcoma are always identical regardless of clinical settings.

- **Patch stage in Kaposi sarcoma:** Tumor is composed of small infiltrative blood vessels lined by endothelial cells surrounding large ectatic blood vessels; dissecting collagen bundles in the upper reticular dermis and infiltrated by lymphocytes and plasma cells.
- **Plaque stage in Kaposi sarcoma:** Tumor is composed of proliferation of relatively bland, vaguely myxoid spindled cells producing slit-like vascular channels showing jagged outlines. There is presence of extravasated red blood cells and extracellular hyaline globule.
- **Papule (nodule) stage in Kaposi sarcoma:** Tumor is well circumscribed cellular nodular polypoidal mass simulating pyogenic granuloma. It is composed of intersecting fascicles of spindle cells with little or without cytologic atypia. Numerous slit spaces contain red blood cells.

Differential Diagnosis

Kaposi sarcoma should be differentiated from acroangiodermatitis, spindle cell hemangioma and spindle cell sarcomas.

Immunohistochemistry Technique

Kaposi sarcoma shows positivity for CD31, CD34, Fli-1, D2–40, VEGFR3 and HHV8 LANA protein (most sensitive marker).

Markers	Expression
CD31	Positive
CD34	Positive
Fli-1	Positive
D2–40	Positive
VEGFR3	Positive
HHV8 LANA protein	Positive

ANGIOSARCOMA

Angiosarcoma is highly malignant tumor showing endothelial differentiation, which affects elderly persons without sex predominance. Characteristics and clinical behavior of angiosarcoma depend on the clinical presentation.

- Angiosarcoma may be caused by radiation, long-standing lymphedema, post-irradiation and in the setting of use of radiocontrast media thorostat, vascular grafts and arteriovenous fistulae. Variants of angiosarcoma based on etiology are given in Table 15.24.
- Cutaneous angiosarcoma presents with violaceous, erythematous plaque and nodule. Angiosarcoma in deep soft tissue presents with hemorrhagic lump. Mammary angiosarcoma presents with rapidly growing mass in the women.
- Patient with angiosarcoma has poor prognosis. Administration of Taxanes chemotherapeutic agents may be of value in cutaneous angiosarcoma related to sun exposed skin.

Surgical Pathology: Angiosarcoma

Gross Morphology

- Gross morphology of angiosarcomas in cutaneous region are variable ranging from erythematous or violaceous plaque or nodule.
- Angiosarcoma in the deep tissue is large hemorrhagic mass.

Light Microscopy

Histologic features of angiosarcoma are variable ranging from well differentiated lesion simulating hemangioma; spindled lesion resembling fibrosarcoma; or epithelioid lesion simulating carcinoma.

- **High-grade angiosarcoma:** Tumor is composed of epithelioid-like endothelial cells with high nuclear grade and sieve-like to solid architectural pattern. Mitoses and necrosis are frequently demonstrated.
- **Well-differentiated angiosarcoma:** Tumor is composed of cells with low nuclear grade lacking mitotic activity and dissecting the adjacent connective tissue.
- **Epithelioid angiosarcoma:** Tumor is composed of epithelioid-like endothelial cells with abundant cytoplasm and uniform high nuclear grade and arranged in sheets. Such lesion is mistaken for carcinoma.

Differential Diagnosis

Angiosarcoma should be differentiated from hemangioma, diffuse angiomatosis, atypical vascular proliferation after radiation, carcinoma, spindle cell carcinoma, melanoma and other spindle cell sarcomas.

Immunohistochemistry Technique	
Angiosarcoma shows positivity for CD31, CD34, Fli-1, vWF, D2–40, VEGFR3 and cytokeratins (low molecular weight) in epithelioid tumors.	
Markers	**Expression**
▪ CD31	▪ Positive (100%)
▪ CD34	▪ Positive (100%)
▪ Fli-1	▪ Positive (100%)
▪ vWF	▪ Positive (100%)
▪ D2–40	▪ Positive (some cases)
▪ VEGFR3	▪ Positive (some cases)
▪ Cytokeratins	▪ Positive (25%)

Table 15.24 Variants of angiosarcoma based on etiology

Variants	Comments
Primary angiosarcoma associated with radiation	Primary angiosarcoma is related to prolonged sun exposure and therapeutic radiation. Elderly patients present with single or multiple lesions in the superficial region of head and neck
Angiosarcoma associated with long-standing lymphedema	Angiosarcoma may arise in the long-standing lymphedema due to Stewart-Treves syndrome, secondary to lymph node dissection and mastectomy performed for breast cancer. Stewart-Treves syndrome is a rare deadly cutaneous angiosarcoma that develops in long-standing chronic lymphedema
Post-irradiation angiosarcoma	Angiosarcoma arises from soft tissue of patient treated by radiation as adjuvant treatment for breast cancer
Angiosarcoma associated with other causes	Angiosarcoma may arise in the setting of use of radiocontrast media thorostat, vascular grafts and arteriovenous fistulae

HYPERTENSION

HYPERTENSION: OVERVIEW

Hypertension is intermittent or sustained elevation of systolic blood pressure >139 mm Hg or diastolic blood pressure >89 mm Hg. It occurs as essential (primary) hypertension or secondary hypertension. Normal blood pressure is <120/80 mm Hg. Optimal blood pressure is <115/80 mm Hg (Table 15.25).

ORGANS DAMAGE

Patient with blood pressure <130–139/80–89 mm Hg is prone to develop hypertension. Hypertension can cause damage to multiple organs such as brain, eyes, cardiovascular system, lungs, kidneys and liver.

ADVERSE EFFECTS

If left untreated, essential hypertension can cause retinal changes, left ventricular hypertrophy, cardiac failure, and benign nephrosclerosis. It can predispose to ischemic heart disease or cerebral stroke.

CLINICAL FEATURES

Patient presents with bruits over abdominal aorta or carotid, renal and femoral arteries, dizziness, confusion, fatigue, blurred vision, nocturia, and edema.

CLASSIFICATION

Hypertension is classified according to etiology and severity described as follows.

Table 15.25 Blood pressure classification by Joint National Committee 7 (JNC-7) and 2017 ACC/AHA hypertension guidelines

Systolic and Diastolic Blood Pressure (mm Hg)	Joint National Committee 7 (JNC-7)	2017 ACC/AHA Hypertension Guidelines
<120 and <80	Normal BP	Normal BP
120–129 and <80	Prehypertension	Elevated BP
130–139 or 80–89	Prehypertension	Stage 1 hypertension
140–159 or 90–99	Stage 1 hypertension	Stage 2 hypertension
>160 or >100	Stage 2 hypertension	Stage 3 hypertension

Etiological Classification

Etiological classification of hypertension is given in Table 15.26.

Essential Hypertension

Essential hypertension is a multifactorial disease of unknown etiology. It accounts for 95% of all diagnosed cases of hypertension.

Secondary Hypertension

Secondary hypertension represents 5% cases of hypertension due to temporary complication of various disorders:

- **Renovascular hypertension:** It occurs due to renal artery stenosis.
- **Endocrinal hypertension:** Primary aldosteronism, Cushing's syndrome, pheochromocytoma, oral contraceptive cause hypertension.
- **Hypertension due to cardiovascular system and blood disorders:** Secondary hypertension can occur due to coarctation of aorta, polyarteritis nodosa, polycythemia vera and excessive blood transfusions.
- **Hypertension due to increased intracranial pressure:** It occurs due to brain tumors, drugs and chemicals, notably amphetamines and steroids (neurologic).

Table 15.26 Etiological classification of hypertension

Primary/Essential Hypertension of Unknown Etiology	
Genetic predisposition (strong familial associations)	▪ Genetic defect of calcium transport increases reactivity of blood vessels to vasoconstrictors ▪ Liddle syndrome, autosomal dominant disorder occurs due to SCNN1B or SCNN1G genes located on chromosome 16 resulting in gain of sodium channel functions and thus hypertension ▪ Syndrome of apparent mineralocorticoids excess increases sodium and water reabsorption and thus hypertension ▪ Glucocorticoid-remediable aldosteronism
Dietary factors	▪ Obesity ▪ High salt intake ▪ Excessive caffeine intake
Humoral factors	Abnormalities in renin-angiotensin-aldosterone system
Neurological factors	Excessive activity of sympathetic nervous system activity
Secondary Hypertension	
Renal parenchymal and vascular disorders	▪ Chronic glomerulonephritis ▪ Chronic pyelonephritis ▪ Polycystic disease of kidney ▪ Renal amyloidosis ▪ Renal artery stenosis
Adrenal gland disorders	▪ Pheochromocytoma ▪ Cushing's syndrome ▪ Conn syndrome (primary hyperaldosteronism) ▪ Congenital adrenal hyperplasia
Other endocrinal disorders	▪ Acromegaly ▪ Thyrotoxicosis ▪ Hyperparathyroidism ▪ Diabetic nephropathy
Cardiovascular disorders	▪ Coarctation of aorta ▪ Arteriovenous fistula
Therapeutic drugs	▪ Oral contraceptive drugs ▪ Anabolic steroids ▪ Corticosteroids ▪ Epinephrine (adrenaline) and related sympathomimetic drugs
Pregnancy associated disorder	Toxemia of pregnancy

Classification of Hypertension According to Severity

Benign Hypertension

Benign hypertension involves diastolic pressure that rises slowly and remains over prolonged period. Untreated cases produce vascular changes in kidneys known as benign nephrosclerosis.

Malignant Hypertension

Malignant hypertension can occur in essential or secondary hypertension, in which diastolic pressure rises quickly over weeks or months. It causes severe damage to circulatory system resulting in cerebral strokes and papilledema. It produces vascular changes (hyperplastic arteriolosclerosis and fibrinoid necrosis) in kidneys known as **malignant nephrosclerosis**.

DETERMINANTS OF HYPERTENSION

Blood pressure depends on the cardiac output and peripheral resistance. Blood pressure may result from increasing the cardiac output or peripheral resistance.

Factors Increasing Cardiac Output

Several factors can affect cardiac output (CO) indirectly by affecting the heart rate (HR) and stroke volume (SV), the primary components of cardiac output determinants often expressed as: CO = HR × SV. Water and sodium retention is regulated by aldosterone and atrial natriuretic factor (ANF). Aldosterone and atrial natriuretic factor/cardionatrin have opposite actions.

- **Aldosterone:** It increases blood volume by enhancing absorption of sodium and water by kidneys. Increased blood volume causes distension of both atria resulting in synthesis of atrial natriuretic factor/cardionatrin by atria.
- **Atrial natriuretic factor/cardionatrin:** It participates in decreasing blood volume by increasing excretion of sodium and water by kidneys. It decreases synthesis of renin and aldosterone. It also decreases venous return.

Factors Affecting Peripheral Resistance

Changes in the arteriolar bed causing increased total peripheral resistance depends on neutral α-adrenergic effect on arterioles, catecholamines and renin-angiotensin-aldosterone system.

- Increased arteriolar thickening by genetic factors and increased arteriolar tone by overactivity of sympathetic nervous system (α-adrenergic effect) lead to increased total arteriolar peripheral resistance.
- Abnormal renin release results in formation of angiotensin II, which constricts the arterioles and increases peripheral blood resistance.

Renin-angiotensin-aldosterone System

Kidneys release renin into the bloodstream. Renin participates in conversion of angiotensinogen to angiotensin I in liver.

- Angiotensin I is converted to angiotensin II (a potent vasoconstrictor) in lungs by angiotensin converting enzyme (ACE). Angiotensin II increases arteriolar peripheral resistance and expansion of blood volume. Angiotensin II causes vasoconstriction resulting to increased peripheral resistance.
- Angiotensin II also stimulates adrenal cortex to synthesize aldosterone, which acts on distal renal tubules resulting in absorption of sodium and water by kidneys, and thus plasma expansion. It also stimulates sympathetic nervous system and thus causes vasoconstriction. It also causes proliferation of smooth muscle cells and thickening (Fig. 15.31).

ESSENTIAL (PRIMARY) HYPERTENSION

Essential hypertension accounts for 95% cases of hypertension, which is of unknown etiology. It represents an interaction of predisposing determinants with a number of exogenous factors such as family history of hypertension, ethnicity (Africans), stress, obesity, smoking, advancing age, sedentary lifestyle, high intake of sodium and saturated fats. Pathogenesis of essential hypertension is discussed below.

- Increased release of vasoconstrictors (e.g. renin, catecholamines and endothelin) in persons result in hypertensive state. In some areas, people salt intake is more thus prone to essential hypertension.
- Obese persons under stress and strain are more prone to develop essential hypertension.

GENETIC DEFECT OF CALCIUM TRANSPORT

Genetic defect of calcium transport in vascular smooth muscle cells leads to increased intracellular concentration of calcium, myosin phosphorylation and ATP production. It leads to increased reactivity of blood vessels to vasoconstrictors and hypertrophy of vascular smooth muscle cells resulting in increased peripheral resistance and essential hypertension.

LIDDLE SYNDROME

Liddle syndrome is an autosomal dominant disorder due to gene mutation on chromosome 16.

- Liddle syndrome is characterized by sustained activation of sodium channels (gain of function), which results in increased absorption of sodium and water at distal convoluted renal tubules independent of action due to mineralocorticoids.

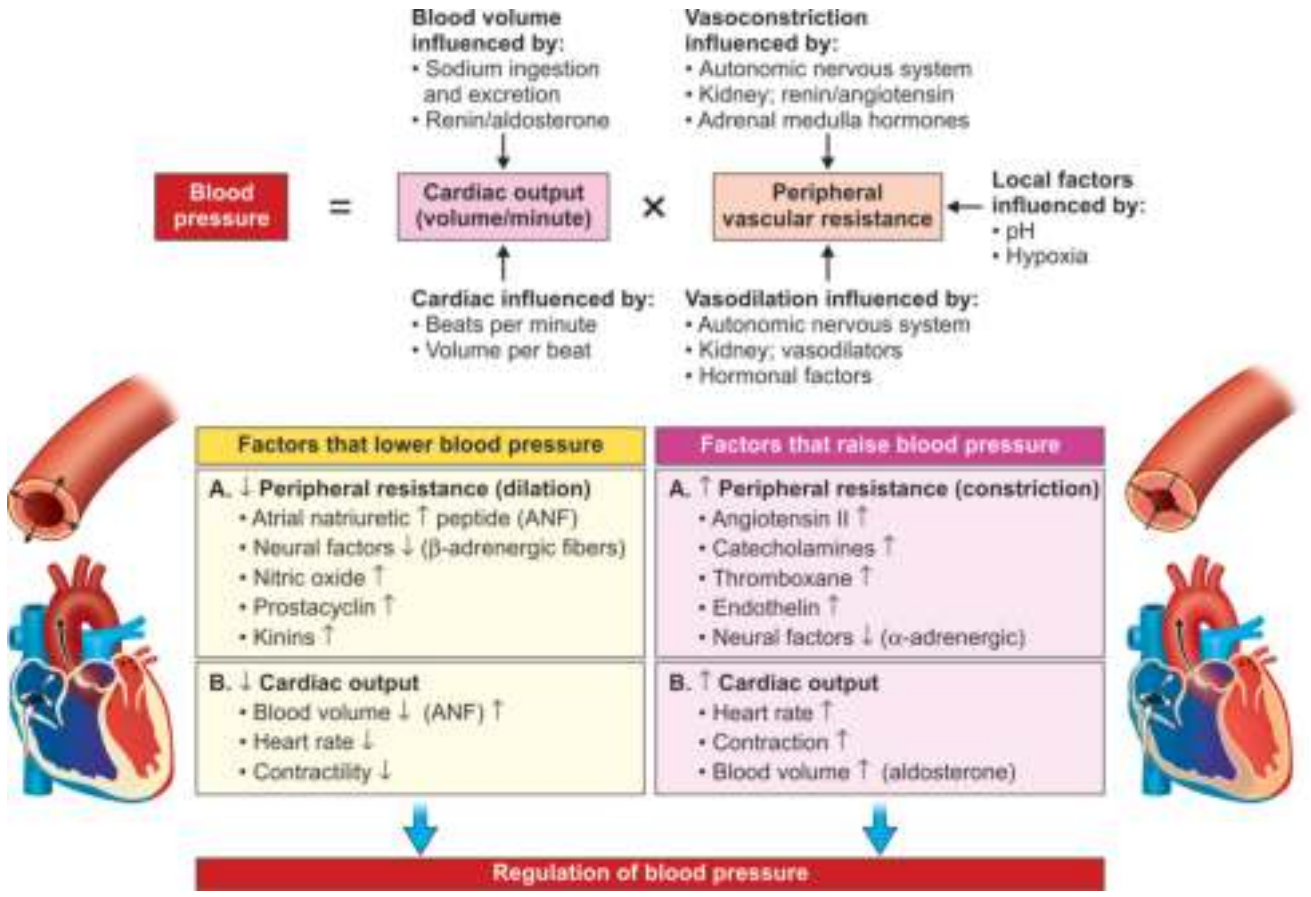


Fig. 15.31: The regulation of blood pressure. Blood pressure is a product of cardiac output (volume of blood flow) and peripheral vascular resistance. Multiple factors influence blood pressure.

- There is expansion of blood volume and thus hypertension. Nephrons are structurally normal.

SYNDROME OF APPARENT MINERALOCORTICOIDS EXCESS (AME)

- **Physiologic state:** Cortisol has weak mineralocorticoids activity. 11β-hydroxysteroid dehydrogenase converts cortisol into cortisone in tubules.
- **Pathologic state:** Gene mutation controlling this 11β-hydroxysteroid dehydrogenase results in accumulation of cortisol, which leads to expansion of blood volume due to sodium and water retention and thus hypertension. Licorice containing glycyrrhetinic acid also inhibits 11β-hydroxysteroid dehydrogenase, thus causes hypertension.

GLUCOCORTICOID-REMEDIABLE ALDOSTERONISM

Glucocorticoid-remediable aldosteronism (GRA) is an autosomal dominant disorder, which causes hypertension.

- **Physiologic state:** Aldosterone synthetase gene and 11β-hydroxylase gene are present on chromosome 8. Aldosterone synthetase acts on zona glomerulosa of adrenal glands to synthesize aldosterone hormones. 11β-hydroxylase gene controls the synthesis of cortisol by zona fasciculata of adrenal glands.
- **Pathologic state:** Mutations of aldosterone synthetase gene and 11β-hydroxylase gene present on chromosome 8 form hybrid genes resulting in ectopic production of aldosterone by zona fasciculata of adrenal cortex. It leads to expansion of blood volume and thus hypertension.

SECONDARY HYPERTENSION

Secondary hypertension accounts for 5% cases of all diagnosed cases of hypertension. Differentiating features of secondary hypertension are given in Table 15.27.

RENOVASCULAR HYPERTENSION

Renovascular hypertension is most important cause of secondary hypertension as a result of increased plasma renin activity. Renal arteriogram reveals narrowing of the ostia of renal arteries due to atherosclerosis. Renal parenchymal diseases and renal artery stenosis result

Table 15.27 Differentiating features of secondary hypertension

Disorder	Specific Clinical and Laboratory Findings
Renal artery stenosis	Bruit
Pheochromocytoma	Episodic hypertension
Conn syndrome	Hypokalemia
Cushing syndrome	▪ Buffalo hump ▪ Truncal obesity ▪ Striae
Coarctation of aorta	Blood pressure elevated in upper extremities than lower extremities
Congenital adrenal hyperplasia	Hirsutism

Investigate secondary causes of hypertension in young <30 or >60 years of age, who fail to control blood pressure within three mediations.

in secondary hypertension. Renal artery stenosis occurs due to atherosclerosis, giant cell arteritis and Takayasu arteritis.

Pathogenesis

- Renovascular hypertension occurs through stimulation of the renin-angiotensin-aldosterone system. Juxtaglomerular cells respond to decreased vascular tone by secreting renin, which facilitates the conversion of angiotensinogen to angiotensin I in liver, which is further converted to angiotensin II in lungs by angiotensin converting enzyme.
- Angiotensin II promotes hypertension by acting both as a vasoconstrictor and as inactivator of aldosterone secretion. Aldosterone promotes sodium and water retention (Fig. 15.32).

Clinical Features

Patient presents with severe uncontrollable hypertension, epigastric bruit (e.g. sound is due to turbulence of blood flow through the narrow renal artery).

ROLE OF HORMONES

Testosterone and estrogens stimulate renin-angiotensin system resulting in expansion of blood volume and vasoconstriction.

- Testosterone and estrogens also increase plasma insulin level, which enhances the synthesis of catecholamines, which increases peripheral resistance and thus secondary hypertension. Insulin increases the peripheral resistance by vasoconstriction of blood vessels and thus secondary hypertension.
- Increased plasma insulin stimulates smooth muscle cells proliferation thus increase peripheral resistance and thus hypertension.

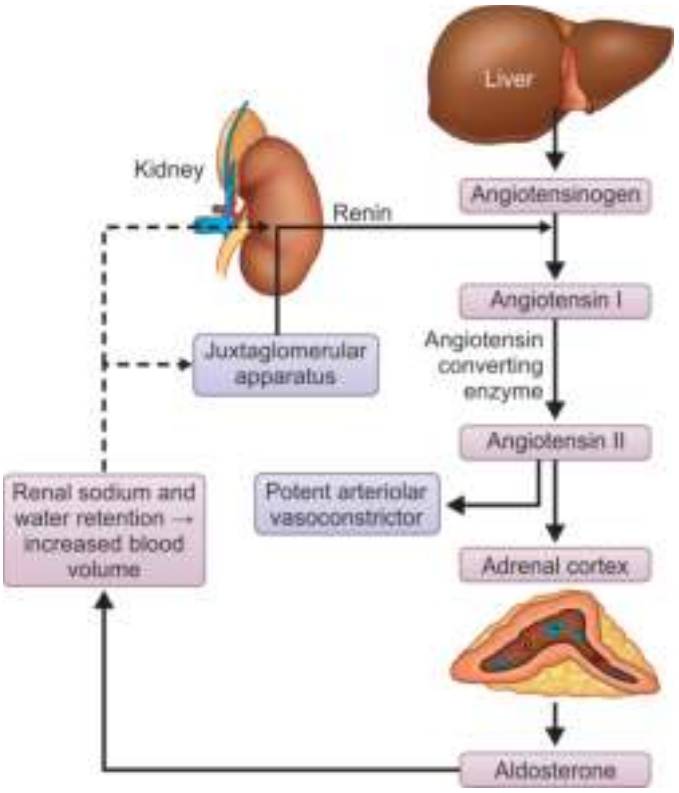


Fig. 15.32: Renin-angiotensin-aldosterone system role in renovascular hypertension.

ENDOCRINE SYSTEM DISORDERS

Endocrine system disorders such as Conn syndrome, pheochromocytoma, or acromegaly, represent the next most common cause. Conn syndrome is an endocrine disorder most commonly caused by an adrenal cortical adenoma or bilateral adrenal hyperplasia. Aldosterone secreted by the adrenal cortical tumor causes secondary hypertension, hypernatremia, and hypokalemia.

CARDIOVASCULAR SYSTEM DISORDERS

Coarctation of aorta is a frequent cause of hypertension limited to the upper extremities. Blood pressure in both arms and one leg must be determined; a pressure difference of >20 mm Hg in favor of the arms may be considered evidence of coarctation of aorta.

TOXEMIA OF PREGNANCY

Pre-eclampsia refers to the triad of hypertension, proteinuria, and edema, complicating third trimester of pregnancy. When these features are complicated by convulsions, the term eclampsia is applied. Early-onset toxemia of pregnancy is attributed to abnormal superficial implantation of placenta resulting in diminished blood supply to the placenta. Late-onset disease occurs due to which release of vasoactive substances lead to endothelial damage. Immunofluorescence microscopy

reveals nonspecific deposits of IgG, IgM and fibrin in renal blood vessels.

Surgical Pathology: Toxemia of Pregnancy

Light Microscopy

- Histopathologic examination of renal biopsy reveals diffuse uniform enlargement of glomeruli and the endothelial cells are swollen (endotheliosis), an appearance that results in an apparently bloodless glomerular tuft.
- Patient blood pressure returns to normal in postpartum period.

Clinical Features

Patient presents with hypertension, proteinuria, and edema usually in the third trimester. Pre-eclampsia is defined as the development of hypertension with proteinuria and generalized edema after 20 weeks gestation. Eclampsia is diagnosed when seizures occur in the setting of pre-eclampsia.

CNS DISORDERS

Hypertension occurs due to increased intracranial pressure due to brain tumors, drugs and chemicals, notably amphetamines and steroids (neurologic).

VASCULAR CHANGES IN HYPERTENSION

VASCULAR CHANGES IN BENIGN HYPERTENSION

Hypertensive nephrosclerosis is identified in 15% of patients with benign hypertension. Even mild-to-moderate hypertension causes benign nephrosclerosis.

- Due to benign hypertension, patient develops increased permeability of vessels resulting into deposition of plasma proteins in subintimal regions of blood vessels. Lumen of the blood vessels is decreased leading to ischemic changes in organs.
- Vascular changes in benign hypertension include hyaline arteriosclerosis and reduplication of elastic lamina. Similar changes are also seen in elderly persons (normal and hypertensive) and diabetes mellitus. Renal changes in benign hypertension are known as benign nephrosclerosis.

Clinical Pearls: Vascular Changes in Benign Hypertension

Gross Morphology

- In benign nephrosclerosis, kidneys are symmetrically reduced in size due to ischemia.
- External surface of the kidneys is finely granular giving grain leather-like appearance.
- V-shaped infarcts may be seen due to occlusion of medium-sized renal vessels.
- On cut section, cortex is thinned out.

Light Microscopy

- In benign nephrosclerosis, renal arterioles exhibit concentric hyaline thickening of the wall with narrowing of lumen.
- It shows tubular atrophy, interstitial fibrosis replacing nephrons and lymphocytic infiltration.

VASCULAR CHANGES IN MALIGNANT HYPERTENSION

Malignant hypertension refers to a severely elevated blood pressure (diastolic blood pressure >130 mm Hg), which results in rapidly progressive vascular disease. It most often occurs in young African-American males. Malignant hypertension affects the brain (cerebral stroke), heart (left ventricular hypertrophy and failure), kidney (renal failure) and retina (focal retinal hemorrhages and papilledema). It can be a complication of either essential (primary) or secondary hypertension (Table 15.28).

Histologic Changes in Blood Vessels

Malignant hypertension causes necrotizing arteriolitis and glomerulitis with fibrinoid necrosis and hyperplastic arteriolosclerosis giving onion skin appearance.

Consequence

Poor perfusion of the kidneys stimulates the release of renin, which serves to elevate systemic blood pressure even further by activation of renin-angiotensin-aldosterone system resulting in renovascular hypertension.

Clinical Pearls: Vascular Changes in Malignant Hypertension

Gross Morphology

Renal changes in malignant hypertension are known as malignant nephroscleosis. Renal arterioles and glomeruli rupture, resulting in '**flea-bitten kidney**', multiple pinpoint petechial hemorrhages on the external surface of kidney.

Light Microscopy

Renal tubules are atrophic. Tubules contain eosinophilic material known as thyroidization of tubules. There is tubular loss in relation to sclerosed glomeruli present. Renal arterioles show fibrinoid necrosis and hyperplastic arteriolosclerosis exhibiting 'onion skin appearance'. Patients develop chronic renal failure with manifestations of uremia.

- **Hyperplastic arteriolosclerosis:** Acute vascular injury in malignant hypertension is followed by smooth muscle proliferation with an increase in concentric layers of smooth muscle cells that yield the so-called 'onion skin appearance'. Smooth muscle hyperplasia occurs as a result of growth factors derived from platelets and inflammatory cells at the site of vascular injury.

- **Fibrinoid necrosis:** Malignant hypertension injures endothelial cells and causes increased vascular permeability, which leads to the insudation of plasma proteins into the vessel wall. Severe endothelial injury causes intravascular coagulation and rupture of blood vessels. Lumen becomes narrow and fixed resulting into ischemia. This morphologic change in renal arterioles is known as fibrinoid necrosis.

Clinical Features

Patient with malignant hypertension develops renal failure and vascular stress that may cause myocardial infarction, congestive heart failure, or cerebral stroke, and increased intracranial pressure, which often produces symptoms such as seizures, headache, nausea, vomiting, visual impairment, and coma.

Physical Examination

Blood pressure is very high >160/≥200 mm Hg, pounding heartbeat on auscultation and bulging optic disc papilledema on fundus examination.

COMPLICATIONS OF HYPERTENSION

- Hypertension is most often called **silent killer**. Initially, patient presents with shortness of breath after mild exercise, headache or dizziness, swelling of the ankles during daytime, excessive sweating and anxiety.
- Long-standing hypertension causes organs damage resulting in coronary artery disease, cerebral stroke, congestive heart failure, end stage renal disease and peripheral vascular disease. These complications may be prevented by treating hypertensive patients by salt restriction, exercise, vasodilators, calcium channel blockers, ACE inhibitors, angiotensin blockers, diuretics, β-blockers and α-blockers (Table 15.29 and Fig. 15.33).

CEREBRAL HEMORRHAGE

Patient may develop cerebral hemorrhages in basal ganglia especially putamen—thalamus region (65%), pons (15%) and cerebellum (10%). Cerebral stroke is common in persons with berry aneurysms undergoing rupture due to hypertension. Cerebral thrombus or embolism may also cause cerebral stroke (Fig. 15.34).

RETINOPATHY

Chronic hypertension results in narrowing of retinal arterioles and hemorrhages in retina producing papilledema giving '**cotton wool spots**' appearance on fundus examination (Fig. 15.35A and B).

Table 15.28 Features of benign and malignant hypertension

Parameters	Benign Hypertension	Malignant Hypertension
Incidence	Very common incidence	Rare incidence
Age group	Begins in <45 years of age and progresses	Young adults of 25–35 years
Sex distribution	Females > males	Male and female equally affected
Etiology	Majority of cases suffers from primary/essential hypertension	Majority of cases develop malignant hypertension due to renal disease, however, a few cases develop in long-standing primary/essential hypertension
Hypertension progression	Very slow progression over many years	Rapid progression within months to one or two years
Blood pressure range	Very slow rise of blood pressure with diastolic blood pressure ranging 90–120 mm Hg	Rapid rise of blood pressure with diastolic blood pressure ≥120 mm Hg
Injury to blood vessels	Mild to moderate injury to blood vessels	Severe injury to blood vessels
Large elastic and medium size arteries involvement	Potentiates slow formation of atheromatous plaque and accelerates arteriosclerosis	Potentiates formation of atheromatous plaque and accelerates arteriosclerosis
Arteriolar changes	Hyaline arteriolar thickening with narrowing of arteriolar lumen and reduplication of elastic lamina	Fibrinoid necrosis of vascular endothelium with occlusion of lumen by thrombus in vessels of kidney and other abdominal viscera
Pathogenesis of arteriolar changes	Benign hypertension increases endothelial permeability of arterioles resulting in deposition of plasma proteins beneath vascular intima and thus ischemia of tissue	Malignant hypertension causes severe damage to vascular endothelium of arterioles resulting in intravascular coagulation and fibrinoid necrosis with fixed vascular lumen

Table 15.29 Complications and systemic effects of hypertension

Parameters	Benign Hypertension	Malignant Hypertension
Blood pressure range and progression		
Blood pressure range	Very slow rise of blood pressure with diastolic blood pressure ranging 90–120 mm Hg	Rapid rise of blood pressure with diastolic blood pressure ≥120 mm Hg
Hypertension progression	Very slow over many years in <45 years of age and progresses	Rapid progression within months to one or two years in young adults of 25–35 years
Cardiovascular manifestations		
Injury to blood vessels	Mild to moderate injury to blood vessels	Severe injury to blood vessels
Large elastic and medium size arteries involvement	▪ Potentiates formation of atheromatous plaque and accelerates arteriosclerosis ▪ Dissection hematoma (low-risk)	▪ Potentiates formation of atheromatous plaque and accelerates arteriosclerosis ▪ Dissection hematoma (high-risk)
Pathogenesis of arteriolar changes	Benign hypertension increases endothelial permeability of arterioles resulting in deposition of plasma proteins beneath vascular intima and thus ischemia of tissue	Malignant hypertension causes severe damage to arteriolar endothelium resulting in intravascular coagulation and fibrinoid necrosis with fixed vascular lumen
Arteriolar morphologic changes	Hyaline arteriolar thickening with narrowing of arteriolar lumen and reduplication of elastic lamina	Fibrinoid necrosis of vascular endothelium with occlusion of lumen by thrombus in vessels of kidney and other abdominal viscera
Cardiac manifestations	▪ Concentric left ventricular hypertrophy (ventricle wall thickness more than 2 cm) ▪ Compensatory left atrium enlargement results in atrial fibrillation ▪ Increased susceptibility to spontaneous cardiac arrhythmias, heart failure, fibrinous pericarditis and ischemic heart disease in 60% of cases ▪ Histology shows myofiber disarray with myocyte hypertrophy and enlarged; box car' nuclei in the background of increased interstitial fibrosis	▪ Concentric left ventricular hypertrophy (ventricle wall thickness more than 2 cm) ▪ Compensatory left atrium enlargement results in atrial fibrillation ▪ Increased susceptibility to spontaneous cardiac arrhythmias, heart failure, fibrinous pericarditis and ischemic heart disease in 60% of cases ▪ Histology shows myofiber disarray with myocyte hypertrophy and enlarged; boxcar nuclei in the background of increased interstitial fibrosis
Brain and retinal manifestations		
Brain	Cerebral hemorrhages and thrombus in basal ganglion (putamen), thalamus, cerebellum	Encephalopathy (fits and loss of consciousness) due to cerebral edema, cerebral hemorrhages and midline shift
Retinal changes on fundus examination	Cotton wool exudates on fundus examination due to ischemia and infarction; and associated with retinal vein thrombosis	Cotton wool exudates on fundus examination due to ischemia and infarction; and associated with retinal vein thrombosis
Renal manifestations		
Renal morphological changes	▪ Benign nephrosclerosis (not usually serious complication) ▪ Grain leather appearance on external surface of kidneys due to ↓ renal blood supply with decrease in thickness of renal cortex and medulla	▪ Malignant nephrosclerosis (serious complications) ▪ Flea bitten appearance due to rupture of renal arterioles, and vascular lumen is narrow and fixed
Pulmonary manifestations		
Lungs	Pulmonary congestion and edema	Pulmonary congestion and edema
Liver changes		
Liver	Chronic venous congestion of liver and nutmeg appearance on cut surface, central veins are dilated congested and later on resulting in cardiac cirrhosis	Rupture of central veins of liver

Patient develops peripheral pitting edema around ankle joints in long-standing case of hypertension.

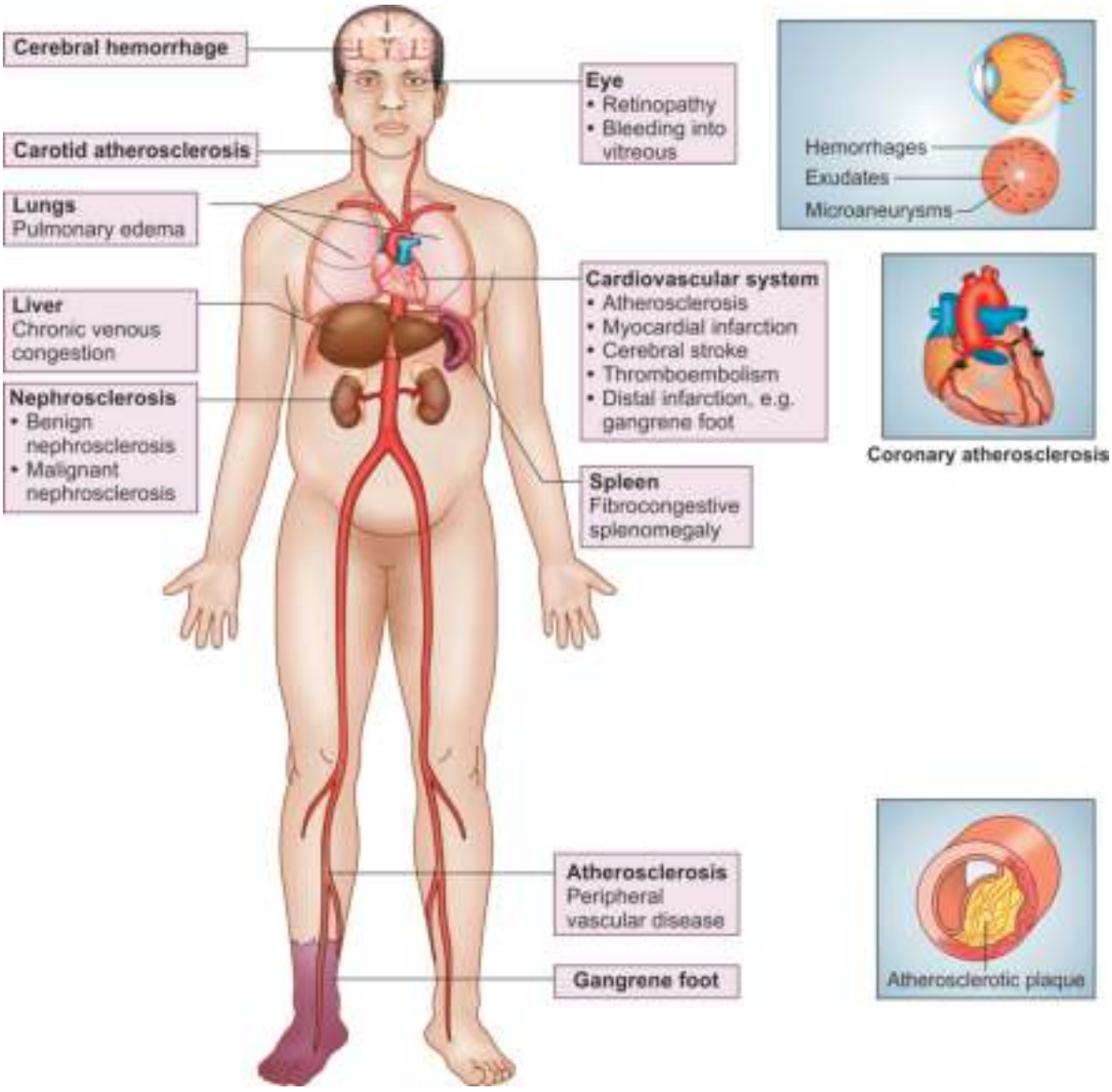


Fig. 15.33: Complications of hypertension. Hypertension is a major risk factor for atherosclerosis.

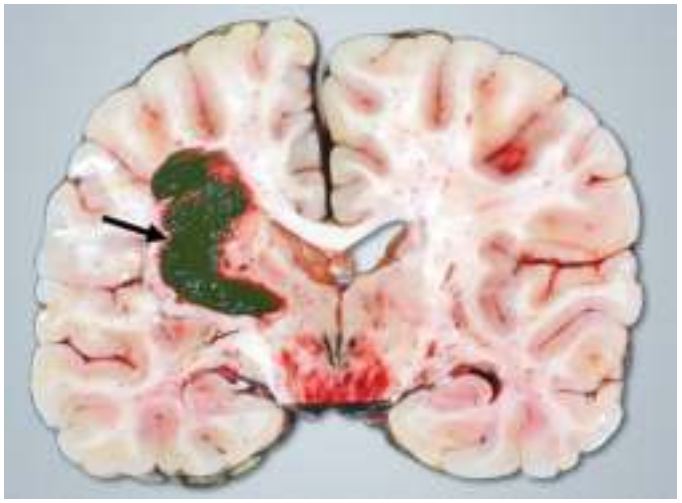


Fig. 15.34: Hemorrhages involving the basal ganglia area (the putamen in particular) tend to be nontraumatic.

CARDIOVASCULAR MANIFESTATIONS

- **Concentric cardiac hypertrophy:** Uncontrolled hypertension results in marked concentric cardiac hypertrophy of left ventricle with decrease in size of left ventricular chamber (Fig. 15.36).
- **Dissecting hematoma (aortic dissection):** Uncontrolled hypertension may tear the intima of aorta. Blood enters and dissects aortic media. External rupture results in massive hemorrhage into the pericardial cavity. Retrograde rupture results in massive hemorrhage into the pericardial cavity. Rarely, internal rupture dissects the aortic wall and reopens at distal region producing '**double-channeled aorta**' (Fig. 15.37).
- **Atherosclerosis:** Chronic hypertension is a major risk factor for atherosclerosis in large elastic and

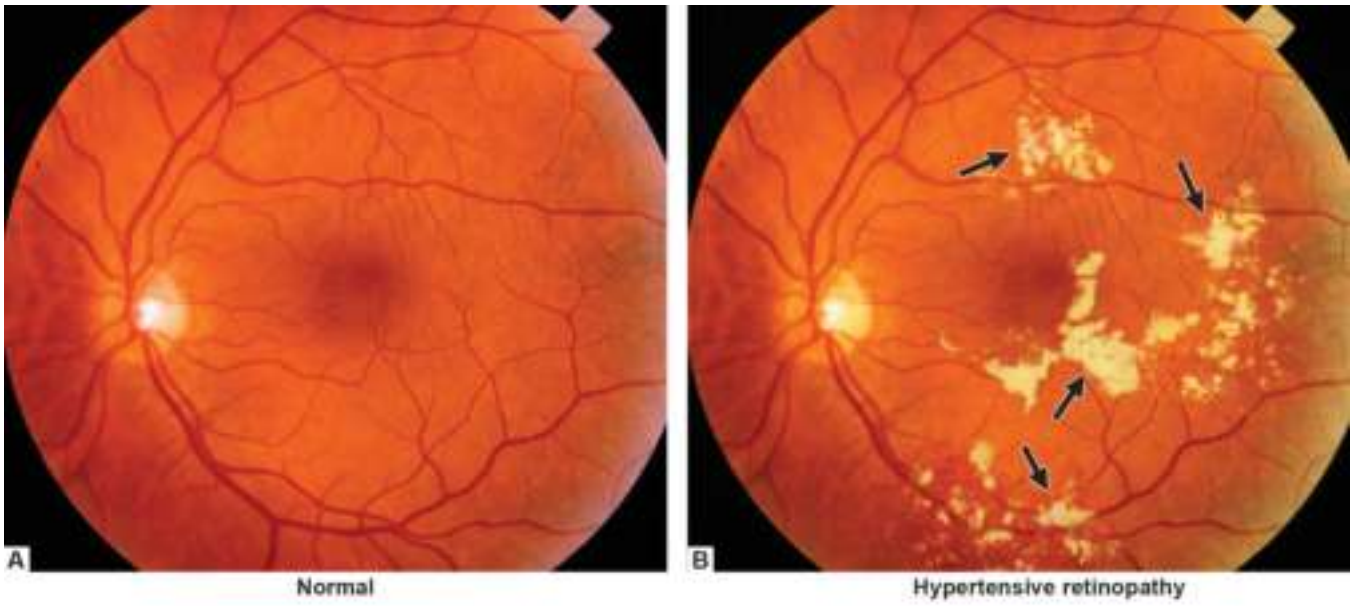


Fig. 15.35A and B: Hypertensive retinopathy. There is arteriolar narrowing and cotton wool spots (arrows). Additional findings can include hemorrhages into the nerve fiber layer and papilledema.

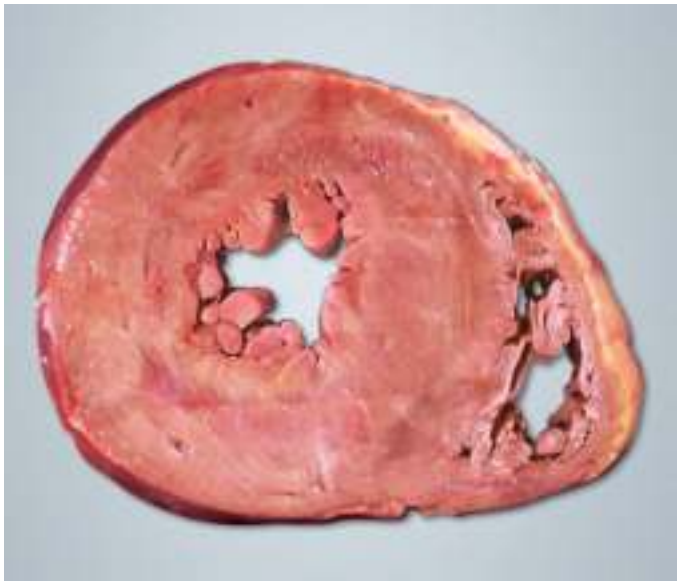


Fig. 15.36: The left ventricle is markedly thickened in this patient with severe hypertension that was untreated for many years. The myocardial fibers have undergone hypertrophy.

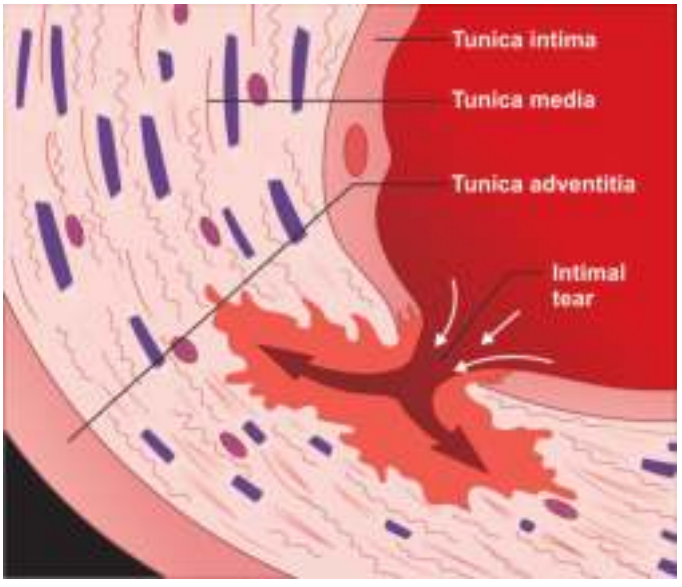


Fig. 15.37: This aortic dissection occurred in patient with uncontrolled hypertension.

medium-sized arteries. Atheromatous plaques consist of central core of lipids covered by fibrous cap, ulceration, thrombus formation, embolism, bleeding into atherosclerotic plaque due to rupture of new vessels formed, calcification and aneurysm. Atheromatous plaques in coronary arteries may cause angina, acute myocardial infarction or chronic ischemic disease (Fig. 15.38).

- **Fibrinous pericarditis:** Chronic hypertension results in deposition of fibrin between visceral and parietal layers of pericardium known as fibrinous pericarditis. Keeping apart of pericardial layers gives bread and butter appearance. On auscultation, friction rub is appreciated. It is worth mentioning that fibrinous pericarditis may also occur in acute myocardial infarction and chronic renal failure.

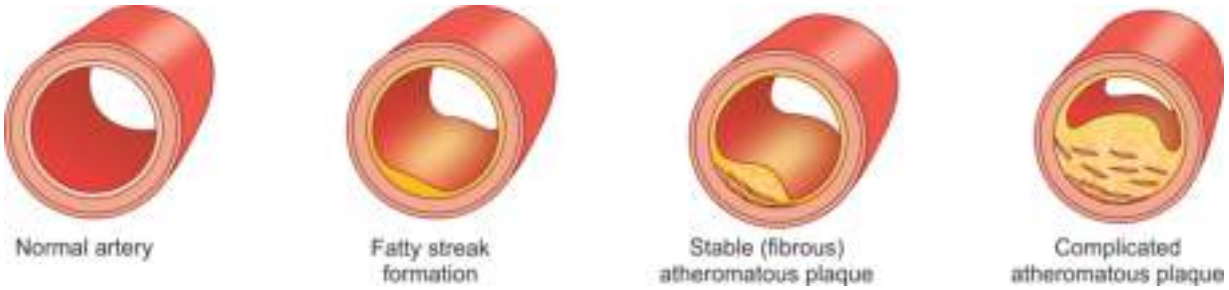


Fig. 15.38: Atheromatous plaques. Uncontrolled hypertension is major risk factor for atherosclerotic plaques in large elastic and medium-sized arteries.

PULMONARY MANIFESTATIONS

Uncontrolled hypertension leads to pulmonary edema. Lungs show passive congestion in alveolar capillaries resulting in accumulation of fluid in the alveoli exhibiting slightly floccular pink material along with 'heart failure cells' (Fig. 15.39A and B).

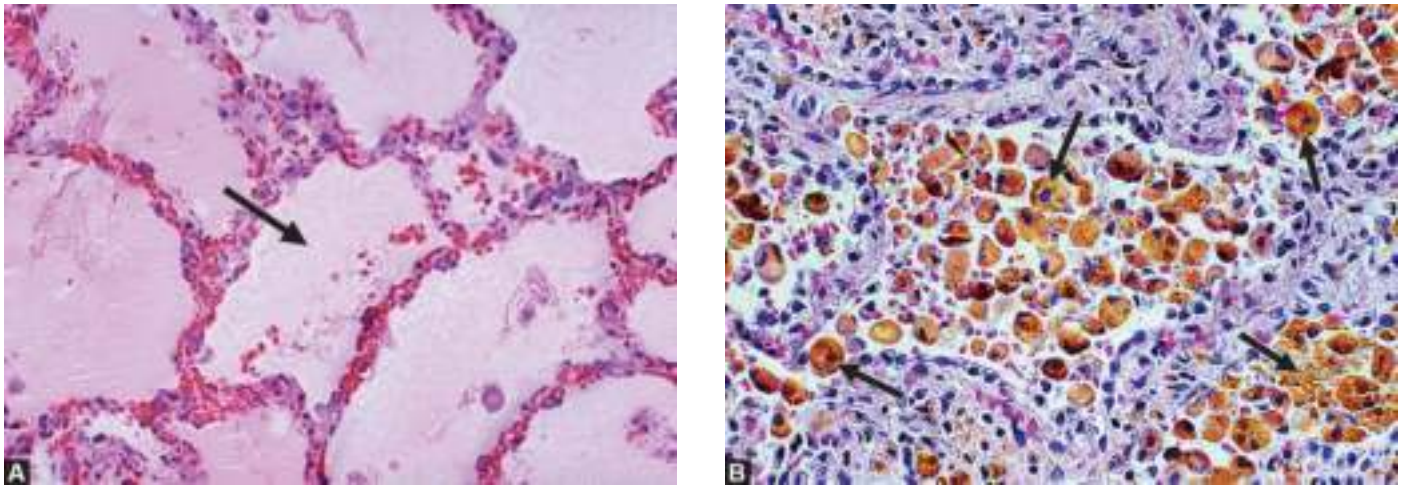


Fig. 15.39: Pulmonary edema. (A) The alveoli in this lung are filled with a smooth to slightly floccular pink material. Alveolar capillaries congested (arrow) (400X), (B) pulmonary edema with heart failure cells (arrows).



Fig. 15.40: Chronic venous congestion in liver. Cut surface shows nutmeg appearance (arrows). The dark red conjusted regions represent accumulation of RBCs in centrilobular regions.

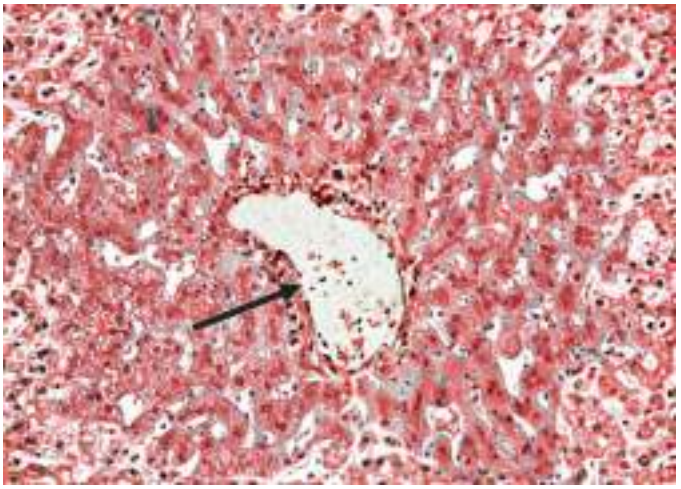


Fig. 15.41: Central venous congestion. Microscopically, the nutmeg pattern results from congestion around the central veins, as seen here (arrow). This is usually due to a right-sided heart failure.

PERIPHERAL PITTING EDEMA

High blood pressure forces fluid out of the capillaries into the interstitial tissues results in pitting type of edema in the dependent parts especially ankles.

RENAL MANIFESTATIONS

- Depending on severity of hypertension, patient may develop benign nephrosclerosis (hyaline arteriolosclerosis with grain leather surface) or malignant nephrosclerosis (flea-bitten kidneys).
- Decreased renal blood supply causes increased renin synthesis by juxtaglomerular apparatus resulting in activation of angiotensin-aldosterone system. Angiotensin II increases peripheral resistance and stimulates aldosterone synthesis. Aldosterone acts on distal tubules and enhances sodium and water retention. These changes further increase the blood pressure.

LIVER MANIFESTATIONS

In long-standing uncontrolled hypertension, patient may also develop right-sided heart failure. Jugular venous pressure is increased.

- Liver shows chronic passive congestion of sinusoids, atrophy of hepatocytes, dilatation of central veins and ischemic changes in zone 3 of liver lobule.
- Long-standing case of chronic venous congestion may develop cirrhosis, known as **cardiac cirrhosis** (Fig. 15.40). Long-standing chronic passive congestion of liver leads to bridging fibrosis in extreme cases resulting in cardiac cirrhosis (Fig. 15.41).

CHAPTER 16

Heart

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

THE NORMAL HEART

ISCHEMIC HEART DISEASE

- Ischemic heart disease: overview
- Angina pectoris
- Myocardial infarction
- Chronic ischemic heart disease

RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

- Rheumatic fever and rheumatic heart disease: overview
  - Pathogenesis
  - Aschoff's nodule
  - Jones diagnostic criteria
- Laboratory diagnosis
- Management

DISORDERS OF MYOCARDIUM

- Myocarditis
- Cardiomyopathies

DISORDERS OF ENDOCARDIUM

- Endocardium and endocarditis: overview
- Infective endocarditis
- Endocarditis in prosthetic valves
- Endocarditis in intravenous drug abuse
- Libman-Sacks endocarditis
- Nonbacterial thrombotic endocarditis
- Endocarditis in rheumatic heart disease
- Right-sided endocarditis in carcinoid syndrome

DISORDERS OF CARDIAC VALVES

- Cardiac valvular disorders: overview
- Mitral valve stenosis
- Mitral valve insufficiency/regurgitation
- Mitral valve prolapse
- Aortic valve stenosis
- Aortic valve insufficiency/regurgitation
- Calcific aortic stenosis
- Tricuspid valve insufficiency
- Pulmonic valve stenosis
- Carcinoid heart disease

DISORDERS OF PERICARDIUM

- Anatomy and physiology
- Pericardial effusion
- Hydropericardium
- Hemopericardium
- Cardiac tamponade
- Pericarditis: overview
- Acute pericarditis
- Chronic pericarditis

CARDIAC HYPERTROPHY AND HEART FAILURE

- Cardiac hypertrophy
- Heart failure
  - Left-sided heart failure
  - Right-sided heart failure

NEOPLASTIC DISORDERS OF HEART

- Neoplasms: classification
- Atrial myxoma
- Cardiac rhabdomyoma

CONGENITAL HEART DISEASES AND BLOOD VESSEL MALFORMATIONS

- Developmental anomalies: overview
- Atrial septal defects
- Ventricular septal defects
- Patent ductus arteriosus
- Coarctation of the aorta
- Tetralogy of Fallot
- Truncus arteriosus communis
- Malformations of CVS

THE NORMAL HEART

ANATOMY AND PHYSIOLOGY

Normal heart weighs 300–350 g (male) and 250–300 g (female). Heart has a critical weight of 500 g termed cor bovinum in tertiary syphilis. Normal left ventricular thickness is 1.2–1.5 cm and 0.3–0.5 cm in the right ventricle measured at the base of papillary muscles. Heart is composed of three layers: epicardium, myocardium and endocardium. Pericardium comprises serous and visceral layers of pericardium.

Histology: Normal Myocardium

Light Microscopy

- Normal myocardium is composed of functional syncytium of myocardial fibers with centrally placed nuclei.
- Numerous capillaries are present in the interstitial tissue between myocardial fibers.

CARDIAC VALVES

Heart consists of atrioventricular valves (mitral and tricuspid). The cardiac valves comprise an annulus,

leaflets, chordae tendineae and papillary muscles. The semilunar valves of aorta and pulmonary artery have three cusps, which meet at commissures.

BLOOD SUPPLY OF HEART

Both coronary arteries are arising from root of aorta, which supply blood to heart during diastole. The principal veins of heart are cardiac vein and the coronary sinus. Most abundant blood supply goes to the myocardium of the left ventricle. Site of myocardial infarct depends on level of occlusion in coronary arteries.

Left Coronary Artery

Left coronary artery divides into left anterior descending (LAD) and left circumflex artery (LCA). Occlusion of left coronary artery at origin site causes produces massive anterolateral acute myocardial infarction.

- **Left anterior descending branch of left coronary artery (LAD):** LAD runs in anterior interventricular sulcus and supplies anterior portion of left ventricle including apex, anterior two-thirds of the interventricular septum, and adjacent area of right ventricle. Stenosis of LAD leads to infarction of anterior part of the left ventricle along with anterior two-thirds of the interventricular septum in 40–50% of cases.
- **Left circumflex artery (LCA):** LCA runs in coronal sulcus is a transverse groove present between the atria and ventricles. It supplies blood to the lateral free wall of the left ventricle. Stenosis of LCA leads to infarction in the lateral wall of the left ventricle in 15–20% of cases.

Right Coronary Artery

- Right coronary artery runs in posterior interventricular sulcus. It nourishes the entire right ventricle, posteroinferior wall of the left ventricle including the posteromedial papillary muscle, and posterior one-third of the interventricular septum. It also supplies 90% of the blood to the SA node (sinoatrial node) and AV node (atrioventricular node).
- Stenosis of right coronary artery causes infarction in the right ventricle, posteroinferior wall of left ventricle, along with posterior one-third of the interventricular septum in 30–40% of cases.

CONDUCTION SYSTEM OF HEART

The conduction system is composed of specialized myocytes, with fewer intercalated discs and higher glycogen content. The conduction system is demonstrated by Masson trichrome, Verhoeff-van Gieson, and Alcian blue histochemical stains.

Sinoatrial Node

Sinoatrial (SA) node measures 0.3 mm and located in the upper right atrial wall near the opening of the superior vena cava. It initiates cardiac action potential at a velocity of 0.3 m/second, which sets basic pace for heart rate and conduction throughout both atria. It possesses high concentration of sodium ions in the extracellular fluid and negative charge inside the nodal fibers.

Atrioventricular Node or Node of Tawara

Atrioventricular (AV) node is located in posterior wall of right atrium along the lower part of interatrial septum, which receives action potential from SA node.

Bundle of His and its Branches

Bundle of His is located in superior portion of interventricular septum and divides into left and right branches, which receives action potential from atrioventricular (AV) node and passes to myofibers through left and right bundle branches.

Conduction Myofibers (Purkinje Fibers)

Purkinje fibers are located in ventricular septum, which extend out to the papillary muscles and lateral walls of ventricles. Purkinje fibers receive action potential from bundle of His and its branches and pass them to ventricular myocardium.

ISCHEMIC HEART DISEASE

ISCHEMIC HEART DISEASE: OVERVIEW

Ischemic heart disease (IHD) is a condition in which there is an inadequate supply of oxygenated blood to the myocardium. It typically occurs when there is an imbalance between myocardial oxygen supply and its demand.

- The most common cause of ischemic heart disease is atheromatous plaque formed in the epicardial artery/arteries, sufficient to cause a regional reduction in myocardial oxygenated blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery.
- Depending on the extent of involvement of coronary artery/arteries, patient may present with angina pectoris (stable, unstable or Prinzmetal variants), myocardial infarction, sudden cardiac death, and chronic IHD with congestive heart failure.

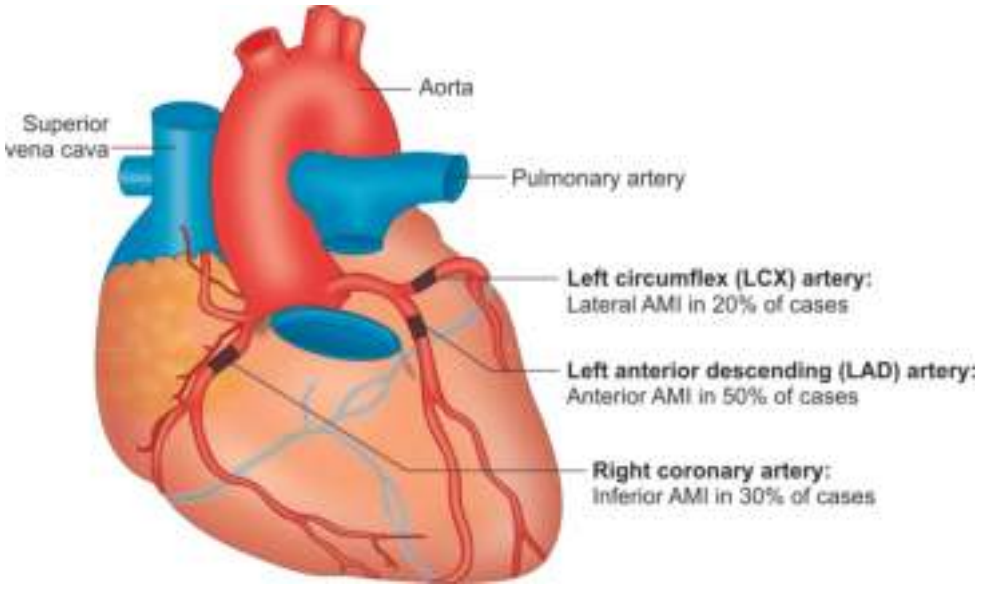


Fig. 16.1: Locations of blockage in the coronary arteries: Left anterior descending artery (50%), right coronary artery (30%) and left circumflex artery (20%).

- Acute coronary syndrome comprises angina pectoris, myocardial infarction and sudden cardiac death.
- Atherosclerosis in epicardial coronary artery is the most important cause of ischemic heart disease.
- Atherosclerosis develops at different rates in different segments of the coronary arteries resulting in segmental reduction in cross-sectional area.
- The coronary arteries involved due to atherosclerosis in descending order include anterior descending branch of left coronary artery (50%) especially in the proximal region, right coronary artery (30%) and circumflex branch of left coronary artery (20%).
- Locations of blockage in the coronary arteries due to atherosclerosis are shown in Fig. 16.1.

RISK FACTORS

Risk factors for atherosclerosis include major modifiable risk factors (hyperlipidemia, hypertension, diabetes mellitus, tobacco smoking), nonmodifiable risk factors (increasing age, male gender, family history of premature atherosclerosis, genetic abnormalities in LDL receptors, ApoA1, ApoAE, hepatic lipase); and minor risk factors (obesity, type A personality, factors affecting thrombosis, high carbohydrate diet, homocysteinemia, infectious agents).

- Approximately 25% of patients either succumb to terminal ventricular arrhythmias and sudden cardiac death prior to reaching hospital or suffer from a similar event in the critical coronary care unit.
- Myocardial ischemia can also occur in left ventricular hypertrophy due to aortic stenosis.
- A reduction in oxygen-carrying capacity in the blood due to severe anemia, presence of carboxyhemoglobin may lower the threshold for myocardial ischemia in patients with moderate coronary obstruction induced by atheromatous plaque. Risk factors for atherosclerosis are given in Table 16.1.

Table 16.1 Risk factors for atherosclerosis

Modifiable Major Risk Factors	Nonmodifiable Major Risk Factors	Minor Risk Factors
▪ Hyperlipidemia ▪ Hypertension ▪ Diabetes mellitus ▪ Tobacco smoking	▪ Increasing age ▪ Male gender ▪ Family history of premature atherosclerosis ▪ Genetic abnormalities (e.g. LDL receptor, ApoA1, ApoB, ApoE, 5-lipoxygenase, hepatic lipase deficiency)	▪ Obesity ▪ Stress (type A personality) ▪ Factors affecting thrombosis (thrombophilia) ▪ High intake of carbohydrate ▪ Homocysteinemia >100 µmol/L ▪ Infectious agents (*Chlamydia pneumoniae*, *Helicobacter pylori*, herpesvirus, cytomegalovirus promote atherosclerosis)

CONSEQUENCE OF MYOCARDIAL ISCHEMIA

During episodes of inadequate perfusion caused by coronary atherosclerosis, myocardial tissue oxygen tension falls, which results in transient disturbances of the mechanical, biochemical and electrical functions of the myocardium. During myocardial ischemia, regional disturbances of ventricular contractility cause segmental hypokinesia, akinesis or dyskinesis in severe cases, which can reduce myocardial pump function. Abrupt development of severe myocardial ischemia due to subtotal or total occlusion of coronary arteries is associated with instantaneous failure of normal cardiac muscle contraction and relaxation. Poor perfusion of the myocardium leads to left ventricular failure. Involvement of papillary muscle causes mitral valve regurgitation.

INCIDENCE OF CORONARY ARTERY DISEASE IN PRESENT SCENARIO

Incidence of ischemic heart disease is decreasing due to various diagnostic and therapeutic advances availability in coronary care units in the present scenario. Diagnostic advances include 12-lead electrocardiogram, coronary angiography, two-dimensional echocardiography, radionuclide scanning, serum cardiac biomarkers (cardiac troponins and enzymes).

- Therapeutic advances include revascularization procedures such as percutaneous coronary artery angioplasty, intracoronary stents and coronary artery bypass graft (CABG) and thrombolytic therapy.
- Modification of major risk factors by treating diabetes mellitus, hypertension, hyperlipidemia (statins lipid lowering agents) and stoppage of tobacco smoking and prophylactic use aspirin reduce the risk of coronary artery disease.
- Diagnostic and therapeutic advances in reducing incidence of coronary artery disease are given in Table 16.2. Revascularization procedures to re-establish coronary artery blood flow to myocardium are given in Table 16.3. 12-lead electrocardiogram (ECG) is given in Table 16.4.

Table 16.2 Diagnostic and therapeutic advances in reducing incidence of coronary artery disease

Advances	Comments
Diagnostic advances	
Coronary artery angiography	Detection of blockage in coronary arteries
12-lead electrocardiogram	Diagnostic in majority of cases
2-dimensional echocardiography	Detection of motion of cardiac chambers
Radionuclide scanning	Detect location of infarct region
Serum cardiac troponins	Defining evidence of myocardial injury
Serum CK-MB isoenzyme	Diagnostic serum cardiac biomarker in myocardial infarction
Revascularization procedures	
Thrombolytic agent therapy (streptokinase or tissue plasminogen activator)	Administered to lyse recently formed thrombus to re-establish blood flow to myocardium
Percutaneous coronary angioplasty	Balloon angioplasty used to vasodilate stenosed coronary artery
Intracoronary stenting	Stents put in the coronary arteries to re-establish blood flow to myocardium, drawback is trauma to myocardium
Coronary artery bypass graft	Internal mammary artery preferred over saphenous vein due to longer life for 5–10 years

Table 16.3 Revascularization procedures to re-establish coronary artery blood flow to myocardium

Revascularization Procedures	Comments
Coronary angioplasty	▪ Coronary angioplasty, also known as balloon angioplasty and percutaneous angioplasty is a minimally invasive endovascular procedure to dilate obstructed coronary artery ▪ A deflated balloon is attached to the catheter and passed over a guidewire into the stenosed coronary artery and then inflated to the fixed size. The balloon forces expansion the blood vessel to re-establish blood flow. Stent can be inserted at the time of ballooning to ensure the blood vessel remains open, and the balloon then deflated and withdrawn ▪ Risks and complications of coronary angioplasty include embolization, hematoma formation in the vessel and arterial rupture from overinflation of balloon ▪ Contraindications of coronary angioplasty include calcified vessel hematoma or a bypass origin

Contd...

Table 16.3 Revascularization procedures to re-establish coronary artery blood flow to myocardium (*Contd...*)

Revascularization Procedures	Comments
Coronary artery bypass grafting (CABG)	▪ Coronary artery bypass grafting is a major surgical operation, where patient's blocked coronary artery with atheromatous plaque is bypassed with harvested arterial or venous conduits ▪ As we know internal mammary arteries are always spared of atheromatous plaque. Therefore, internal mammary graft having longer life is preferred over saphenous vein graft ▪ Saphenous vein graft undergoes adaptive arterialization with development of atheromatous plaque in a span of 5–10 years ▪ Atheromatous plaque formed in saphenous vein graft is indistinguishable from that formed in the native coronary arteries, a process referred to as restenosis of the graft
Thrombolytic agents administration	Administration of thrombolytic agents such as streptokinase or tissue plasminogen activator (tPA) cause lysis of recently formed thrombus to re-establish the coronary blood flow and reduce further myocardial damage

Table 16.4 12-lead electrocardiogram (ECG)

In a 12-lead electrocardiogram (ECG), leads II, III and aVF demonstrate activity from the inferior cardiac wall, while leads I, aVL, V_5 and V_6 reflect activity of the lateral cardiac wall. Leads V_1–V_6 represent the anterior cardiac wall. Changes in the axis of the ECG can indicate conduction blockage or right ventricular hypertrophy. Increasing amplitude of electrical impulses is indicative of cardiac hypertrophy. P wave results from atrial contraction. QRS complex is produced by septal conduction and ventricular contraction. T wave is caused by repolarization of the ventricle

- **ST-segment elevation:** It is indicative of acute myocardial ischemia in myocardial infarction and Prinzmetal angina
- **ST segment depression:** It is indicative of stable and unstable angina
- **Q wave appearance:** It is usually indicative of transmural myocardial infarct
- **Non-Q wave myocardial infarcts:** These are indicative of subendocardial infarcts

ANGINA PECTORIS

Angina pectoris is episodic chest pain caused by inadequate oxygenation of the myocardium. Three variants of angina pectoris include: stable, unstable and Prinzmetal angina. Comparison of stable angina, unstable crescendo angina and Prinzmetal variant angina is given in Table 16.5. Management of angina pectoris is given in Table 16.6.

STABLE ANGINA

Stable angina is most common form of angina pectoris and predictable, which occurs at a fixed level of exercise.

- Due to severe atheromatous plaque in coronary arteries with reduction in cross sectional area by 70% and luminal diameter <1 mm in epicardial arteries (fixed stenosis) and decreased blood flow are not able to meet out the metabolic demand of myocardium during exercise.

Table 16.5 Comparison of stable angina, unstable crescendo angina and Prinzmetal variant angina

Characteristics	Stable Angina	Unstable Crescendo Angina (also known as Preinfarction Angina)	Prinzmetal Angina
Etiology	Occurs due to fixed coronary atherosclerosis with reduction in cross-sectional by 70% and luminal diameter <1 mm in epicardial arteries	Occurs due to rupture of an atheromatous plaque with superimposed thrombosis, incomplete occlusion of coronary arteries and microembolization	Occurs due to coronary artery vasospasm
Precipitating factors	Chest pain related to strenuous physical activity and emotional excitement of patient	Chest pain not related to strenuous physical activity, but prone to acute myocardial infarction	Chest pain not related to strenuous physical activity
Pathology	Represents reversible injury to myocytes without necrosis in subendocardial region	Represents reversible injury to myocytes without necrosis in subendocardial region	Represents reversible injury to myocytes without necrosis in transmural region
Electrocardiogram	ST segment depression due to subendocardial ischemia	ST segment depression due to subendocardial ischemia	ST-segment elevation due to transmural ischemia

Contd...

Table 16.5 Comparison of stable angina, unstable crescendo angina and Prinzmetal variant angina (*Contd...*)

Characteristics	Stable Angina	Unstable Crescendo Angina (also known as Preinfarction Angina)	Prinzmetal Angina
Clinical features	Chest pain in substernal region lasting <20 minutes, that radiates to the left arm and/or jaw, diaphoresis, and shortness of breath	Chest pain in the substernal region especially in the morning, that lasts for longer duration, high risk of progression to myocardial infarction	Intermittent chest pain in the substernal region even during rest
Management	Modification of risk factors, low-dose of aspirin, nitroglycerin, β-blockers and calcium channel blockers, percutaneous coronary intervention using balloon angioplasty, or coronary artery bypass grafting (CABG)	Potential thrombolysis or percutaneous coronary intervention using balloon angioplasty	Administration of sublingual nitroglycerin or calcium channel blockers
Outcome	Responds to medication vasodilator nitroglycerin	Requires hospitalization with interventional techniques to prevent myocardial infarction	Responds to medication vasodilator nitroglycerin

Table 16.6 Management of angina pectoris

Angina Pectoris Variants	Comments
Stable angina	▪ Stable angina can be managed by modification of risk factors, combination of drug therapy with low-dose of aspirin, nitroglycerin, β-blockers and calcium channel blockers ▪ Percutaneous coronary intervention using balloon angioplasty is performed to dilate coronary artery stenosis. ▪ Coronary artery bypass grafting (CABG) may be indicated in severe occlusive atheromatous plaque in the coronary artery
Unstable angina	▪ Patient with unstable angina should be hospitalized for ECG evaluation and potential thrombolysis or percutaneous coronary intervention using balloon angioplasty ▪ Such active measures reduce progression of myocardial infarction. Unstable angina is included in the non-ST-segment elevation myocardial infarction (NSTEMI) category of acute coronary syndrome
Prinzmetal crescendo angina	▪ Treatment during an episode of Prinzmetal crescendo angina is to relieve pain and shorten its duration by administration of sublingual nitroglycerin ▪ Patient is advised to take calcium channel blockers or long acting nitrates to reduce the frequency of episodes. β-Blockers are contraindicated, as these may worsen the attacks by blocking β_2 receptors

- Stable angina represents reversible injury to myocytes without necrosis. Patient presents with chest pain in substernal region lasting for <20 minutes, that radiates to the left arm and/or jaw, diaphoresis, and shortness of breath. Pain is relieved by taking rest and sublingual nitroglycerin. Electrocardiogram shows ST-segment depression due to subendocardial ischemia.
- About 20–30% of patients may develop unstable angina and myocardial infarction within one year of diagnosing stable angina.
- Stable angina can be managed by modification of major risk factors for atherosclerosis, combination of drug therapy with low-dose of aspirin, nitroglycerin, β-blockers and calcium channel blockers.
- Percutaneous coronary intervention using balloon angioplasty is performed to dilate coronary artery stenosis. Coronary artery bypass grafting (CABG) may be indicated in severe occlusive atheromatous plaque in the coronary arteries.

UNSTABLE ANGINA (CRESCENDO OR PREINFARCTION ANGINA)

Unstable angina is caused due to rupture of an atheromatous plaque with superimposed thrombosis, incomplete occlusion of coronary arteries and micro-embolization.

- Occluded coronary arteries are unable to supply oxygenated blood to support the increased myocardial demands during exertion. It usually begins as stable angina. It is an acute unpredictable event and unrelated to exercise, which represents reversible injury to myocytes without necrosis.
- Electrocardiogram shows ST segment depression due to subendocardial ischemia.
- Patient presents with recurrent chest pain in the substernal region especially in the morning, that lasts for longer duration. In patient with unstable angina there is high risk for progression to myocardial infarction.

- Patient with unstable angina should be hospitalized for ECG evaluation and potential thrombolysis or percutaneous coronary artery intervention using balloon angioplasty. Such active measures reduce progression of unstable angina to myocardial infarction. Unstable angina is included in the non-ST-segment elevation myocardial infarction (NSTEMI) category of acute coronary syndrome.

PRINZMETAL ANGINA

Prinzmetal angina is caused by vasospasm of coronary artery due to increased synthesis of thromboxane A_2 or decreased synthesis of endothelin vasodilator by vascular endothelium. It represents reversible injury to myocytes.

- Electrocardiogram shows ST-segment elevation due to transmural ischemia.
- Patient presents with intermittent chest pain in the substernal region even on rest. Pain is relieved by nitroglycerin. Disorder is self-limited. Sometimes chest pain may be severe. It may rarely result in myocardial infarction.
- Treatment during an episode of Prinzmetal angina is to relieve pain and shorten its duration by administration of sublingual nitroglycerin.
- Patient is advised to take calcium channel blockers or long-acting nitrates to reduce the frequency of episodes. β-Blockers are contraindicated, as these may worsen the attacks by blocking β_2 receptors.

MYOCARDIAL INFARCTION

Myocardial infarction is the most important cause of morbidity and mortality due to cardiac arrhythmias, cardiac failure, mitral valve regurgitation due to necrosis of the papillary muscle and hemopericardium. Mortality is about fourfold higher in elderly patients aged >75% as compared with young patients.

- Most myocardial infarcts occur in the left ventricle and interventricular septum, which receive blood supply from both the coronary arteries.
- Complete occlusion of the coronary artery produces transmural infarction from endocardium to epicardium.
- Subendocardial infarct involves inner third to inner half of myocardium as a result of prolonged ischemia induced by partially occluded coronary artery (Figs 16.2 to 16.4).
- Severe reduction in blood flow leads to circumferential myocardial infarct. It can be averted by immediate coronary artery angioplasty and administration of thrombolytic agent.
- Myocardial infarct undergoes a series of progressive gross and histologic changes. Altered permeability of necrosed myocytes releases cardiac troponins and enzymes into the circulation. Neutrophils, macrophages and fibroblasts participate in the evolution of myocardial infarct.
- Patients with acute coronary syndrome are classified into two groups to facilitate evaluation and management, namely patients with acute myocardial infarction with ST-segment elevation (**STEMI**) on electrocardiogram and those with non-ST-segment elevation (**NSTEMI**) with evidence with myocyte necrosis.
- Relative evidence of STEMI is declining due to greater use of aspirin, lipid lowering agents 'statins' and stoppage of tobacco smoking.
- When patients with prolonged myocardial ischemic discomfort at rest are examined, the working clinical diagnosis is that they are suffering from acute coronary syndrome. The 12-lead electrocardiogram is a pivotal diagnostic tool, that helps to decide pathway for management of the patients.
- Following disruption of a vulnerable atheromatous plaque, patients may experience ischemic discomfort

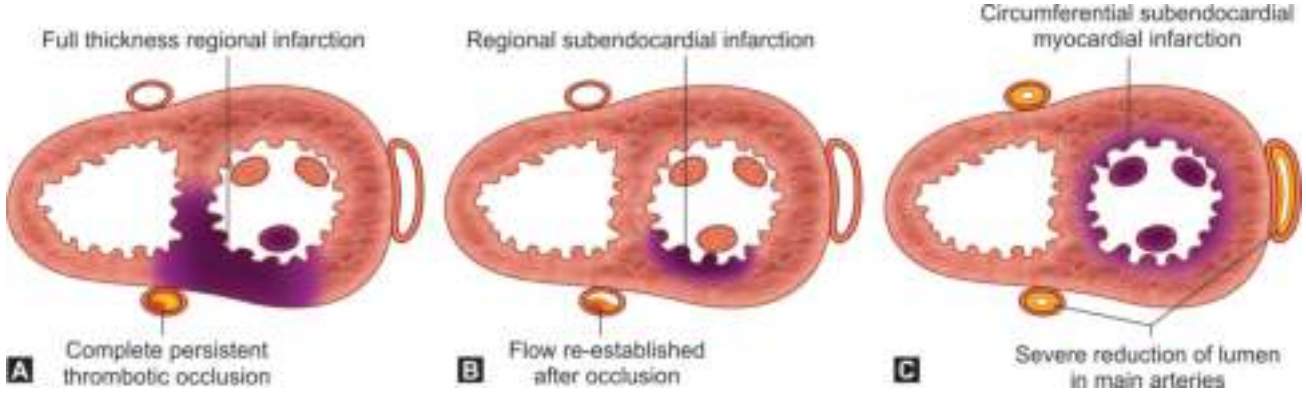


Fig. 16.2: Patterns of myocardial infarcts. (A) Transmural infarct due to persistent complete obstruction of coronary atherosclerosis with superimposed thrombus, (B) regional subendocardial infarct occurs as a result of re-establishment of blood flow after occlusion of coronary artery, (C) circumferential subendocardial infarct occurs due to svere reduction of lumen of coronary artery/arteries.

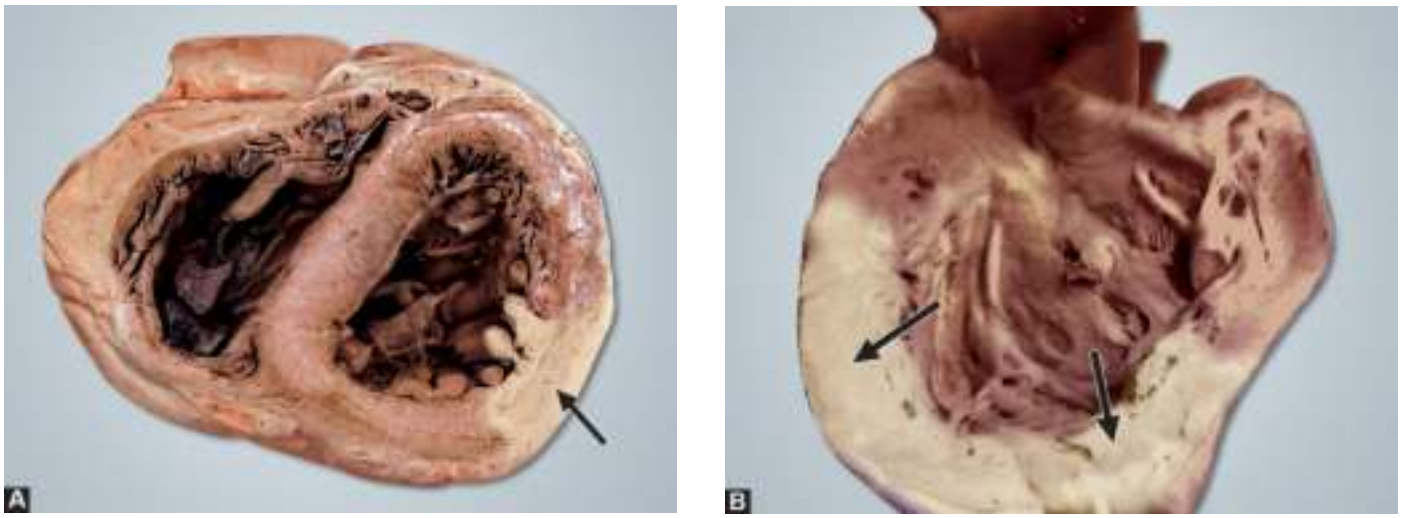


Fig. 16.3A and B: Transmural infarct due to persistent complete obstruction of coronary atherosclerosis with superimposed thrombus (arrows).

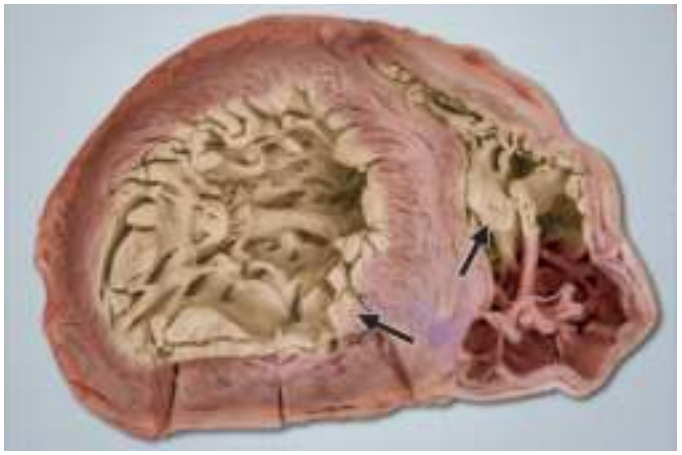


Fig. 16.4: Regional subendocardial infarct occurs as a result of re-establishment of blood flow after occlusion of coronary artery (arrows).

resulting from a reduction of oxygenated blood flow through the affected epicardial coronary artery/arteries. The reduction in blood flow may occur either due to complete occlusion or partial occlusion of the coronary artery.

- Patient may present with ischemic discomfort with or without ST-segment elevation.
- Patients with ST-segment elevation, the majority do not develop a new 'Q wave' on the electrocardiogram (Q wave MI), while a minority of cases do not develop a new 'Q wave' MI (NQMI). The patients who present without non-ST-segment elevation MI (NSTEMI), a distinction is based on the analysis of serum cardiac biomarkers such as CK-MB enzyme and cardiac troponins.

PATHOPHYSIOLOGY

Acute myocardial infarction with ST-segment elevation myocardial infarction (STEMI) usually occurs when the coronary blood flow decreases abruptly after a thrombotic occlusion of coronary artery previously affected by atheromatous plaque.

- Slowly developing severe coronary artery stenosis does not precipitate STEMI due to the presence of a rich collateral vascular network overtime. Instead, STEMI occurs when coronary artery thrombosis develops rapidly over disrupted atheromatous plaque.
- The amount of myocardial damage caused by coronary artery occlusion depends on the territory supplied by the affected coronary artery, partial or complete occlusion of the vessel, duration of coronary occlusion, quantity of blood supplied by the collateral blood vessels, demand of oxygen of the myocardium, endogenous factors and perfusion-induced injury.

Role of Acute Atheromatous Plaque Disruption

In most cases, STEMI occurs due to the disruption of an atheromatous plaque resulting in exposure of its contents to the blood favoring thrombogenesis. Formation of mural thrombus at the site of atheromatous plaque disruption leads to occlusion of the coronary artery/arteries. Histologic studies indicate that the coronary atheromatous plaques are prone to disruption. Causes of myocardial infarction are given in Table 16.7.

Role of Platelets

After an initial formation of platelet monolayer at the site of atheromatous plaque, various agonists (collagen, ADP, epinephrine, serotonin) promote platelet activation. After agonist activation of platelets, thromboxane A_2, a potent local vasoconstrictor is released by activatd platelets for further platelet activation. Formation of primary hemostatic plug by platelet adhesion activation, aggregation and degranulation is shown in Fig. 16.5.

Table 16.7 Causes of myocardial infarction

Lesions	Comments
Atheromatous lesions (most common)	
Complicated atheromatous plaque with rupture	Complicated atheromatous plaque fissuring leads to adhesion and aggregation of platelets and activation of coagulation cascade
Nonatheromatous lesions (less common)	
Vasculitis	Polyarteritis nodosa, systemic lupus erythematosus, Kawasaki disease
Embolization to coronary arteries	Emboli arising from left-sided heart due to infective endocarditis; or paradoxical emboli from right side of heart through patent foramen ovale resulting in blockage of coronary arteries.
Thrombotic disorders	Antithrombin III deficiency and polycythemia vera
Vasospasm of coronary arteries	Cocaine abuse causes sudden vasospasm of coronary arteries
Hemodynamic alterations	Hypotension due to spinal anesthesia and hypotensive shock
Congenital anomaly of origin of left anterior descending branch of left coronary artery	Congenital anomaly of origin of left anterior descending branch of left coronary artery results in blood flow obstruction to myocardium
Aortic dissection	Aortic dissection may obstruct coronary arteries
Trauma to coronary arteries	Direct trauma to the coronary arteries on road side accidents may obstruct blood flow in coronary arteries

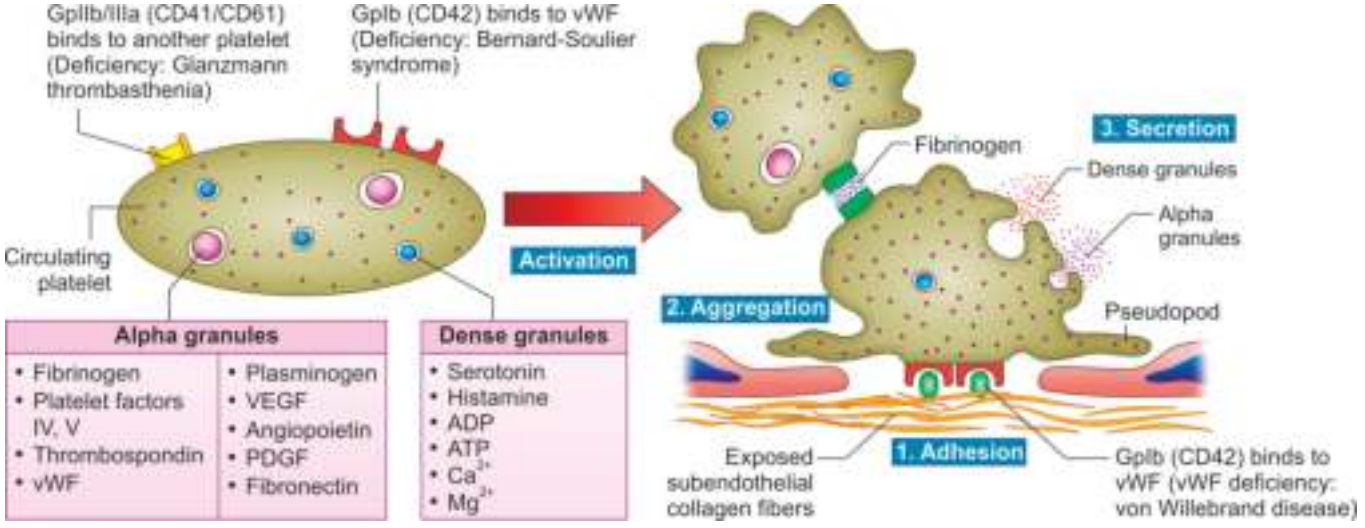


Fig. 16.5: Formation of hemostatic plug by platelet adhesion activation, aggregation and degranulation. Platelet activation involves three overlapping mechanisms. 1. Adhesion of platelets to the exposed subendothelium is mediated by the binding of von Willebrand factor (vWF) to glycoprotein (Gp) Ib/IX (CD42) resulting in initiation of signal for activation of platelets. 2. Exposure of GpIIb/IIIa (CD41/61) to the fibrinogen receptor on the platelet surface permits for platelet aggregation. 3. At the same time, platelets synthesize their granule contents, which facilitate further activation.

- **Platelet adhesion:** Tissue injury causes platelets to adhere to subendothelial collagen. Shape change, secretion of granule contents, and aggregation follow. Within seconds of tissue injury, vWF (von Willebrand factor), released from the Weibel-Palade of vascular endothelium and a granule of platelets, binds to the platelet using GP1a receptors resulting in adhesion of the platelets to the exposed collagen fibers of damaged blood vessels.
- **Platelet activation and degranulation:** The platelets become activated and release ADP and thromboxane A_2 (TXA_2). The ADP and TXA_2 attract additional platelets resulting in platelet aggregation and primary hemostatic plug formation. Platelet shape change after activation by an agonist by ADP and thromboxane A. Agonist is a substance, which initiates a physiologic response when combined with a receptor. Pseudopodia develop on the surface of platelet. The pseudopodia contain a network of actin and myosin. The microtubule circumferentially contracts resulting in activation of phospholipids and glycoprotein IIb/IIIa receptors. Internal biochemical changes occur in the platelets resulting in granule secretion.

- **Primary and secondary hemostasis:** Platelets aggregate at the site of injury via GpIIb/IIIa using fibrinogen from the plasma as a linking molecule; resulting in formation of platelet plug (primary hemostasis). Platelet plug is weak; coagulation system cascade (secondary hemostasis) stabilizes it.

Blood Coagulation System

- Activators and inhibitors of coagulation system and fibrinolysis keep the coagulation system in balance. Clotting occurs when blood vessels are injured and exposed to activators of coagulation factors. Clotting is controlled because fibrinolysis process is initiated in response to activation of coagulation factors. Inhibitors of both clotting and fibrinolysis serve to bring the coagulation system back to balance. An imbalance in the activation or inhibition of either blood clotting and/or fibrinolysis causes thrombosis.
- The terminal steps in both intrinsic and extrinsic coagulation pathways remain same. Both coagulation pathways lead to the activation of factor X. Intrinsic pathway is initiated by activation of circulating factor XII. Extrinsic pathway is activated by a cellular lipoprotein known as tissue factor that becomes exposed when tissues are injured. Calcium, factor X, factor V, and platelet phospholipids combine to form prothrombin activator, which then converts prothrombin to thrombin. The interaction causes conversion of fibrinogen into fibrin strands that create the insoluble and stable blood clot (Fig. 16.6).

MYOCARDIAL INFARCTS: TYPES

- Gross morphology of myocardial infarct depends on extent of involvement of myocardium. There are three types of myocardial infarcts (transmural, subendocardial and circumferential infarcts).
- ECG is diagnostic of AMI in 75–80% of cases (appearance of new Q wave). Appearance of new Q wave on ECG is dependent on the volume of myocardial infarct area (transmural infarction). That is the reason that ST segment study in the electrocardiogram is important, which differentiates transmural and subendocardial infarcts (Table 16.8).

Transmural Myocardial Infarct

Transmural myocardial infarct involves the entire left ventricular wall from endocardium to epicardium including involvement of interventricular septum.

- **Pathogenesis:** Transmural infarct is caused by disrupted atheromatous plaque with superimposed thrombus. Blood supply to the heart is not restored after ischemia. In small number of cases, transmural infarct is related to thromboemboli or vasospasm.
- **ECG study:** It reveals STEMI and appearance of new Q wave. ST-segment elevation indicates area of injury and loss of normal myocardial cell membrane ion pumps. Appearance of new Q wave indicates extent of myocardial infarct.
- **Therapeutic correlation:** Patients with transmural myocardial infarction require emergency treatment to reopen the obstructed coronary artery either by coronary angioplasty or thrombolytic drugs that

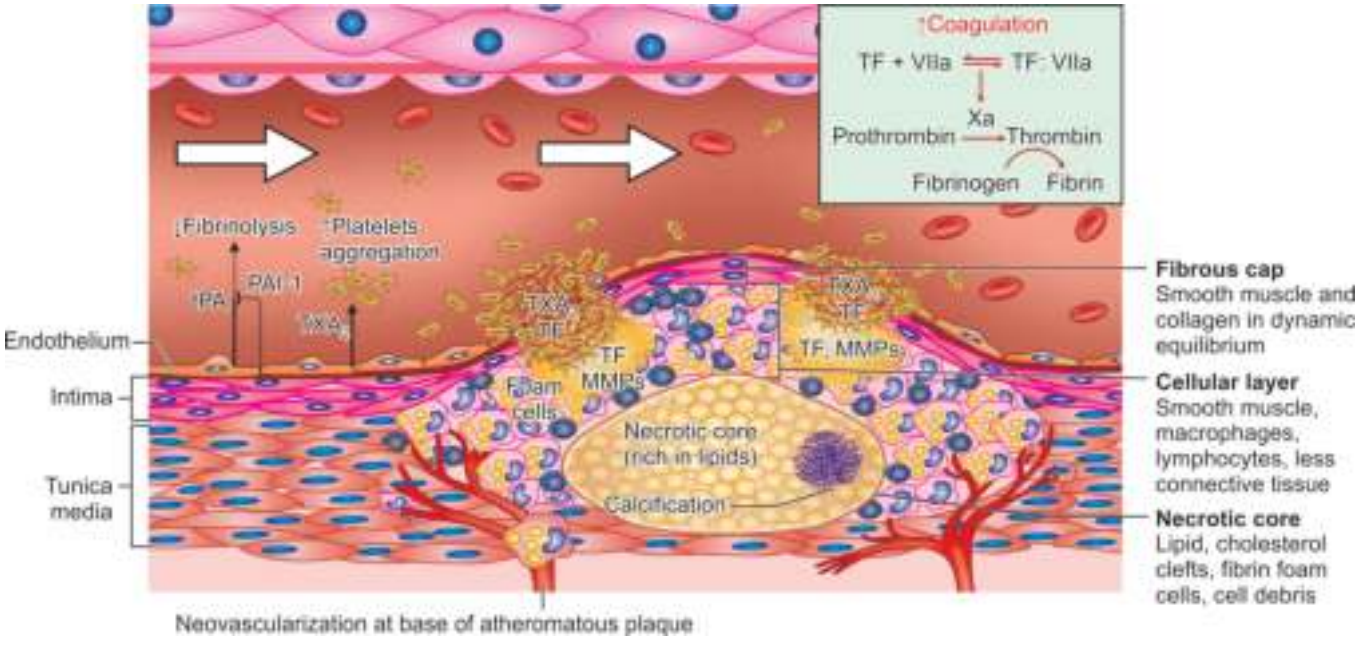


Fig. 16.6: When atheromatous plaque ruptures, the balance between fibrinolysis and coagulation is shifted to greater thrombosis, and occlusive thrombus formation (TF: Tissue factor, tPA: Tissue plasminogen activator, TXA_2: Thromboxane A_2, MMPs: Matrix metalloproteinases).

Table 16.8 Differences between transmural and subendocardial infarcts

Characteristics	Transmural Infarct	Subendocardial Infarct
Extent of myocardium involved due to infarction	Entire left ventricular wall from endocardium to epicardium including interventricular septum	One-third to inner-half of the ventricular thickness
Frequency	More common (85%)	Less common (5%)
Nature of lesion	Unifocal and solid area of coagulative necrosis	Multifocal and patchy areas of coagulative necrosis in circumferential distribution
Distribution	Area supplied by specific coronary artery	Circumferential pattern
Thrombosis superimposed on atheromatous plaque in the coronary artery	Common as a result of thrombosis superimposed on atheromatous plaque in the coronary artery resulting in persistent deprivation of blood supply to myocardium	▪ No evidence of thrombus superimposed ▪ Uncommon as a result of atheromatous plaque disruption followed by thrombus formation and lysis resulting in restoration of blood supply to myocardium
Cardiogenic shock	Often causes cardiogenic shock	Often results from hypotension or shock
Pericarditis	Common	Absent
Cardiac aneurysm	May be observed	Does not develop
ECG changes	▪ Elevation of ST segment and appearance of new Q wave ▪ ST elevation infarct (STEMI)	Non-ST elevation infarct (NSTEMI)

Coagulative necrosis occurs only 20–40 minutes after ischemia. Gross and microscopic changes develop only hours to days after the onset of ischemia.

activate the plasminogen system to lyse thrombosis in coronary arteries.

Subendocardial Myocardial Infarct

Under physiologic state, subendocardial region of heart receives oxygen and nutrients by diffusion directly from the circulating blood in the ventricular cavity. Subendocardial myocardial infarct involves inner one-third to inner-half of the left ventricular wall or sometimes right ventricle. It exhibits multifocal areas of coagulative necrosis. Isolated infarcts of right ventricle and right atrium are extremely rare.

- **Pathogenesis:** Typically, subendocardial myocardial infarct occurs as a result of decreased perfusion of myocardium in hypotensive shock, and restoration of blood supply.
- **ECG study:** It reveals absence of ST-segment elevation myocardial infarction (NSTEMI) as well as appearance of a new Q wave. In minority of cases, ECG study reveals absence of ST-segment elevation and appearance of a new Q wave.
- **Diagnostic approach:** These patients require estimation of cardiac troponins for establishing diagnosis.

REPERFUSION-INDUCED MYOCARDIAL INJURY

Reperfusion of the myocardial infarct by therapeutic administration of thrombolytic agents may cause further injury to myocardium by synthesis of oxygen-derived free radicals.

Oxygen-derived Free Radicals

Initially, myocardial ischemia produced cellular damage results in generation of oxygen-derived free radicals.

- Subsequently, myocardial reperfusion by thrombolytic agents provides abundant molecular oxygen (O_2) to generate more oxygen-derived free radicals through the xanthine oxidase pathway in activated neutrophils.
- These oxygen-derived free radicals injure viable myocytes.

Histologic Alterations

Reperfusion injury histologically alters irreversibly damaged myocardial cells. It produces contraction band necrosis of myofibrils in dying cells due to influx of calcium into the cytosol.

Removal of Necrosed Myocytes

Removal of irreversibly damaged myocytes improves short- and long-term function and survival. It prevents further damage to myocardial cells and limits the size of myocardial infarct.

Morphologic Changes: Myocardial Infarction

Gross Morphology

- No gross changes are evident within 24 hours of myocardial infarction.
- In autopsy specimen, myocardial infarct area can be appreciated by applying triphenyltetrazolium chloride (TTC) to slices of myocardial infarct (2–3 hours old). Due to depletion of lactate dehydrogenase (LDH) enzyme, infarct area of myocardium remains unstained.
- Normal myocardium is rich in LDH enzyme, hence gives uniform brownish discoloration to the dye TTC.

Light Microscopy

Irreversible injury to myocardium occurs in 20–40 minutes after cessation of blood supply. Myocardium shows wavy myocardial fibers within 1–3 hours of myocardial infarction. There may be eosinophilia of myocardial fibers.

- **Acute inflammatory changes:** Neutrophils accumulate at the peripheral zone and then infiltrate the necrotic tissue within 18–24 hours. Two days after myocardial infarction, the affected heart muscle demonstrates coagulative necrosis with loss of cross striations, contraction bands, edema, and hemorrhage. Neutrophils are replaced by macrophages.
- **Healing stage:** Proliferation of fibroblasts results in fibrosis of infarct area. Myocardial cells being terminally differentiated permanent cells are unable to regenerate and healing occurs by fibrosis. However, the patient may die due to electrical disturbances before the myocardial infarct heals. Examination of the heart in such cases at autopsy will reveal the typical macroscopic features of a myocardial infarct at various stages of evolution and/or healing (Figs 16.7, 16.8 and Table 16.9).

CLINICAL COMPLICATIONS

Clinical complications of acute myocardial infarction (AMI) depend upon the size and location of the myocardial infarct, as well as pre-existing myocardial damage. Ventricular arrhythmia is the most common cause of death in early 0–4 hours of AMI. Risk of myocardial rupture is fatal complication within first 3–7 days. Ventricular aneurysm is common in scarred area after 7 weeks of AMI.

Cardiogenic Shock

Cardiogenic shock is commonly associated with large transmural infarcts, which occurs in the first 24 hours in 10–15% of cases. Weakening of the myocardium leads to cardiac failure. Revascularization improves survival.

Cardiac Arrhythmias

- Cardiac arrhythmias account for 50% of all deaths within the first 24 hours after an AMI. The risk of cardiac arrhythmia is high within the first 6 hours after AMI.
- Acute myocardial infarction (AMI) is often associated with premature ventricular beats, ventricular tachycardia, complete heart block (anterior or posterior region), ventricular fibrillation and cardiogenic shock with fatal outcome within 2 days of AMI.

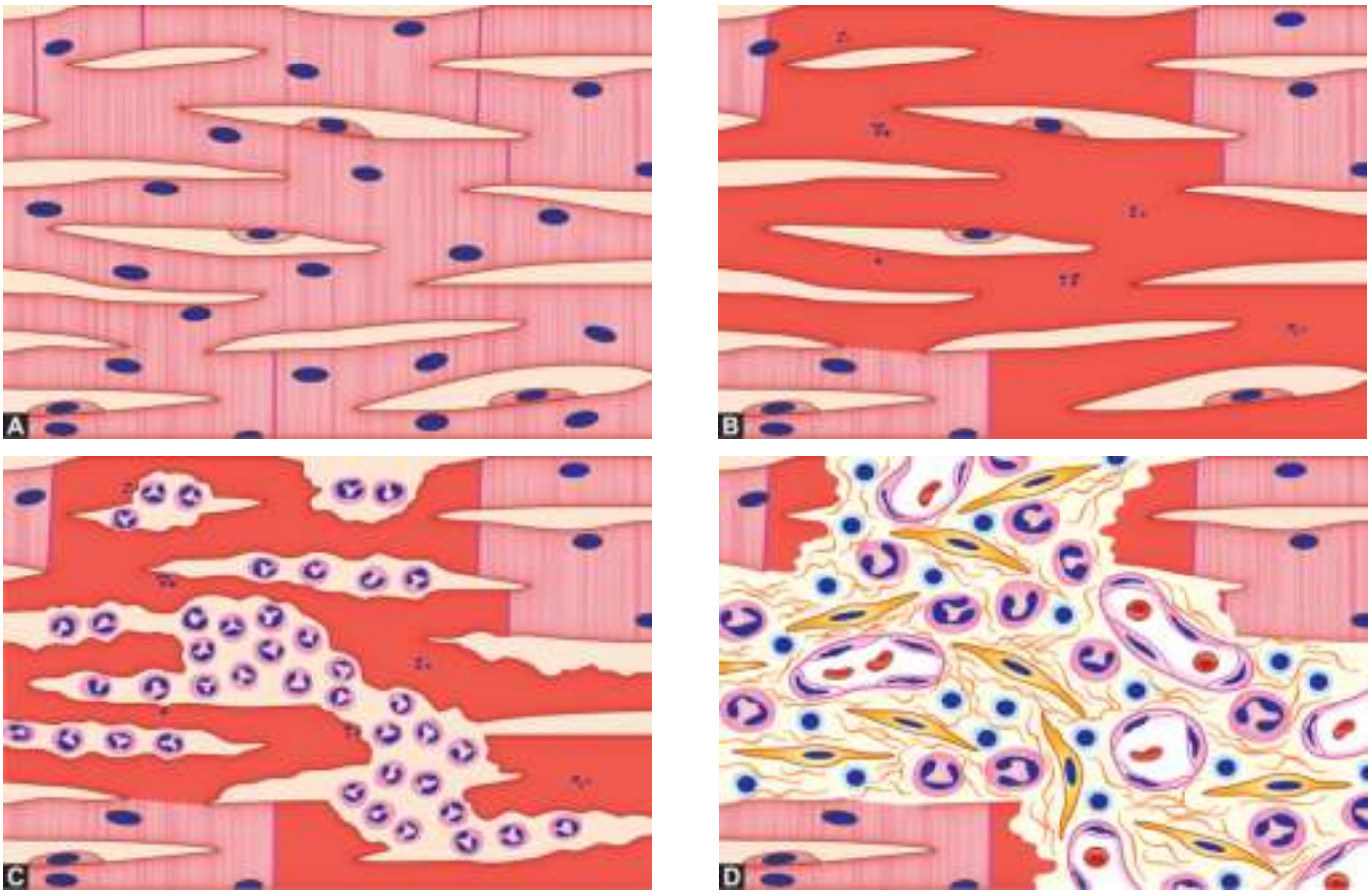


Fig. 16.7: Morphologic changes in acute myocardial infarction. (A) Normal heart muscles, (B) coagulative necrosis of acute myocardial infarction (AMI) after 12–18 hours, (C) PMN cells about 24 hours after acute myocardial infarction (AMI), (D) granulation tissue is formed after 3 weeks of acute myocardial infarction (AMI).

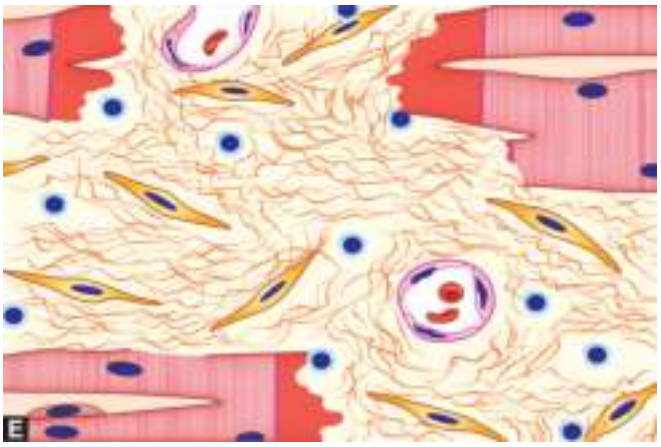


Fig. 16.7E: Morphologic changes in acute myocardial infarction—after 3 months or more, the infarct myocardium has been replaced by scar tissue.

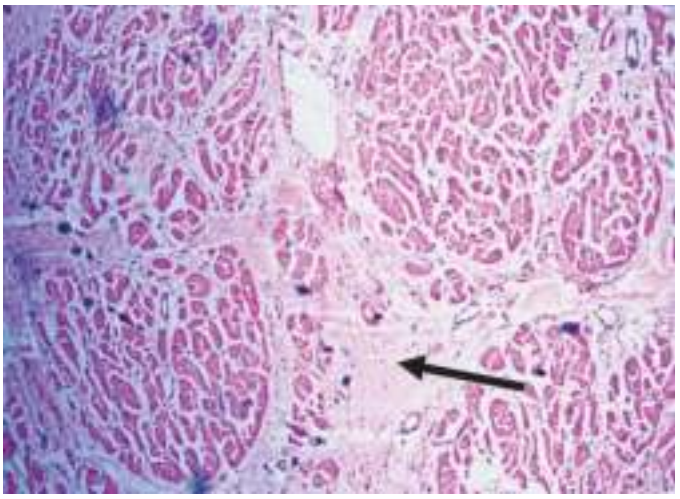


Fig. 16.8: Healed myocardium after myocardial infarction (arrow).

Table 16.9 Progressive morphologic changes in myocardial infarction

Time Post-AMI	Gross Morphology	Microscopic Examination	Laboratory Findings
0–4 hours	No change	▪ Wavy myocardial fibers ▪ Staining defect with triphenyl tetrazolium chloride in 2–3 hours	No change
4–12 hours	No change	Coagulative necrosis begins with loss of cross striations, contraction bands, edema, hemorrhage and early neutrophilic infiltrate	▪ CK-MB (4–8 hours) ▪ LDH (10 hours) ▪ Aspartate aminotransferase (AST) (6–12 hours)
12–24 hours	Early pallor of myocardium due to coagulative necrosis	▪ Continued coagulative necrosis with nuclear pyknosis, marginal contraction bands, loss of cardiac striations with eosinophilic cardiac fibers and neutrophilic infiltrate in interstitial tissue. ▪ Cytoplasm displays affinity for acidophilic dyes	▪ CK-MB (4–8 hours) and peaks in 24 hours ▪ LDH (10 hours) ▪ Aspartate aminotransferase (AST) (6–12 hours)
1–3 days (24–72 hours)	Definite pallor of the infarct area with zone of hyperemia	▪ On second day, total loss of striations and nuclei along with heavy neutrophilic infiltrate. ▪ Some macrophages start appearing in the infarct area	▪ CK-MB starts disappearing ▪ LDH flip peaks in 2–3 days ▪ Aspartate aminotransferase (AST) peaks 1–2 days
3–7 days	On 4th day, central yellow infarct area with well demarcated hyperemic border. There is maximum softness and danger of ventricular rupture	▪ On 4th day, there is influx of macrophages from hyperemic area, which slowly replace neutrophils and remove the dead myocardial cells ▪ At end of first week, it shows fibrovascular response with formation of granulation tissue surrounding area of infarct	LDH starts disappearing
7–10 days	Same findings as seen in 1–3 days. Central yellow area and well demarcated hyperemic border. There is maximum softness and danger of ventricular rupture	▪ By the end of first week, neovascularization marks the beginning of granulation tissue formation, i.e. repair with collagen synthesis. ▪ Macrophages infiltration is present	▪ CK-MB is absent, as it disappears in 3–4 days ▪ LDH disappears in 5–9 days ▪ Aspartate aminotransferase (AST) disappears
10–21 days	Maximum yellow and soft central area with vascular margins by third week (21 days)	▪ During second week, fibrovascular response begins to form granulation tissue to replace the yellow necrotic myocardium	No enzyme or cardiac troponins are detected in blood after 2 weeks

Contd...

Table 16.9 Progressive morphologic changes in myocardial infarction (*Contd...*)

Time Post-AMI	Gross Morphology	Microscopic Examination	Laboratory Findings
		▪ By third week, granulation tissue slowly transforms into a fibrous scar, i.e. repair. Phagocytosis of necrotic debris by macrophages is complete. Inflammatory infiltrate recedes	
7 weeks	White fibrosis	▪ Infarct tissue is replaced by white, patchy and non-contractile scar tissue by sixth week ▪ Ventricular aneurysm may occur in scarred area	No enzyme or cardiac troponins are detected in blood after 2 weeks

Sudden Cardiac Death

Most often, sudden cardiac death occurs due to ventricular arrhythmia and other electrical disturbances. It occurs within an hour of onset of symptoms in acute myocardial infarction. It occurs more frequently in the morning hours, when hypercoagulability is at its peak.

- **Pathogenesis:** Disrupted atheromatous plaque releases thromboplastin, which initiates thrombus formation in affected coronary artery. It results in complete occlusion of the coronary artery with superimposed thrombus (>80% lumen occlusion) resulting in myocardial ischemia. Ventricular arrhythmia is most common cause of death in these patients.
- **Autopsy findings:** Most patients have severe, fixed atherosclerotic coronary artery disease involving one or more coronary arteries. However, a grossly obvious coronary thrombosis or acute changes in the myocardium (coagulative necrosis) are not usually present on autopsy.

Congestive Heart Failure

Congestive heart failure usually occurs within the first 24 hours following acute myocardial infarction. Patient develops breathlessness due to pulmonary edema.

Fibrinous Pericarditis with or without Effusion

Acute fibrinous pericarditis occurs with transmural infarction usually within 2–10 days as a result of increased blood vessel permeability in the pericardium with pouring of fibrinous exudate in the pericardium (Fig. 16.9).

- Precordial friction rub is heard on auscultation.
- Fibrinous pericarditis may lead to obliteration of the pericardial cavity and formation of fibrous adhesions in the healing stage of the disease.
- Substernal chest pain is relieved by leaning forward and aggravated by leaning backward.

Myocardial Rupture

Rupture of the heart occurs within 3–7 days after myocardial infarction, when the necrotic area has the least tensile strength. It occurs in transmural infarction due to softening of the necrosed myocardium by increased neutrophil activity before organization is established (Figs 16.10 and 16.11).

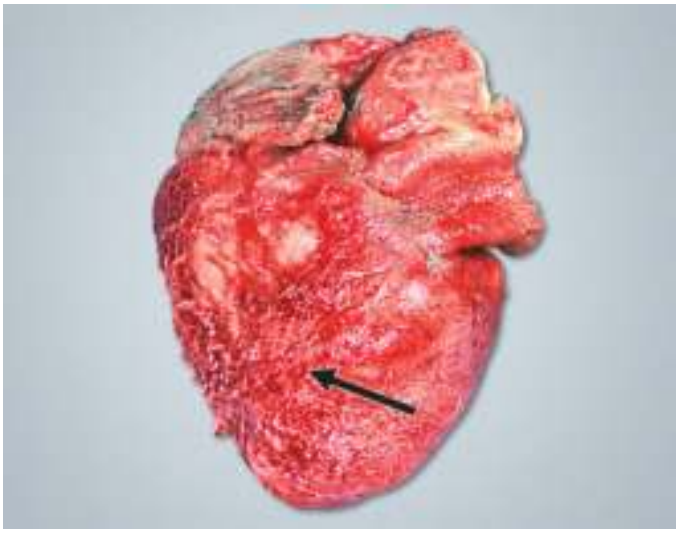


Fig. 16.9: Fibrinous pericarditis in rheumatic pancarditis (arrow).

- **Rupture of anterior wall of left ventricle:** The anterior wall of the heart is the most frequent site of rupture, usually resulting in fatal cardiac tamponade, which occurs in transmural infarct. High pressure in left ventricle blows out the necrotic myocardium resulting in rupture of anterior wall of heart and escape of massive blood into the pericardial cavity called hemopericardium. Patient dies of cardiac tamponade.
- **Posteromedial papillary muscle necrosis/dysfunction:** It is most often associated with right coronary artery thrombosis and inferior myocardial infarction. Since the damaged papillary muscle cannot contract, hence causes acute onset of mitral valve insufficiency/regurgitation resulting in left ventricular failure.
- **Interventricular septum rupture:** It is most often associated with thrombus formation in anterior descending branch of left coronary artery. Rupture of interventricular septum produces a left-to-right shunt resulting in right heart failure and pulmonary hypertension. Hence, there is increased O_2 saturation and pressure in right ventricle.

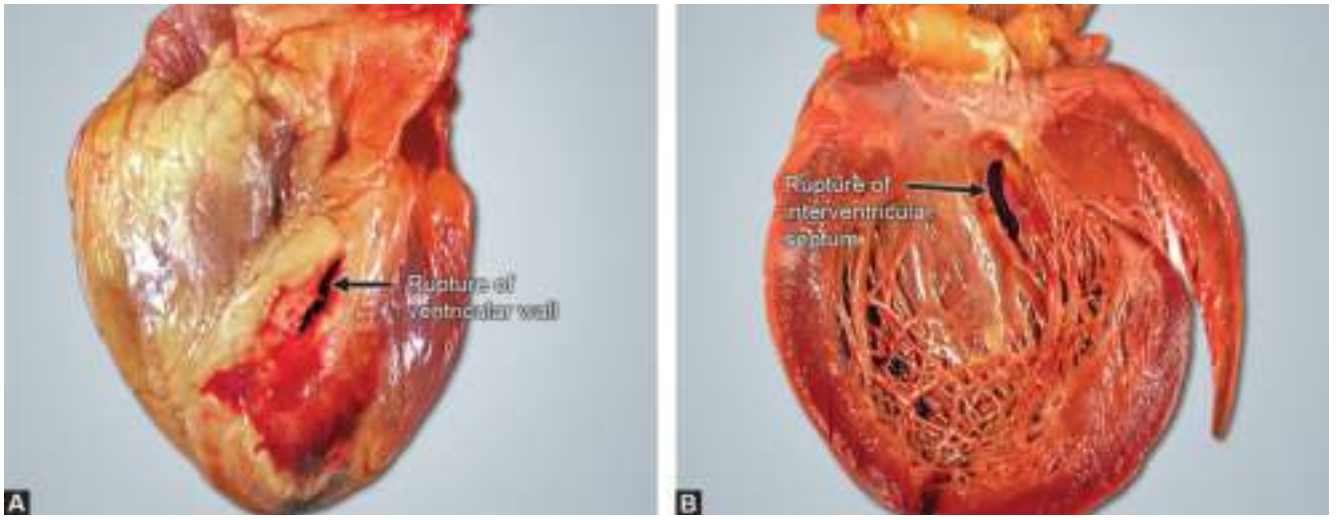


Fig. 16.10: Acute myocardial infarction. (A) Rupture of ventricular wall, (B) rupture of interventricular septum.

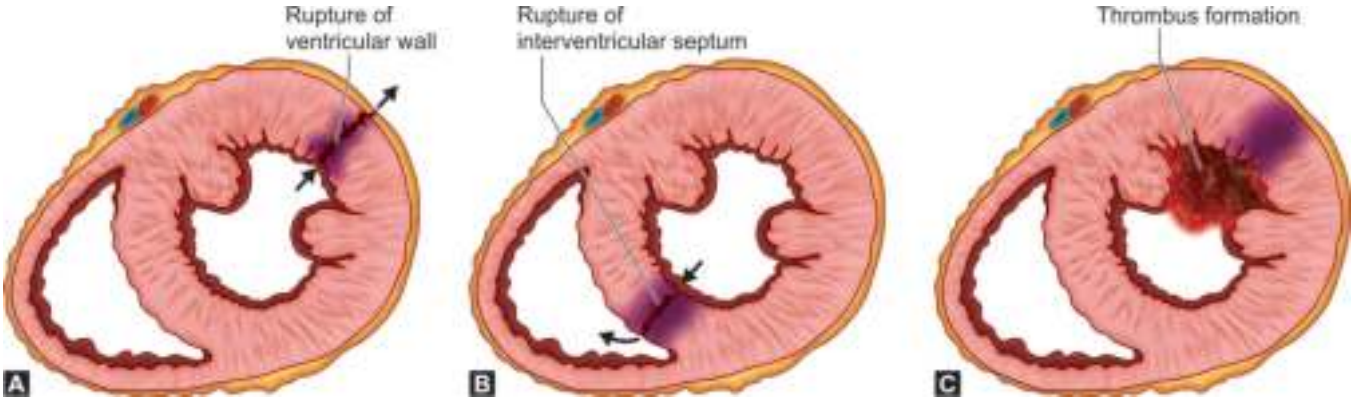


Fig. 16.11: Acute myocardial infarction. (A) Rupture of ventricular wall, (B) rupture of interventricular septum, (C) thrombus formation.

Mural Thrombi with Possible Embolization

Patient with acute myocardial infarction may develop thrombi in left ventricle, left atria and leg veins (Fig. 16.12).

- **Ventricular mural thrombi:** These are formed during first week of acute myocardial infarction as a result of left anterior descending (LAD) artery disease. Thrombi are attached to endocardium overlying myocardial infarct area of left ventricle. These thrombi have the potential for embolization to brain, intestine, kidney and lower limbs.
- **Atrial thrombi:** These develop in atrial appendages, especially if atrial rhythm is disturbed due to irregular blood flow inside the dilated and irregularly contracting left atrium, i.e. atrial fibrillation and atrial flutter.
- **Leg vein thrombosis:** It may develop due to prolonged bed rest and venous stasis. Patient presents with pain and swelling in the affected leg. Patient may have pulmonary embolism with fatal outcome.

Autoimmune Pericarditis

Dressler's syndrome is a complication of transmural myocardial infarct after 2–10 weeks, clinically presenting with pericardial pain associated with pleural effusion, joints and fever (i.e. symptoms suggestive of serum sickness).

- Autoantibodies are formed against pericardial proteins released from the necrotic cells resulting in pericarditis.
- Cortisone treatment is beneficial, also supporting an immunologic explanation.
- Other causes of immune-mediated pericarditis include systemic erythematosus, scleroderma and rheumatic heart disease.

Ventricular Aneurysm

- Ventricular aneurysm may develop in the large myocardial fibrotic scar within 3–6 months after myocardial infarction in 10–15% cases. During each systole, healed myocardial scar tissue does not

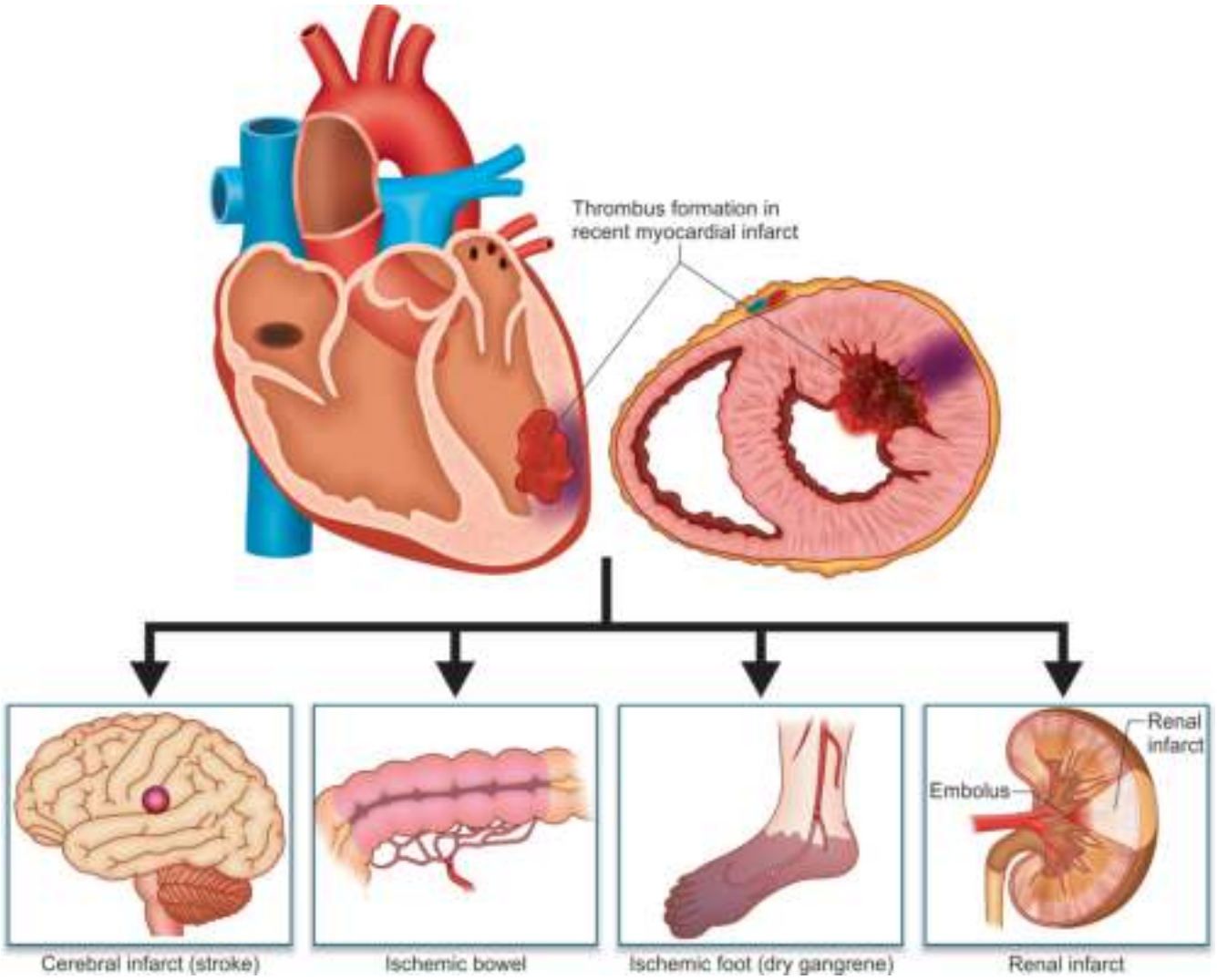


Fig. 16.12: Myocardial infarction. Thromboembolism phenomenon produces infarcts in vital organs.

contract, and actually bulges out resulting in ventricular aneurysm.

- Mural thrombi also form over ventricular aneurysms, which are found at the site of healed transmural myocardial infarct resulting in embolization. Aneurysm rupture is uncommon as scar tissue in healed myocardial infarct has good tensile strength.

Right Ventricular Infarct

Right ventricular infarct occurs in one-third of patients in the inferior region. It is associated with right coronary artery thrombosis. It is clinically significant in 30% of cases. Patient presents with hypotension, right heart failure and preserved left ventricular function.

Recurrent Myocardial Infarction

Recurrent myocardial infarction is characterized by the reappearance of CK-MB enzyme in blood after 3 days of acute myocardial infarction.

Expansion of Myocardial Infarct Area

Patient may develop expansion of myocardial infarct area with few hours of acute myocardial infarction.

Systemic Venous Thrombosis

Patient may develop systemic venous thrombus formation with fatal outcome.

Angina Pectoris (>12 Hours)

Patient may present with symptoms of angina pectoris after 12 hours of myocardial infarction.

CLINICAL FEATURES

Patient with acute myocardial infarction may be symptomatic (majority of cases) or asymptomatic (silent). Silent acute myocardial infarction occurs due to high pain threshold as a result of nervous system involvement in diabetes mellitus and elderly persons.

Table 16.10 Clinical complications of acute myocardial infarction

Complication	Interval	Diagnostic Tests	Treatment
Mechanical complication			
Cardiac failure due to ventricular dysfunction (cardiogenic shock)	Usually within hours	ECHO, Swan-Ganz catheter (right heart)	ACE inhibitors, urgent revascularization
Structural complications			
Mitral valve incompetence due to papillary muscle dysfunction, necrosis or rupture	First few days	ECHO	ACE inhibitors, intra-aortic balloon pump as a bridge that sits in aorta 2 cm away from the left subclavian
Myocardial wall rupture	3–5 days	ECHO	Pericardiocentesis, urgent cardiac repair
Interventricular septum and papillary muscles rupture	3–5 days	ECHO, Swan-Ganz catheter (right heart)	ACE inhibitors, nitroprusside, urgent surgery
Aneurysm at myocardial infarct	≥4 weeks	ECHO	Conservative treatment
Right ventricular infarction	Usually within hours	ECG	Fluid loading
Electrical complications			
Cardiac arrhythmias	First several hours	ECG (sinus bradycardia)	Atropine followed by pacemaker if symptoms persist
Conduction abnormalities (third degree block)	First several hours	ECG (cannon 'a' wave)	Atropine followed by pacemaker if symptoms resolve
Other complications			
Fibrinous pericarditis	2–4 days due to transmural infarct	ECHO	Conservative treatment
Dressler's syndrome (chest pain, pericardial effusion) due to autoimmune mechanism	Weeks to a few months	ECHO	Conservative treatment
Mural thrombi due to abnormal endocardial surface due to infarct	≥1 week	ECHO	Thrombolytic therapy
Progressive myocardial necrosis results in persistent pain	12 hours to a few days	ECHO	Conservative treatment
Myocardial infarct expansion	Usually within hours	ECHO	Conservative treatment

All the complications of myocardial infarction results in hypotension.
Septal rupture results in systolic murmur. Left ventricular pressure is greater than right ventricular pressure. This causes left-to-right shunt of oxygenated blood. Oxygen saturation in the right ventricle is markedly increased compared with right atrium.
Thrombolytic agents are only administered if there is ST-segment elevation or a new LBBB within 12 hours of the onset of chest pain.

Clinical complications of acute myocardial infarction are given in Table 16.10.

Chest Pain

Patient presents with sudden onset of severe crushing substernal chest pain for 30–45 minutes in central portion of chest, which is not relieved by nitroglycerin. Chest pain is often precipitated by exertion. It occurs most often in the morning hours due to catecholamine burst after awakening. Pain radiates down the left arm in 30% of cases to the shoulders or to the jaw or epigastrium or beneath xiphoid process or occipital region. Pain never radiates below umbilicus.

Constitutional Symptoms

Patient develops breathlessness, fever, discomfort, giddiness. Severe distress is marked by symptoms such as nausea, sweating and vomiting.

Clinical Examination

Clinical examination reveals pallor and cold extremities, tachycardia (sympathetic overactivity), bradycardia (parasympathetic overactivity), apical pulse difficult to palpate, apical systolic murmur due to mitral regurgitation as a result of papillary muscle necrosis pericardial friction rub—fibrinous pericarditis and raised jugular venous pressure due to right heart failure.

LABORATORY DIAGNOSIS

The prognosis of acute myocardial infarction (AMI) depends on the degree of left ventricular dysfunction. The electrocardiogram (ECG), cardiac enzyme (CK-MB), and cardiac-specific troponin I assays are the main diagnostic tools in confirming an AMI.

Electrocardiogram (12-Lead)

Electrocardiogram (ECG) (12-lead) gives a good guide as to which coronary artery is narrowed and the extent of myocardial damage. It is diagnostic of AMI in 75–80% of cases during the first 2 days of onset of myocardial infarction. Acute myocardial infarcts are now classified according to presence or absence of ST-segment elevation and appearance or absence of a new Q wave on ECG.

- **Transmural infarct:** ECG shows ST elevation (STEMI) along with appearance of new Q wave in 75–80% cases of AMI. It is more dependent on the volume of myocardial infarct area rather than transmural infarction (Fig. 16.13A and B).
- **Subendocardial infarct:** ECG shows absence of non-ST-segment elevation MI (NSTEMI), as well as appearance of a new Q wave. These patients require estimation of cardiac troponins for diagnosis.

Serum Cardiac Enzymes

Cardiac enzymes like creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) are estimated to diagnose myocardial infarction (Table 16.11).

- **Creatine kinase-MB (CK-MB):** It exists in two isoforms, i.e. CK-MB1 and CK-MB2 with sensitivity in 95% of cases. If ratio of these two isoforms (CK-MB1 and CK-MB2) is 1.5 or more, it is an excellent indicator of early acute myocardial infarction. Its half-life is 16 hours. CK-MB enzyme appears within 4–8 hours, peaks at 24 hours and disappears within 1.5–4 days.

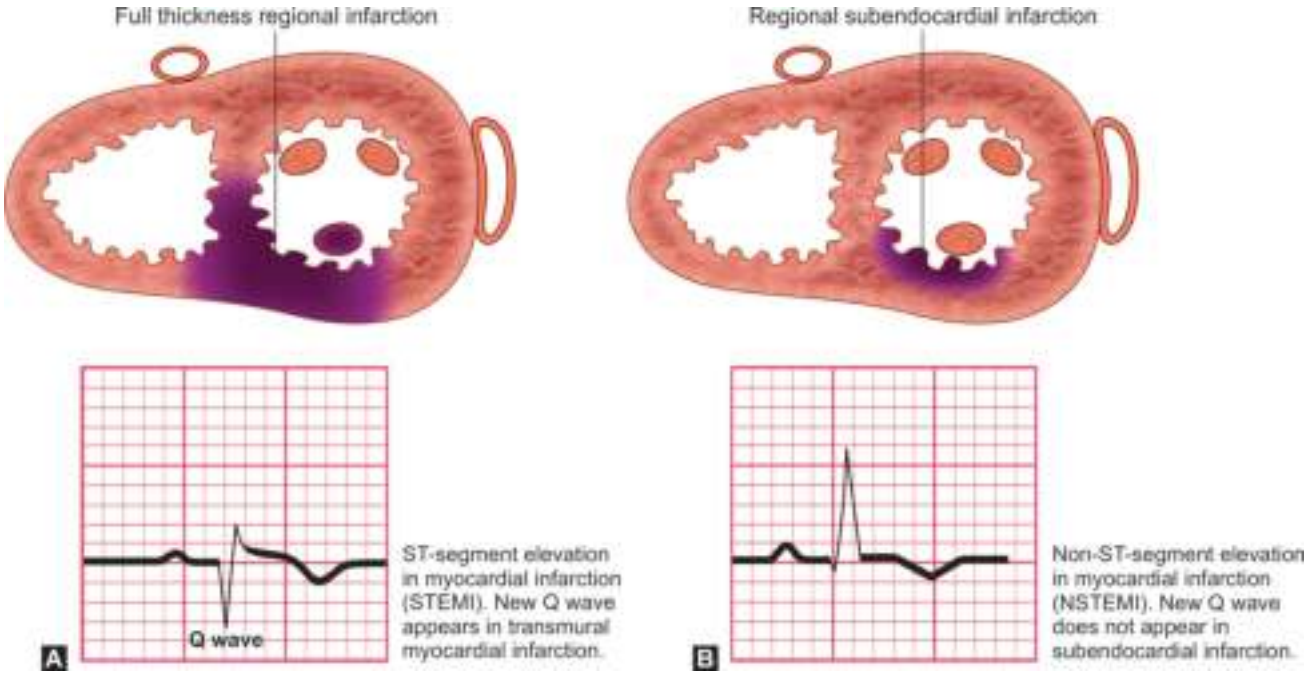


Fig. 16.13A and B: ECG changes in acute myocardial infarction.

Table 16.11 Serum estimation of cardiac enzymes in acute myocardial infarction

Cardiac Enzymes/Troponins	Half Life	Abnormal Activity Detectable	Peak Value Abnormality	Baseline
Troponin T or I	Not applicable	4–6 hours	12–24 hours	7–10 days
Creatine kinase-MB (CK-MB) isoenzyme	16 hours	3–10 hours	12–24 hours	Within 4 days
Cardiac-specific LDH	54 hors	8–16 hours	30–48 hours	7–10 days
SGOT (aspartate aminotransferase)	20 hours	12–24 hours	18–36 hours	3–4 days

Cardiac troponins and/or CK-MB are used to asses for myocardial infarction. Troponin C binds calcium. It is bound to both troponin T and troponin I. Troponin T is bound to tropomyosin. Troponin I is bound to actin. It holds the troponin–tropomyosin complex in one place.

Serial testing for a period of 9–12 hours after AMI will provide a pattern to determine whether CK-MB is rising, indicative of myocardial injury. Subsequent elevation of serum CK-MB after 4 days of acute myocardial infarction is indicative of recurrent myocardial infarction.

- **Lactate dehydrogenase (LDH):** It is an excellent indicator of acute myocardial infarction. Its half-life is 54 hours. It begins to rise within 12–24 hours, peaks at 2–3 days and disappears within 7–14 days due to its more half-life. It exists in two isoforms, i.e. LDH-1 and LDH-2. Ordinarily, ratio of LDH-1–LDH-2 is >1. But in AMI, this pattern is flipped and ratio of LDH-1–LDH-2 becomes higher.
- **Aspartate aminotransferase (AST):** Aspartate aminotransferase first appears in 6–12 hours, peaks in 1–2 days, and disappears within 3–4 days as half-life of AST is 20 hours. Remember AST analysis is not analyzed to diagnose acute myocardial infarction.

Cardiac Troponins (TnI and TnT)

Cardiac troponins (TnI and TnT) are structural components of cardiac muscle, which regulate calcium mediated contractions. Troponin T is bound to tropomyosin. Troponin I is bound to actin, that holds the troponin–tropomyosin complex in one place. Troponin C is bound to both troponin T and troponin I. Elevation of cardiac troponins level occurs due to myocardial infarction (Table 16.12).

- **Cardiac troponins analysis:** Following acute myocardial infarction, cardiac troponins are released into the bloodstream within 3–12 hours. Its level attains peak at 24 hours disappear within 7–10 days. Troponin I (TnI) is more specific indicator of myocardial injury. Troponin T lacks some specificity because its elevation can also appear in skeletal myopathies as well as in renal failure.

Table 16.12 Elevation of cardiac troponins due to myocardial injury but not due to myocardial infarction

Myocardial Injury not Related to Myocardial Ischemia
▪ Cardiac surgery, cardiac contusion, pacing, defibrillator shocks
▪ Rhabdomyolysis with cardiac involvement
▪ Myocarditis
▪ Cardiotoxic drugs
Multifactorial or Indeterminate Myocardial Injury
▪ Congestive heart failure
▪ Severe pulmonary embolism or pulmonary hypertension
▪ Renal failure
▪ Sepsis
▪ Subarachnoid hemorrhage
▪ Strenuous exercise

- **Serial cardiac troponins testing:** Serial testing of troponin I (TnI) in blood is a diagnostic tool in AMI patients. Its sensitivity is 84–96% and specificity is 80–95%. False positive results are usually related to myocardial ischemia in patients with unstable angina. CK-MB is used in conjunction in cardiac troponin I (TnI) to diagnose AMI. CK-MB detects re-infarction of myocardium, while troponins do not (Fig. 16.14).

Cardiac Imaging Techniques

- **Two-dimensional echocardiography:** This technique is useful for demonstration of abnormally contracting ventricular myocardium and visualizing thrombi or rupture in the wall of the myocardium. It is difficult to differentiate between acute myocardial infarct and old myocardial infarct. Doppler echocardiography is done to detect ventricular septal defect (VSD) and mitral valve regurgitation due to AMI.
- **High resolution MRI:** A standard imaging agent gadolinium is administered and analyzed on high resolution MRI. As normal cardiac myocytes are tightly packed, hence imaging agent does not penetrate. But the agent penetrates into the expanded intercellular junction of acute myocardial infarct zone.
- **Radionuclide scanning (99mTc) stannous pyrophosphate:** It is performed 2–5 days after AMI. It is a chemical agent which emits radioactive γ-rays after intravenous dose or oral dose. More active cells of myocardium take up more of the radionuclide hence, emit radioactive γ-rays as (hot spots) on the picture of the computer monitor. Poor blood flow to organs, emit low levels of γ-rays that may be shown as blue cold spots.
- **Perfusion scintigraphy:** Thallium 201 or Tc 99m sestamibi is administered. It is concentrated in viable myocardium. It is difficult to differentiate acute myocardial infarct from old myocardial infarct.

Other Important Parameters

- **Hyperhomocysteinemia:** Serum homocysteine level >100 μm/L is associated with increased risk of coronary arterial disease and peripheral vascular disease.
- **C-reactive protein:** C-reactive protein (CRP) synthesized by liver causes opsonization of bacteria and activation of complement. Its level is increased in patients with disrupted coronary atheromatous plaques resulting in acute myocardial infarction. It may be a strong predictor of cardiovascular events, e.g. acute myocardial infarction, cerebral stroke and peripheral vascular disease than serum LDL level.

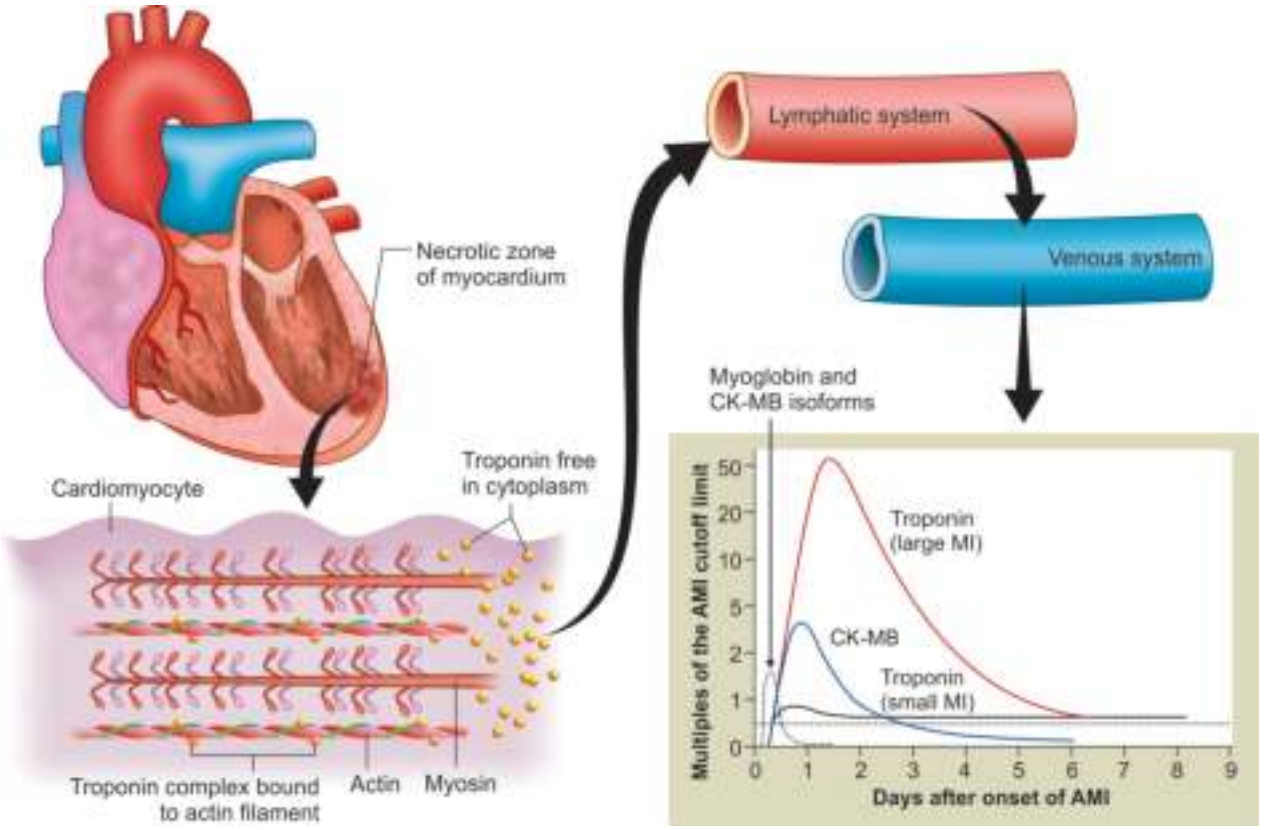


Fig. 16.14: Acute myocardial infarction (AMI)—CK-MB and cardiac troponins.

- **Nonspecific indexes of tissue necrosis:** Leukocytosis (neutrophilia) occurs within 6 hours of AMI and persists for 3–7 days. Patient has fever 39°C before a new Q wave appears and persists for a week. ESR rises more slowly and persists for 1–2 weeks in these patients.

Clinical Pearls: Management of ST-segment Elevation Myocardial Infarction (STEMI)

- A 50-year-old man with history of diabetes mellitus and hypertension comes to the emergency with crushing, substernal chest pain that radiates to his left arm for 30 minutes to several hours in duration. Past history revealed that he had chest pain on exertion before cardiac CK-MB and troponin levels are increased. 12-lead electrocardiogram shows STEMI. C-reactive proteins are raised.
- After diagnosing STEMI, oxygen, morphine and heparin should be administered. Heparin administration reduces mortality. It potentiates the effect of antithrombin. Antithrombin inhibits every step of coagulation system cascade. Therefore, heparin does not work with antithrombin deficiency. Heparin only prevents formation of new blood clots.
- Thrombolytic agent (e.g. streptokinase) is only administered if there is ST-segment elevation or new LBBB develops within 12 hours of the onset of chest pain. Failure to streptokinase therapy, patient should be immediately transferred for rescue percutaneous coronary intervention (PCI).
- Percutaneous coronary intervention is most effective, if performed within 12 hours of onset of chest pain following myocardial infarction. Earlier is the percutaneous coronary intervention, better the prognosis.
- Heparin anticoagulant should be started to prevent myocardial reinfarction and reduce thromboembolic phenomenon.
- Complete bed rest is advised for first 24–48 hours in the coronary care unit.
- Later, patient should be put on aspirin, β-blockers, angiotensin converting enzyme (ACE) inhibitors and statins (lipid lowering drugs).

Troponin C binds to calcium to activate actin–myosin interaction. Troponin T binds to tropomyosin. Troponin I blocks or inhibits actin–myosin interaction.

Clinical Pearls: Recombinant Tissue Plasminogen Activator use in Clinical Practice

- Tissue plasminogen activator (tPA) functions by catalyzing the conversion of plasminogen to plasmin, the primary enzyme involved in dissolving blood clots.
- Recombinant biotechnology has allowed to manufacture tissue plasminogen activator (tPA) such as alteplase, reteplase and teneplase in the laboratories.
- Recombinant tissue plasminogen activators are administered in AMI within 3 hours before percutaneous transluminal coronary angioplasty, severe pulmonary thromboembolism causing severe instability due to pressure in heart and deep vein thrombosis.

CHRONIC ISCHEMIC HEART DISEASE

Chronic ischemic damage to myocardial tissue results in progressive congestive heart failure.

- Focal replacement of injured myocardium results in non-contractile scar tissue.
- Myocardial fibrosis tends to be progressive, reflecting the progression of atheromatous plaque in coronary arteries.
- Loss of myocardium in scar tissue is accompanied by increased oxygen demand.

Clinical Features

Patient presents with symptoms related to congestive cardiomyopathy, congestive heart failure (CHF) and coronary artery insufficiency such as chest pain, after physical exertion, dyspnea, swelling over feet or ankles.

RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE: OVERVIEW

- **Rheumatic fever:** Rheumatic fever is a multisystem autoimmune disorder which affects periarteriolar connective tissue. It usually occurs 1–4 weeks after an episode of exudative pharyngitis caused by group A Streptococcus β-haemolyticus. Patient develops pancarditis, transient mild migratory polyarthritis, Sydenham chorea, erythema marginatum and subcutaneous nodules.
- **Rheumatic heart disease:** Rheumatic heart disease (RHD) comprises spectrum of cardiac lesions from pericarditis, myocarditis, and valvulitis during rheumatic fever to chronic valvular lesions that evolve over years following one or more episodes of acute rheumatic fever. Damage to cardiac valves may be chronic and progressive, resulting in cardiac decompensation.

AGE GROUP

Rheumatic fever most often affects children between 5 and 15 years of age (80%). It may affect 20% of adults. It rarely affects children <4 years and adults >40 years. Poverty and overcrowding contribute to increased incidence of under developed countries.

ETIOLOGY

- Rheumatic fever usually occurs 1–4 weeks after an episode of exudative pharyngitis caused by group A Streptococcus β-haemolyticus. An elevated titer of antistreptolysin *O* (ASO) is evidence of a recent streptococcal infection.
- Rheumatic fever is believed to be caused by antibody cross-reactivity, with antigens in multiple systems, which mimic streptococcal antigen that can affect heart, big joints, skin and brain.

MULTIPLE SYSTEMS INVOLVEMENT

Shortly after group A Streptococcus β-haemolyticus infection, signs appear within 2–3 weeks (1–5 weeks) in the heart (pancarditis), joints (migratory polyarthritis), skin (erythema marginatum), basal ganglia (Sydenham's chorea) and subcutaneous nodules in soft tissues.

PATHOGENESIS

The pathogenesis of rheumatic fever, RHD and complications is known to involve molecular mimicry and genetic predisposition (Fig. 16.15).

Genetic Predisposition

HLA-DR7 located on chromosome 6 is most often associated with development of cardiac valvular lesions in rheumatic heart disease. Exact mechanism is unknown. It is suggested that HLA molecules may play role in presenting antigens to CD4+ helper T cells, thus triggering an immune response. There is increased expression of TNF-α cytokine in HLA-DR7 patients, which may exacerbate cardiac valvular tissue inflammation, contributing to pathogenesis of RHD.

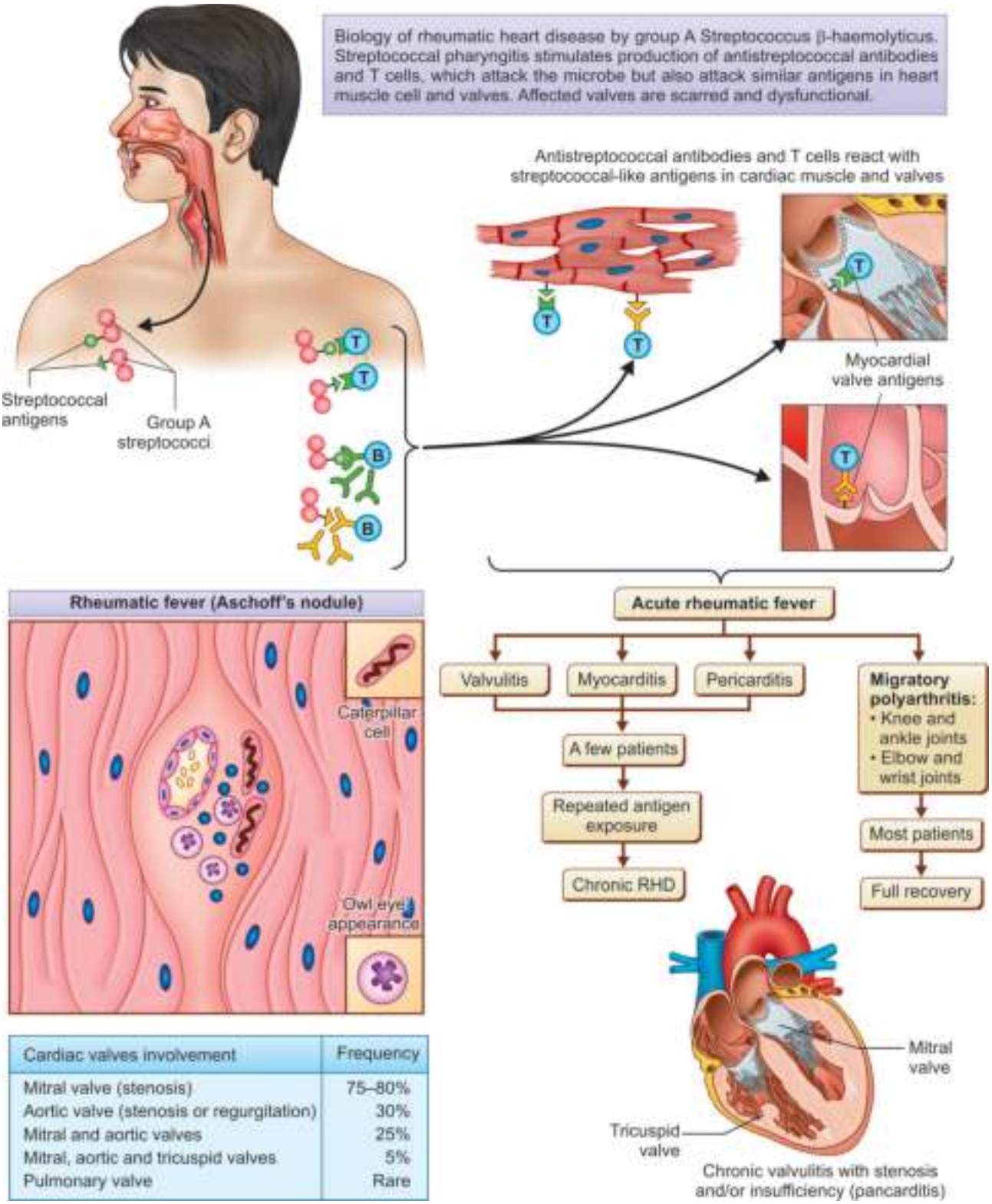
Mannose-binding Lectin 2 (MBL-2)

Mannose-binding lectin is an inflammatory protein involved in pathogen recognition. Increased synthesis of mannose-binding lectin protein is associated with mitral stenosis in rheumatic heart disease.

Type 2 Hypersensitivity Reaction

Molecular mimicry involves epitope sharing between host antigens and highly antigenic streptococcal M proteins.

- Development of antibodies against streptococcal capsular M protein antigens (virulence factor), cross react with some proteins in human connective tissue (e.g. heart–myosin, joints, skin, brain, arteries), and induce cytokine release resulting in tissue destruction in rheumatic fever.
- Antistreptolysin *O* (ASO), anti-DNase and hyaluronidase are estimated to establish streptococcal etiology of rheumatic fever.
- An elevated antistreptolysin *O* (ASO) titer is a proof of previous streptococcal infection.



Cardiac valves involvement	Frequency
Mitral valve (stenosis)	75–80%
Aortic valve (stenosis or regurgitation)	30%
Mitral and aortic valves	25%
Mitral, aortic and tricuspid valves	5%
Pulmonary valve	Rare

Fig. 16.15: Pathogenesis of rheumatic fever and heart disease and clinical manifestations.

Type 4 Hypersensitivity Reaction

Type 4 hypersensitivity reaction has been implicated in pathogenesis of rheumatic fever, as cardiac valvular lesions predominantly contain CD4+ helper T cells and macrophages. Cardiac valves display increased expression of VCAM-1, a protein that mediates the adhesion of CD4+ helper T cells.

ASCHOFF'S NODULE

Aschoff's nodule is the classic lesion of rheumatic fever. It develops after several weeks after the onset of symptoms.

Composition

Aschoff's nodule is composed of swollen eosinophilic collagen fibers, central fibrinoid necrosis surrounded by macrophages (Anitschkow myocytes), lymphocytes, plasma cells and occasional multinucleated giant cells demonstrated on light microscopy. It is globular, elliptic, or fusiform microscopic structure (Figs 16.16 and 16.17).

Phases of Aschoff's Nodule

- **Early phase of Aschoff's nodule:** Early phase of Aschoff's nodule is also known as **exudative phase**, which persists up to the 4th week of rheumatic fever. It is characterized by swelling of collagen fibers around blood vessels.
- **Granulomatous phase of Aschoff's nodule:** Granulomatous phase of Aschoff's nodule is also known as **proliferative phase**, which is evident during the 4th–13th weeks of the disease. It assumes granulomatous appearance.
- **Late phase of Aschoff's nodule:** Late phase of Aschoff's nodule is also known as **fibrous or healing phase**. Eventually Aschoff's nodule is replaced by formation of scar tissue.

Anitschkow Cells

Normally, cardiac macrophages are small in number. But in rheumatic fever, macrophage number as well as size is increased. Macrophages participating in formation of Aschoff's nodule are known as Anitschkow cells. Nuclei of these **Anitschkow cells** contain a central band of clumped chromatin, that gives **caterpillar-like appearance** in longitudinal sections (also called caterpillar cells); and owl-eyed appearance in cross sections (Fig. 16.18).

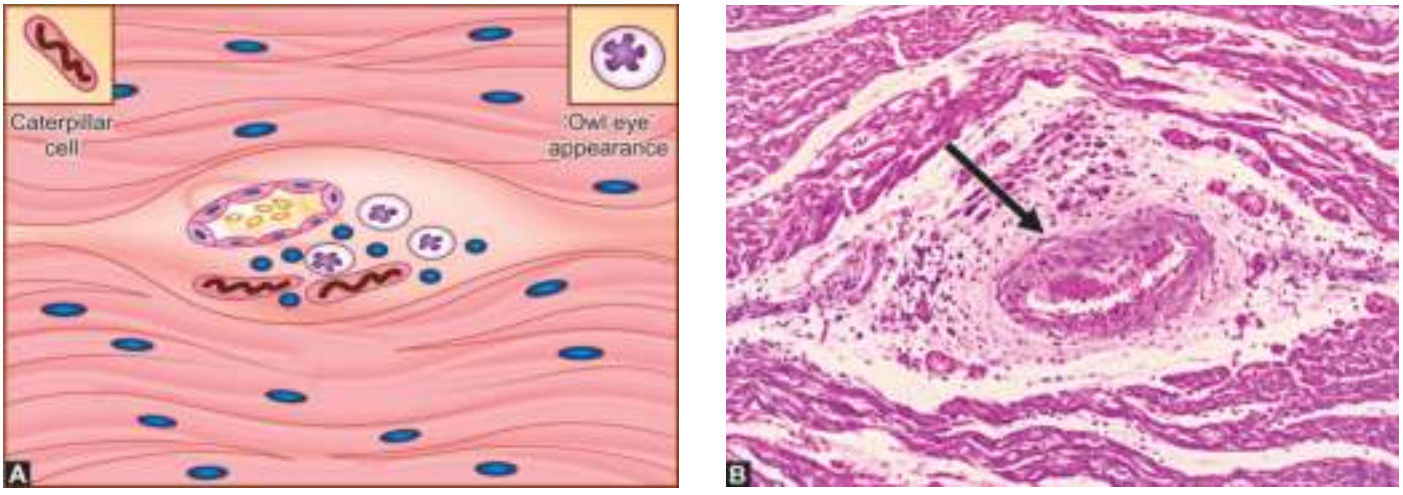


Fig. 16.16: (A) Aschoff's nodules schematic, (B) Aschoff's nodules in myocardium—these are centered in interstitium around vessels as shown here. The myocarditis may be severe enough to cause congestive heart failure (arrow) (100X).

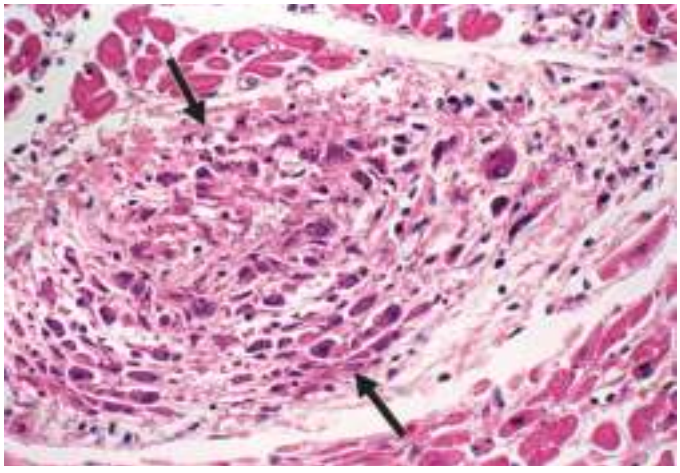


Fig. 16.17: Aschoff's nodules in myocardium (arrows) (400X).

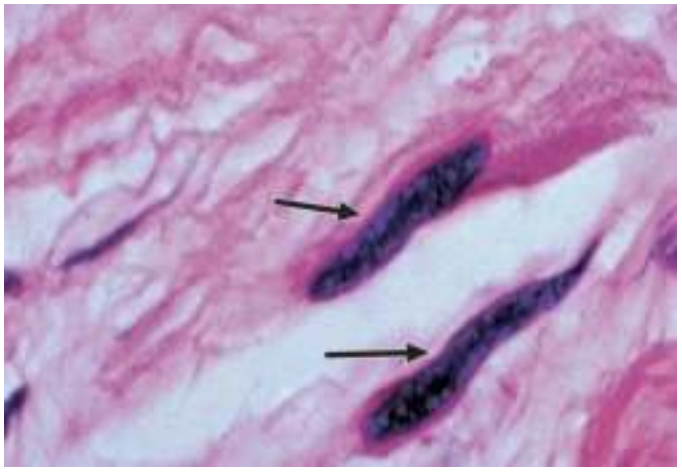


Fig. 16.18: Anitschkow/caterpillar cell in myocardium (arrows) (400X).

Giant Aschoff's Bodies

Fusion of multiple Aschoff's nodules produces giant Aschoff's bodies, located over bony prominences and tendons in subcutaneous regions.

Locations

In rheumatic fever, Aschoff's nodules can be seen in periarteriolar tissue of any layer of the heart (most common in myocardium) and hence called pancarditis. Occasionally, these are present in the pericardium. Aschoff's nodules have also been described in the adventitia of the aorta.

Nature and Lesions

As Aschoff's nodules in big joints are more exudative and less proliferative in nature, thus inflammatory changes in the joints completely resolve and hence no residual effect like joint deformity. On the other hand, Aschoff's nodules in heart are more proliferative and less exudative type, resulting into fibrosis of cardiac valves (stenosis/incompetence of cardiac valves).

JONES DIAGNOSTIC CRITERIA

Diagnostic criteria for rheumatic fever were first published in 1944 by T Duckett Jones. According to revised Jones criteria by American Heart Association, the diagnosis of rheumatic fever can be made when two of the major criteria, or one major criterion plus two minor criteria, are present along with evidence of streptococcal infection: elevated or rising antistreptolysin *O* titer or DNase. Exceptions are Sydenham chorea and indolent carditis, each of which by itself can indicate rheumatic fever. Jones criteria for diagnosis of rheumatic fever are given in Table 16.13.

Major Jones Diagnostic Criteria

- **Migratory polyarthritis (≤75%):** It is a temporary migrating inflammation of the large joints, usually starting in the legs and migrating upwards in the upper extremities.
- **Pancarditis (<35%):** Inflammation of the cardiac muscle (myocarditis) can manifest as congestive heart failure with shortness of breath, pericarditis with a friction rub, or a new cardiac murmur.
- **Subcutaneous nodules (<10%):** Patient develops painless, firm collections of collagen fibers over bony prominences or tendons in adults, i.e. wrists, elbows, scapula, ankles, knees, patella margins and spinous processes of vertebrae.
- **Skin rashes (erythema marginatum):** Skin rashes have bathing suit distribution in <10% of cases. A long-lasting reddish skin rash that begins on the trunk or arms as macules, which spread outward and clear in the middle to form rings, which continue to spread and coalesce with other rings, ultimately taking on a snake-like appearance. Skin rashes typically spare the face and become worse with heat.
- **Sydenham's chorea:** It is known as **St. Vitus dance** in <10% of cases. A characteristic series of random rapid involuntary and purposeless movements of face and arms. This can occur very late in the disease for at least three months from onset of infection.

Minor Jones Diagnostic Criteria

- **Fever:** Patient has 38.2–38.9°C (101–102°F)
- **Arthralgia:** Patient presents with joint pain without swelling. It cannot be included if polyarthritis is present as a major symptom.
- **Increased acute phase reactants:** ESR and C-reactive protein are raised.

Table 16.13 Jones criteria for diagnosis of rheumatic fever

Category of Jones Criteria	Diagnostic Criteria
Major diagnostic criteria	▪ Pancarditis (pericarditis, myocarditis, endocarditis) ▪ Migratory polyarthritis ▪ Subcutaneous nodules over bony prominences ▪ Sydenham chorea ▪ Erythema chronicum migrans
Minor diagnostic criteria	▪ Fever ▪ Arthralgia ▪ Previous history of rheumatic fever ▪ Chronic rheumatic heart disease ▪ Raised erythrocyte sedimentation rate ▪ C-reactive proteins (CRP) elevated ▪ Prolonged PR interval on ECG (first degree AV block)

Patients have two major criteria, or one major criterion and two minor criteria.

- **Total leukocyte count:** Peripheral smear examination shows leukocytosis (neutrophilia).
- **ECG study:** It shows features of first degree heart block such as a prolonged PR interval. The minor diagnostic criteria cannot be included if pancarditis is present as a major symptom.
- **Past history:** Previous episode of rheumatic fever or inactive RHD is minor diagnostic criteria.

Other Signs and Symptoms

There is preceding streptococcal infection of rheumatic fever: recent scarlet fever raised antistreptolysin *O* or other streptococcal antibody titer, or positive throat culture. Supportive evidence of streptococcal infection is absent in ≤10% of cases.

SYSTEMIC MANIFESTATIONS

Migratory Polyarthritis

Migratory polyarthritis is most common initial presentation of acute rheumatic fever up to 75% of cases.

- Patient develops temporary migrating inflammation of the large joints, usually starting in the lower extremities (knees and ankles) and migrating upwards in the upper extremities, i.e. elbows and wrists. It reaches maximum severity in 12–24 hours.
- Affected joints are painful, swollen, warm, erythematous, and limited in their range of motion.
- Articular cartilage and synovial membrane are never involved; hence there is no permanent joint deformity. As the Aschoff's nodules in big joints are more exudative and less productive in nature, thus inflammatory changes in the joints completely resolve and hence there is no residual effect such as joint deformity.
- Aspirin improves symptoms in affected joints and prevents further migration of the arthritis. Complete resolution of the symptoms typically occurs with improvement in 1–2 weeks and full recovery of joints in 2–3 months.

Pancarditis

Pancarditis is the most common and serious complication of rheumatic fever (50% of cases). It involves all three layers of heart.

- Degree of involvement of cardiac valves depends on severity of the initial attack and number of recurrent episodes by group A Streptococcus β-haemolyticus.
- If cardiac murmur is not cleared within three weeks time after an episode of rheumatic fever; prognosis is poor due to cardiac valve deformity. New or changing cardiac murmurs are considered necessary for diagnosis of rheumatic valvulitis.
- Doppler echocardiography evidence of mitral valve insufficiency in association with aortic valve involvement may be sufficient for diagnosis of pancarditis.

Myocarditis

- Myocardium is first to be involved in acute rheumatic fever. During early stage of rheumatic fever, myocarditis may lead to congestive heart failure associated with fatal outcome.
- Aschoff's nodules are present in periarteriolar interstitial tissue of myocardium, which usually heal and transform into fibrous scar. Spread of inflammation results into serofibrinous pericarditis and endocarditis.

Serofibrinous Pericarditis

- Inflammation may cause a serofibrinous pericardial exudate described as 'bread and butter pericarditis' due to deposition of fibrin giving shaggy appearance (Fig. 16.19).
- Patient presents with precordial pain. On auscultation, friction rub can be heard.
- Other causes of serofibrinous pericarditis must be excluded which include uremic pericarditis, systemic lupus erythematosus, acute myocardial infarction, uremia and chest radiation.
- Resolution of serofibrinous pericarditis is uncommon, which most often gets organized and resulting in constrictive pericarditis.

Endocarditis

- Endocarditis leads to cardiac valvular damage. Usually left-sided cardiac valves are inflamed, i.e.

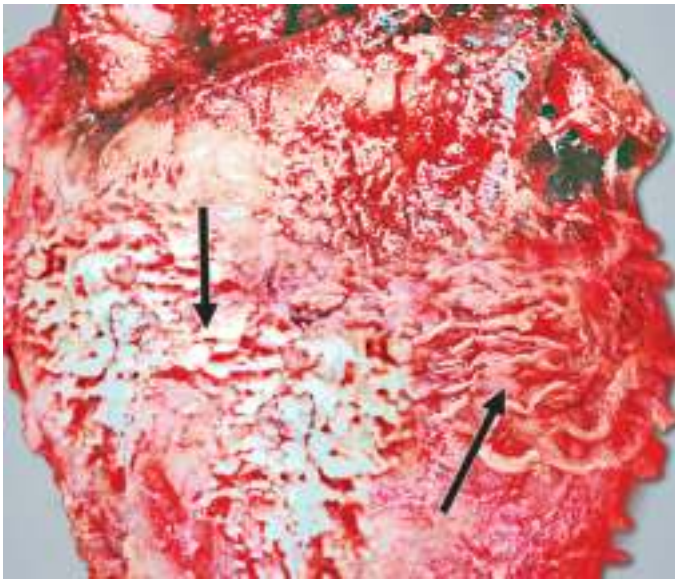


Fig. 16.19: Serofibrinous pericarditis (arrows).

mitral valve (75–80%), aortic valve (30%), mitral and aortic valve (25%), and tricuspid valve (<5%).

- Residual fibrosis effects occur in mitral and aortic valves. Mitral stenosis is marked by high diastolic pressure in the left atrium than in the left ventricle.
- The aortic valve is affected most often along with the mitral valve. Aortic valve may undergo stenosis or insufficiency.
- The tricuspid valve is affected along with the mitral valve and aortic valves (trivalvular involvement) in approximately 5% of cases of rheumatic heart disease. The pulmonary valve is rarely involved (Table 16.14).
- In acute phase of the disease, all the cardiac valves and chordae tendineae are swollen due to inflammation. Mitral valve regurgitation occurs in acute phase, while mitral valve stenosis in chronic phase of disease.

Table 16.14 Cardiac valves involvement in rheumatic fever and RHD

Cardiac Valves Involvement	Frequency
Mitral valve (stenosis)	75–80%
Aortic valve (stenosis or regurgitation)	30%
Mitral and aortic valves	25%
Mitral, aortic and tricuspid valves	5%
Tricuspid	<5%
Pulmonary valve	Rare

- Fibrin-rich exudate forming tiny, warty, bead-like, rubbery sterile cardiac vegetations measuring 1–2 mm in size are present along the line of opposition of cardiac valvular cusps and chordae tendineae; but never present behind cusps.
- Cardiac vegetations are nonfriable and hence not source of peripheral emboli. These cardiac valvular changes resolve with minimal fibrosis (Figs 16.20 to 16.22).

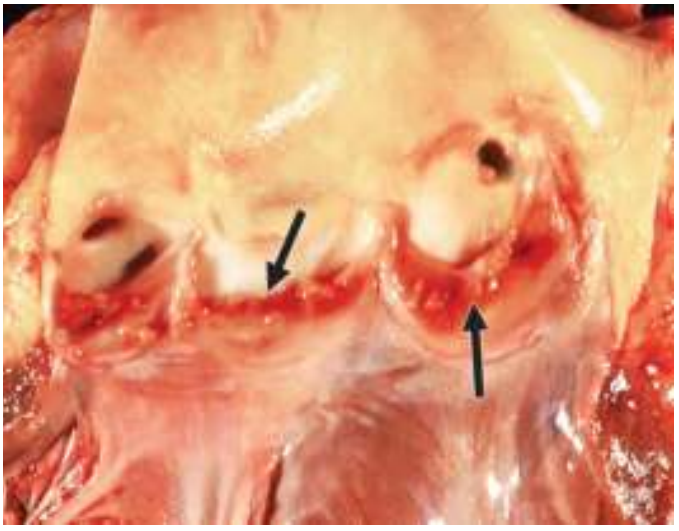


Fig. 16.20: Acute rheumatic endocarditis. Small verrucous vegetations measuring a few mm are seen along the closure line of this mitral valve (arrows).

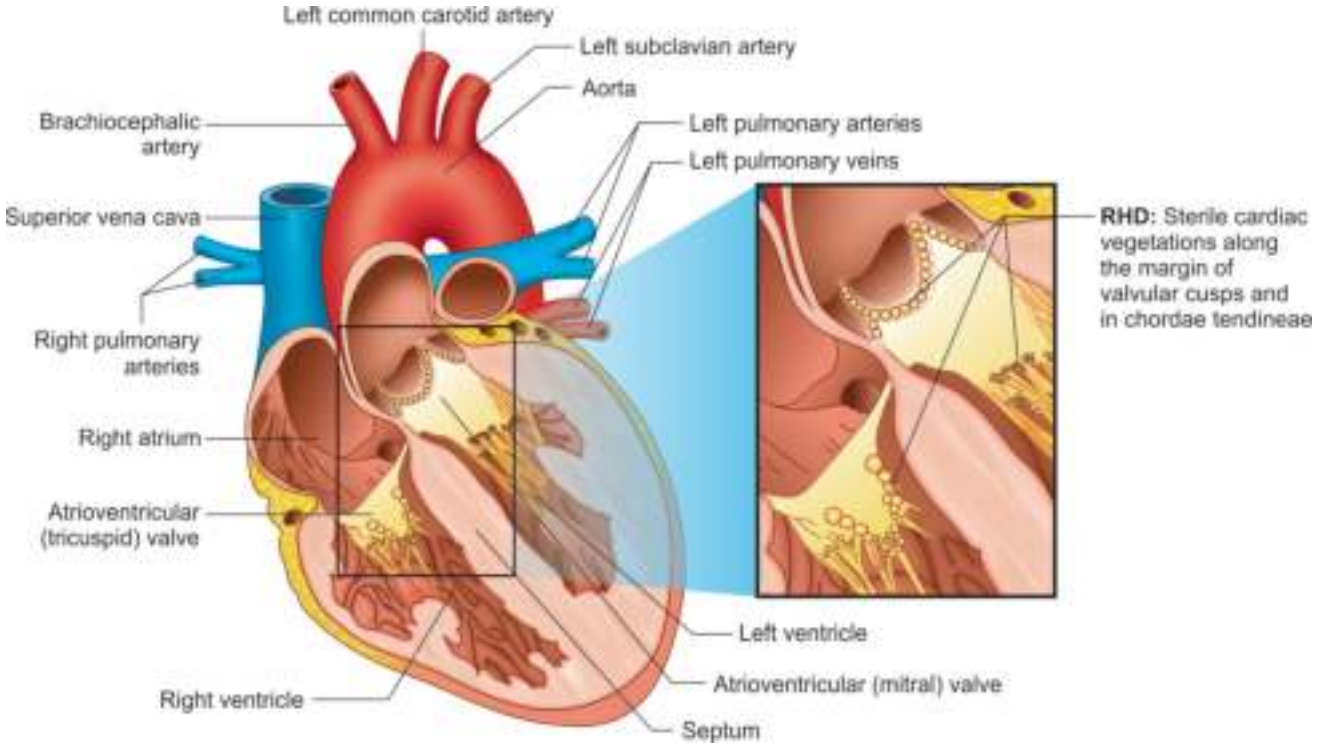


Fig. 16.21: Cardiac vegetations in rheumatic heart disease.

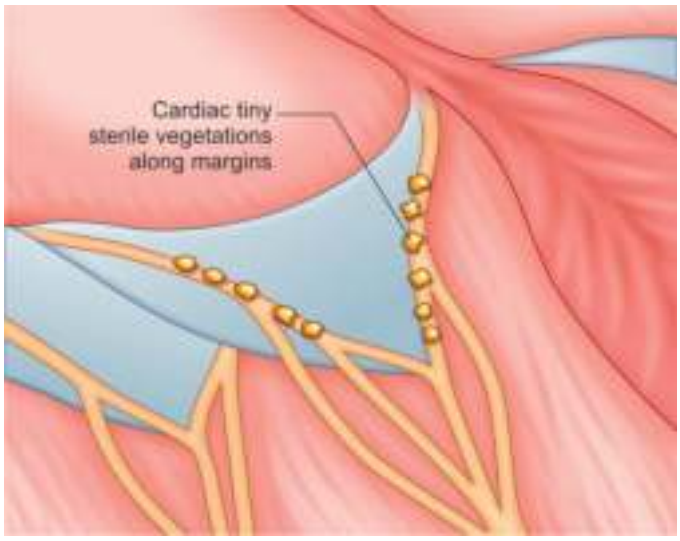


Fig. 16.22: Cardiac vegetations in rheumatic heart disease. Cardiac vegetations are tiny, few mm in diameter present along the margin of the cardiac valves and chordae tendineae.

- McCallum plaque is a healed lesion located in posterior wall of left atrium. Blood regurgitation jets result in subendocardial thickening known as McCallum plaque.
- Aschoff's nodules in heart are more proliferative and less exudative lesions, and hence resulting in valvular leaflet fibrosis, commissural fusion, thickening and shortening of the chordae tendineae. Aschoff's nodules are replaced by fibrosis hence not demonstrated on light microscopy.
- Buttonhole stenosis of mitral valve with dystrophic calcification results in fish-mouth appearance (Fig. 16.23). Mitral valve stenosis is accompanied by cardiac murmurs that can be heard on auscultation.
- In advanced cases, patients may manifest with congestive heart failure, dyspnea, mild to moderate chest discomfort, pleuritic chest pain, edema, cough, or orthopnea or a new cardiac murmur.
- Residual cardiac vegetations often become infected even with low virulent bacteria and known as subacute infective endocarditis.
- Superimposed infection may cause ulceration of the cusps, perforation of cardiac valves, and rupture of chordae tendineae. The infected cardiac vegetations are more likely to be detached and cause embolism resulting in infarction of organs (Figs 16.24 and 16.25). Sites of radiation of cardiac murmurs are given in Table 16.15.

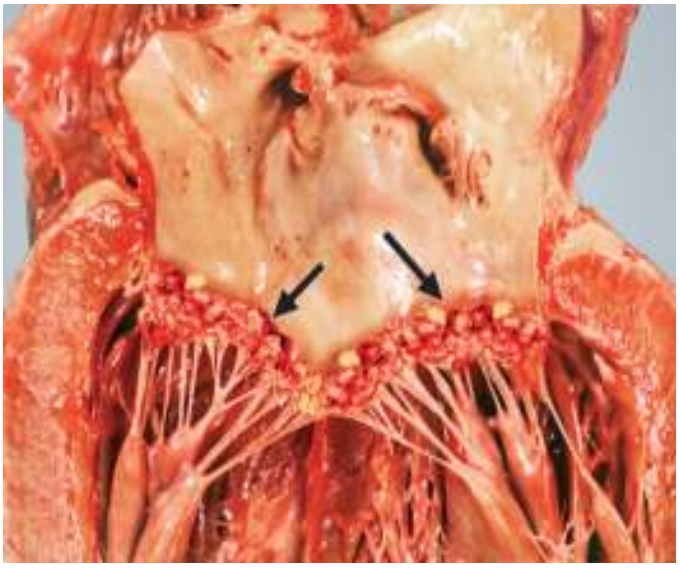


Fig. 16.24: Development of endocarditis in RHD (arrows). Cardiac vegetations are tiny, few mm in diameter present along the margin of the cardiac valves and chordae tendineae.

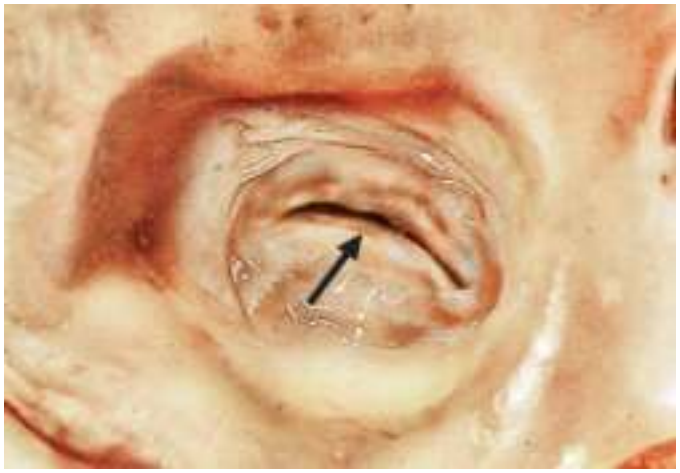


Fig. 16.23: Severe mitral stenosis in RHD shows fish-mouth appearance. Cusps are rigid, fibrotic and calcified with narrowing of orifice (arrow).

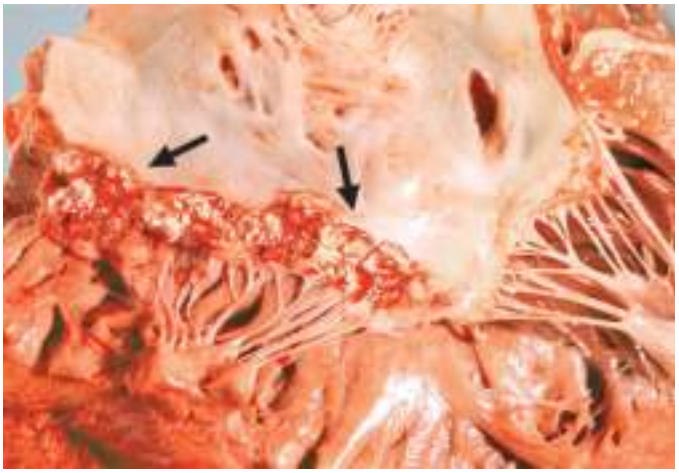


Fig. 16.25: Vegetations on the mitral valve in subacute bacterial endocarditis. These are also a source of peripheral emboli (arrows).

Table 16.15 Sites of radiation of cardiac murmurs

Cause	Primary Site of Cardiac Murmur	Radiation of Cardiac Murmur
Mitral stenosis	Apex	Cardiac murmur does not radiate
Mitral regurgitation	Apex	Cardiac murmur radiates to left axilla beneath left scapula
Aortic stenosis	Apex	Cardiac murmur radiates towards upper right sternal edge over carotids
Aortic regurgitation	Left sternal edge	Cardiac murmur radiates down left sternal edge towards apex
Ventricular septal defect	Left sternal edge	Cardiac murmur radiates all over precordium
Pulmonary stenosis	Left sternal edge in upper part	Cardiac murmur radiates towards left clavicle beneath left scapula

Clinical Pearls: Long-term Complications of Pancarditis

- **Infective endocarditis:** Valvular stenosis and/or incompetence and predisposes to infective endocarditis.
- **Congestive heart failure:** Patient develops congestive heart failure in long-standing cases secondary to severe valve insufficiency or myocarditis. Patient presents with dyspnea, orthopnea, and peripheral edema, pleuritic chest pain, cough and rheumatic pneumonitis. On clinical examination, patient presents with right heart failure and shows signs of raised jugular venous pressure, hepatomegaly and peripheral edema. Right ventricular hypertrophy is very common in mitral stenosis, and left ventricular hypertrophy seen in aortic regurgitation.
- **Cardiac arrhythmias (atrial fibrillation):** Patient may develop atrial fibrillation, tachycardia, and arrhythmias.
- **Thromboembolism:** Mural thrombi are very common in left atrium. Patient is prone to thromboembolism associated with infarction of affected organs.
- **Constrictive pericarditis:** There is deposition of fibrinous exudate between two layers of pericardium giving shaggy appearance. It is known as bread and butter pericarditis. Its resolution is uncommon. It most often gets organized resulting in constrictive pericarditis. Patient presents with precordial pain. On auscultation, friction rub can be heard.
- **Other organs involvement:** Blood vessels and lungs may be involved in rheumatic fever, i.e. coronary arteries, renal arteries, mesenteric arteries, cerebral arteries, aorta, pulmonary arteries and lungs (pneumonitis and pleuritis). A few patients may develop abdominal pain and nose bleeds.

Subcutaneous Nodules

Subcutaneous nodules are formed due to fusion of multiple Aschoff's nodules. Patient develops a few mm to 1–2 cm size firm, painless subcutaneous nodules, over bony prominences or tendons in adults, i.e. wrists, elbows, scapula, ankles, knees, patella margins, scalp and spinous processes of thoracic and lumbar vertebrae especially in adults in 10–60% of cases.

Skin Rashes (Erythema Marginatum)

Skin rashes exhibit bathing suit distribution in ≤10% of cases. Patient presents with 1–3 cm ring-shaped reddish rashes such as macules/papules on the trunk and extremities (bathing suit distribution), which coalesce to exhibit snake-like appearance. These rashes typically spare the face and becoming worse with heat.

Sydenham's Chorea (St. Vitus Dance)

Sydenham's chorea is delayed manifestation of rheumatic fever. This can occur at least 3 months from onset of infection up to 10% of cases.

- Patient presents with series of random rapid involuntary purposeless muscular movements of the face and arms. Patient has difficulty in writing, drawing, handwork, and talking.
- Patient develops hyperextension of joints, hypotonia of muscles, diminished deep tendon reflexes, and relapsing grip (e.g. alternate increase and decrease in tension).
- Patient starts improving in 1–2 weeks, and full recovery occurs in 2–3 months. CSF examination reveals no pathologic findings.

LABORATORY DIAGNOSIS

JONES DIAGNOSTIC CRITERIA

Rheumatic fever is diagnosed clinically using Jones criteria, which are divided into two groups: major and minor manifestations. The diagnosis is established by presence of two major and one minor; or one major and two minor criteria as discussed.

ANTISTREPTOLYSIN *O* TITER (ASO TITER)

- Antistreptolysin *O* titer (ASO titer) is estimated in patient with rheumatic fever. Peak level is achieved at 4–5 weeks after streptococcal infection. High ASO titers are supportive but not diagnostic.

- ASO titer is expressed in Todd units. ASO titers >400 Todd units in adults and >300 Todd units in children >5 years of age are significant.

ANTI-DNase B/ANTI-HYALURONIDASE TITER

Anti-DNase B titer is less reliable diagnostic parameter than ASO titer. It is raised in cases of Sydenham's chorea.

ANTISTREPTOZYME TEST

- Antistreptozyme test is very sensitive in recent case of rheumatic fever.
- Hemagglutination test value >200 units/ml is associated with polyarthritis suggestive of acute rheumatic fever.

ELECTROCARDIOGRAM

Electrocardiogram (ECG) study demonstrates first-degree block and prolonged PR interval.

IMAGING TECHNIQUES

Echocardiography is done to visualize cardiac motion. Chest radiograph is done to evaluate heart and lungs.

BLOOD CULTURE

- Blood culture is done to diagnose or rule out infective endocarditis in these patients. Blood culture is positive in 95% of cases due to presence of bacterial colonies not covered by fibrin (Fig. 16.26).
- Presence of deep seated bacterial colonies covered by fibrin leads to negative blood culture (Fig. 16.27).

ACUTE PHASE REACTANTS

Serum complement, mucoproteins, α_2-globulin and β-globulin levels are increased. Peripheral blood smear examination shows leukocytosis. Erythrocyte sedimentation rate and C-reactive protein (CRP) levels are raised in migratory polyarthritis.

DECREASED ERYTHROPOIESIS

Chronic inflammatory changes suppress erythropoiesis leading to anemia.

MANAGEMENT

- **Aspirin:** Aspirin is the drug of choice for treatment of migratory polyarthritis by reducing inflammation. Patients with positive throat cultures should be treated with antibiotics.
- **Corticosteroids:** These drugs are reserved for cases where there is evidence of pancarditis. Use of corticosteroids prevents further scarring of tissue and development of mitral stenosis. Unlike congestive heart failure, rheumatic heart disease responds well to corticosteroids.
- **Penicillin:** Basic aim of monthly injections of long-acting penicillin is to prevent development of infective endocarditis in rheumatic heart disease. It must be administered for a period of five years in patients having one attack of rheumatic fever. If there is evidence of carditis, the length of long acting penicillin therapy may be continued up to 40 years of age.
- **Other drugs:** Angiotensin converting enzyme (ACE) inhibitors, diuretics, β-blockers, and digoxin drugs are used for treatment of carditis, which manifests as congestive heart failure.

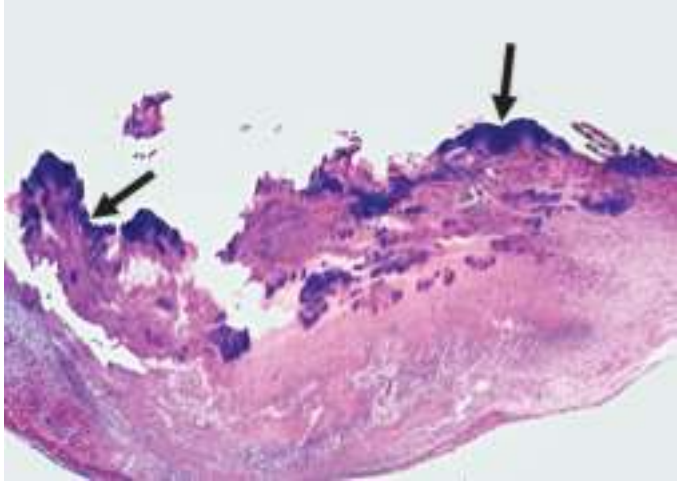


Fig. 16.26: Infective endocarditis. Valves are relatively avascular, so high dose antibiotic therapy is needed to eradicate the infection. Blue bacterial colonies are extending into the pink fibrin, platelets and connective tissue of the valve. Blood culture is positive (arrows) (400X).

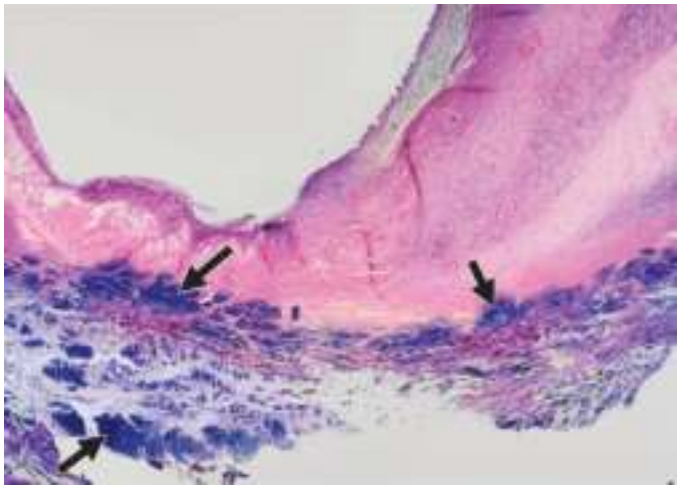


Fig. 16.27: Infective endocarditis. Valves are relatively avascular, so high dose antibiotic therapy is needed to eradicate the infection. Blue bacterial colonies are extending into the pink fibrin, platelets and connective tissue of the valve. Blood culture is negative (arrows) (400X).

DISORDERS OF MYOCARDIUM

MYOCARDITIS

Myocarditis is focal or diffuse inflammation of the myocardium affecting any age group. Myocarditis constitutes 10% of cases of new onset cardiac dysfunction. If myocarditis is not fatal, it often progresses to congestive cardiomyopathy. Causes of myocarditis include viral infections, autoimmune disorders, environmental toxins and adverse reactions to medications.

- Diagnosis of active myocarditis is based on inflammatory infiltrate usually lymphocytes and myocyte necrosis not of ischemic etiology.
- Patient presents with congestive heart failure, tachypnea, peripheral edema, and hepatomegaly. Atrial or ventricular arrhythmias are common resulting in fatal outcome.
- Electrocardiogram (ECG) may show low voltage QRS complex, ST elevation, or heart block. Serial 24-hour Holter recordings should be obtained in all patients.
- Chest radiograph shows pulmonary edema. Echocardiography is extremely useful in excluding congenital structural cardiac defects; pericardial effusion with cardiac tamponade.
- Cardiac catheterization with endomyocardial biopsy remains the gold standard for the diagnosis of myocarditis.

PRIMARY VIRAL MYOCARDITIS

Primary viral myocarditis is most often caused by Coxsackie B virus. It may be caused by HIV, EB virus, poliovirus, cytomegalovirus. Infectious virus triggers an autoimmune, cellular, and humoral reaction resulting in viral myocarditis. Primary viral myocarditis has four clinicopathologic manifestations described as under.

Fulminant Myocarditis

Patient with fulminant myocarditis develops ventricular dysfunction without dilatation within 2 weeks of viral infection. Complete histological and functional recovery occurs. Some patients die within 2 weeks.

Surgical Pathology: Fulminant Myocarditis
Light Microscopy
Histopathologic examination of myocardium in fulminant myocarditis shows multiple foci of active inflammation comprising lymphocytes and necrosis (Fig. 16.28).

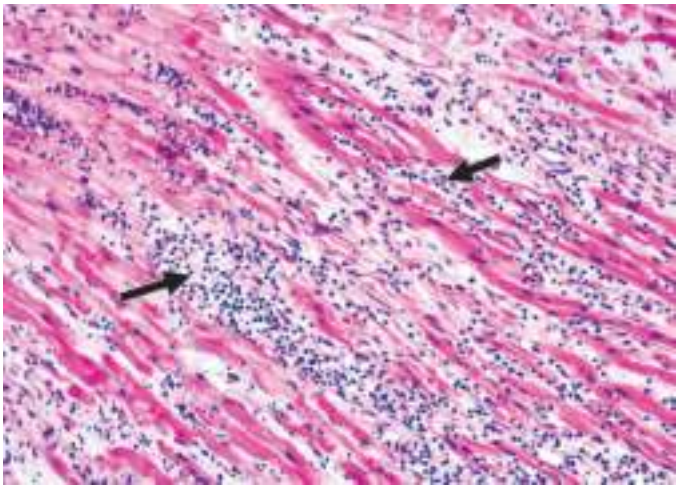


Fig. 16.28: Viral myocarditis. It is caused by Coxsackie B virus showing heavy but focal increase of lymphocytes in the interstitial tissue of the myocardium. Outcome is subclinical or fatal (arrows) (100X).

Chronic Active Myocarditis

Patient has an indistinct onset with moderate cardiac dysfunction. Patient may develop restrictive cardiomyopathy within 2–4 years of clinical manifestations.

Surgical Pathology: Chronic Active Myocarditis
Light Microscopy
Histologic examination shows active or borderline myocarditis.

Eosinophilic Myocarditis

Eosinophilic myocarditis is attributed to allergic reactions, which leads to left ventricular dysfunction. It is linked to treatment with methyldopa, penicillin, para-aminosalicylic acid, streptomycin, anticonvulsants and antidepressants.

Surgical Pathology: Eosinophilic Myocarditis
Light Microscopy
▪ Histologic examination of eosinophilic myocarditis shows infiltration by eosinophils and occasional giant cells. ▪ There is presence of a little myocardial necrosis and infiltration by lymphocytes and histiocytes around blood vessels. In a few cases, inflammation is present in subendocardium with poorly formed epithelioid cell granulomas.

Idiopathic Giant Cell Myocarditis

Idiopathic giant cell myocarditis is also known as **Friedler's myocarditis** and associated with autoimmune diseases such as inflammatory bowel disease. It affects young adults of myocardium in idiopathic giant cell myocarditis.

Table 16.16 Comparison of cardiomyopathies (CMPs)

Parameters	Congestive CMP	Hypertrophic CMP	Restrictive CMP
Etiology	Alcohol toxicity Peripartum postviral genetic mutation	▪ Autosomal dominance with gene mutation encoding beta heavy chain on chromosome 14 (50%) ++++ ▪ Troponin T gene mutation on chromosome 1 (15%) ▪ α-Tropomyosin gene mutation on chromosome 15 (3–5%)	▪ Unknown in majority of cases ▪ Amyloid deposition in subendocardial region ▪ Hemosiderin deposition in subendocardial region
Age	Any age	Young age	Infants, children
Valves motion	Normal motion	Anterior motion of mitral valve during systole	Normal motion
Heart wall and chambers	All chambers are dilated and congested	Left ventricle is hypertrophied	Endocardial thickening is marked
Heart contraction	Poor contraction	Marked contraction	Poor contraction due to heart rigid
Congestive heart failure	Slow onset	Rapid onset	Rapid onset
Cardiomegaly	++++ (marked)	++ (moderate)	+ (less)
Arrhythmias	++	Absent	++++ (marked)
Atrial fibrillation	Absent	++++ (marked)	Absent
Thrombus	Common	Common	Absent
Infective endocarditis	Absent	Present	Absent
Prognosis	Poor	Poor	Poor

Surgical Pathology: Idiopathic Giant Cell Myocarditis

Light Microscopy

- Histologic examination shows infiltration of myocardium by lymphocytes, macrophages, and multinucleated giant cells in the absence of granulomas.
- Giant cells have immunohistochemical profile of histiocytes.

CARDIOMYOPATHIES

Cardiomyopathy refers to noninflammatory disease of myocardium. It is diagnosed by exclusion of all cases of hypertension, ischemic heart disease, valvular diseases and congenital heart diseases. It is characterized by unexplained ventricular dysfunction unresponsive to digitalis.

- Primary cardiomyopathies include congestive (dilated), hypertrophic and restrictive types. Secondary cardiomyopathies occur due to specific causes (e.g. hemochromatosis, amyloidosis, and alcohol and thiamine deficiency).

Table 16.17 Etiology of secondary cardiomyopathies

Causes	Example
Infection and inflammation	Myocarditis
Toxic and metabolic disturbances	▪ Alcohol ▪ Adriamycin ▪ Catecholamines
Multisystem diseases	▪ Diabetes mellitus ▪ Amyloidosis ▪ Hemochromatosis ▪ Thyroid dysfunction
Primary skeletal muscle disorders	▪ Muscular dystrophy ▪ Mitochondrial cytopathy

- Diagnostic evaluation of cardiomyopathies is based on clinical history, clinical examination, imaging techniques, ECG, cardiac catheterization and myocardial biopsy examination (Table 16.16).
- Etiology of secondary cardiomyopathies is given in Table 16.17. Selected genetic defects associated with cardiomyopathy are given in Table 16.18.

Table 16.18 Selected genetic defects associated with cardiomyopathy

Gene Features	Gene Product	Inheritance	Cardiac Phenotype
Sarcomere	▪ MYH7 (myosin heavy chain 7) ▪ MYBPC3 (myosin binding protein C3) ▪ MYL2 (myosin regulatory light chain 2) ▪ MYL3 (myosin essential light chain 3) ▪ TNNT2 (troponin T2) ▪ TNNI3 (troponin I3) ▪ TPMα_1 (tropomyosin α_1) ▪ TNNC1 (troponin C1) ▪ TTN (titin)	▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant/autosomal recessive ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant	▪ HCM, DCM, LVNC ▪ HCM ▪ HCM ▪ HCM ▪ HCM, DCM, LVNC ▪ HCM, DCM, LVNC ▪ HCM, DCM ▪ DCM ▪ DCM
Sarcolemmal membrane	▪ DMD (Duchenne muscular dystrophy) ▪ DMPK (dystrophica myotonica protein kinase) ▪ SGCD (δ-sarcoglycan)	▪ X-linked disorder ▪ Autosomal dominant ▪ Autosomal dominant	▪ DCM ▪ DCM ▪ DCM
Z-disc and cytoskeleton	▪ DES (desmin) ▪ ACTN2 (α-actin-2) ▪ CRYAB ($\alpha\beta$-crystallin) ▪ FLNC (filamin C) ▪ ANKRD1 (CARP) ▪ CSRP3 (MLP)	▪ Autosomal recessive ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant	▪ DCM ▪ DCM ▪ DCM ▪ DCM ▪ DCM, RCM ▪ HCM (DCM)
Nuclear membrane	▪ LMNA ▪ EMD	▪ Autosomal dominant/autosomal recessive ▪ X-linked disorder	▪ CDDC ▪ CDDC
Mitochondria	Mitochondrial DNA	Maternal transmission	DCM, HCM
Desmosome	▪ DSP (desmoplakin), JUP (junctional plakoglobin) ▪ DSG2 (desmoglein 2), DSC2 (desmocollin 2), PKP2 (plakophilin 2)	▪ Autosomal dominant/autosomal recessive ▪ Autosomal dominant	▪ ARVC ▪ ARVC
Cellular metabolism	▪ PRKAG2 (γ-subunit of AMP kinase) ▪ TMEM43 (transmembrane protein 43) ▪ FXN (frataxin) ▪ LAMP2 (lysosomal-associated membrane protein 2) ▪ TAZ (taffazin)	▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal recessive ▪ X-linked disorder ▪ X-linked disorder	▪ HCM ▪ ARVC ▪ HCM ▪ HCM ▪ DCM, LVNC
Other examples	▪ RBM20 (RNA-binding motif protein 20) ▪ ALK3 (α-kinase 3) ▪ BAG3 (BCL-2-associated athanogene 3) ▪ PSEN1 (presenilin 1)	▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant ▪ Autosomal dominant	▪ DCM ▪ HCM ▪ DCM ▪ DCM

DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; RCM: Restrictive cardiomyopathy; LVNC: Left ventricular noncompaction; CDDC: Conduction disease dilated cardiomyopathy, ARVC: Arrhythmogenic right ventricular cardiomyopathy.

CONGESTIVE CARDIOMYOPATHY

Congestive cardiomyopathy is most common form of cardiomyopathy (CMP). Damage to cardiac muscle fibers results in poor contraction. The resulting loss of myocardium tone dilates all four chambers of the heart, resulting in cardiomegaly (>500 g) and giving the heart, a globular shape (Figs 16.29 and 16.30).

- Patient is prone to thrombus formation and cardiac arrhythmias (atrial and ventricular). Mural thrombi may fragment and enter circulation in the form of emboli.
- Emboli from left side of heart are carried to brain, kidney, coronary arteries and peripheral tissues resulting in interruption of blood supply to organs.

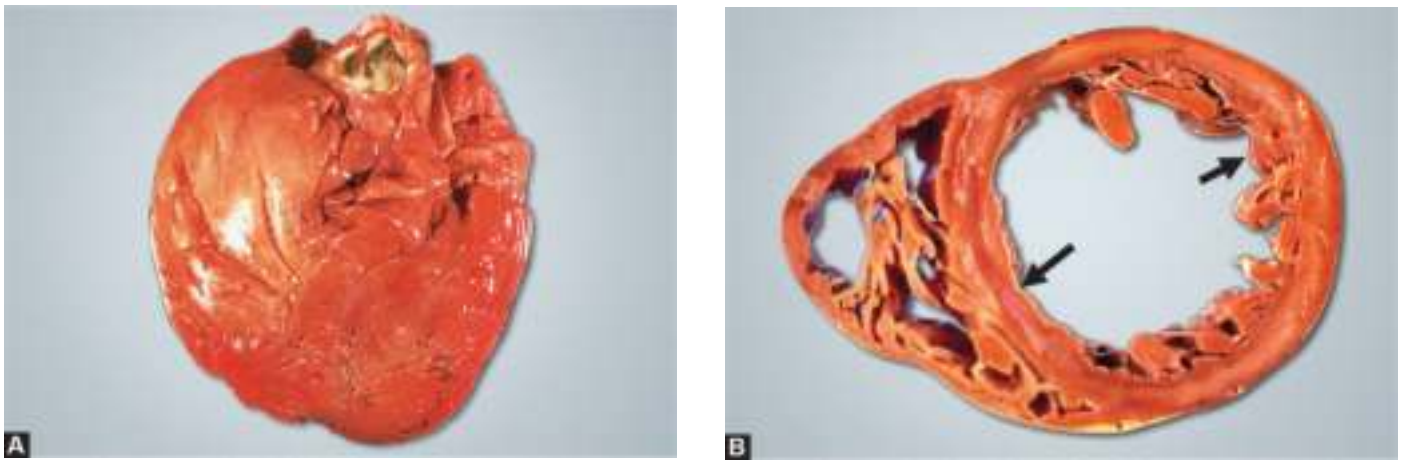


Fig. 16.29: Congestive cardiomyopathy. (A) Heart has a globoid shape because all of the chambers are dilated, (B) heart is very flabby, large and the myocardium is poorly contractile (arrows).

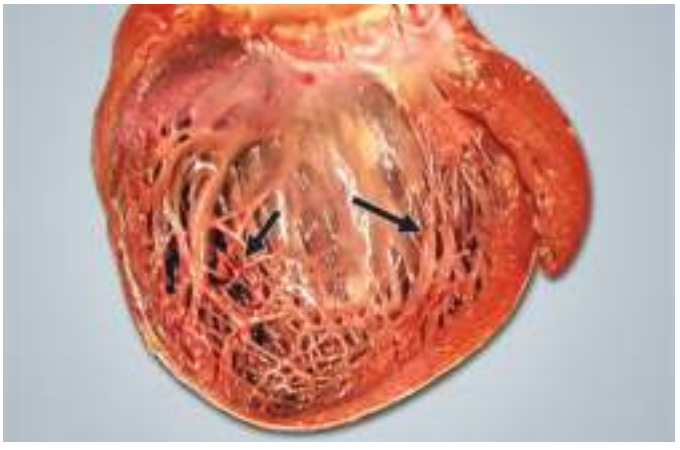


Fig. 16.30: Congestive cardiomyopathy. Cut opened chamber is showing marked dilatation. Heart is very flabby, large and the myocardium is poorly contractile (arrows).

- Emboli from right-sided heart reach pulmonary artery resulting in pulmonary infarction.

Etiology

Etiology of congestive cardiomyopathy (CMP) is most often unknown. In some cases, it is associated with chronic alcoholism, thiamine deficiency (beriberi heart), peripartum period (last trimester or within 6 months in postpartum period), end stage of viral myocarditis, myxedema, drug toxicity (e.g. doxorubicin, daunorubicin, cocaine) and Friedreich ataxia.

- Some cases of congestive CMP are associated with mutant cytoskeletal proteins (i.e. dystrophin or desmin), sarcomeric proteins (troponin), cardiac myosin heavy chain; and other muscle proteins, such as actin.
- Mutations in mitochondrial genes have also been implicated in congestive CMP. Causes of congestive cardiomyopathy are given in Table 16.19.

Table 16.19 Causes of congestive cardiomyopathy

Inflammatory Myocarditis
▪ Infective myocarditis
▪ Viral myocarditis (coxsackievirus, adenovirus, hepatitis C, HIV)
▪ Parasitic myocarditis (*Trypanosoma cruzi*—Chagas' disease, toxoplasmosis)
▪ Systemic fungal disease
▪ Noninfective myocarditis
▪ Sarcoidosis
▪ Giant cell myocarditis
▪ Eosinophilic myocarditis
▪ Collagen vascular diseases
▪ Polymyositis
▪ Dermatomyositis
▪ Checkpoint inhibitor chemotherapy
▪ Transplant rejection
Toxic Agents
▪ Alcohol
▪ Cocaine abuse
▪ Catecholamines: Amphetamines
▪ Interferon
▪ Therapeutic drugs (chloroquine, hydroxychloroquine)
▪ Drugs of misuse (anabolic steroids, emetine)
▪ Heavy metals (mercury, lead)
▪ Occupational exposure (hydrocarbons, arsenic)
Metabolic Disorders
▪ Nutritional deficiencies (thiamine, carnitine, selenium)
▪ Electrolyte deficiencies (calcium, phosphate, magnesium)
▪ Thyroid diseases
▪ Diabetes mellitus
▪ Pheochromocytoma
▪ Hemochromatosis
▪ Obesity
Miscellaneous Disorders
▪ Peripartum cardiomyopathy
▪ Tachycardia-related cardiomyopathy

Surgical Pathology: Congestive Cardiomyopathy
Gross Morphology
▪ The heart assumes a globular shape. ▪ There is pronounced ventricular dilatation, diffuse endocardial thickening and atrial enlargement often with thrombi in the appendages.
Light Microscopy
▪ Normal myocytes are filled with myofibrils, hence exhibit a uniform pink cytoplasm. ▪ Damaged myocytes undergo loss of myofibrils in myocytes, hence exhibit empty spaces within cytoplasm. There is presence of focal myocyte necrosis, interstitial fibrosis, and infiltration by T cells and macrophages (Fig. 16.31A and B).

Clinical Features

Patient presents with slow onset congestive heart failure, shortness of breath, orthopnea, dyspnea on exertion, fatigue and dry cough at night.

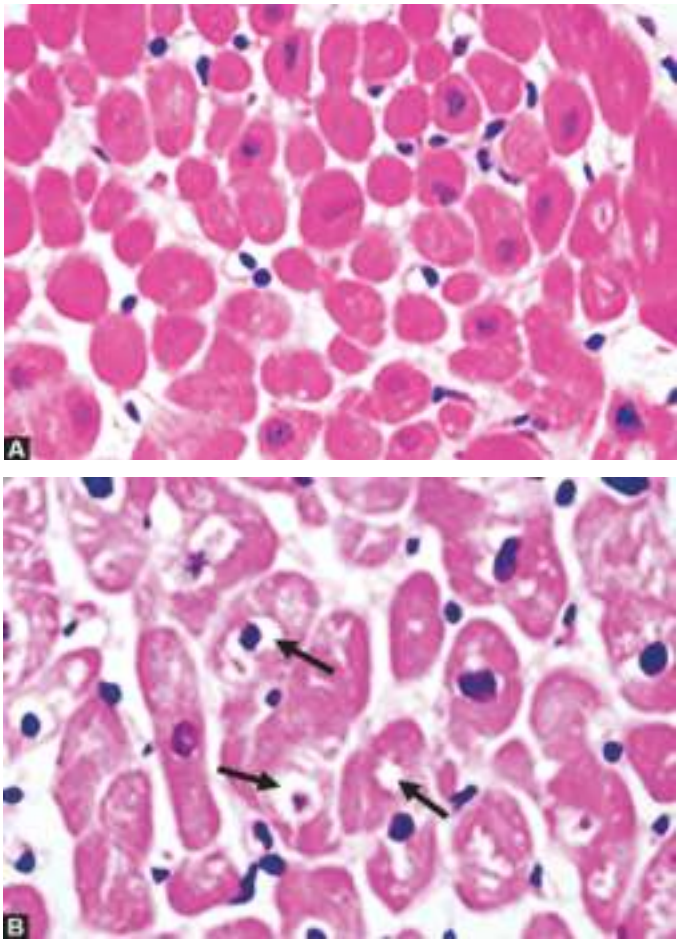


Fig. 16.31: (A) Each normal myocyte in cross section has a uniform pink cytoplasm owing to the majority of the cell being filled by myofibrils, (B) congestive cardiomyopathy. The myocytes have lost myofibrils and empty spaces have appeared within the cytoplasm (arrows).

Physical Examination

Physical examination reveals jugular vein distension, peripheral edema, hepatomegaly, peripheral cyanosis, irregular pulse and tachycardia.

Auscultation

Congestive CMP weakens papillary muscles resulting in mitral and tricuspid valve insufficiency. On auscultation, pansystolic murmur is heard.

Clinical Course

Prognosis is very poor and death occurs within 2 years from onset of symptoms. Cardiac transplantation is recommended for congestive CMP patients.

HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy is also known as idiopathic hypertrophic subaortic stenosis (**IHSS**) and transmitted as an autosomal dominant trait in 50% of cases. It is most common form of cardiomyopathy with hypercontracting heart affecting younger adults.

Genetic Basis of HCMP

Hypertrophic cardiomyopathy is inherited as an autosomal dominant trait in >50% of cases. Gene mutations implicated in this disorder include gene coding for β-myosin heavy chain mapped on chromosome 14 (most common and severe form in 50% of cases), cardiac troponins T and I (less severe form in 15% of cases), α-tropomyosin gene on chromosome 15 (less severe in 3–5% of cases), myosin-binding protein C, and myosin light chain.

Surgical Pathology: Hypertrophic Cardiomyopathy
Gross Morphology
▪ Heart shows hypertrophy of all the chambers especially asymmetric interventricular septum hypertrophy. It becomes stiff and noncompliant. It is unable to relax during ventricular filling. ▪ Lumen of the left ventricle is markedly reduced assuming banana-shaped chamber (Fig. 16.32).
Light Microscopy
▪ The myocytes in normal myocardium are arranged in parallel, and within the myocyte myofibrils. ▪ But in hypertrophic cardiomyopathy, the myocytes are arranged in disoriented tangled criss-cross whorls losing their normal parallel arrangement (Figs 16.33 and 16.34).



Fig. 16.32: Hypertrophic cardiomyopathy. Left ventricular hypertrophy with asymmetrical septal hypertrophy, systolic anterior motion of mitral valve, banana-shaped left ventricular chamber and hypercontracting heart (arrow).

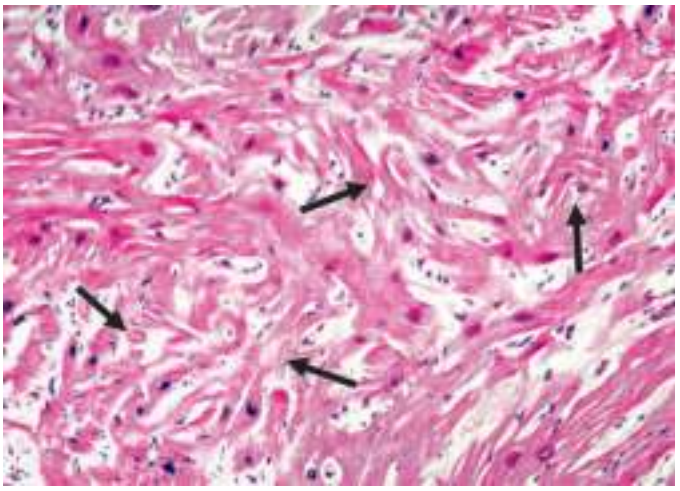


Fig. 16.34: Hypertrophic cardiomyopathy. The cells arranged in whorls around foci of connective tissue. Higher power magnification shows the myofibrils within the cell criss-cross whorls losing their normal parallel arrangement (arrows) (400X).

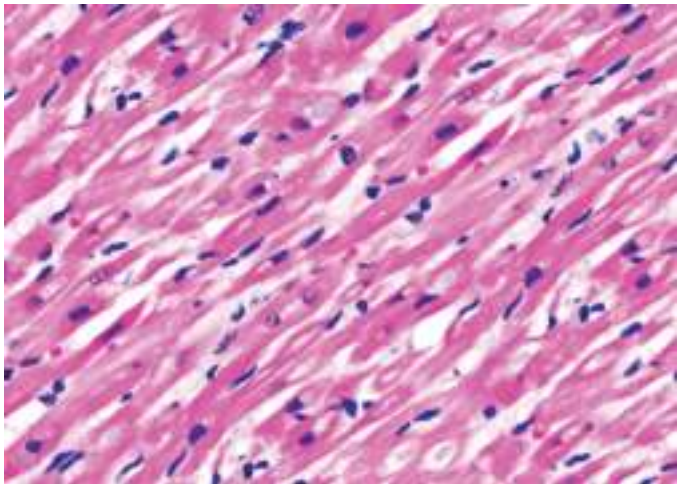


Fig. 16.33: Normal cardiac muscle biopsy. The myocytes in normal myocardium are arranged in parallel, and within the myocyte myofibrils (400X).

Pathophysiology

In patient, asymmetrical hypertrophy of the interventricular septum is responsible for subaortic stenosis resulting in obstruction to blood flow and systolic motion of anterior leaflet of mitral valve toward the interventricular septum. It impairs ventricular filling resulting in increased pressure in left atrium and pulmonary veins. It causes unexpected death in young athletes.

Clinical Features

Patient presents with breathlessness on exertion, dizziness, fainting, precordial pain, and cardiac arrhythmias. The obstruction to blood flow from the left ventricle increases the ventricle's work, and harsh systolic ejection cardiac murmur may be heard along the left sternal border and at the apex. Patient develops abrupt irregular arterial pulse due to atrial fibrillation.

Complications

Patient may develop cardiac arrhythmia (most common), atrial fibrillation, infective endocarditis, congestive heart failure and thrombus formation with fatal outcome.

RESTRICTIVE CARDIOMYOPATHY

Restrictive cardiomyopathy is least common but serious disorder, which affects infants and children. Infiltration of extraneous material in the ventricles causes left ventricular hypertrophy with decreased ventricular chamber lumen and endocardial fibrosis. The stiffness reduces the ventricle's ability to relax and filling during diastole. The rigid myocardium fails to contract completely during systole. As a result, cardiac output falls. ECG shows low-voltage QRS complex.

Etiopathogenesis

In adults, cardiac amyloidosis and hemochromatosis cause restrictive cardiomyopathy, which is characterized by reduced diastolic filling and right-sided heart failure. Infiltration of the conduction system by amyloid material can result in cardiac arrhythmias or sudden cardiac death. Causes of restrictive cardiomyopathy are given in Table 16.20.

Table 16.20 Causes of restrictive cardiomyopathy

Infiltrative Causes between Myocytes
▪ Amyloidosis • Primary amyloidosis (light chain) • Familial (abnormal transthyretin) • Senile amyloidosis (normal transthyretin or atrial peptides) ▪ Inherited metabolic defects
Storage Disorders within Myocytes
▪ Hemochromatosis (primary or secondary) ▪ Inherited metabolic disorders • Glycogen storage disease (II and III) • Fabry's disease
Fibrotic Causes
▪ Scleroderma ▪ Radiation
Endomyocardial Causes
▪ Possibly related to fibrotic disease • Hypereosinophilic syndrome (Loffler's endocarditis) • Tropical endomyocardial fibrosis ▪ Carcinoid syndrome ▪ Drugs (serotonin, ergotamine) ▪ Radiation
Overlap with other Cardiomyopathy
▪ Hypertrophic cardiomyopathy ▪ Sarcoidosis ▪ Minimally dilated cardiomyopathy • Early-stage dilated cardiomyopathy • Partially recovery from dilated cardiomyopathy

Surgical Pathology: Restrictive Cardiomyopathy

Gross Morphology

- Gross examination of the heart shows bilateral dilatation due to increased filling pressures.
- Ventricles are relatively of normal size.
- Ventricular cavity is within normal range.
- Ventricular wall thickness is abnormal with a rubbery texture.
- Thickening of the endocardium may be observed.

Light Microscopy

- Amyloid appears amorphous, glassy, and eosinophilic material in extracellular compartment.
- Amyloid is readily documented using Congo red stain, which demonstrates green birefringence when viewed under polarized light.
- Prussian blue staining is done to demonstrate iron in hemochromatosis involving heart (Fig. 16.35).

Clinical Features

In restrictive cardiomyopathy (RCM), myocardium of ventricles become stiff leading to enlarged atria and impairment of ventricular filling during diastole.

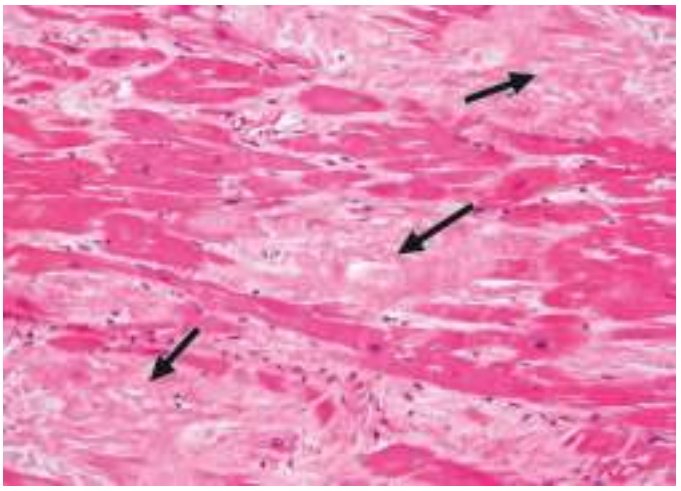


Fig. 16.35: Restrictive cardiomyopathy. Pale pinkish amorphous material is deposited between myocardial fibers due to amyloid deposition. Arrhythmias occur during surgery (arrows).

Symptoms of RCM vary depending on severity such as congestive heart failure and cardiac arrhythmias especially during surgery. Patient presents with dyspnea, orthopnea, cough, chest pain, fatigue, and swelling of feet and ankles. Physical examination reveals distended neck veins, peripheral edema, hepatomegaly, lung crackles, peripheral cyanosis, and pallor. Auscultation reveals S3 or S4 gallop rhythm. Restrictive cardiomyopathy may appear like constrictive pericarditis. Cardiac catheterization may help confirm the diagnosis.

ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

- Arrhythmogenic right ventricular cardiomyopathy is an autosomal dominant disorder of cardiac muscle. In arrhythmogenic right ventricular cardiomyopathy (ARVC), fibrofatty tissue gradually replaces normal myocardium of right ventricular apex, base region, and right ventricle outflow tract. Patient has left bundle branch block morphology. Pathology of ARVC may also extend to involve left ventricle associated with left ventricular dysfunction. ARVC interrupts normal electrical signals in the heart and may cause right-sided heart failure, irregular heartbeat, chest pain, and potentially life-threatening cardiac arrhythmias. Osteoglycin (OGN) gene has been observed in patients with ARVC. Mutations in desmosomal gene encoding defective desmosome-associated protein have been linked to ARVC and Nexos syndrome. Patient with Nexos syndrome presents with ARVC and hyperkeratosis of plantar and palmar skin surfaces.

DISORDERS OF ENDOCARDIUM

ENDOCARDIUM AND ENDOCARDITIS: OVERVIEW

The endocardium is the innermost layer of the heart and lines the cardiac chambers that extends over the cardiac valves, chordae tendineae, and papillary muscles. It serves as a barrier between cardiac muscles and the blood stream. Endocardium is thicker in the atria than in ventricles, and is more thick in left than right cardiac chambers, and in the outflow tracts than in the inflow tracts. Depending on etiology, endocarditis is categorized in two groups—infective and noninfective endocarditis.

- **Infective endocarditis:** Acute endocarditis affects normal heart due to highly virulent bacteria. Subacute endocarditis occurs in previously diseased heart due to even low virulent bacteria. Systemic symptoms of acute infective endocarditis are fever, weight loss and malaise due to synthesis of cytokines. Patient develops skin petechiae and microhemorrhages around the fingernails due to deposition of immune complex in small blood vessels in these regions. Splenomegaly and anemia are present due to persistent bacteremia. Finger clubbing, Janeway lesions and/or Osler lymph nodes are present.
- **Noninfective endocarditis:** Examples of noninfective endocarditis are Libman-Sacks endocarditis due to systemic lupus erythematosus (SLE), nonbacterial thrombotic endocarditis due to mucin secreting carcinoma of colon and pancreas, and small sterile verrucous vegetations in rheumatic heart disease.

INFECTIVE ENDOCARDITIS

Infective endocarditis is an infection of the endocardium, cardiac valvular surfaces, or cardiac prosthesis resulting from bacterial (most common) or fungal invasion. Infective endocarditis may be acute or subacute. There are, however, no strict criteria for separating clinically acute bacterial endocarditis from subacute bacterial endocarditis (Fig. 16.36 and Table 16.21). Predisposing conditions for infective endocarditis are rheumatic heart disease (RHD), valve and myxomatous aortic valve.

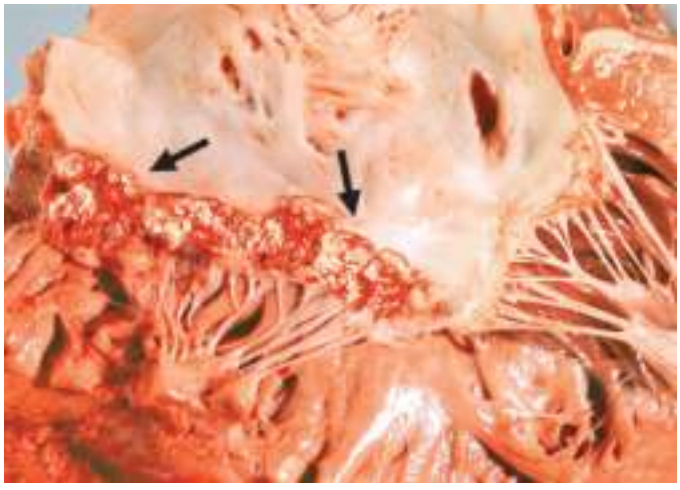


Fig. 16.36: Infective endocarditis. Large friable vegetations are seen along the margin of cusps and chordae tendineae of mitral valve. Patient presents with a 4-week history of fever, fatigue and weight loss. Physical examination revealed petechial hemorrhages and clubbing of fingers (arrows).

Table 16.21 Differences between acute and subacute bacterial endocarditis

Characteristics	Acute Bacterial Endocarditis	Subacute Bacterial Endocarditis
Heart status	Previously normal	Previously diseased
Onset	Rapid onset	Gradual onset
Pathogen's virulence	Highly virulent organisms, e.g. *Staphylococcus aureus*	Less virulent organisms β-hemolytic streptococci
Cardiac vegetations size and location	More bulky and friable, present along edges of cusps	Less bulky and present along edges of cusps myocardium
Nature of lesions	More erosive and destructive lesions result in perforation of cusps (quite common) and chordae tendineae	Less erosive and destructive lesions may cause perforation of cusps (less common)
Myocardial involvement	Myocardium microabscesses ++++	Myocardium microabscesses ++
Clinical features	Fever high-grade with chills and arthralgia ++	Fever slight (flu-like syndrome)
Physical examination	▪ Splenomegaly present ▪ Splinter hemorrhages due to microemboli beneath nails known as Janeway's lesions	▪ No splenomegaly ▪ No splinter hemorrhages beneath nails (Janeway's lesions absent)
Auscultation	Cardiac murmurs are quite common	Cardiac murmurs may be present

- **Cardiac valves affected:** Mitral valve is most frequently involved, followed by the aortic, tricuspid, and the pulmonary valves in infective endocarditis cases. The mitral valve along with the aortic valve is involved in about 40% of cases. The tricuspid valve is involved in >50% of cases of endocarditis due to intravenous drug abuse, in whom endocarditis is most often caused by *Staphylococcus aureus* infection.
- **Risk factors:** Infective endocarditis occurs most commonly secondary to bacterial pathogens. Predisposing factors include dental procedures, immunodeficiency, intravenous drug addicts, surgery, tooth extractions, venous access devices, such as central or peripheral catheters and artificial heart valves.
- **Source of infection:** Organisms reach the endocardium by hematogenous route from oropharynx flora (dental procedures), skin flora, gastrointestinal tract flora, and genitourinary procedure and lung infections.
  - **Highly virulent bacteria:** *Staphylococcus aureus* produces abscess at multiple sites in >30% of cases.
  - **Low virulent bacteria:** These include streptococci group A and B (55%), oropharyngeal flora HACEK (e.g. Haemophilus, Actinobacillus, Cardiobacterium, Eikenella, Kingella), enterococci-penicillin resistant (6%) by genitourinary manipulations. *Streptococcus viridans* and Staphylococcus may be indolent or associated with minimal symptoms.

ACUTE ENDOCARDITIS

Acute endocarditis is caused by highly virulent bacteria. *Staphylococcus aureus* produces an acute bacterial endocarditis in previously normal heart in 50% of cases. Acute endocarditis is often secondary to infection occurring elsewhere in the body.

Cardiac Vegetations

Cardiac vegetations are large, bulky, destructive, and extremely friable on the cardiac valve cusps that can extend onto the chordae tendineae, hence predisposing to systemic embolization producing infected infarcts in various organs in patients with acute endocarditis.

Clinical Features

The symptoms are more pronounced which develop acute endocarditis much rapidly. Fever is most consistent sign in 95% of cases.

Blood Cultures

Blood cultures are positive in 95% of cases. Negative blood culture is either due to deep seated organisms covered by fibrin, or prior antibiotic therapy and endocarditis caused by fungus, Coxiella or Chlamydia.

SUBACUTE ENDOCARDITIS

Subacute endocarditis is caused by low-virulent bacteria Streptococcus viridans in >50% of cases or fungi (e.g. Candida and Aspergillus).

Predisposing Factors

Subacute endocarditis tends to occur in patients with congenital heart disease or pre-existing valvular heart disease or acquired.

- Congenital heart diseases such as atrial septal defect (ASD), ventricular septal defect (VSD), patent ductus arteriosus, coarctation of aorta, tetralogy of Fallot, and mitral valve prolapse lead to subacute endocarditis.
- Acquired causes include chronic rheumatic endocarditis, prosthetic valve, syphilitic aortitis, calcific aortic stenosis, arteriovenous fistula and degenerative heart disease.

Cardiac Vegetations

Cardiac vegetations are smaller, less fragile, and associated with fewer destructive lesions in subacute endocarditis. Thrombus formation and septic embolization can subsequently occur.

Clinical Features

Patient presents with gradual onset of nonspecific symptoms such as slight fever (flu-like syndrome).

Pathology Pearls: Complications of Infective Endocarditis

Complications of infective endocarditis include destruction of cardiac valves, dissemination of bacteria, embolization and immune complex formation (Figs 16.37 and 16.38). Remote embolic complications of infective endocarditis are given in Table 16.22.

Destruction of Cardiac Valves

- Endocarditis may impede the blood flow and cause turbulence reflecting cardiac murmurs. As the disease advances, bacteria may destroy parts of the cardiac valve and cause cardiac valve perforation, rupture of chordae tendineae, or abscesses at the base of the cusps.
- All these pathologic changes adversely affect the function of cardiac valve and may cause valvular insufficiency. Stenosis of cardiac valve may also develop, even though more often it is found in the healing stages of the disease, characterized by fibrosis and dystrophic calcification of cusps. Cardiac vegetations are demonstrated by echocardiography.

Dissemination of Bacteria

- The entry of bacteria into the circulation results in bacteremia that causes bronchopneumonia. The patient typically has symptoms of sepsis.
- Positive blood culture will reveal bacteria as the cause of these relatively nonspecific symptoms.

Embolization

- Fragments of infected cardiac vegetations are carried by the blood, lodging in terminal arteries.
- Such microemboli interrupt the blood supply to the affected areas resulting in septic infarcts in various organs, i.e. brain (cerebral strokes), retina, spleen, kidney (glomerulonephritis and renal infarct) and digits.
- Thromboemboli from tricuspid valve endocarditis may result in bronchopneumonia and pulmonary infarcts.

Immune Complex Deposition

- Antibodies formed against bacterial antigens are released in the infected endocardial vegetations. These immune complexes circulate in the blood and are deposited in the wall of glomeruli and small blood vessels resulting in rupture.
- Patients develop Roth's retinal hemorrhages, Osler's nodes (painful nodules on pads of the fingers and toes), glomerulonephritis and Janeway's lesions.
- Janeway's lesions are splinter hemorrhages (linear red streaks) on palms, soles beneath nails; due to rupture of small vessels by microemboli.

Clinical Pearls: Laboratory Diagnosis of Infective Endocarditis

- **Blood culture:** Serial blood cultures two to six specimens over 48 hours have been shown to confirm the diagnosis of infective endocarditis in 95% of cases. Blood culture is negative either due to deep seated colonies of organisms, covered by fibrin or prior antibiotic therapy and infective endocarditis due to organisms like fungus, Coxiella or Chlamydia.
- **Electrocardiogram study (ECG):** ECG may show prolongation of the P-R interval or bundle branch block.
- **Echocardiography:** Echocardiography is done to localize the lesion in heart.
- **Blood Investigations:** Peripheral smear shows leukocytosis and anemia. Erythrocyte sedimentation rate (ESR) is raised.



Fig. 16.38: Complications of infective endocarditis. It shows splinter hemorrhage on index finger and thumb.

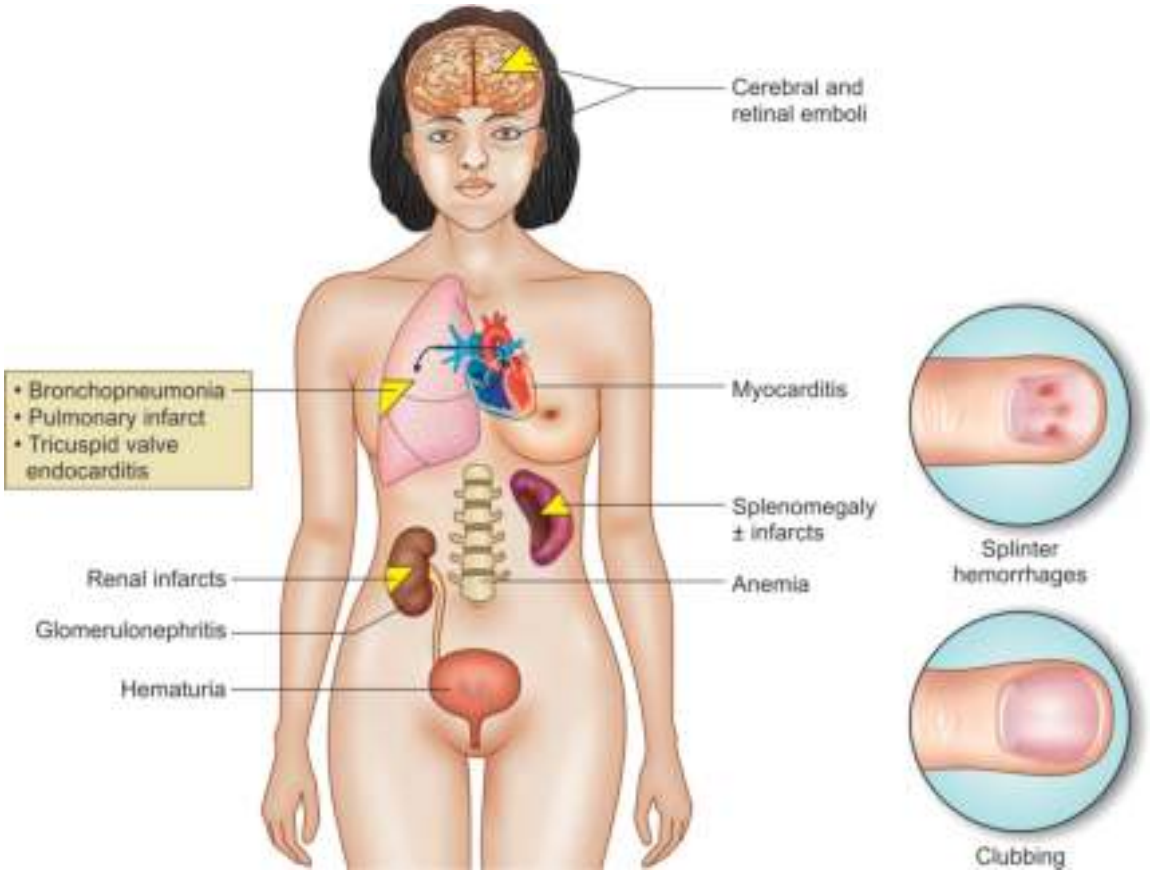


Fig. 16.37: Complications of infective endocarditis. Septic emboli may lodge in various organs causing infarcts, bronchopneumonia and splinter hemorrhages.

ENDOCARDITIS IN PROSTHETIC VALVES

Prosthetic valve endocarditis accounts for approximately 20% of all clinically diagnosed cases of endocarditis. Aortic valve is commonly affected along the suture of prosthetic valve, which occurs in two forms, i.e. early-onset and late-onset.

- Early-onset endocarditis occurs within 60 days of cardiac valve surgery (i.e. plastic, metal and bioprosthesis of animal origin) in 2 or 3% of patients. It occurs either due to contamination during surgery or with drug resistant pathogens in postoperative period. *Staphylococcus aureus* and *Staphylococcus epidermidis* account for >50% of infections, gram-negative bacteria and fungi (Candida, Aspergillus).
- Late-onset endocarditis occurs after 60 days of cardiac valve replacement in 0.5% of patients. It is usually related to transient bacteremias. It is most often caused by Staphylococcus (50%) and *Streptococcus viridans* (40%).
- The most important complications of prosthetic valve include thromboembolism, paravalvular leaks, mechanical malfunction, mechanical deterioration (rupture and calcification of valves), microangiopathic hemolytic anemia with hemoglobinuria and mechanical dysfunction with heart failure. The thrombi developing on the prosthetic valves may also become infected.

ENDOCARDITIS IN INTRAVENOUS DRUG ABUSE

Intravenous drug abuse persons develop endocarditis due to *Staphylococcus aureus* (50% of cases), streptococci (15% of cases), enterococci and opportunistic fungal infections, i.e. Candida and Aspergillus.

- Tricuspid valve (55% of cases) is most often affected in drug addicts followed by bicuspid valve (20% of cases) and aortic valve (25% of cases) (Fig. 16.39).

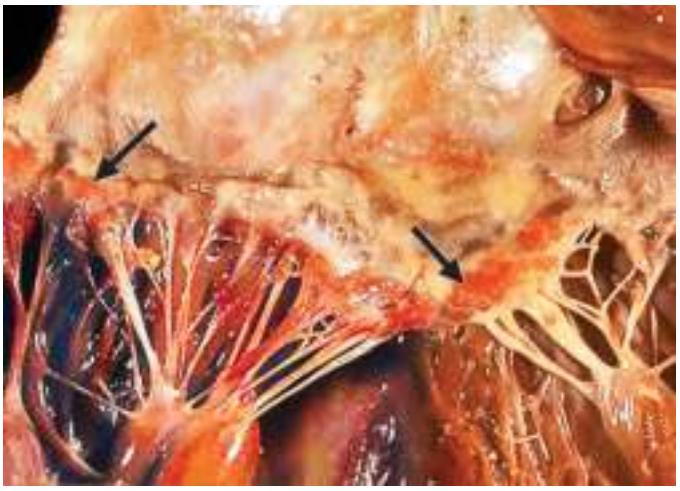


Fig. 16.39: Libman-Sacks endocarditis in SLE. The verrucous 1 to 2 mm, multiple, sterile widely spaced vegetations along margins of the mitral valve and less commonly tricuspid valve in 30–50% of cases of systemic lupus erythematosus. These vegetations usually heal without residual effect (arrows).

- Patient with prosthetic valve endocarditis may develop cellulitis, thrombophlebitis and embolic episodes in femoral arteries and lungs. It is worth mentioning that tricuspid valve may also be affected in patients with indwelling catheters, cardiac surgery, ventricular septal defects and carcinoid tumors.

LIBMAN-SACKS ENDOCARDITIS

In patient with systemic lupus erythematosus (SLE), endocarditis is the most striking cardiac lesion, termed Libman-Sacks endocarditis in 10–30% of patients. Immune complex-mediated injury of the valvular endocardium results in formation of sterile cardiac vegetations.

- Cardiac vegetations in Libman-Sacks endocarditis are 1–4 mm in size widely spaced, present on the undersurface of the mitral valve (most common) close to the origin of the leaflets from the valve ring.

Table 16.22 Remote embolic complications of infective endocarditis

Organs	Clinical Manifestations
Brain	Infarction of brain with secondary hemorrhage from embolus to anterior cerebral arteries, small infarcts of basal ganglion
Retina	Embolus in ocular fundus with retinal infarction and petechial hemorrhages
Hands skin and fingers	Multiple petechial hemorrhages of skin and clubbing of fingers
Mucous membrane	Petechial hemorrhages of mucous membrane
Kidneys	Petechial hemorrhages
Spleen	Mycotic aneurysms of splenic arteries and infarct of spleen. It shows splenomegaly

Embolization is a serious complications of infective endocarditis.

Cardiac vegetations are less often present in tricuspid valve and adjacent endocardium.

- Mitral valve deformity and regurgitation may occur in Libman-Sacks endocarditis. There is fibrinoid necrosis of small vessels with focal degeneration of interstitial tissue. These cardiac vegetations can be identified in living patients by echocardiography.
- Libman-Sacks endocarditis is not a serious complication during the course of the disease. Cardiac vegetations usually heal without residual effect, hence are clinically of limited significance.

NONBACTERIAL THROMBOTIC ENDOCARDITIS

Nonbacterial thrombotic endocarditis (**NBTE**), also known as marantic endocarditis, refers to the presence of sterile cardiac vegetations on apparently normal cardiac valves, almost always in association with metastatic cancer or debilitating disorders. The principal danger of sterile cardiac vegetations is embolization to distant organs.

PATHOGENESIS

The cause of nonbacterial thrombotic endocarditis is poorly understood, but it has been attributed to increased blood coagulability and immune complex deposition.

- **Mechanical injuries during intracardiac procedures:** Thrombi are formed on sites of minute mechanical injuries of the endothelium due to intracardiac procedures, turbulent blood flow, deformed valves, and old scars in chronic rheumatic heart disease.
- **Malignant tumors:** Sterile cardiac vegetations may develop as part of a paraneoplastic syndrome in malignancies associated with procoagulant effect of circulating mucin secreting tumors of colon, pancreas and lung.
- **Cachexia:** In debilitated people, cancer, chronic infection, and defective normal repair mechanisms increase risk for NBTE. Small thrombi formed are not removed by plasminogen activators and fibrinolytic substances. These thrombi grow and transform into large excrescences.

CARDIAC VEGETATIONS

Cardiac vegetations are composed of fibrin, platelets, and red blood cells which resemble thrombi. These cardiac vegetations are sterile, measuring 1–5 mm in size, and nondestructive (single or multiple) loosely attached randomly (Fig. 16.40).

- Cardiac vegetations are present on superior surface of aortic, mitral, tricuspid and pulmonary valves as a result of mild inflammation and associated with endothelial damage.

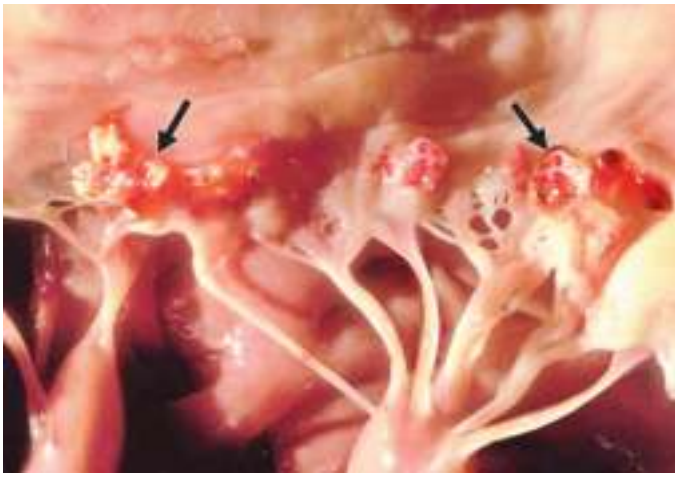


Fig. 16.40: Nonbacterial thrombotic endocarditis—sterile large vegetations are loosely attached along margins of normal cardiac valves on both sides (arrows). These are associated with cancer and wasting diseases. There is principal risk of bacterial endocarditis and embolization to brain, heart, lungs and kidneys.

- Cardiac vegetations may cause peripheral embolization to brain, heart, lungs and kidneys.

THERAPEUTIC SIGNIFICANCE

Sterile cardiac vegetations in NBTE require treatment with anticoagulants. Remember anticoagulant therapy is contraindicated in bacterial infective endocarditis. It is important to distinguish these two diseases. Administration of anticoagulants in infective endocarditis may result in fragmentation of cardiac vegetations and embolization to distant organs.

ENDOCARDITIS IN RHEUMATIC HEART DISEASE

Cardiac vegetations in rheumatic heart disease are sterile measuring 1–2 mm in diameter, present along the line of opposition of cusps and chordae tendineae. These cardiac vegetations are never present behind cusps (Fig. 16.41).

CARDIAC VALVES INVOLVED

Frequency of cardiac valves involved in rheumatic heart disease includes: (a) mitral valve (75–80%), (b) aortic valve (30%), (c) mitral and aortic valves (25%), (d) mitral and tricuspid valves (<5%) and (e) pulmonary valve (rare).

- These cardiac vegetations with super-added infection lead to subacute bacterial endocarditis.
- Subacute bacterial endocarditis in RHD patients is caused by *Streptococcus viridans* and treated with high doses of intravenous antibiotics.

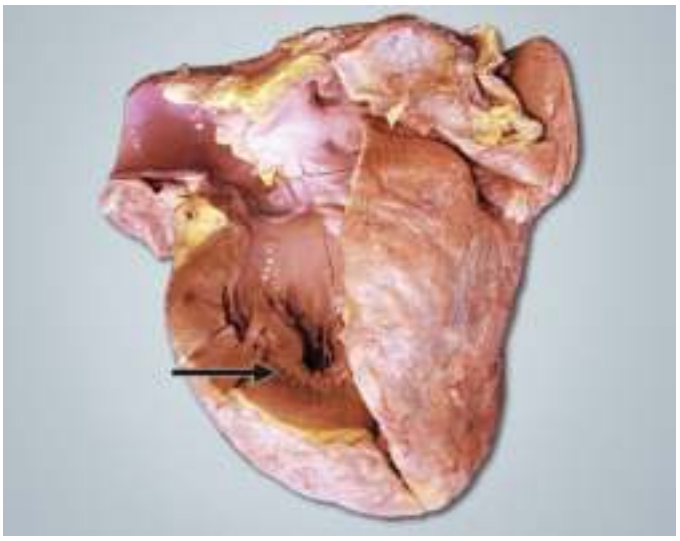


Fig. 16.41: Rheumatic heart disease. Sterile vegetations are seen along margin of mitral valve (arrow).

RIGHT-SIDED ENDOCARDITIS IN CARCINOID SYNDROME

Right-sided endocarditis in carcinoid syndrome is caused by secretory products of intestinal carcinoid tumors, i.e. vasoactive amines (serotonin) and vasoactive peptide hormones (somatostatin and glucagon).

PATHOGENESIS

- The secretory products of nonmetastasizing intestinal carcinoid tumor are inactivated and metabolized in liver and lungs.
- In case of metastases of carcinoid tumor to liver, the secretory products of carcinoid tumor enter circulation, reach to right-sided heart and cause tricuspid valve regurgitation and pulmonary trunk stenosis.
- Cardiac valves on the left side of the heart are rarely involved, because serotonin and other carcinoid secretory products are detoxified in the lung. These valvular lesions heal by fibrosis in cases of carcinoid syndrome.

CLINICAL FEATURES

Patient presents with cutaneous flushing, diarrhea, and valvular disease (tricuspid regurgitation and pulmonary trunk stenosis). Comparison of cardiac vegetations in various disorders involving endocardium is shown in Fig. 16.42A to D and Table 16.23.

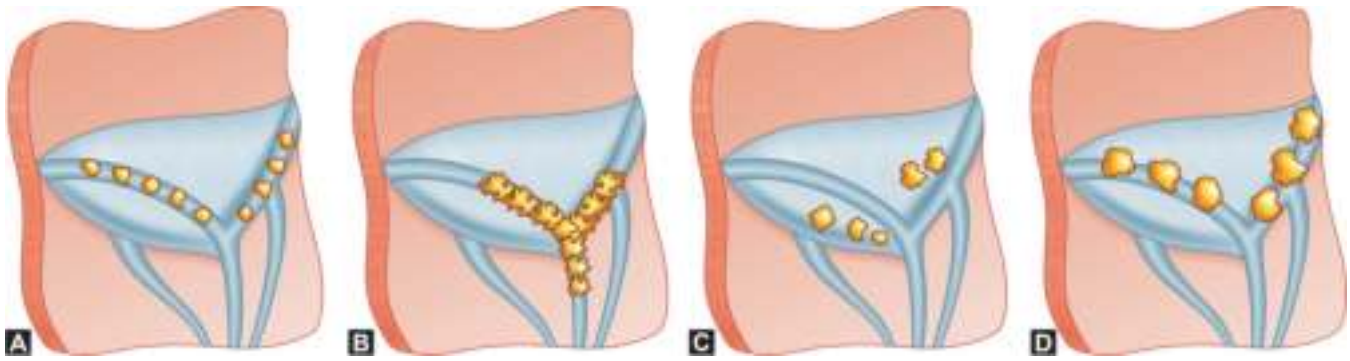


Fig. 16.42: Cardiac vegetations. (A) Cardiac vegetation in RHD, (B) infective endocarditis (IE), (C) Libman-Sacks endocarditis (LSE), (D) nonbacterial thrombotic endocarditis (NBTE).

Table 16.23 Comparison of cardiac vegetations in various disorders involving endocardium

Parameters	Rheumatic Fever	Nonbacterial Thrombotic Endocarditis	Libman-Sacks Endocarditis (SLE)	Infective Endocarditis
Etiology	Group A Streptococcus β-haemolyticus	Hypercoagulable state, mucin secreting carcinoma of colon and pancreas	Autoimmune disorder	Infective organisms
Mechanism	Type 2 and Type 4 hypersensitivity reaction (molecular mimicry)	Noninflammatory disorder	Immune complex mediated by DNA-anti-DNA autoantibody	Acute inflammation
Size of cardiac vegetations	1–2 mm size firm and warty	1–5 mm single/multiple friable results in embolism	1–4 mm single or multiple widely spaced	Large, friable bulky loosely attached to valves

Contd...

Table 16.23 Comparison of cardiac vegetations in various disorders involving endocardium (*Contd...*)

Parameters	Rheumatic Fever	Nonbacterial Thrombotic Endocarditis	Libman-Sacks Endocarditis (SLE)	Infective Endocarditis
Nature	Sterile, nondestructive	Sterile nondestructive	Sterile small and non-destructive	Infective, destructive
Composition	Aschoff's nodule	Fibrin, platelets, and RBCs	Fibrin, platelets, and RBCs	Acute inflammation in PMN with bacteria
Location	Along the lines of closure of valvular cusps	Along the lines of closure of cusps or both sides of valvular cusps	Present on both sides of mitral valvular cusps. Most common on inferior surface. Pocket of valves. Less common on mural endocardium	Upper surface of valvular cusps. It can extend to the chordae tendineae
Valves affected	Mitral valve (75–80%), aortic valve (30%), mitral valve and aortic valve (25%), tricuspid valve and pulmonary valve <5%	Any cardiac valve	Mitral valve (most common), tricuspid valve +ve	Aortic, tricuspid and pulmonary values
Chances of embolization	Low incidence	High incidence	Absent	High incidence
Blood test	Antistreptolysin *O* titer raised	Not diagnostic	LE cells preparation	Blood culture positive (95%)

DISORDERS OF CARDIAC VALVES

CARDIAC VALVULAR DISORDERS: OVERVIEW

Cardiac valvular diseases most often occur as a result of rheumatic heart disease or secondary to various inflammatory processes.

- Prosthetic valves are the site of thrombus formation or development of infective endocarditis.
- Prosthetic valves cause mechanical disruption of red blood cells which result in hemolytic anemia with formation of schistocytes. Major causes of cardiac valve disease are given in Tables 16.24 to 16.28.

Table 16.24 Pathological causes and clinical manifestations of mitral and aortic valvular diseases

Cardiac Valvular Disease	Cause	Effects on Organs	Physical Findings
Mitral valve stenosis	Rheumatic heart disease	▪ Left heart failure with predisposition to atrial thrombus ▪ Left atrial dilated ▪ Pulmonary edema ▪ Right heart failure	▪ Left atrium and right ventricle hypertrophy ▪ Rumbling late diastolic murmur following a loud S1 sound open snap ▪ Fine crepitations on auscultation
Mitral valve regurgitation	▪ Acute myocardial infarction involving papillary muscle ▪ Rheumatic heart disease ▪ Floppy mitral valve prolapse syndrome ▪ Hypertrophic obstructive CMP ▪ Blunt injury to mitral valve	▪ Left heart failure ▪ Pulmonary edema ▪ Left ventricular hypertrophy and dilation resulting in left heart failure ▪ Progressive left heart failure ▪ Impairs ventricular filling and resulting in increased pressure in left atrium and pulmonary veins	▪ Variable hemodynamic effects ▪ Pansystolic murmur

Contd...

Table 16.24 Pathological causes and clinical manifestations of mitral and aortic valvular diseases (*Contd...*)

Cardiac Valvular Disease	Cause	Effects on Organs	Physical Findings
Aortic valve stenosis	▪ Rheumatic heart disease ▪ Calcification in congenital bicuspid aortic valve ▪ Senile cardiac valve degeneration	▪ Progressive left ventricular hypertrophy ▪ Left heart failure ▪ Pulmonary congestion ▪ Coronary arteries insufficiency	▪ Early systolic ejection murmur reaching peak intensity in mid- and late-systole at apex of left sternal border ▪ Narrow pulse pressure ▪ Slow rising of carotid artery pressure ▪ Angina pectoris, syncope and sudden cardiac death
Aortic valve regurgitation	*Acute aortic valve regurgitation due to:* ▪ Endocarditis ▪ Chest trauma ▪ Prosthetic valve dysfunction ▪ Aortic dissection ▪ Cystic medial necrosis ▪ Marfan's syndrome *Chronic aortic regurgitation occurs due to:* ▪ Rheumatic heart disease ▪ Hypertension ▪ Marfan's syndrome ▪ Syphilitic aortitis ▪ Ankylosing spondylitis ▪ Ventricular septal defect	▪ Left ventricular hypertrophy and dilation ▪ Progressive development of left heart failure	▪ High pitched blowing diastolic murmur following an open snap ▪ Collapsing pulse ▪ Wide pulse pressure

Pulmonary artery stenosis occurs in the setting of tetralogy of Fallot, carcinoid heart disease and rarely in rheumatic heart disease.
Calcific aortic stenosis occurs due to rheumatic heart disease, senile calcific aortic stenosis and congenital aortic stenosis.

Table 16.25 Major causes of mitral valve disease

Mitral Stenosis		
▪ Rheumatic heart disease ▪ Infective endocarditis with large vegetations ▪ Congenital (parachute valve, cor triatriatum)	▪ Severe mitral annular calcification with leaflet involvement ▪ Systemic lupus erythematosus	▪ Rheumatoid arthritis ▪ Myxoma tumor
Mitral Regurgitation		
▪ Acute mitral regurgitation ▪ Infective endocarditis ▪ Papillary muscle rupture following myocardial infarction ▪ Chordae tendineae rupture/leaflet flail (mitral valve prolapse, infective endocarditis) ▪ Blunt injury to mitral valve ▪ Chronic causes primarily involving leaflets and chordae tendineae	▪ Rheumatic heart disease ▪ Healed infective endocarditis ▪ Myxomatous change (mitral valve prolapse, Barlow's forme fruste) ▪ Congenital mitral regurgitation ▪ Chronic causes involving leaflets, chordae tendineae as 'innocent bystanders' ▪ Ischemic cardiomyopathy	▪ Congestive cardiomyopathy ▪ Hypertrophic cardiomyopathy (HCM) ▪ Chronic atrial fibrillation with left atrial enlargement and annular dilatation ▪ Mitral annular calcification

MITRAL VALVE STENOSIS

Mitral valve is most commonly affected in chronic rheumatic heart disease, which undergoes fibrosis and dystrophic calcification, with fusion of the commissures resulting in stenosis giving fish-mouth appearance (Fig. 16.43).

CONSEQUENCES

Mitral valve stenosis hampers normal blood flow from left atrium to left ventricle during diastole. It leads to hypertrophy of the left atrium, pulmonary congestion and brown induration; and hypertrophy of right ventricle.

Table 16.26 Major causes of aortic valve disease

Aortic Stenosis
▪ Rheumatic heart disease ▪ Senile calcific aortic stenosis
Aortic Regurgitation
▪ Valvular causes • Endocarditis • Rheumatic fever • Aortic prolapse (myxomatous change) • Traumatic injury • Syphilitic aortitis • Ankylosing spondylitis ▪ Aortic root disease • Aortic dissection • Marfan syndrome • Bicuspid aortic valve • Systemic hypertension • Aortitis ▪ Nonsyndromic familial aneurysm

Table 16.27 Causes of tricuspid valve disease

Tricuspid Stenosis
▪ Rheumatic heart disease ▪ Congenital mitral valve stenosis
Tricuspid Regurgitation
▪ Primary (structural) causes • Rheumatic heart disease • Endocarditis (intravenous drug abuse) • Myxomatous changes causing tricuspid valve prolapse • Carcinoid heart disease • Radiation induced tricuspid regurgitation • Congenital tricuspid regurgitation (Ebstein's anomaly) • Traumatic injury • Papillary muscle injury following myocardial infarction
▪ Secondary (functional) causes producing right ventricular and tricuspid annular dilatation due to multiple causes of right ventricular enlargement due to the following: • Long-standing pulmonary hypertension • Remodeling of post-right ventricular hypertrophy following myocardial infarction • Left sides valve disease • Cardiomyopathy • Atrial fibrillation

CLINICAL FEATURES

The patient presents with chest pain, palpitations, fatigue, dizziness, breathlessness, orthopnea and hemoptysis secondary to pulmonary congestion, dysphagia for solids owing to left atrial enlargement, abdominal pain due to hepatomegaly and leg swelling.

- Mural thrombi in left atrium result in systemic embolization dislodged by atrial fibrillation.

Table 16.28 Causes of pulmonic valve disease

Pulmonic Stenosis
▪ Congenital pulmonic stenosis ▪ Carcinoid heart disease ▪ Endocarditis ▪ Tumor
Pulmonic Regurgitation
▪ Primary pulmonic valve disease • Congenital pulmonic valve regurgitation • Post-valvotomy • Endocarditis • Carcinoid heart disease ▪ Annular enlargement • Pulmonary hypertension • Idiopathic dilatation of pulmonic valve • Marfan's syndrome

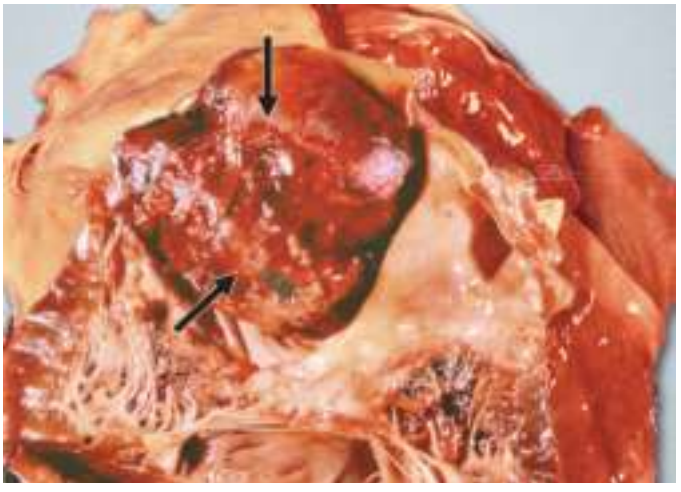


Fig. 16.43 Mitral valve stenosis. Ball thrombus (arrows) in the left atrium is complication in mitral stenosis and atrial fibrillation.

- Turbulent blood flow through the mitral stenosis leads to mid-diastolic murmur heard best at apex.

MITRAL VALVE INSUFFICIENCY/ REGURGITATION

Mitral valve insufficiency/regurgitation usually occurs due to rheumatic heart disease. It can also result from mitral valve prolapse, hypertrophic obstructive cardiomyopathy, damage to papillary muscle in myocardial infarction, ruptured chordae tendineae and transposition of great arteries. It can be secondary to left ventricular dilatation, with stretching of the mitral valve ring. Blood from the left ventricle flows back into the left atrium during systole, causing the atrium enlargement to accommodate the backflow. Patient eventually leads to left-sided and right-sided heart failure.

CLINICAL FEATURES

Patient presents with exertional dyspnea, paroxysmal nocturnal dyspnea, orthopnea, weakness, fatigue and palpitations. Physical examination reveals peripheral edema, jugular vein distension, ascites, and hepatomegaly due to right-sided heart failure. On auscultation, crackles and a loud S1 or opening snap and holosystolic murmur are heard at the apex.

MITRAL VALVE PROLAPSE

Mitral valve prolapse is the most frequent valvular lesion resulting in mitral valve insufficiency. Normally, mitral valve closes during systole. Mitral valve prolapse commonly affects young women. It most often occurs patients with Marfan's syndrome (90%) (Fig. 16.44).

PATHOGENESIS

Mitral valve prolapse shows myxoid degeneration of ground substance due to stromal accumulation of glycosaminoglycans. Architecture of the mitral valve is maintained and shows elongation and thinning of chordae tendineae of mitral valve. It leads to stretching of the posterior valve leaflet resulting in floppy cusp, i.e. parachute deformity with prolapse into the left atrium during systole.

CONSEQUENCES

Regurgitation of the blood across the incompetent mitral valve leads to reflux of blood from left ventricle into left atrium. Back pressure is transmitted to lungs resulting in pulmonary congestion and then right-sided heart failure.

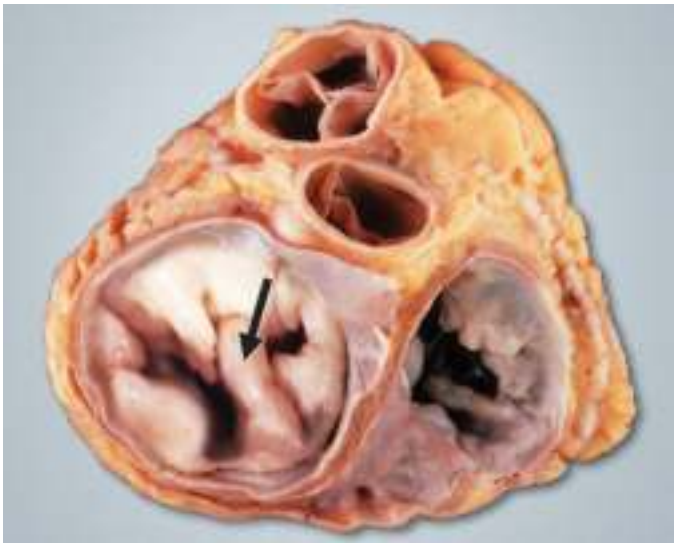


Fig. 16.44: Mitral valve prolapse. Many patients are asymptomatic. There is increased risk of cerebral embolism (arrow).

CLINICAL FEATURES

Patient presents with pulmonary symptoms, i.e. breathlessness and cough occurring late in the disease. Regurgitation of the blood across mitral valve prolapse presents with pansystolic murmur radiating into the axilla heard best at the apex on auscultation and increases in intensity on expiration. Mid-systolic click can be heard on auscultation in cases of mitral valve prolapse.

COMPLICATIONS

Mitral valve prolapse is often associated with cardiac arrhythmias. Infective endocarditis with arterial embolization is a possible consequence of mitral valve prolapse. Antibiotic prophylaxis for dental procedures is recommended.

AORTIC VALVE STENOSIS

Aortic valve is frequently involved in rheumatic heart disease. Normally, aortic valve is closed during diastole. Aortic valve stenosis prevents the outflow of blood from left ventricle into the aorta during systole. Aortic valve stenosis may result from rheumatic fever, idiopathic aortic valve fibrosis and calcification of aortic valve in old age >60 years and congenital aortic bicuspid valve.

CLINICAL FEATURES

Aortic valve stenosis increases blood pressure inside the left ventricle resulting in concentric ventricular hypertrophy associated with decreased chamber size.

- Patient develops left ventricular failure leading to pulmonary congestion and right heart failure.
- Patient presents with breathlessness, cough, syncope, chest pain or sudden death. High-pitched ejection systolic murmur is heard on auscultation.

AORTIC VALVE INSUFFICIENCY/ REGURGITATION

Aortic valve insufficiency/regurgitation is the incomplete closure of the aortic valve. During diastole, blood from aorta flows back into the left ventricle resulting in dilatation of chamber and eccentric ventricular hypertrophy.

- Stroke volume is increased often >100 ml. Widening of pulse pressure leads to hyperdynamic circulation, i.e. water-hammer pulse.
- Ultimately, it leads to left-sided heart failure and pulmonary edema. Aortic valve insufficiency can be acute or chronic and usually caused by scarring or retraction of valve leaflets.

ETIOLOGY

- **Acute aortic valve insufficiency:** It occurs due to endocarditis, chest trauma, prosthetic valve malfunction, acute ascending aortic dissection and non-dissecting aneurysm due to cystic medial necrosis.
- **Chronic aortic valve insufficiency:** It occurs due to hypertension, chronic rheumatic heart disease, Marfan's syndrome, ankylosing spondylitis, syphilitic aortitis and ventricular septal defect.

CLINICAL FEATURES

Patient develops left-sided heart failure and pulmonary congestion, and presents with dyspnea, orthopnea, paroxysmal nocturnal dyspnea, fatigue, palpitation and exercise intolerance. Auscultation reveals S3 heart sound and high-pitched blowing diastolic murmur at left sternal border.

CALCIFIC AORTIC STENOSIS

Calcific aortic stenosis refers to narrowing of the aortic valve orifice as a result of the deposition of calcium in the valve cusps and ring (Fig. 16.45).

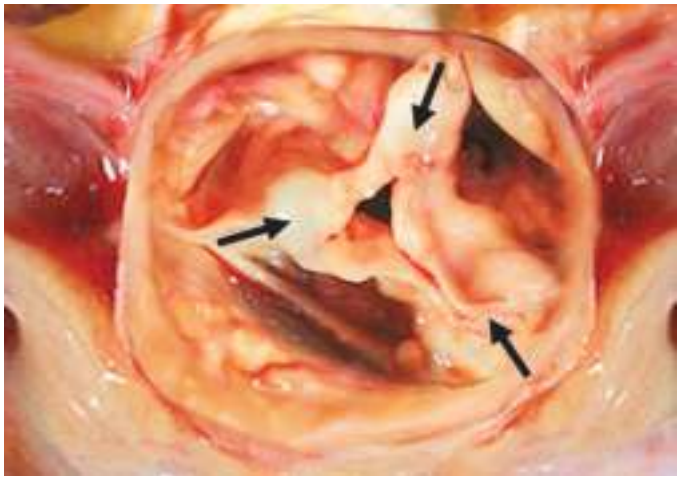


Fig. 16.45: Calcific aortic stenosis. A 75-year-old man presented with history of recurrent syncope underwent surgery for aortic valve disease. Valve was markedly deformed and calcified (arrows).

PATHOGENESIS

Calcific aortic stenosis occurs due to rheumatic disease, senile calcific stenosis, and congenital aortic stenosis. It is related to the cumulative effect of years of trauma due to turbulent blood flow around the aortic valve. It becomes progressively narrowed with some degrees of regurgitation.

TRICUSPID VALVE INSUFFICIENCY

Tricuspid valve insufficiency secondary to bacterial endocarditis is one of the most common complications of intravenous drug abuse. It may be involved together with the mitral and aortic valves in 5% cases of rheumatic heart disease. The cardiac lesions consist of plaque-like deposits of dense fibrous tissue on the tricuspid and pulmonary valves.

PULMONIC VALVE STENOSIS

Pulmonic valve stenosis usually occurs due to congenital malformations either alone or in tetralogy of Fallot. Pulmonic valve stenosis may be involved in the carcinoid syndrome.

CARCINOID HEART DISEASE

The cardiac lesions in carcinoid syndrome consist of plaque-like deposits of dense fibrous tissue on the tricuspid and pulmonary valves in the carcinoid syndrome.

CLINICAL FEATURES

Patient develops tricuspid valve insufficiency and pulmonary valve stenosis in carcinoid heart disease due to release of active tumor products, characterized by diarrhea, flushing, bronchospasm, and skin lesions. Increased excretion of 5-HIAA (5-hydroxyindoleacetic acid), a metabolite of serotonin in urine, is diagnostic of carcinoid syndrome.

DISORDERS OF PERICARDIUM

ANATOMY AND PHYSIOLOGY

Pericardium of heart consists of outer parietal and inner visceral layers, which are intimately related to the myocardium.

- Pericardial cavity contains 20–50 ml of fluid, which provides protection to the heart and prevents intracardial pressure changes associated with postural variation and respiration.
- Pericardium surrounds heart and helps it function properly. It also covers the roots of major blood vessels as they extend from heart.
- Pericardium minimizes friction of pericardial layers.

- Pericardium facilitates atrial filling during ventricular systole, prevents sudden dilatation of chambers during exercise and increased blood volume.
- Pericardium prevents displacement of heart and kinking of the blood vessels and prevents spread of infection from lung and pleura to heart.

PERICARDIAL EFFUSION

Pericardial effusion is sudden excessive collection of fluid in the pericardial sac without cardiac chamber enlargement beyond the 20–50 ml normally present. There is filling of the retrosternal space, and effacement of the normal cardiac borders giving a 'water bottle configuration'. Causes of transudative pericardial effusion include cardiac surgery, congestive heart failure, uremia, myxedema and collagen vascular diseases.

- Pericardial exudative effusions are caused by pericarditis (e.g. fibrinous, suppurative, hemorrhagic or tubercular), metastatic deposits from lung carcinoma, breast carcinoma or lymphomas. Tubercular pericardial effusion is straw/pale-colored fluid formed by Mycobacterium tubercle bacilli.
- Laboratory diagnosis of pericardial effusion includes imaging techniques (such as X-ray chest showing water bottle configuration) and echocardiography (to demonstrate amount of fluid), complete urine analysis for proteins and sugar, liver function tests, kidney function tests, blood sugar, and fluid cytology.

HYDROPERICARDIUM

Accumulation of serous transudate in the pericardial cavity is known as hydropericardium. It is caused by congestive heart failure and hypoproteinemia. Malnutrition, chronic liver disease and nephrotic syndrome are important causes of hypoproteinemia.

HEMOPERICARDIUM

Accumulation of blood in the pericardial cavity is known as hemopericardium. It may cause cardiac tamponade with fatal outcome.

- Hemopericardium occurs due to traumatic perforation of the heart or dissecting aortic hematoma, myocardial rupture associated with acute myocardial infarction or malignant neoplasm.
- As the first part of the aorta (root) lies within the pericardial sac, rupture of the aortic wall due to aortic dissection may cause intrapericardial bleeding.

CARDIAC TAMPONADE

Rapid accumulation of excessive fluid or blood in pericardial cavity leads to cardiac tamponade, resulting in interference in cardiac filling (Figs 16.46 and 16.47).

- Cardiac tamponade is characterized by cardiogenic shock (hypotension), reduced cardiac output and raised jugular venous pressure.
- Pulsus paradoxus, the hallmark of cardiac tamponade is >10 mm falls in systolic blood pressure during inspiration.

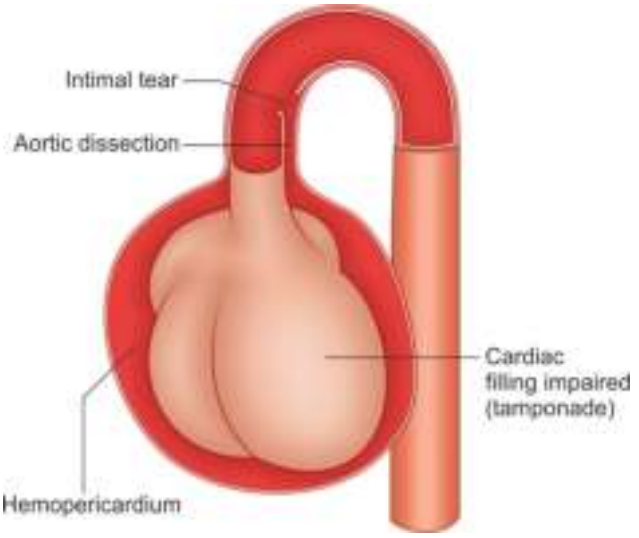


Fig. 16.46: Hemopericardium—pericardial cavity is filled with blood.

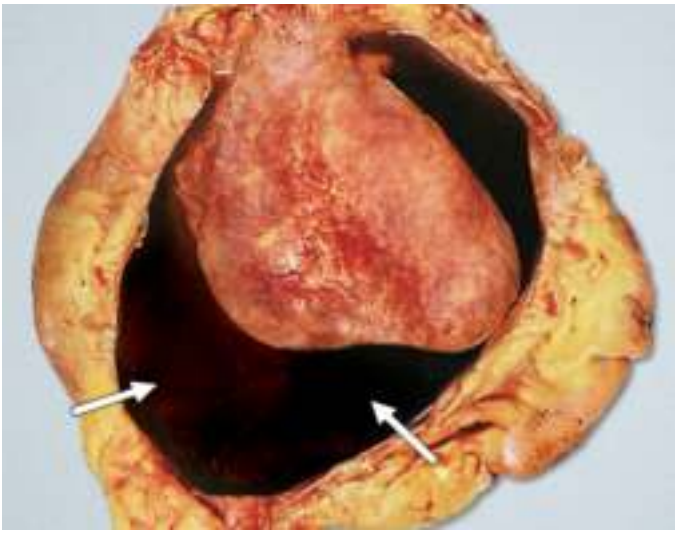


Fig. 16.47: Hemopericardium—aortic dissection may lead to hemopericardium when blood dissects through the media proximally. Such a massive amount of hemorrhage can lead to cardiac tamponade (arrows).

PERICARDITIS: OVERVIEW

Pericarditis is inflammation of the pericardium. In most instances, pericarditis is mild and unrecognized. Acute pericarditis may contain fibrinous exudate, purulent, serous, or hemorrhagic exudate. Chronic constrictive pericarditis is characterized by dense, fibrous pericardial thickening. Common symptoms of pericarditis are pain at the sternum and shallow and rapid respiration.

ETIOLOGIC CLASSIFICATION

Pericarditis is an inflammation of the pericardium that develops suddenly and may last up to several months.

- **Infective pericarditis:** Infective pericarditis can be caused by pyogenic bacteria, Mycobacterium tubercle bacilli, *Treponema pallidum*, viruses and fungi. Infection may occur after cardiac surgery.
- **Noninfective pericarditis:** Noninfective pericarditis known as 'bread and butter pericarditis' is associated with uremia, and acute myocardial infarction (Dressler syndrome), and rheumatic fever. Other causes include high dose radiation to mediastinum, or metastasis (lung, breast or lymphomas), sarcoidosis, myxedema and chylopericardium.
- **Pericarditis presumably related to hypersensitivity or autoimmunity:** Causes of pericarditis presumably related to hypersensitivity or autoimmunity include rheumatic pancarditis, rheumatoid arthritis, systemic lupus erythematosus (very common), scleroderma and drugs (e.g. procainamide and hydralazine).

Clinical classification and etiologic classification of pericarditis are given in Tables 16.29 and 16.30.

Table 16.29 Clinical classification of pericarditis

Acute Pericarditis (<6 Weeks)
▪ Fibrinous pericarditis ▪ Effusive pericarditis (serous or sanguineous type)
Subacute Pericarditis (6 Weeks to 6 Months)
▪ Effusive constrictive pericarditis ▪ Constrictive pericarditis
Chronic Pericarditis (>6 Weeks)
▪ Constrictive pericarditis ▪ Adhesive pericarditis (nonconstrictive type)

Table 16.30 Etiologic classification of pericarditis

Infective Pericarditis

- Viral pericarditis (Coxsackievirus A and B, herpesviruses, mumps, echovirus, adenovirus, HIV, hepatitis viruses)
- Pyogenic pericarditis (staphylococci, streptococci, pneumococci, *Haemophilus influenzae*, Chlamydia, Legionella, Neisseria)
- Tuberculous pericarditis (spread from mediastinal lymph node)
- Fungal pericarditis (*Histoplasma capsulatum*, Coccidioidomycosis, Candida, Blastomycosis)
- Pericarditis caused by other organisms (*Treponema pallidum*, Protozoa and parasites)

Noninfective Pericarditis

- Acute idiopathic pericarditis
- Uremic pericarditis in chronic renal failure
- Pericarditis induced by tumors
- Primary tumors of heart
- Metastatic tumors invading pericardium (lung carcinoma, breast carcinoma, lymphoma, leukemia)
- Traumatic penetrating chest injury or nonpenetrating injury to pericardium following open heart surgery
- Aortic dissection results in involvement of pericardium
- Myocardial infarction inducing fibrinous pericarditis
- Post-irradiation pericarditis
- Metabolic pericarditis (myxedema, cholesterol)
- Familial pericarditis seen in Mulibrey nanism
- Familial Mediterranean fever

Pericarditis Presumably Related to Hypersensitivity or Autoimmunity

- Fibrinous pericarditis in rheumatic fever
- Collagen vascular disorders induced pericarditis
- Systemic lupus erythematosus
- Rheumatic fever, scleroderma
- Rheumatoid arthritis
- Ankylosing spondylitis
- Wegener granulomatosis
- Drug-induced pericarditis
  - Phenytoin
  - Isoniazid
  - Procainamide
  - Hydrazine
  - Anticoagulants
  - Methysergide
  - Minoxidil
- Postcardiac injury induced pericarditis
- Post-traumatic
- Postmyocardial infarction (Dressler's syndrome)
- Postpericardiotomy

PATHOGENESIS

Pericardial tissue damaged by injurious agent releases chemical mediators of inflammation into the surrounding tissue. Chemical mediator such as histamine increases vascular permeability resulting in leakage of inflammatory exudate.

- Neutrophils, macrophages begin to phagocytose and degrade the injurious agent, remove necrotic tissues and dead inflammatory cells which are eventually reabsorbed into healthy tissue.
- Friction rub is heard on auscultation due to inflamed pericardial layers rubbing against each other.

CLINICAL FEATURES

Patient presents with sharp sudden pain at the sternum radiating to the neck, shoulders, and arms; shallow and rapid respiration, mild fever, dyspnea and orthopnea.

PHYSICAL EXAMINATION

Patient may develop congestive heart failure, i.e. elevated jugular pressure, hepatomegaly and peripheral edema. Patient develops Beck's triad (e.g. hypotension, elevated jugular venous pulse, and muffled distant heart sounds). Friction rub is appreciated on auscultation. Apex pulse may vanish. Dullness beneath angle of left scapula is known as Ewart's sign.

ACUTE PERICARDITIS

Acute pericarditis is characterized by rapid accumulation of fluid or exudate in the pericardial cavity known as pericardial effusion and usually diagnosed by echocardiography. Several pathologic forms of pericarditis are described below.

SEROUS PERICARDITIS

Serous pericarditis is associated with viral infections, rheumatic heart disease and systemic lupus erythematosus. Pericardial cavity contains clear, straw-colored, protein-rich exudate containing small numbers of inflammatory cells. These patients may develop chest pain due to pericardial effusion (Fig. 16.48).

FIBRINOUS OR SEROFIBRINOUS PERICARDITIS

Fibrinous or serofibrinous pericarditis is characterized by fibrin-rich exudate due to increased vascular permeability. It may be caused by chronic renal failure (uremia), acute myocardial infarction, or acute rheumatic fever or systemic lupus erythematosus.

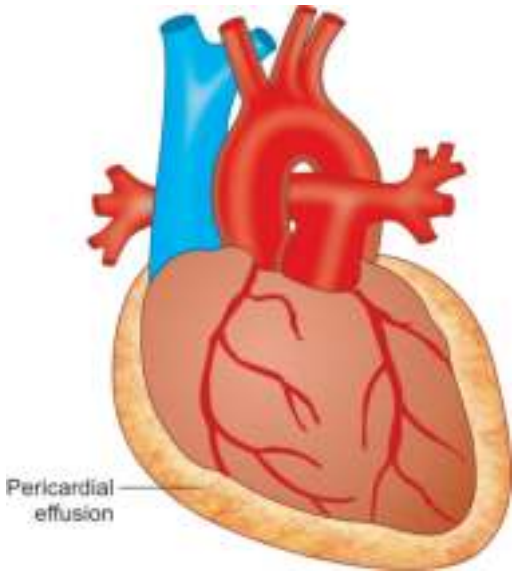


Fig. 16.48: Pericardial effusion shows accumulation of serous fluid in pericardial cavity.

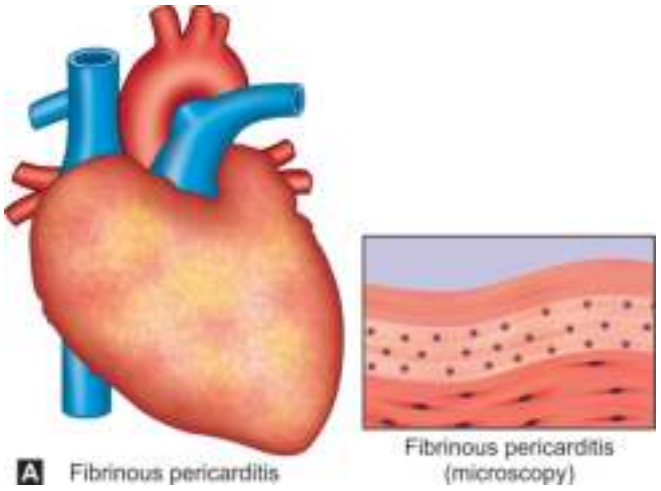
- Fibrin deposited in pericardium exhibits bread and butter appearance. Friction rub is heard on auscultation.
- Fibrinous debris may be removed by fibrinolysis and macrophages resulting in restoration of normal tissue structure.
- When fibrinogen is not removed, there is danger of obliteration of pericardial cavity due to fusion of parietal and visceral layers (Figs 16.49 and 16.50).

PURULENT OR SUPPURATIVE PERICARDITIS

Pus or purulent exudate accumulates in the pericardial cavity. It is almost always caused by pyogenic bacterial infection as a consequence of direct spread from lung infection due to *Staphylococcus aureus* or *Streptococcus pneumoniae*. Pericardium exhibits yellowish microabscesses. On histologic examination, microabscess consists of blue bacterial colonies and is surrounded by acute inflammatory cells (Fig. 16.51).

HEMORRHAGIC PERICARDITIS

Accumulation of bloody inflammatory exudate in the pericardial cavity is known as hemorrhagic pericarditis, which usually results from invasion of the pericardium by lung cancer, esophageal cancer, breast cancer or lymphomas in 5–10% of cases. Surface of the epicardium shows pale white-tan nodules of metastatic tumor. Cardiac surgery and tubercular etiology may also result in hemorrhagic pericarditis (Fig. 16.52).



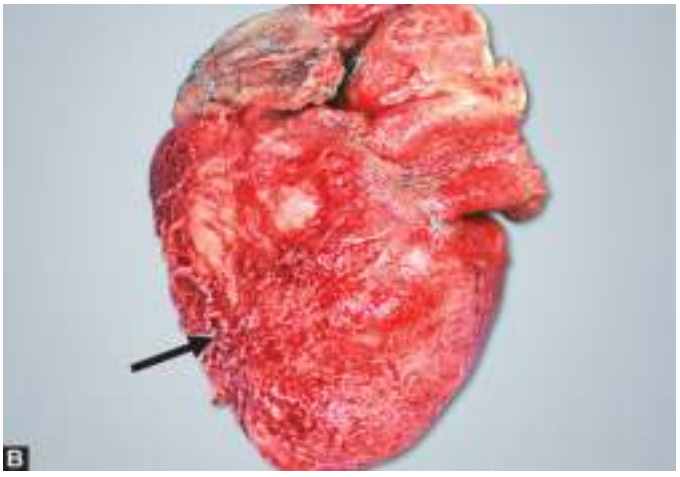


Fig. 16.49: Fibrinous pericarditis after acute myocardial infarction (arrow).

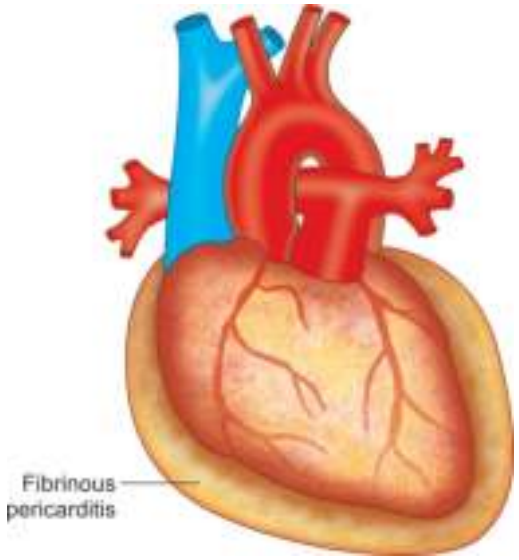


Fig. 16.50: Fibrinous pericarditis shows deposition of fibrin in pericardial sac.

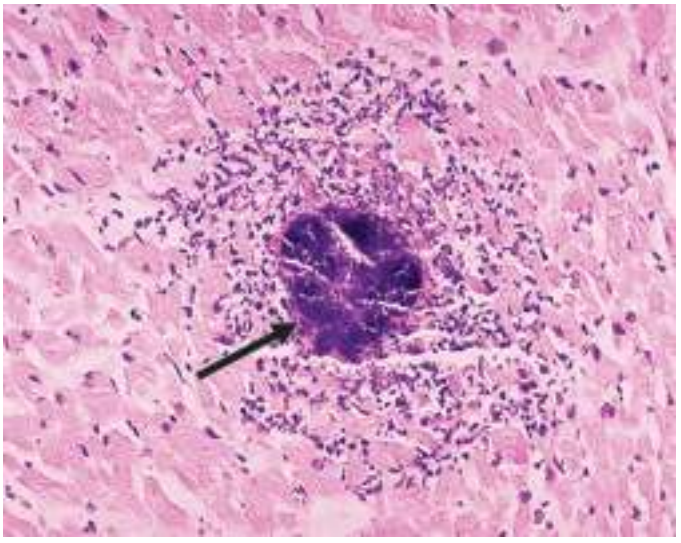


Fig. 16.51: Purulent or suppurative pericarditis. Yellowish purulent exudate is visible on the surface of pericardium. Bacterial colonies are shown (arrow).

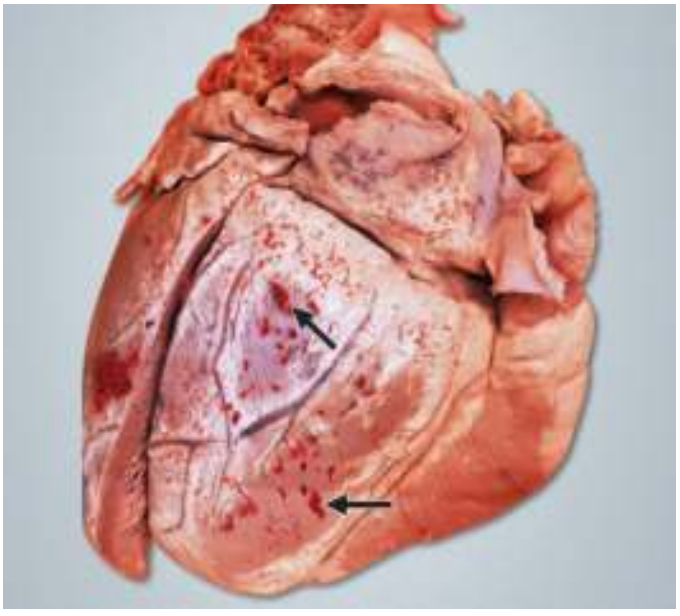


Fig. 16.52: Petechial hemorrhages on epicardium. It occurs due to low platelets count and sudden hypoxia (arrows).

CHRONIC PERICARDITIS

Patient develops chronic pericarditis, when fluid or fibrinous exudate is not removed from pericardial sac.

- Chronic pericarditis is characterized by thickening and scarring of the pericardium with resultant loss of elasticity. Proliferation of fibrous tissue with occasional small foci of calcification is marked. This prevents the pericardium from stretching and thus interferes

with cardiac function and venous return, often mimicking the signs and symptoms of right-sided heart failure.

- Patient develops obliteration of pericardial cavity due to fusion of parietal and visceral layers, known as constrictive pericarditis.
- Pericardial adhesions are formed in cases of constrictive pericarditis, which impairs cardiac filling by limiting the total cardiac volume compliance and diastolic filling function.

CONSTRICTIVE PERICARDITIS

Constrictive pericarditis represents a chronic phase of fibrous scarring, pericardial thickening and obliteration of the pericardial cavity. It can result in restriction of diastolic cardiac filling. Acute pericardial injury and exuberant healing response by forming granulation tissue leads to fusion of parietal and visceral layers of pericardium (Fig. 16.53).

Etiopathogenesis

Constrictive pericarditis may be caused by bacteria, viruses, or fungi. Previous radiation therapy to mediastinum and cardiac surgery account for one-third of cases.

- Tuberculosis is the most common cause of constrictive pericarditis. Stiffness of the pericardium is often enhanced by dystrophic calcification.
- Constrictive pericarditis restricts the heart's ability to pump effectively. The return of venous blood to the right heart is also compromised. Thickened pericardium compresses epicardial coronary artery resulting in under perfusion of the heart. Constrictive pericarditis should be differentiated from angina pectoris.

Clinical Features

Patient presents with chest pain, cough, exertional dyspnea and orthopnea. Elevated systemic venous pressure results in raised jugular venous pressure, hepatomegaly, marked ascites and peripheral edema.

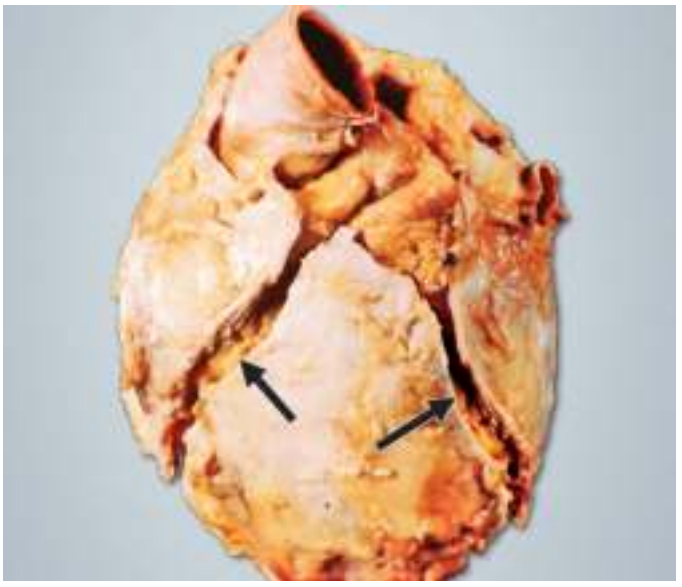


Fig. 16.53: Constrictive pericarditis in a case of tuberculosis. Patient presents with dyspnea, chest pain and abdominal discomfort. Physical examination revealed hepatomegaly, ascites and pitting edema. Cardiac auscultation revealed pericardial rub, respiratory crackles in both lungs and bases (arrows).

Reduced cardiac output causes fatigue and tachycardia (Table 16.31).

Cardiac MRI Techniques

Cardiac magnetic resonance imaging (MRI) in constrictive pericarditis demonstrates pericardial thickening, i.e. 4 mm, pericardial calcification, reduced right ventricular volume, a dilated right atrium, superior vena cava and inferior vena cava, hepatomegaly and ascites.

ADHESIVE PERICARDITIS

In some cases, adhesions formed between pericardium and thoracic cage leads to systolic retraction of thoracic cage and cardiac hypertrophy (cardiomegaly demonstrated by X-ray). Differences between constrictive and adhesive pericarditis are shown in Table 16.32.

Table 16.31 Clinical features of constrictive pericarditis

Systemic Changes	Clinical Features
Reduced cardiac output	Fatigue, hypotension, reflex tachycardia
Elevated systemic venous pressure	Jugular venous distension, hepatomegaly with marked ascites, peripheral edema
Pulmonary venous congestion	Exertional dyspnea, cough and orthopnea
Chest pain typical of angina may be related to under perfusion of the coronary arteries or compression of an epicardial coronary artery by the thickened pericardium	▪ Because the most impressive physical findings are often the insidious development of hepatomegaly and ascites, such patients are often mistakenly thought to suffer from hepatic cirrhosis or an intra-abdominal tumor ▪ It is only after a careful inspection of the jugular veins that a cardiac source is identified

Table 16.32 Differences between constrictive and adhesive pericarditis

Parameters	Constrictive Pericarditis	Adhesive Pericarditis
Etiology	▪ Bacteria, viruses, parasites and fungi ▪ Rheumatic fever, AMI, uremia and SLE causing fibrinous pericarditis leads to constrictive pericarditis	▪ Bacteria, viruses, parasites and fungi ▪ Rheumatic fever, AMI, uremia and SLE causing fibrinous pericarditis leads to adhesive pericarditis
Pathology	Parietal and visceral layers are fused	Adhesions between pericardium and thoracic cage
Clinical effects	It restricts the heart's ability to pump effectively	Cardiac hypertrophy results in cardiomegaly

CARDIAC HYPERTROPHY AND HEART FAILURE

CARDIAC HYPERTROPHY

Cardiomyocytes exit the cell cycle and become terminally differentiated soon after the birth. Cardiac hypertrophy initially develops as an adaptive response to physiologic and pathologic stimuli, that augments myocardial contractile strength by increase in the cardiomyocytes number in response to an increased workload during adult life.

- There is distinction between physiologic and pathologic cardiac hypertrophy. Physiologic cardiac hypertrophy develops in highly trained athletes. On the other hand, pathologic cardiac hypertrophy occurs due to injury or disease.
- Pathologic cardiac hypertrophy is a compensatory response to hemodynamic overload, which occurs in association with chronic systemic hypertension or cardiac valvular stenosis (pressure overload), myocardial injury, valvular insufficiency (volume workload), that increase the cardiac overload.
- Cardiac hypertrophy is initially a compensatory and possibly a reversible phenomenon, but with persistent stress, the myocardium becomes irreversibly increased in thickness and dilated resulting in heart failure.
- The overall weight of the heart increases exceeding 375 g in men and 350 g in women. The thickness of the left ventricle increases exceeding (normal 1.2–1.5 cm) and right ventricle (normal 3–5 mm).
- Hypertrophic cardiomyocytes exhibit increased in size containing hyperchromatic rectangular nuclei. Left ventricular hypertrophy in systemic hypertension is shown in Fig. 16.54. Histology of cardiac hypertrophy is shown in Fig. 16.55.

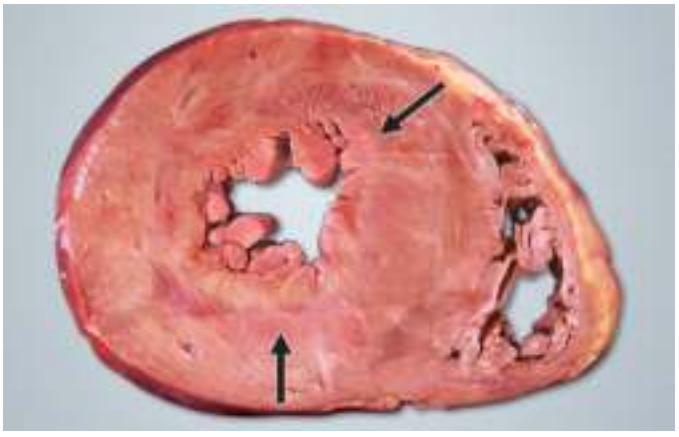


Fig. 16.54: Left ventricular hypertrophy in systemic hypertension. The wall of the left ventricle is markedly thickened (normal thickness 1.2–1.5 cm) as a result of increase in size of the cardiac myocytes (arrows).

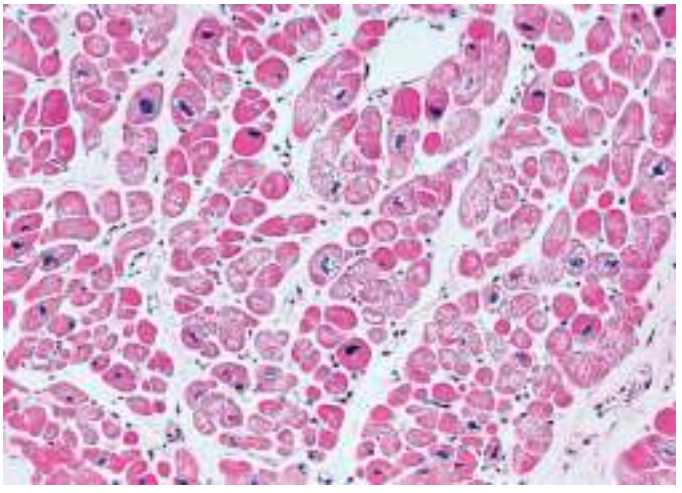


Fig. 16.55: Histology of cardiac hypertrophy. It shows marked hypertrophy of cardiac myocytes with interstitial fibrosis (400X).

MOLECULAR PATHOGENESIS

Numerous mechanisms are involved in the pathogenesis of cardiac hypertrophy affecting gene transcription, calcium handling, protein synthesis, cellular metabolism, autophagy, oxidative stress and inflammation. New mediators participating in the pathogenesis of cardiac hypertrophy include signaling proteins, microRNAs, long noncoding RNAs and calcineurin and calcium/calmodulin-dependent protein kinase.

Signaling Pathways

Each form of cardiac hypertrophy is regulated by distinct cellular signaling pathways. Receptor-mediated myocardial events in response to mechanical stimuli promote the hypertrophic response regulated by autocrine and paracrine mechanisms.

- Cardiomyocytes respond to mechanical stimuli release various ligands such as angiotensin II, endothelin 1 (ET-1), norepinephrine, insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), transforming growth factor 1 (TGF-1) and transforming growth factor β (TGF-β). These ligands activate signaling pathways to induce cardiac hypertrophy.
- Some of these ligands (mediators) may also act on interstitial fibroblasts in the myocardium to promote synthesis and deposition of extracellular matrix.
- These ligands bind to G-coupled receptors and receptor tyrosine kinases to initiate intracellular signaling cascades.
- G-coupled receptors activate various signaling pathways such as mitogen-activated protein kinases (MAPKs), phosphoinositide 3-kinases (PI3Ks) and protein kinase C (PKC).
- Calcium calmodulin-dependent protein kinase pathways are regulated by calcium.
- Events mediated by β-adrenergic receptors are implicated in the transition from compensatory cardiac hypertrophy to congestive heart failure.

Expression of Fetal Genes

Gene expression varies during prenatal and postnatal life. During prenatal life, fetal ventricles synthesize atrial natriuretic factor (ANF), however synthesis of ANF is shut off in normal adult tissue.

- Cardiac hypertrophy and congestive heart failure can result in reactivation of some embryonic genes encoding ANF and fetal isoforms of creatine kinase and myosin light chains.
- Changes in fetal gene expression are mediated by mechanical stress, which induces synthesis of cytokines and hormones, that redirect gene expression.
- Cardiomyocytes sense mechanical stress through integrin-mediated signaling through PI3K, RAS, AKT signaling pathways. Induction of some cardiac-specific transcription factors may be important in programmed change in gene expression.

PATHOLOGY

Structural damage or gene mutation may lead to heart failure. Ischemic heart disease is the most important cause of heart failure. Cardiomyopathies and congenital heart diseases lead to heart failure. Ventricular hypertrophy is demonstrated in virtually all conditions associated with chronic heart failure.

Table 16.33 Causes of left-sided and right-sided heart failure

Left-sided Heart Failure: Causes	Right-sided Heart Failure: Causes
Ischemic heart disease	Left heart failure (most common)
Systemic hypertension	Pulmonary hypertension
Aortic valve disease	Tricuspid valve disease
Mitral valve disease	Pulmonic valve disease
Myocarditis	Myocarditis
Cardiomyopathies	Cardiomyopathies
Chemotherapy treatment for cancer	Intrinsic pulmonary disease

- Initially left cardiac hypertrophy occurs as in compensated hypertensive heart disease. But when the left ventricle fails to perform normal functions, right ventricular hypertrophy follows as a result of increased workload on the right ventricle.
- The distribution of end-organ involvement is dependent on whether the heart failure is predominantly left-sided or right-sided. In heart failure, heart is unable to pump out blood to meet the body's metabolic needs resulting in poor perfusion of tissues.
- In adults, acute heart failure occurs after acute myocardial infarction, while chronic heart failure due to long-standing systemic hypertension.
- Children develop heart failure due to congenital heart diseases and blood vessel malformations. Causes of left-sided and right-sided heart failure are given in Table 16.33. Clinical manifestations in heart failure are shown in Fig. 16.56.

HEART FAILURE

LEFT-SIDED HEART FAILURE

Left-sided heart failure is most common and caused due to cardiac injury (ischemic heart disease, systemic hypertension) primarily affect the left ventricle.

- To compensate for left ventricular failure, venous pressures in left atrial and pulmonary vein increase, resulting in passive pulmonary congestion. Alveolar septal capillaries filled with blood rupture resulting in escape of red blood cells. As a result, alveoli contain numerous hemosiderin macrophages, so-called heart failure cells. If capillary hydrostatic exceeds plasma oncotic pressure, fluid leaks from alveolar capillaries into the alveoli. Resultant pulmonary edema may be massive with alveoli being filled with transudate.
- Clinical examination eveals dyspnea, orthopnea, decreased breath sounds, crackles and dull percussion note.
- Presence of congestion in pulmonary capillaries over an extended period results in interstitial pulmonary

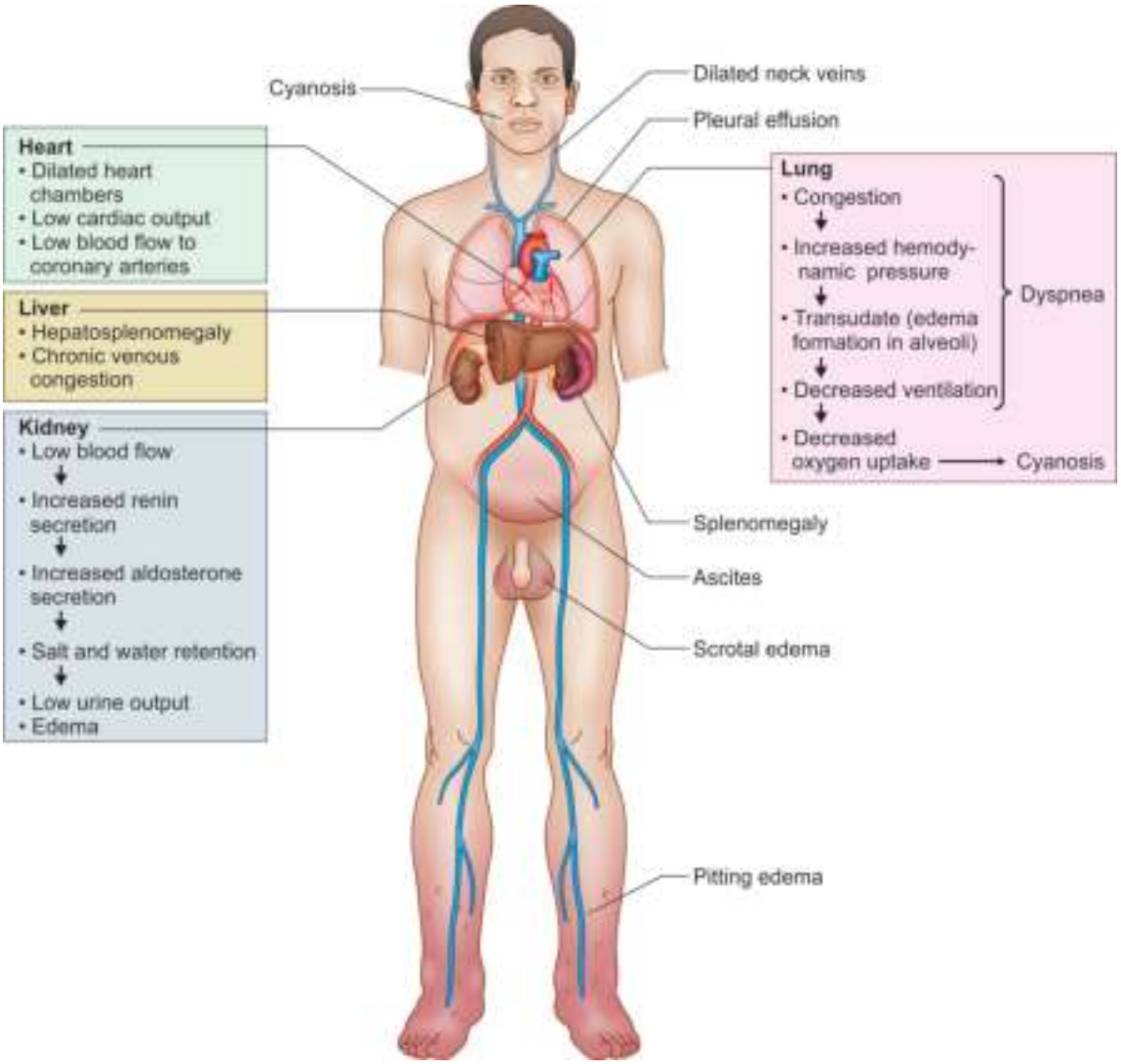


Fig. 16.56: Clinical manifestations in heart failure. Left-sided heart failure results in pulmonary congestion, dyspnea on exertion, dyspnea on lying down (orthopnea), awakening from sleep (paroxysmal nocturnal dyspnea) and cough with frothy sputum. Right-sided heart failure results in systemic venous congestion, peripheral edema over ankles, hepatosplenomegaly and raised jugular venous pressure.

fibrosis. Pulmonary edema with heart failure cells is shown in Fig. 16.57A and B.

Clinical Features

Patient developing left-sided heart failure presents with dyspnea on exertion, dyspnea on lying down (orthopnea), awakening from sleep (paroxysmal nocturnal dyspnea) and cough with frothy sputum.

- Dyspnea on exertion is related to increased pulmonary congestion as a result of higher end-diastolic pressure in the left atrium and left ventricle.
- Orthopnea and paroxysmal nocturnal dyspnea occur, when pulmonary blood volume increases owing to reduced blood volume in the legs during recumbent (lying down) position.
- Many of the clinical manifestations of heart failure can be explained on the basis of venous congestion (backward failure) and inadequate perfusion of vital organs (forward failure).
- Majority of patients with left-sided heart failure retain sodium and water (peripheral edema) owing to poor renal perfusion, decreased glomerular filtration rate and activation of renin-angiotensin-aldosterone system.
- Inadequate cerebral perfusion results in confusion, memory loss and disorientation.
- Inadequate perfusion of skeletal muscles leads to weakness and fatigue.
- On clinical examination, patient has weak pulse and cool moist skin as a result of vasoconstriction, and shunting of blood to vital organs.

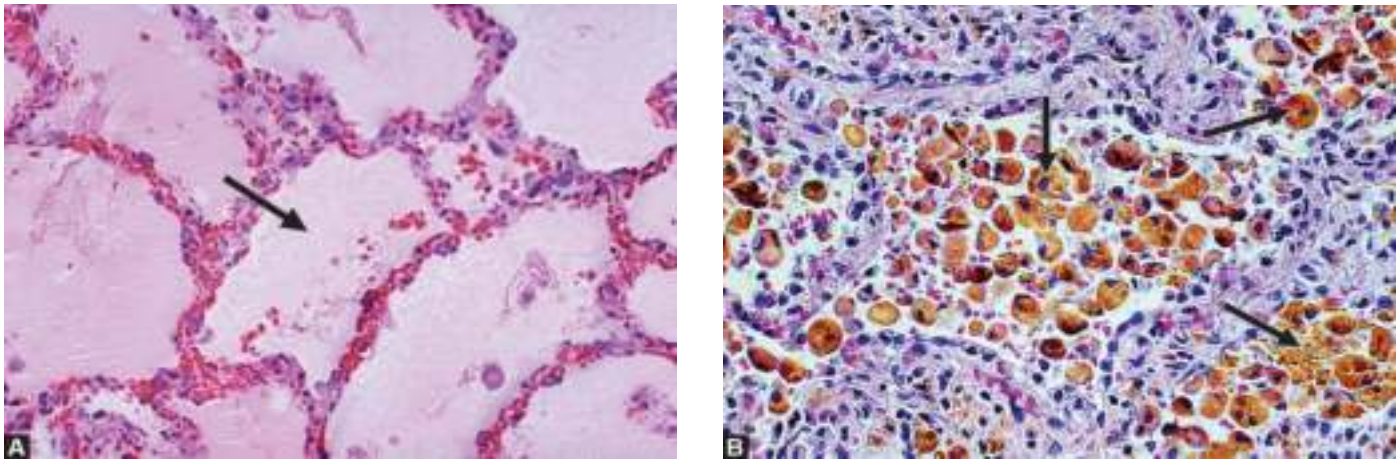


Fig. 16.57: (A) Pulmonary edema. Histologic examination of lung demonstrates congested alveoli filled with a smooth to slightly floccular pink material (arrow) (400X), (B) pulmonary edema with hemosiderin laden alveolar macrophages called heart failure cells (arrows) (400X).

RIGHT-SIDED HEART FAILURE

Right-sided heart failure most commonly complicates left-sided heart failure, which can also develop independently due to intrinsic pulmonary disease or pulmonary hypertension.

- Pulmonary hypertension creates resistance to blood flow through the lung resulting in increase in right atrial pressure and systemic venous pressure. Jugular veins are congested and distended. Increased pressure in vena cava results in congestion of liver and spleen; and pitting edema in legs.
- Hepatic congestion in heart is characterized by congestion and distension of central veins and sinusoids. Cut surface of the liver is dark red giving '**nutmeg appearance**' when compared to the cut surface of a nutmeg.
- Fibrocongestive splenomegaly occurs in right-sided heart failure. Increased pressure results in hemorrhage in the spleen, that becomes organized and calcified (dystrophic calcification) with formation of Gamna-Gandy bodies.
- Chronically injured cardiomyocytes lose myofibrils. Regardless of the nature of injury, dysfunctional cardiomyocytes lose sarcomeres and correspondingly increased glycogen in cytosol. Cardiomyocytes become vacuolated termed **cardiomyocytolysis**.

Liver Manifestations

In long-standing right-sided heart failure, increased pressure results in chronic passive congestion and dilatation of the central veins and sinusoids. Hepatocytes undergo ischemic changes in zone 3 of hepatic lobule.

- Long-standing chronic venous congestion in liver may result in cardiac cirrhosis. Cut surface of liver due to chronic venous congestion exhibits dark red areas against ischemic areas giving '**nutmeg appearance**'. Histologic examination demonstrates congestion and dilatation of central veins and sinusoids of liver.
- Chronic venous congestion in gross specimen in a case of right-sided heart failure is shown in Fig. 16.58. Histology of chronic venous congestion of spleen is shown in Fig. 16.59.

Fibrocongestive Splenomegaly

Fibrocongestive splenomegaly occurs in right-sided heart failure. Increased pressure results in hemorrhage in the spleen, that becomes organized and calcified (dystrophic calcification) to form Gamna-Gandy bodies.

- Gross examination of spleen shows irregular tan-white fibrous plaques over the purple capsular surface.
- Light microscopy shows firm and brown fibrotic nodules termed **Gamna-Gandy bodies** which are organized hemorrhage forming nodule with dystrophic calcification and hemosiderin pigment.
- Histology of chronic venous congestion of spleen in long-standing right-sided heart failure is shown in Fig. 16.60.



Fig. 16.58: Chronic venous congestion in gross specimen in a case of right-sided heart failure. Cut surface demonstrates nutmeg appearance (arrows).

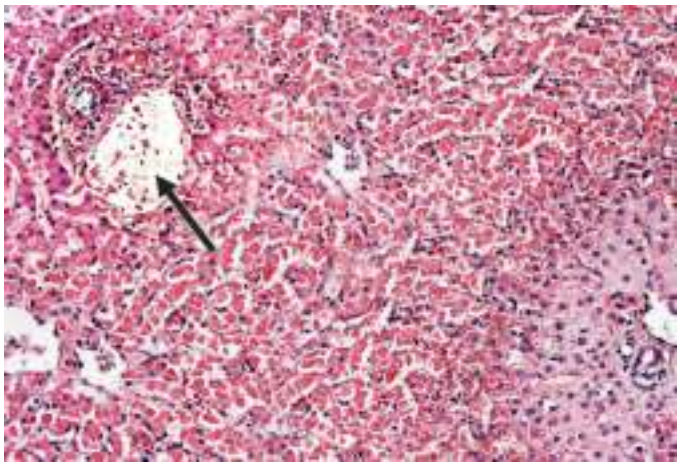


Fig. 16.59: Histology of chronic venous congestion of liver. It demonstrates central veins and sinusoids congested and dilated. At places, the hepatocytes have become atrophied (arrow) (400X).

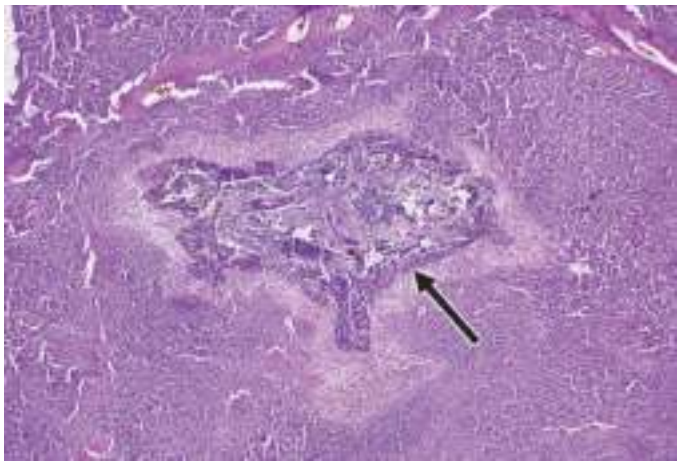


Fig. 16.60: Histology of chronic venous congestion of spleen in long-standing right-sided heart failure. It demonstrates Gamna-Gandy bodies (arrow) (400X).

DIASTOLIC HEART FAILURE

Diastolic heart failure most often occurs in elderly persons, which has become an important clinical problem. The ventricles become progressively stiffer with advancing age and require great diastolic pressure.

Some patients having normal systolic contractile function develop signs and symptoms of heart failure. These patients with normal heartbeats are unable to tolerate increased blood volume resulting in pulmonary edema in response to a fluid challenge. The patients demonstrate interstitial fibrosis in heart.

NEOPLASTIC DISORDERS OF HEART

NEOPLASMS: CLASSIFICATION

Cardiac neoplasms are classified into primary (benign or malignant) and secondary neoplasms. Myxoma is the most common primary cardiac neoplasm in adults. Secondary neoplasms are more common than primary.

PRIMARY NEOPLASMS

- **Benign neoplasms:** Benign cardiac neoplasms include atrial myxoma and rhabdomyoma. Myxoma of the left atrium is most common primary benign neoplasm of heart. Other tumors include papillary fibroelastoma and fibroma.
- **Malignant neoplasms:** Malignant cardiac neoplasms are extremely rare, which include angiosarcoma and mesothelioma. Cardiac angiosarcoma most commonly occurs in right atrium.

SECONDARY NEOPLASMS

Secondary neoplasms of heart are more common than primary. Tumors metastasizing to heart include lung carcinoma, breast carcinoma and malignant melanoma. Epicardial surface is covered by pale white-tan multiple nodules of metastases. Metastases may lead to a hemorrhagic pericarditis (Fig. 16.61).

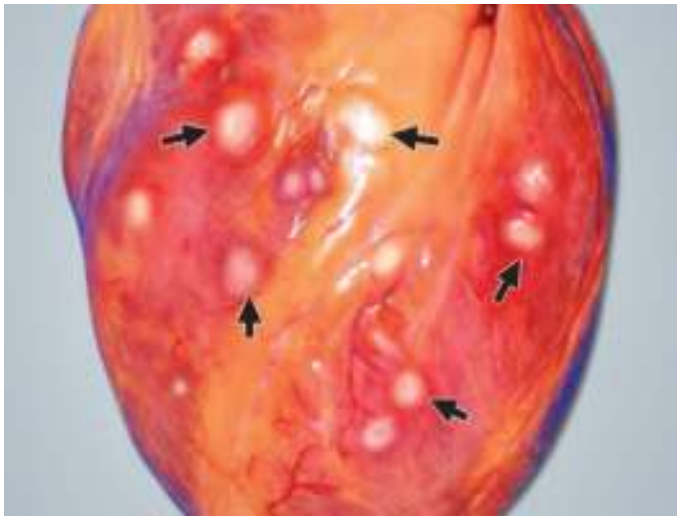


Fig. 16.61: Metastatic tumors to heart. It shows multiple metastatic pale, white-tan deposits on the pericardium (arrows).

ATRIAL MYXOMA

Atrial myxoma is most common benign primary cardiac neoplasm. It accounts for >50% of all primary tumors. Most cardiac myxomas are sporadic, but some occur as a part of Carney syndrome characterized by skin tumors and schwannomas (Fig. 16.62).



Fig. 16.62: Cardiac myxoma. 'Ball valve' effect of myxoma is partially obstructing mitral valve intermittently. It is diagnosed by echocardiography (arrow).

ORIGIN AND LOCATION

Cardiac myxoma arises from multipotent mesenchymal cells which exhibits endothelial and neural differentiation. Tumor is most often demonstrated in left atrium in 90% of cases, which arises from the left atrial septal wall around the fossa ovalis in 80% of cases, on valves and in ventricles.

PATHOLOGY

Most cardiac myxomas are pedunculated, however some myxomas are broad based and sessile. The size varies, between 5 mm to 5 cm. Ball valve effect of myxoma partially obstructs mitral valve intermittently, which prevents diastolic filling of the left ventricle simulating mitral valve stenosis.

Clinical Features

Patient presents with episodes of syncope and non-specific symptoms (e.g. fever, anemia and elevated ESR). Tumor may fragment and give rise to arterial emboli to the brain.

Surgical Pathology: Atrial Myxoma	
Gross Morphology	
On gross examination, cardiac myxomas appear gelatinous (myxoid).	
Light Microscopy	
Myxoma is composed of spindle-shaped cells with scant pink cytoplasm surrounded by loose myxoid matrix containing chondroid sulphate and hyaluronic acid.	
Immunohistochemistry Technique	
Tumor cells show positivity for vimentin, calretinin, CD31, CD34, and α-antitrypsin.	
Markers	**Expression**
▪ Vimentin	▪ Positive
▪ Calretinin	▪ Positive
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ α-Antitrypsin	▪ Positive

DIAGNOSIS

Echocardiography is the most useful technique for visualizing myxoma. The left atrium is located in posterior region of heart.

CARDIAC RHABDOMYOMA

Cardiac rhabdomyoma is second most common primary neoplasm of the heart in infants and children. It is associated with tuberous sclerosis in adults. It arises from striated muscle of myocardium. At autopsy, a large firm, white tumor mass is found filling much of the left ventricle.

CONGENITAL HEART DISEASES AND BLOOD VESSEL MALFORMATIONS

DEVELOPMENTAL ANOMALIES: OVERVIEW

Developmental anomalies of the heart and the major blood vessels are seen in 6–8 per 1000 newborns. Some congenital defects become clinically apparent at birth or in infancy, others are recognized in early childhood. Patients with mitral valve prolapse are diagnosed in early adulthood.

- Most common congenital heart diseases (CHD) are ventricular septal defect (30% of cases), tetralogy of Fallot (10% of cases), atrial septal defect (8% of cases), patent ductus arteriosus (8% cases), coarctation of the aorta (6% of cases) and transposition of the great arteries (5% of cases).
- The most common acyanotic heart disease is ventricular septal defect, whereas the most common cyanotic heart disease is tetralogy of Fallot (Table 16.34). Clinical manifestations of congenital heart disease and blood vessel malformations are given in Table 16.35.

Table 16.34 Major groups of congenital heart diseases and blood vessel malformations

Left-to-right Shunt (Noncyanotic)	Right-to-left Shunt (Cyanotic)	Obstructive Congenital Defects
Ventricular septal defect (VSD)	Tetralogy of Fallot	Coarctation of aorta
Atrial septal defect (ASD)	Transposition of great arteries	Pulmonary artery stenosis
Patent ductus arteriosus (PDA)	Persistent truncus arteriosus	Aortic stenosis/atresia
Atrioventricular septal defect	Total anomalous venous connection	

Table 16.35 Clinical manifestations of congenital heart disease and blood vessel malformations

Cardiac Valvular Disease	Cause	Effects on Organs	Clinical Findings
Ventricular septal defect (VSD)	VSD nonclosure of interventricular septum within first two years of life	▪ Left to right shunt ▪ Pulmonary hypertension ▪ Right heart failure	▪ High-pitched holosystolic murmur over lower left sternal border and loud pulmonic S2 ▪ VSD spontaneous closure within the first six months
Atrial septal defect (ASD)	Congenital defect due to nonclosure of interatrial septum	▪ Most common type: patent foramen ovale ▪ Majority symptomatic	Loud S1, wide fixed splitting of S2, systolic ejection murmur along left upper sternal border
Patent ductus arteriosus (PDA)	Congenital defect due to nonclosure of ductus arteriosus at birth in premature and maternal rubella infection	Pulmonary hypertension	▪ Wide pulse pressure, bounding arterial pulses ▪ Continuous loud machine-like murmur
Coarctation of the aorta	Coarctation of the aorta at origin of left subclavian artery in 98% of cases	Blood pressure higher in arms than legs	▪ Bounding pulses in arms and decreased pulses in legs ▪ Stabilized by surgery
Tetralogy of Fallot	Most common congenital heart disease (ventricular septal defect, right ventricular hypertrophy, right outflow obstruction and overriding aorta)	Right to left shunt	▪ Substernal right ventricular impulse, systolic thrill along left sternal border ▪ Intermittent hyperpnea, cyanosis with decreased intensity of murmur, irritability ▪ Surgical repair at 4–12 months of age

Pulmonary artery stenosis occurs in the setting of tetralogy of Fallot, carcinoid heart disease and rarely in rheumatic heart disease.
Calcific aortic stenosis occurs due to rheumatic heart disease, senile calcific aortic stenosis and congenital aortic stenosis.

ETIOLOGY AND ASSOCIATIONS

- **Chromosomal disorders:** Patients with chromosomal disorders are associated with congenital heart disease. Child with Down syndrome is associated with atrial and ventricular septal defects. Turner syndrome has been associated with coarctation of the aorta.
- **Genetic predisposition:** Due to the multifactorial inheritance, tetralogy of Fallot can cluster in families.
- **High altitude:** There is increased incidence of patent ductus arteriosus (PDA) in persons living at high altitude, suggesting an association with decreased supply of oxygen to fetus.
- **Rubella infection (German measles):** Intrauterine exposure to rubella virus during the first trimester of pregnancy is important cause of congenital heart disease.
  - Newborn develops congenital rubella syndrome, characterized by cardiovascular defects, microcephaly with mental retardation, deafness, cataracts, and growth retardation. Cardiovascular defects include ventricular septal defect, aortic stenosis, patent ductus arteriosus, valvular stenosis and tetralogy of Fallot.
  - It is mandatory to determine mother's immune status to rubella. Presence of antirubella IgM antibodies indicates recent primary infection, and IgG antibodies indicate past infection. Gene defects associated with congenital heart diseases are given in Table 16.36.

FUNCTIONAL ABNORMALITIES OF CHD

- **Noncyanotic diseases (left-to-right shunt):** These include atrial septal defect (ASD), ventricular septal

Table 16.36 Gene defects associated with congenital heart diseases*

Disorder	Gene	Gene Product Function
Nonsyndromic disorders		
ASD or conduction defects	NKX2.5	Transcription factor
ASD or VSD	GATA4	Transcription factor
Tetralogy of Fallot	ZFPM2 or NKZ2.5	Transcription factor
Syndromic disorders**		
Alagille syndrome: Pulmonary artery stenosis or tetralogy of Fallot	JAG1 OR N0TCH2	Signaling proteins or receptors
Char syndrome: PDA	TFAP2B	Transcription factor
CHARGE syndrome: ASD, VSD, PDA, or hypoplastic right side of the heart	CHD7	Helicase-binding protein
DiGeorge syndrome: ASD, VSD, or outflow tract obstruction	TBX1	Transcription factor
Holt-Oram syndrome: ASD, VSD, or conduction defect	TBX5	Transcription factor
Noonan syndrome: Pulmonary valve stenosis, VSD, or hypertrophic cardiomyopathy	PTPN11, KRAS, S0S1	Signaling proteins

ASD—atrial septal defect, VSD—ventricular septal defect, PDA—patent ductus arteriosus, CHARGE—coloboma, heart defects, choanal atresia, growth retardation, genital and ear abnormalities.
**Different mutations can cause the same phenotype, and mutations in some genes can cause multiple phenotypes (e.g. NKX2.5). Many of these congenital lesions also can occur sporadically, without specific genetic mutation.*
***Only the cardiac manifestations of the syndrome are listed; the other skeletal, facial, neurologic and visceral changes are not.*

defect (VSD) and patent ductus arteriosus (PDA). In atrial septal defects, both pressure and oxygen saturation may be equalized between the two atria.

- **Cyanotic diseases (right-to-left shunt):** These include tetralogy of Fallot and transposition of the great vessels.
- **Malformations without left-to-right shunt:** These include hypoplastic left heart syndrome, coarctation of the aorta and total anomalous pulmonary venous return.

ATRIAL SEPTAL DEFECTS

Atrial septal defects (ASDs) account for 10% of all congenital heart diseases. Normally, the left atrial pressure keeps the foramen ovale closed. Fossa ovalis represents the remnant of foramen ovale on right interatrial septum. Patent foramen ovale is usually clinically insignificant. Depending on the location, there are three types of atrial septal defects. Complications of atrial septal defects include cyanosis, right-sided heart failure, bacterial endocarditis and paradoxical thrombi.

SEPTUM SECUNDUM

Septum secundum accounts for 90% of all atrial septal defects. It is a true defect and should not be confused with patent foramen ovale.

- Atrial septal defect occurs in the middle portion of septum varying in size to involve entire fossa ovalis. It is most common disease usually diagnosed in adult life.
- Large atrial septal defects cause dilatation and hypertrophy of the right atrium and ventricle resulting in pulmonary hypertension. The defect may allow a thrombus formation from right atrium to left atrium known as **paradoxical embolus**, so-called because a thromboembolus arising from the venous circulation can enter in the systemic circulation.
- Lutembacher syndrome is characterized by atrial septal defect with mitral stenosis.

SEPTUM PRIMUM

Large defect in the lower part of septum is known as septum primum. It may be associated with deformities of atrioventricular valves.

SINUS VENOSUS

Defect in the upper part of the septum near the entrance of the superior vena cava is known as **sinus venosus**.

VENTRICULAR SEPTAL DEFECTS

The interventricular septum usually closes down spontaneously in the first 2 years of life. Ventricular septal defects (VSDs) occur within the membranous (80%) or muscular (20%) portions of interventricular septum that produce a left-to-right shunt. Ventricular septal defects may vary in size.

- Small ventricular septal defects constitute 50% of cases, which may close spontaneously. VSDs are recognized by high-pitched holosystolic cardiac murmur.
- Large ventricular septal defects produce more severe disease. Left-to-right shunt may lead to pulmonary hypertension and eventual right-sided heart failure. Reversal of the shunt occurs when the pulmonary hypertension exceeds the pressure in the aorta in Eisenmenger syndrome resulting in cyanosis. Patient develops left ventricular hypertrophy and dilatation.

PATENT DUCTUS ARTERIOSUS

During intrauterine life, ductus arteriosus permits the blood from the venous system to bypass the lungs. During fetal life, its patency is maintained by combined effects of low oxygen tension and prostaglandin synthesis. Patent ductus arteriosus (PDA) normally closes soon after birth. If the ductus arteriosus remains open, left-to-right shunt develops (Fig. 16.63).

- Failure of closure of the fetal ductus arteriosus leads to pulmonary hypertension and right ventricular hypertrophy. The left-to-right shunt is associated with a loud machinery-like cardiac murmur during systole and diastole.
- Patent ductus arteriosus (PDA) can be closed surgically or pharmacologically treated with injection indomethacin (prostaglandins inhibitor).

COARCTATION OF THE AORTA

Coarctation of the aorta accounts for 6% of all cases of congenital heart disease. It is localized narrowing of the aortic lumen leading to outflow obstruction.

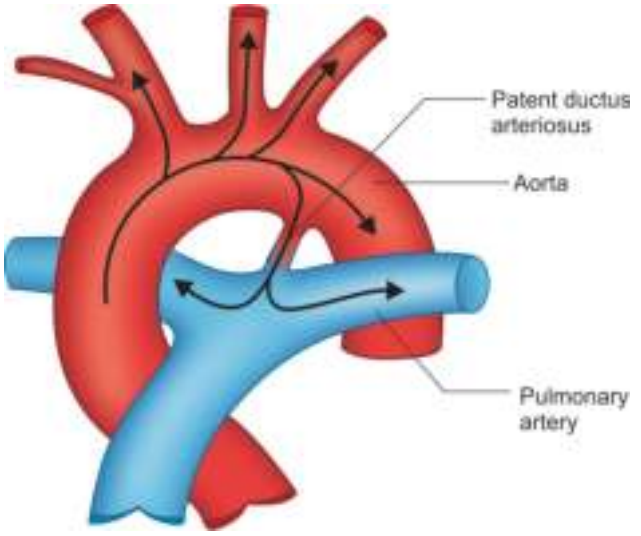


Fig. 16.63: Patent ductus arteriosus. There is persistence of patency of duct communicating aorta and pulmonary trunk.

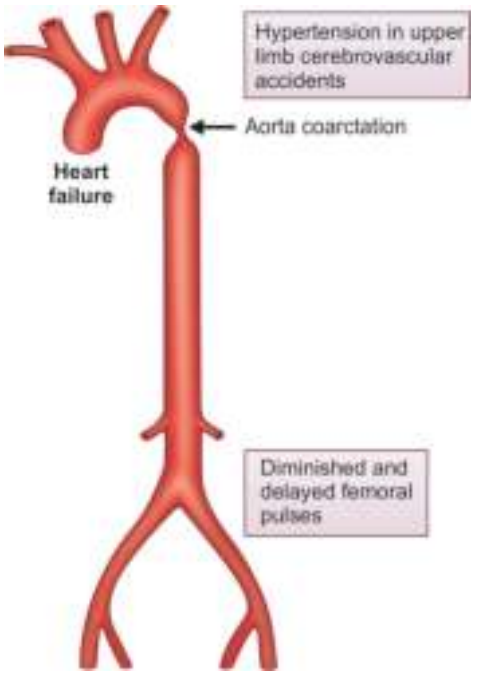


Fig. 16.64: Coarctation of the aorta leads to diminished blood flow to lower extremities.

- Disorder usually occurs in the thoracic portion of the descending aorta, distal to the take off of the left subclavian artery at the site of the ductus arteriosus.
- The cardinal physical finding is diminution or absence of femoral pulses and hypertension in the upper extremities. It occurs in two types—infantile and adult (Fig. 16.64).

INFANTILE TYPE OF COARCTATION OF THE AORTA

Infantile type of coarctation of the aorta constitutes 70% of cases. Ascending aorta and aortic arch is markedly narrowed with persistence of ductus arteriosus.

- Coarctation of the aorta is generally present at birth. Symptoms can range from mild to severe.
- Most infants do not survive without surgical correction of the defect.

ADULT TYPE OF COARCTATION OF THE AORTA

Adult type of coarctation of the aorta is distal to the origin of the subclavian arteries leading to hypertension.

Hypertension is limited to the upper extremities and cerebral vessels.

- The parts of the body below the obstruction become hypoxic, and the blood pressure measured on the legs is lower than the pressure measured on the arms.
- Hypertension increases risk for developing dissecting hematoma in aorta and berry aneurysms in the cerebral vessels. Hypertension causes proximal aortic dilatation resulting in aortic regurgitation. Collaterals that involve the intercostal arteries cause notching of the ribs seen on radiograph.

- Diastolic hypertension occurs due to activation of the renin-angiotensin-aldosterone system from reduced blood flow.

TETRALOGY OF FALLOT

Tetralogy of Fallot is the most common cyanotic disease in newborn. Tetralogy of Fallot comprises four pathologic findings, i.e. (a) ventricular septal defect (membranous type), (b) overriding aorta sitting over the ventricular septal defect, (c) pulmonary artery stenosis (below pulmonary valve), and (d) right ventricular hypertrophy (Fig. 16.65).

PATHOPHYSIOLOGY

The obstruction to right ventricular outflow creates a right-to-left shunt that leads to cyanosis since birth in tetralogy of Fallot.

- Pulmonary artery stenosis does not permit entry of venous blood into the lungs for oxygenation.
- Overriding aorta sitting over the ventricular septal defect receives blood from both ventricles results in cyanosis.

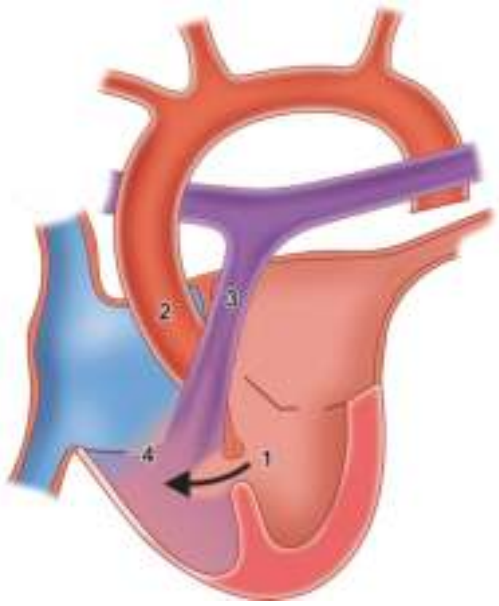


Fig. 16.65: Tetralogy of Fallot comprises four pathologic findings, i.e. (1) ventricular septal defect, (2) overriding aorta sitting over the ventricular septal defect, (3) pulmonary artery stenosis results in, and (4) right ventricular hypertrophy.

CLINICAL FEATURES

Newborn develops bluish discoloration of the skin and mucosa, i.e. cyanosis at birth. In most children, cyanosis is evident by the age of 4 months. Child presents with hypoxemic spells, i.e. faint or loss of consciousness, retarded growth, squatting position and clubbing of fingers.

PROGNOSIS

Some children may die in the first year of life. Death occurs due to hypoxia, infections and cerebrovascular accidents related to thromboembolism. Surgery is recommended as the treatment of choice for most cases. But it is associated with 3–15% mortality rate.

TRUNCUS ARTERIOSUS COMMUNIS

Truncus arteriosus communis refers to common trunk for the origin of the aorta, pulmonary arteries, and coronary arteries. Truncus arteriosus communis occurs due to incomplete separation of the aortic and pulmonary outflows and forming single vessel overriding both ventricles during development. It allows mixing of oxygenated and deoxygenated blood with right-to-left shunting.

MALFORMATIONS OF CVS

TRANSPOSITION OF THE GREAT VESSELS

The aorta arises from the right ventricle, and the pulmonary artery arises from the left ventricle. There is right-to-left shunting. It accounts for ≤5% of congenital heart disease. Its incidence is increased in maternal diabetes mellitus. Compensatory anomaly such as patent ductus arteriosus is necessary for survival.

HYPOPLASTIC LEFT HEART SYNDROME

There is varying degree of hypoplasia or atresia of the aortic and mitral valves, along with a small to absent left ventricular chamber.

TOTAL ANOMALOUS PULMONARY VENOUS RETURN

The pulmonary veins do not directly connect to the left atrium, but drain into left innominate vein, coronary sinus, or some other sites, leading to possible mixing of blood and right-sided overload.

CHAPTER

17

Nasal Cavity, Nasopharynx, Paranasal Sinuses, Ear, Larynx and Neck

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

DISORDERS OF NASAL CAVITY, NASOPHARYNX AND PARANASAL SINUSES

- Inflammation and infections
  - Acute rhinosinusitis
  - Chronic rhinosinusitis
  - Wegener's granulomatosis
  - Allergic polyps
  - Fungal sinusitis
- Sinonasal neoplastic disorders
  - Schneiderian sinonasal papillomas
  - Sinonasal undifferentiated carcinoma
  - Nasopharyngeal carcinoma
  - Sinonasal squamous cell carcinoma
  - Sinonasal melanoma
  - Olfactory neuroblastoma
  - Nasopharyngeal angiofibroma
  - Alveolar rhabdomyosarcoma
  - Sinonasal plasmacytoma
  - Sinonasal adenocarcinoma
  - Sinonasal hemangioblastoma
- Miscellaneous sinonasal disorders
  - Rhinosporidiosis
  - Rhinoscleroma

DISORDERS OF EAR

- Anatomy of ear
- Ear: disorder
  - External ear disorders
    - Cauliflower ear
    - Chondrodermatitis nodularis chronica
    - Malignant neoplasm
  - Middle ear disorders
  - Inner ear disorders
    - Presbycusis
    - Toxic damage of inner ear
    - Acoustic neuroma

DISORDERS OF LARYNX

- Anatomy and histology
- Inflammatory disorders
- Tumors and tumor-like disorders

DISORDERS OF NECK

- Neck swellings: overview
- Cervical lymphadenopathy
- Branchial cyst
- Thyroglossal cyst
- Paraganglioma (carotid body tumor)

DISORDERS OF NASAL CAVITY, NASOPHARYNX AND PARANASAL SINUSES

INFLAMMATION AND INFECTIONS

ACUTE RHINOSINUSITIS

Upper respiratory tract infections are most common cause of rhinosinusitis, which occur as a result of extension of infection from nasal cavity or dental region. Blockage of paranasal sinuses results in accumulation of inflammatory exudates in ethmoid sinus in children and maxillary sinus in adults.

- Acute rhinosinusitis is caused by bacteria (*Streptococcus pneumoniae*, *Staphylococcus aureus*, or *Haemophilus influenzae*), viruses (adenovirus) and allergy (allergens).
- Acute rhinosinusitis caused by bacteria may be superimposed on acute viral or allergic rhinitis resulting in injury to mucosal cilia of airways.
- Adenovirus causes coryza (runny nose), sneezing, nasal congestion, and mild sore throat. Allergic rhinosinusitis is characterized by nasal discharge and peripheral eosinophilia. Respiratory epithelium is infiltrated by necrotic material, apoptotic neutrophils and nuclear debris.
- Diagnostic modalities include bacterial culture of sinus aspirate, sinus radiographs and CT scan (most sensitive).

CHRONIC RHINOSINUSITIS

Predisposing factors for chronic rhinosinusitis include allergy, upper respiratory tract infections, cystic fibrosis, and immotile cilia syndrome (Kartagener's syndrome) especially secondary to obstruction of paranasal sinuses outflow. It commonly affects children. Submucosa is

infiltrated by lymphocytes, plasma cells and eosinophils. Complications of chronic rhinosinusitis include superadded bacterial infection and inflammatory polyp.

WEGENER'S GRANULOMATOSIS

Wegener's granulomatosis is an autoimmune disorder, which shares some features with lethal midline granuloma. Both diseases are characterized by necrotizing, ulcerated, mucosal lesions of upper respiratory tract.

- Lethal midline granuloma is a sign of underlying lymphomas. Wegener's granulomatosis is characterized by necrotizing vasculitis (fibrinoid necrosis) involving arterioles, small arteries and veins. Wegener's granulomatosis also involves respiratory system and kidney.
- Patient suffering from Wegener's granulomatosis presents with rhinorrhea, sinusitis, headache, nasal obstruction and symptoms due to eustachian tube obstruction. More than 90% of patients with Wegener's granulomatosis exhibit antineutrophil cytoplasmic antibody (ANCA).

Surgical Pathology: Wegener's Granulomatosis

Light Microscopy

Histologic examination of lesion shows chronic inflammatory infiltrate, granulomas and necrotising vasculitis.

ALLERGIC POLYPS

Allergic polyps are most common non-neoplastic lesions in nasal cavity. Long-standing chronic allergic rhinitis causes non-neoplastic mucosal and submucosal projections forming nasal polyps.

- Allergic polyps most often affect adults. In case of nasal polyps in children, sweat test must be done to rule out cystic fibrosis.
- Patient presents with nasal obstruction and rhinorrhea. Unilateral or bilateral multiple polyps arise from lateral nasal wall or ethmoid recess. Allergic polyps may cause deviated nasal septum, destruction of bone and rarely extend to nasopharynx (Fig. 17.1).



Fig. 17.1: Nasal polyp.

Surgical Pathology: Allergic Polyps

Gross Morphology

- Allergic polyps may measure up to several centimeters.
- These polyps are boggy, gelatinous, and translucent with broad base (Fig. 17.2A and B).

Light Microscopy

- Histologic examination of allergic polyps reveals intact respiratory epithelium, edematous, myxomatous stroma, infiltrated by lymphocytes, plasma cells and eosinophils.
- There is absence of seromucinous glands (Fig. 17.3).

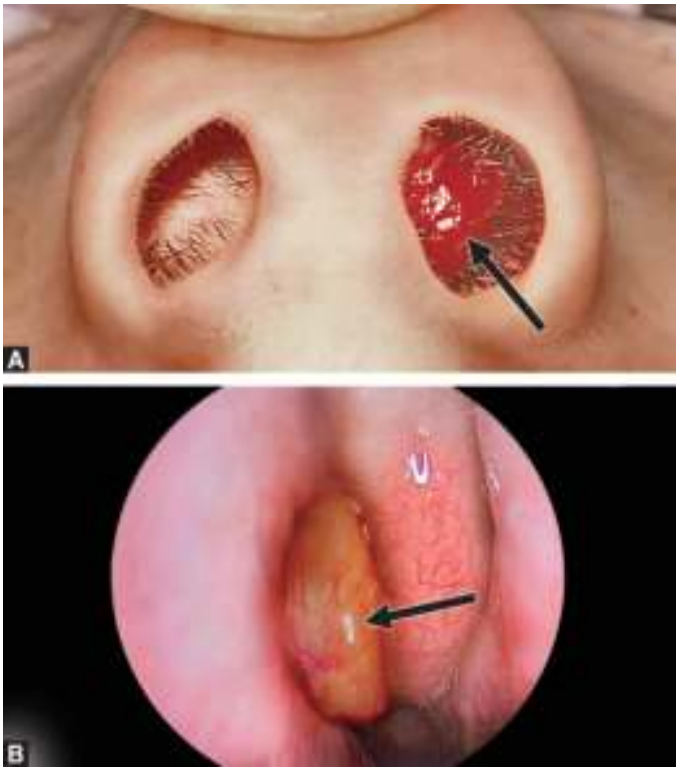


Fig. 17.2A and B: Nasal polyp (arrows).

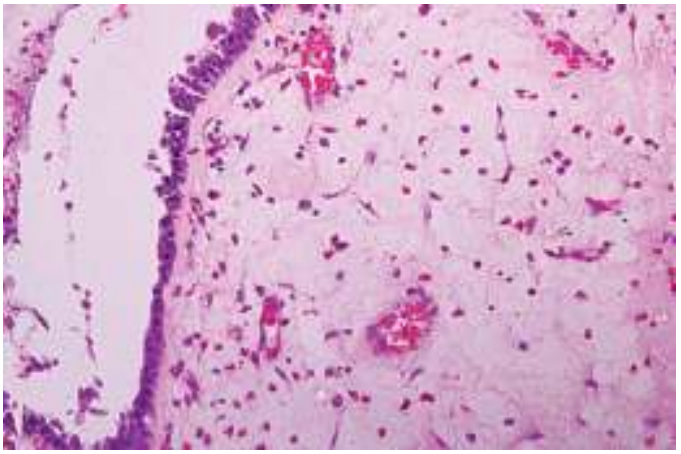


Fig. 17.3: Allergic nasal polyp. It shows dense eosinophilic infiltrate (400X).

FUNGAL SINUSITIS

Fungi may cause noninvasive or invasive sinusitis.

- Patient with noninvasive fungal sinusitis presents with polyps formation involving single or multiple paranasal sinuses.
- Patient with invasive fungal acute sinusitis presents with severe acute sinusitis, fever, and nasal discharge, ocular or neurologic deficits. Chronic invasive sinusitis is characterized by slowly progressive sinusitis with neurologic or orbital defects and mass.
- Fungi are demonstrated by Grocott's methenamine silver stain. Features of fungal sinusitis are given in Table 17.1.

SINONASAL NEOPLASTIC DISORDERS

A wide variety of neoplasms occurs in the sinonasal region (Fig. 17.4A and B). WHO classification of tumors of the nasal cavity, paranasal sinuses and skull base is given in Table 17.2.

SCHNEIDERIAN SINONASAL PAPILLOMAS

Schneiderian papillomas are the most common benign tumors of the nasal cavity, which are derived from Schneiderian membrane. Histologic variants of Schneiderian sinonasal papillomas are exophytic, endophytic and oncocytic. Histologic examination is essential due to their different clinical behavior and risk for development of invasive carcinomas.

Table 17.1 Features of fungal sinusitis

Entity	Clinical Manifestations	Light Microscopy	Causative Organism
Noninvasive fungal sinusitis			
Fungal sinusitis	Allergic polyps involving multiple sinuses	Eosinophils, Charcot-Leyden crystals and hyphae	Bipolaris, Curvularia, Alternaria, *Aspergillus dematiaceous*, Cladosporium
Mycetoma (fungal ball)	Nonallergic polyps involving single sinus	Fungal hyphae	Mycetoma
Invasive fungal sinusitis			
Acute invasive fungal sinusitis	Severe acute sinusitis, fever, nasal discharge, ocular or neurologic deficits	Fungal hyphae in blood vessels, granulomas, necrosis	Mucor, Rhizopus, Absidia, Cunninghammella *Aspergillus dematiaceous* (uncommon)
Chronic invasive sinusitis	Slowly progressive sinusitis onset, neurologic or orbital defects, mass	Fungal hyphae in blood vessels, granulomas, necrosis	*Aspergillus dematiaceous*

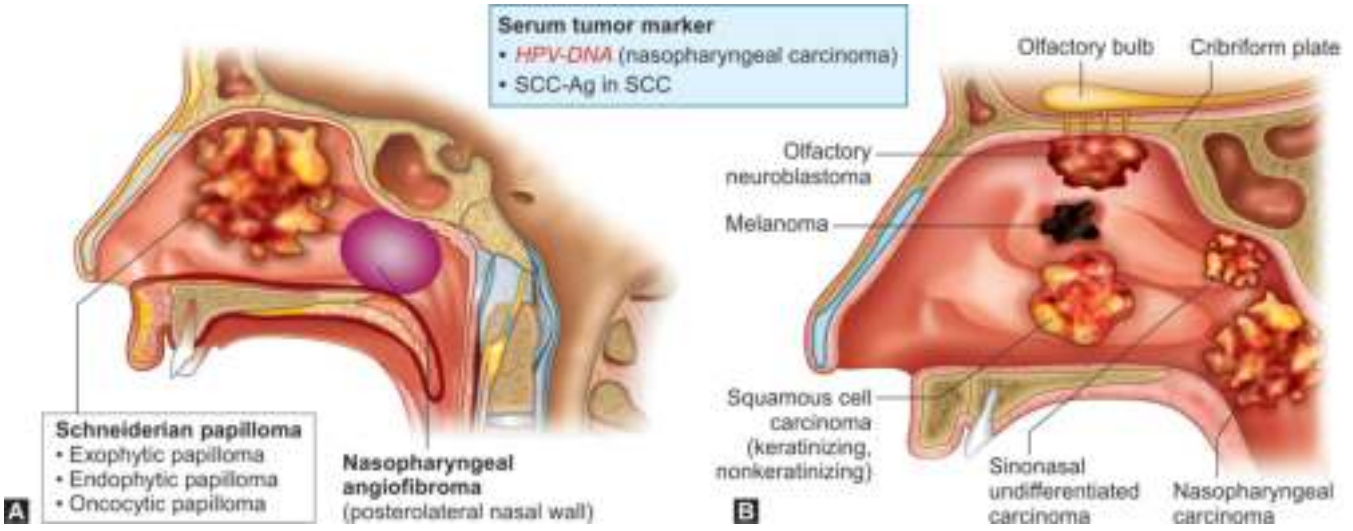


Fig. 17.4: Tumors of nasal cavity and paranasal sinuses. (A) Benign tumors, (B) malignant tumors.

Table 17.2 WHO classification of tumors of the nasal cavity, paranasal sinuses and skull base

Carcinomas
- Keratinizing squamous cell carcinoma
- Nonkeratinizing squamous cell carcinoma
- Spindle cell variant of squamous cell carcinoma
- Lymphoepithelial carcinoma
- Sinonasal undifferentiated carcinoma
- NUT carcinoma
- Neuroendocrine carcinomas
  - Small cell neuroendocrine carcinoma
  - Large cell neuroendocrine carcinoma
- Adenocarcinomas
  - Intestinal-type adenocarcinoma
  - Nonintestinal-type adenocarcinoma
- Teratocarcinoma

Benign Tumors
- Sinonasal papilloma
  - Sinonasal papilloma, inverted type
  - Sinonasal papilloma, oncocytic type
  - Sinonasal papilloma, exophytic type
- Respiratory epithelial lesions
  - Respiratory epithelial adenomatoid hamartoma
  - Seromucinous hamartoma
- Salivary gland tumor
  - Pleomorphic adenoma

Malignant Soft Tissue Tumors
- Fibrosarcoma
- Undifferentiated pleomorphic sarcoma
- Leiomyosarcoma
- Rhabdomyosarcoma, not otherwise specified
- Alveolar rhabdomyosarcoma
- Pleomorphic rhabdomyosarcoma, adult type
- Spindle cell rhabdomyosarcoma
- Angiosarcoma
- Malignant peripheral nerve sheath tumor
- Biphenotypic sinonasal sarcoma
- Synovial sarcoma

Borderline/Low Grade Malignant Soft Tissue Tumors
- Desmoid-type fibromatosis
- Sinonasal glomangiopericytoma
- Solitary fibrous tumor
- Epithelioid hemangioendothelioma

Benign Soft Tissue Tumors
- Leiomyoma
- Hemangioma
- Schwannoma
- Neurofibroma

Other Tumors
- Meningioma
- Sinonasal ameloblastoma
- Chondromesenchymal hamartoma

Hematolymphoid Tumors
- Extranodal NK/T cell lymphoma
- Extraosseous plasmacytoma

Neuroectodermal/Melanocytic Tumors
- Ewing's sarcoma/primitive neuroectodermal tumor
- Olfactory neuroblastoma
- Mucosal neuroma

Contd...

Sinonasal Exophytic Papilloma

Exophytic papilloma most often arises from nasal septum in 20–50 years males. Tumor is solitary and discrete. Human papillomavirus (6 and 11) has been demonstrated in large number of cases. Patient presents with epistaxis, unilateral nasal obstruction or symptomatic nasal mass. Recurrence of exophytic papilloma is common after surgery. There is no risk for development of carcinoma in these patients.

Surgical Pathology: Sinonasal Exophytic Papilloma

Gross Morphology

Tumor is gray pink, translucent mass with broad base (Fig. 17.5).

Light Microscopy
- Tumor is composed of exophytic papillary fronds lined by thickened epithelium (squamous to respiratory or transitional or features of both).
- Scattered mucin secreting cells are also present.
- Neutrophil collections are present in epithelial fronds. Surface keratinization is absent except in rare case.
- Tumor cells do not show atypia (Fig. 17.6).

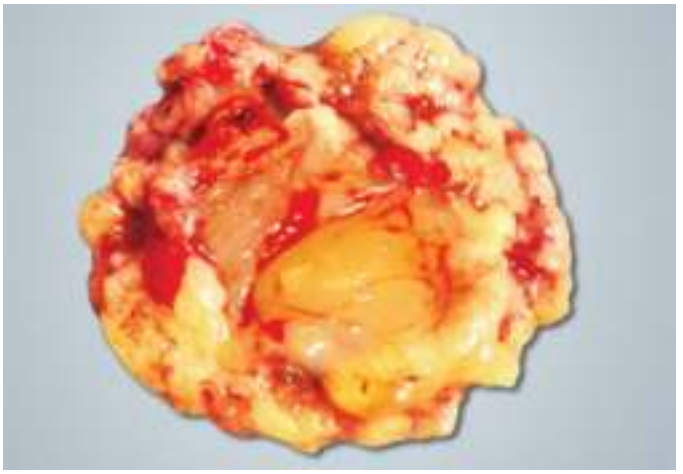


Fig. 17.5: Exophytic papilloma. Tumor is gray pink, translucent mass with broad base.

Sinonasal Endophytic Papilloma

Endophytic papilloma most often occurs in 40–70 years adults. Tumor arises from lateral nasal wall in the region of middle turbinate and ethmoid recesses. It may also arise from nasal septum in 8% of cases. Human papillomavirus (6 and 11) has been demonstrated in 50% of

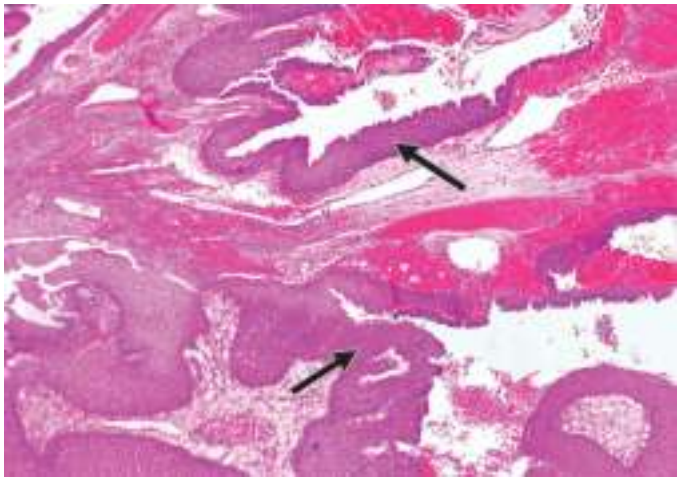


Fig. 17.6: Sinonasal exophytic papilloma: It shows exophytic papillary fronds lined by thickened epithelium (squamous to respiratory or transitional or features of both). Tumor cells do not show atypia (arrows) (100X).

cases. Patient presents with unilateral nasal obstruction or symptomatic nasal mass. Recurrence is common in 5–20% of cases after surgery. There is increased risk for development of carcinoma in 10–15% of cases.

Surgical Pathology: Sinonasal Endophytic Papilloma
Gross Morphology
Tumor is fleshy pink tan with papillary or polypoid (Fig. 17.7).
Light Microscopy
▪ Tumor is composed of numerous basement membrane enclosed, rounded ribbons of thickened epithelium ranging from squamous to transitional with minimal mitoses without atypia. ▪ Neutrophils are present in the epithelium (Figs 17.8 and 17.9).



Fig. 17.7: Sinonasal endophytic papilloma. It is fleshy pink-tan with papillary fronds.

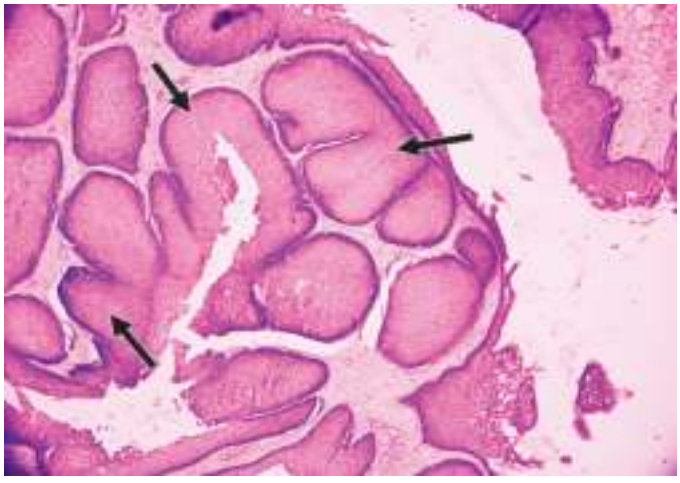


Fig. 17.8: Sinonasal endophytic (inverted) papilloma. Tumor is composed of numerous basement-enclosed, rounded ribbons of thickened epithelium ranging from squamous to transitional with minimal mitoses without atypia (arrows) (100X).

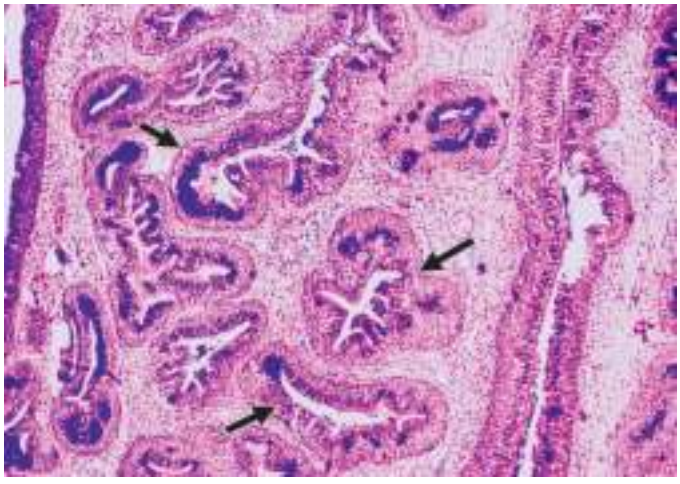


Fig. 17.9: Sinonasal endophytic (inverted) papilloma. It shows numerous rounded ribbons of thickened epithelium ranging from squamous to transitional with minimal mitoses without atypia (arrows) (200X).

Sinonasal Oncocytic Papilloma

Oncocytic papilloma is least common equally affecting men and women. HPV has not been demonstrated in oncocytic papilloma. Patient presents with unilateral nasal obstruction or symptomatic nasal mass. Recurrence is common after surgery. There is increased risk for development of carcinoma in 5–15% of cases.

Surgical Pathology: Sinonasal Oncocytic Papilloma
Light Microscopy
Tumor is composed of exophytic and endophytic nests of columnar, oncocytic cells with abundant, granular eosinophilic cytoplasm; intraepithelial neutrophils and microcysts (Fig. 17.10). Features of Schneiderian sinonasal papilloma are given in Table 17.3.

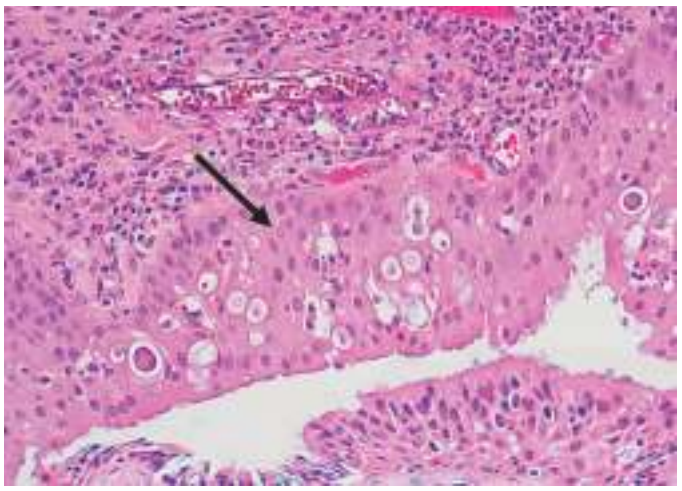


Fig. 17.10: Sinonasal oncocytic papilloma. Tumor is composed of nests of oncocytic cells with abundant, granular eosinophilic cytoplasm, and intraepithelial neutrophils (arrow) (400X).

SINONASAL UNDIFFERENTIATED CARCINOMA

Sinonasal undifferentiated carcinoma is an aggressive tumor. It most often occurs in elderly persons in their 6th decade. It arises from superior region of nasal cavity or maxillary or ethmoid sinuses. Patient presents with nasal obstruction, epistaxis, orbital or cranial nerve deficits.

Surgical Pathology: Sinonasal Undifferentiated Carcinoma
Gross Morphology
This bulky tumor has no unique gross features.
Light Microscopy
▪ Tumor is composed of small- to moderate-sized uniform cells with hyperchromatic to vesicular nuclei and prominent nucleoli. ▪ Tumor cells are arranged in nests, lobular or trabeculae pattern. ▪ There is abundant apoptosis, mitoses and necrosis in the tumor (Fig. 17.11).
Immunohistochemistry Technique
Panel of markers is employed on histologic sections obtained from tumor.

Markers	Expression
▪ Pancytokeratins (7, 8, 19) ▪ Epithelial membrane antigen (EMA) ▪ Neuron-specific enolase (NSE)	▪ Positive ▪ Positive ▪ Positive (30%)
Neuroendocrine markers: Tumor expresses NSE, but negative for chromogranin A and synaptophysin.	

NASOPHARYNGEAL CARCINOMA

Nasopharyngeal carcinoma is most common tumor of nasopharynx. It is associated with Epstein-Barr virus (EB virus). It is more prevalent in males especially in Chinese and African population. It metastasizes to cervical lymph nodes. Most patients have anti-EBV IgA in their serum.

Clinical Features

Patient presents with sinonasal mass, nasal obstruction, epistaxis and serous otitis media due to blockage of eustachian tube. Infrequently, patient may be asymptomatic even with neck metastases.

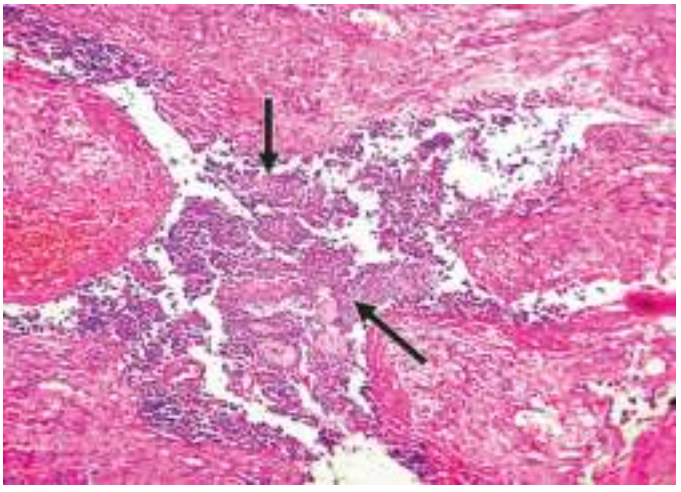


Fig. 17.11: Sinonasal undifferentiated carcinoma. Tumor is composed of atypical cells with hyperchromatic nuclei and prominent nucleoli arranged in nests (arrows) (100X).

Table 17.3 Features of Schneiderian sinonasal papillomas

Histologic Variants	Common Location	Pathology	Behavior after Surgical Excision	Malignant Transformation to Invasive Carcinoma
Exophytic papilloma	Nasal septum	Exophytic fronds of squamous or transitional, epithelium containing neutrophils	Local recurrence	No risk of malignancy
Inverted papilloma	Lateral nasal wall; paranasal sinuses	Endophytic fronds of squamous or transitional, epithelium containing neutrophils	Local recurrence	10–15%
Oncocytic papilloma	Lateral nasal wall; paranasal sinuses	Exophytic and endophytic nests of columnar, oncocytic cells with abundant, granular eosinophilic cytoplasm; intraepithelial neutrophils and microcysts	Local recurrence	5–15%

Surgical Pathology: Nasopharyngeal Carcinoma

Gross Morphology

Tumor is gray white to tan. Cut surface is gray white with areas of hemorrhage (Fig. 17.12).

Light Microscopy

WHO classification of nasopharyngeal carcinoma is given in Table 17.4. EB virus is strongly linked in the oncogenesis of nonkeratinizing nasopharyngeal carcinoma (types: WHO-2a and 2b).

- **Keratinizing squamous cell carcinoma (WHO-1):** Tumor has morphology similar to keratinizing squamous cell carcinoma elsewhere in head and neck and graded as well to poorly differentiated carcinoma (Fig. 17.13).
- **Nonkeratinizing differentiated carcinoma (WHO-2a):** Tumor has surface disease with stratified cells that have similar morphology to that of urothelial carcinoma of urinary bladder.
- **Nonkeratinizing undifferentiated carcinoma (WHO-2b):** Tumor is the most common variant of nasopharyngeal carcinoma. It is composed of tumor cells arranged in sheets/syncytial aggregates. Tumor cells contain eosinophilic cytoplasm, large vesicular nuclei and prominent nuclei. These tumor cells are admixed with chronic inflammatory cells (Fig. 17.14).
- **Basaloid squamous cell carcinoma (WHO-3):** Tumor is least common variant of nasopharyngeal carcinoma. It has similar morphology that occurs in head and neck.

Immunohistochemistry Technique

These are immunoreactive with antibodies to cytokeratins.

Clinical Course

- **Nonkeratinizing undifferentiated carcinoma:** It most often metastasizes to cervical lymph nodes in posterior triangle (level 5). The tumor responds well to systemic therapy. Radiotherapy is the first line of treatment. Surgery is reserved for salvage therapy.



Fig. 17.12: Squamous cell carcinoma. Tumor is arising from maxillary sinus.

Table 17.4 WHO classification of nasopharyngeal carcinoma

Histologic Variants	Types
Keratinizing squamous cell carcinoma	1
Nonkeratinizing differentiated carcinoma	2a
Nonkeratinizing undifferentiated carcinoma	2b
Basaloid squamous cell carcinoma	3

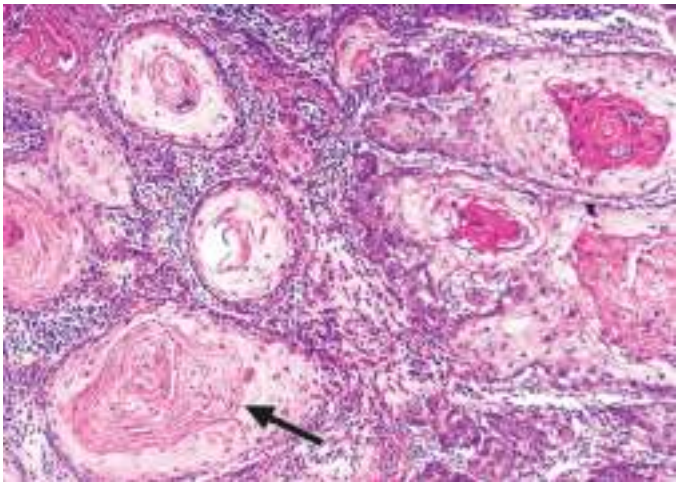


Fig. 17.13: Keratinizing squamous cell carcinoma (WHO-1). Tumor shows areas of keratinization (arrow) (400X).

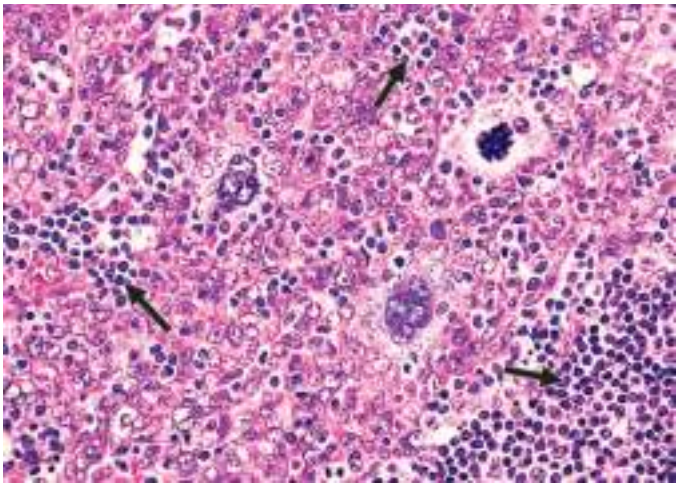


Fig. 17.14: Nonkeratinizing undifferentiated carcinoma most common variant (WHO-2b)—tumor cells contain eosinophilic cytoplasm, large vesicular nuclei and prominent nuclei, arranged in sheets syncytial aggregates and admixed with chronic inflammatory cells (arrows) (100X).

- **Keratinizing squamous cell carcinoma:** Patient most often presents with localized disease. It does not respond to systemic therapy.

SINONASAL SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma is the most common tumor in head and neck. It constitutes 65% of carcinomas in the

nasal cavity and paranasal sinuses. Histologic variants of squamous cell carcinoma include keratinizing and nonkeratinizing squamous cell carcinoma.

- **Keratinizing squamous cell carcinoma:** It is associated with tobacco smoking, exposure to nickel, textile dusts and chlorophenols.
- **Nonkeratinizing squamous cell carcinoma:** It has papillary configuration. Tumor is composed of ribbons of pleomorphic cells lacking keratinization. It has been associated with HPV. This variant has better prognosis.

SINONASAL MELANOMA

Melanoma of sinonasal region constitutes 1% of all melanomas. It occurs in the nasal cavity and paranasal sinuses especially in middle or inferior turbinate in patients over 50 years. The tumor may be sessile/polypoidal growth with mucosal ulceration and heavily pigmented (brown or black). It metastasizes to sinonasal region. Prognosis of melanoma is poor.

Surgical Pathology: Sinonasal Melanoma

Light Microscopy

- Melanoma has a wide range of morphologic features. Tumor is composed of pleomorphic melanocytes. Some tumors are composed of mixture of epithelioid cells and spindle cells.
- There is also presence of junctional component with pagetoid spread of melanocytes in the intact mucosa.

Immunohistochemistry Technique

Panel of markers is employed on histologic sections obtained from tumor.

Markers	Expression
S-100	Positive
HMB-45	Positive
Melan-A	Positive
CAM 5.2	Positive
Ki-67	Positive

OLFACTORY NEUROBLASTOMA

Olfactory neuroblastoma most often occurs in the upper nasal cavity wall and superior turbinate of men between 30 and 40 years unlike pediatric neuroblastoma, which most often occurs in the adrenals/abdomen of infants and young children. Tumor arises from olfactory mucosa of the cribriform plate in the upper nasal cavity wall and superior turbinate. Patient presents with unilateral polypoid mass in nasal cavity causing nasal obstruction and epistaxis.

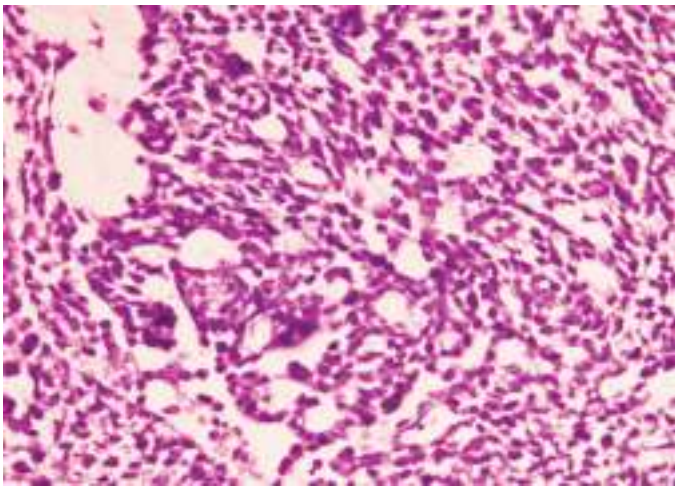


Fig. 17.15: Olfactory neuroblastoma (diffuse pattern) with neurofibrillary background (100X).

Surgical Pathology: Olfactory Neuroblastoma

Light Microscopy

- Tumor is composed of small round blue cells set in a neurofibrillary matrix.
- Tumor cells contain round uniform nuclei with uniform delicate stippled chromatin (Fig. 17.15).

Immunohistochemistry Technique

Panel of markers is used on histologic section to demonstrate expression by tumor cells.

- CD45 rules out lymphoma
- CD99 rules out Ewing/PNET
- Myogenin/MyoD1 rules out rhabdomyosarcoma
- Pituitary hormone markers rule out pituitary adenoma.

Markers	Expression
Synaptophysin	Positive
Chromogranin A	Positive
Neuron-specific enolase (NSE)	Positive
Calretinin	Positive
CAM 5.2	Positive

NASOPHARYNGEAL ANGIOFIBROMA

Nasopharyngeal angiofibroma is a rare vascular tumor, which arises from fibrovascular nidus in the posterolateral nasal wall adjacent to the sphenopalatine foramen. It may occur in maxilla and ethmoid bones in elderly females. It most often affects adolescent males between 10 and 20 years. It is histologically benign but locally aggressive tumor. It may destroy nearby bone and extend in cranial cavity. Patient presents with nasopharyngeal mass bulging in nasopharynx and nasal obstruction and epistaxis.

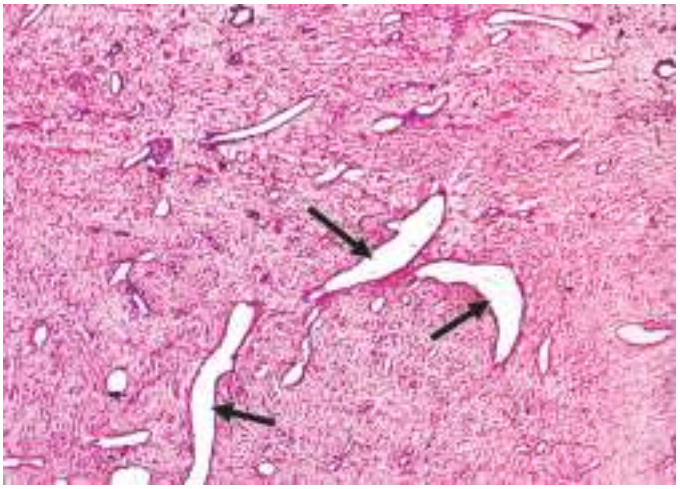


Fig. 17.16: Nasopharyngeal angiofibroma. It shows vascular channels lined by flat to plump endothelial cells of variable size dispersed in collagenized stroma (arrows) (100X).

Surgical Pathology: Nasopharyngeal Angiofibroma
Gross Morphology
Tumor is gray white to tan, smooth with lobulated appearance. Cut surface is homogenous.
Light Microscopy
▪ Tumor is composed of vascular channels of variable size dispersed in collagenized stroma containing scattered spindle and stellate cells. ▪ Vascular channels are lined by flat to plump endothelial cells without cytologic atypia. Stromal cells contain vesicular nuclei with vesicular chromatin without cytologic atypia (Fig. 17.16).
Immunohistochemistry Technique
▪ Vascular endothelium is positive with CD31 and CD34. ▪ Fibroblastic component expresses cytoplasmic vimentin.

ALVEOLAR RHABDOMYOSARCOMA

Alveolar rhabdomyosarcoma is an aggressive mesenchymal malignancy most common in young children. Tumor is always characterized by two characteristics translocations. Translocation (1;13) leading to PAX7-FKHR fusion transcription factor in 20% of cases is associated with better prognosis only in setting of metastatic disease. Translocation (2;13) causes PAX3-FKHR fusion transcription factor in 80% of cases.

Surgical Pathology: Alveolar Rhabdomyosarcoma
Gross Morphology
Tumor has fleshy to firm tan-gray appearance.
Light Microscopy
▪ Tumor is composed of small- to medium-sized cells with hyperchromatic nuclei and scant eosinophilic cytoplasm. ▪ Tumor cells are arranged in loosely cohesive sheets separated by fibrous septa.
Immunohistochemistry Technique
Panel of markers is used on histologic section to demonstrate expression by tumor cells.

Markers	Expression
▪ Desmin	▪ Positive
▪ Muscle-specific actin	▪ Positive
▪ Myoglobin	▪ Positive
▪ MyoD1	▪ Positive

SINONASAL PLASMACYTOMA

Plasmacytoma is a plasma cell tumor in its extraosseous form, present in the upper respiratory tract, which lacks radiographic or morphologic evidence of bone marrow involvement. Tumor is composed of plasma cells and their precursors at various stages of development. Accordingly, immunohistochemical reactivity for CD138 can be useful to confirm the diagnosis.

SINONASAL ADENOCARCINOMA

Nasal adenocarcinoma accounts for 5% of malignant tumor of the nose and throat. Histologic examination shows intestinal-type and nonintestinal-type of adenocarcinoma of nasal cavity.

SINONASAL HEMANGIOBLASTOMA

Hemangioblastoma is a benign tumor of nose. Tumor is treated by surgical excision. Tumor is composed of fascicles of spindle cells with indistinct borders arranged around blood vessels. Tumor may exhibit storiform pattern or whorled appearance.

MISCELLANEOUS SINONASAL DISORDERS

RHINOSPORIDIOSIS

Rhinosporidiosis is caused by *Rhinosporidium seeberi*. Patient presents with polypoidal masses containing sporangia in the nose (most common site), larynx, trachea, skin and ear.

Surgical Pathology: Rhinosporidiosis
Light Microscopy
▪ Nasal mucosa may undergo squamous metaplasia. Thick-walled sporangia containing numerous endospores are present beneath epithelium.

- Sporangia are surrounded by mixed inflammatory cells with numerous eosinophils.
- These sporangia may rupture resulting in formation of thick hyalinized wall (Fig. 17.17).

Histochemistry

Sporangia are demonstrated by PAS and silver methenamine stains.

RHINOSCLEROMA

Rhinoscleroma is caused by gram-negative Klebsiella rhinoscleromatis by droplet infection. It most often occurs in nose, which may extend to pharynx and upper respiratory tract. Patient presents with tumor mass in the nasal cavity protruding from the nostril and extending in the nasopharynx.

Surgical Pathology: Rhinoscleroma

Light Microscopy

- Rhinoscleroma is composed of large foamy histiocytes laden with organisms known as Mikulicz cells.
- Mikulicz cells are admixed with numerous plasma cells (Fig. 17.18).

Histochemistry

The organisms are demonstrated by Giemsa, PAS and silver impregnation stains.

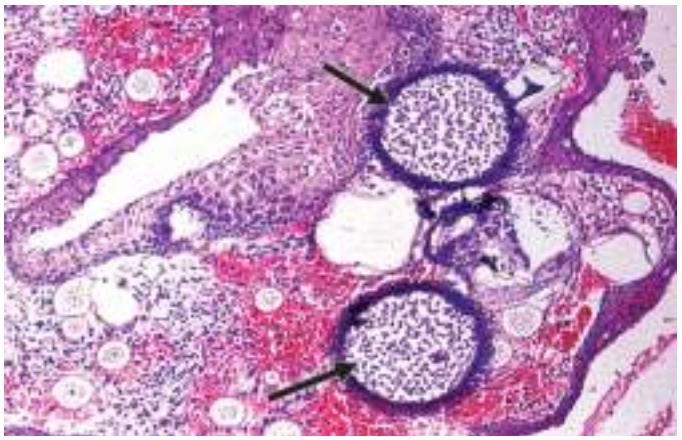


Fig. 17.17: Rhinosporidiosis. It shows many sporangia containing spores (arrows) (100X).

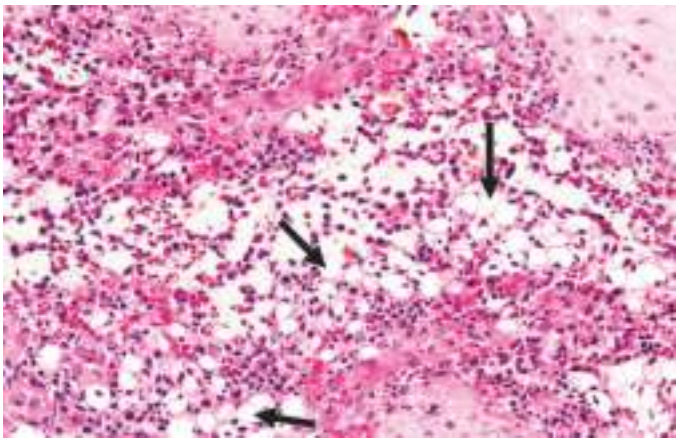


Fig. 17.18: Rhinoscleroma is composed of large foamy histiocytes laden with organisms known as **Mikulicz cells** (arrows) (400X).

DISORDERS OF EAR

ANATOMY OF EAR

Ear is composed of external, middle and internal portions. External ear consists of auricle, external auditory canal, and eardrum (tympanic membrane).

- The external auditory meatus extends up to the tympanic membrane. It is lined by wax secreting sebaceous glands.
- Middle ear consists of the auditory (eustachian tube), ossicles (malleus, incus and stapes), oval window and round window. Sound waves travel down the external auditory canal and strike tympanic membrane. Vibrations from eardrum pass to three tiny bones of the middle ear called ossicles.
- Eustachian tube or the auditory connects the middle ear cavity with the nasopharynx. It helps in equalizing the pressures on either sides of the eardrum.
- Inner ear consists of bony labyrinth, membranous labyrinth and vestibular apparatus. Common diseases affecting ear are shown in Fig. 17.19.

EAR: DISORDERS

EXTERNAL EAR DISORDERS

Preauricular Sinus

Preauricular sinus is commonest disorder arising as a result of abnormal fusion of facial folds, which leads to formation of blind-ending track opening on the skin just anterior to the external auditory meatus. Obstruction of sinus opening results in information of keratin-filled epidermal cyst. Sinus tract may undergo superadded infection resulting in abscess formation.

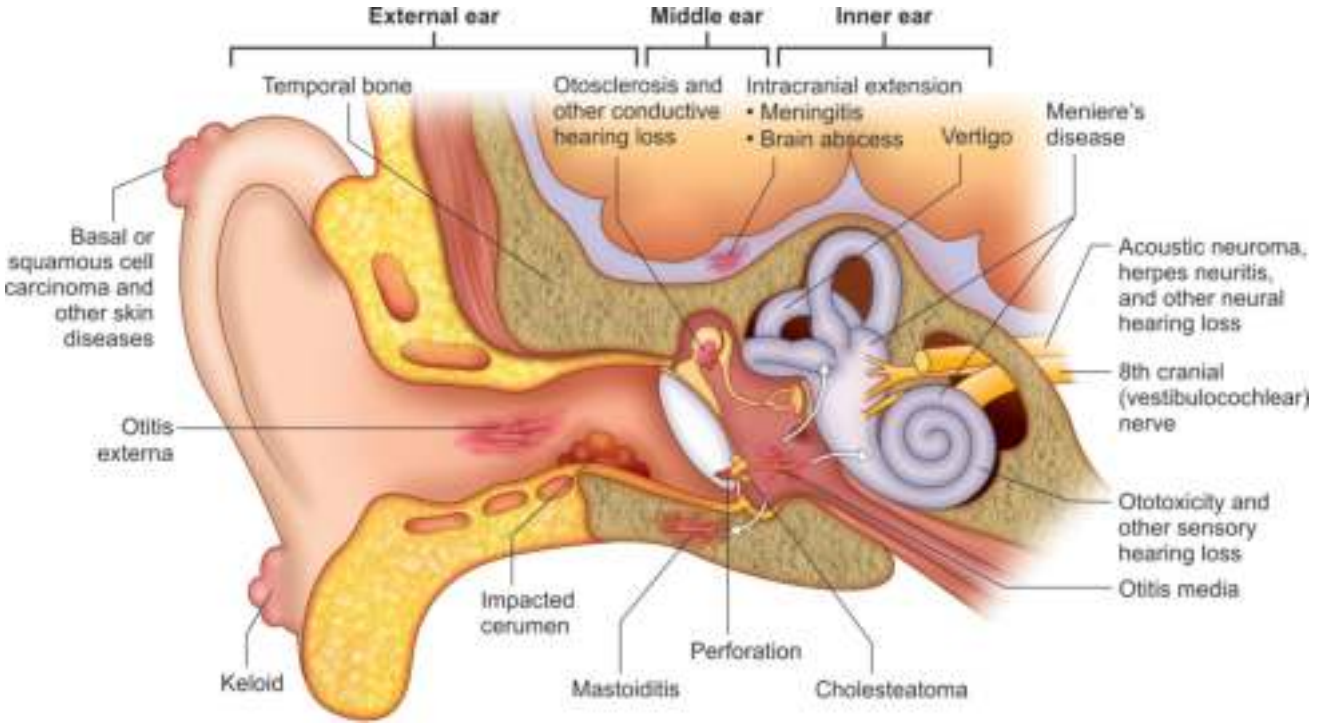


Fig. 17.19: Common diseases of the external, middle and internal ear.

Diagonal Ear Lobe Crease

Some persons show a crease of the ear lobe running downwards from the external auditory meatus. There is increased risk for development of cerebrovascular disease, most notably ischemic heart disease.

Cauliflower Ear

Cauliflower ear is characterized by thickening of the pinna due to partial organization of repeated hematoma resulting from trauma. It is commonly seen in boxers.

Chondrodermatitis Nodularis Chronica

Chondrodermatitis nodularis chronica is characterized by painful nodule on the superior portion of the helix. Skin ulceration extends to cause degenerated eosinophilic cartilage associated with inflammation of the perichondrium. Trauma is considered to be underlying etiology.

Relapsing Polychondritis

Relapsing polychondritis is characterized by involvement of cartilage, eye and heart. Patient presents with reddening, swelling and painful pinna followed by atrophy and destruction of cartilage. It is considered to be an autoimmune disorder related to autoimmune vasculitis.

Inflammation of Pinna and External Auditory Meatus

Inflammation may affect pinna and external auditory meatus caused by bacteria, viruses or fungi.

- *Pseudomonas aeruginosa* produces very severe otitis externa. Herpes simplex 1 and 2 produce painful blisters in the auditory canal and meatus.
- Varicella zoster lying dormant in ganglion involves pinna and meatus.
- Occasionally, varicella zoster may involve 8th cranial nerve ganglion leading to disturbances in hearing and balance known as **Ramsay Hunt syndrome**. Foreign bodies within tissues of the external ear produce non-infective inflammatory disease.

Ceruminous Gland Adenoma

Cerumic gland adenoma most often presents with blockage of the external auditory canal and unilateral deafness.

Malignant Neoplasms

Malignant neoplasms are rare in external ear.

MIDDLE EAR DISORDERS

Acute Serous Otitis Media

Acute serous otitis media is common in children most often associated with bacterial infection, i.e. *Streptococcus pneumoniae* and *Haemophilus influenzae* at the orifice of the eustachian tube, which may be precipitated by viruses, allergens or sudden changes in atmospheric pressure during flying in an aircraft or deep-sea diving resulting in obstruction of the eustachian tube.

- Repeated bouts of otitis media in early childhood often contribute to unsuspected hearing loss due to residual sterile fluid in the middle ear. Acute suppurative otitis media is unlikely without fever.
- In untreated cases, infection may spread to mastoid air spaces within bone, which further induce meningitis, encephalitis or brain abscess.

Chronic Otitis Media

Chronic otitis media most often develops without a preceding acute phase. It is usually caused by Proteus, *Pseudomonas aeruginosa*.

- Mastoid cells and tubotympanic region of the middle ear are most commonly involved. Chronic inflammation leads to formation of abundant granulation tissue that protrudes through tympanic membrane perforation as a polypoid mass, i.e. aural polyp. This process triggers scarring and new bone formation. Cholesteatoma and cholesterol granulomas are not associated with chronic otitis media.
- Patient presents with persistent earache, conduction deafness and chronic discharge from the external auditory meatus.

Cholesterol Granuloma

Cholesterol granuloma most often occurs as a consequence of previous hemorrhage. The lipid is being derived from cell membrane. Patient develops yellow nodules within tympanic membrane and mastoid. Histologic examination shows chronic inflammatory cells and foreign body giant cells.

Tympanosclerosis

Tympanosclerosis is special form of scarring associated with chronic otitis media. It is characterized by laying down of large amounts of dense hyalinized collagen fibers within the middle ear lining adversely affecting tympanic membrane and crura of stapes. There is presence of spotty calcification and new bone formation within plaques of fibrous tissue.

Otitis Media with Effusion (Glue Ear)

Otitis media with effusion is common cause of hearing loss in children. Patient develops thick effusion in the middle ear behind nonperforated tympanic membrane. As the drainage via eustachian tube is impaired, otitis media with effusion is treated by insertion of groomets through the tympanic membrane.

Cholesteatoma

Cholesteatoma refers to formation of actively growing, keratinizing, stratified squamous epithelium in the external ear canal resulting in extension into the middle ear through perforated eardrum and mastoid air cells.

- The keratin mass frequently becomes infected and shields the bacteria from antibiotics. Cholesteatoma is a complication of chronic suppurative otitis and rupture of the eardrum.
- Cholesteatoma may exist either as a closed cystic mass or open cystic mass, in which patient presents with keratin squames discharge into the middle ear cavity, which may show clinical evidence of bone destruction.

Classification

- **Congenital cholesteatoma:** It arises from an epithelial rest in the developing middle ear. Most common site of congenital cholesteatoma is, where middle ear epithelium joins eustachian tube.
- **Acquired cholesteatoma:** It most often occurs in the upper and posterior portion of middle ear filling most of middle ear cavity. Clinical significance of cholesteatoma is erosion of bony structures such as ossicles or the labyrinth and surrounding soft tissue especially facial nerve.

Surgical Pathology: Cholesteatoma

Light Microscopy

- Cholesteatoma is identical to epidermal inclusion cysts surrounded by granulation tissue and fibrosis.
- It erodes ossicles, labyrinth and soft tissues, especially facial nerve (Fig. 17.20).

Otosclerosis

Otosclerosis is commonest disorder of the cochlea and the footplates of stapes. It is an autosomal dominant disorder, which affects young and middle-aged adults in the United States. Approximately, 90% of cases are asymptomatic. Otosclerosis is characterized by formation

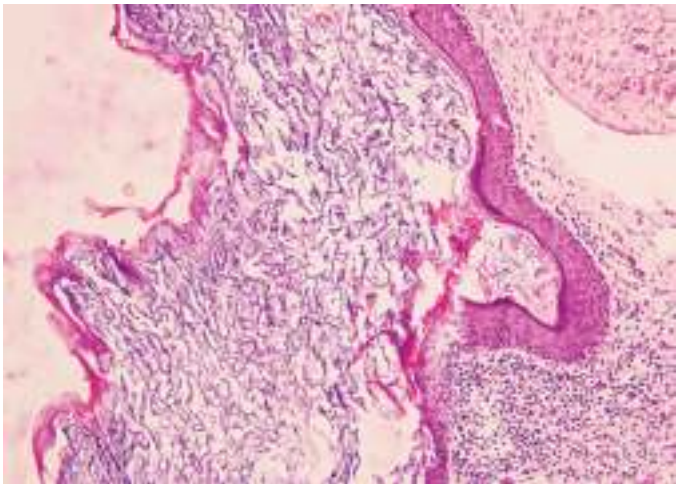


Fig. 17.20: Cholesteatoma is surrounded by granulation tissue and fibrosis. It is worth mentioning that it is identical to epidermal inclusion cysts. Squamous cell carcinoma rarely occurs in the ear (400X).

of new spongy bone over the stapes and the oval window, resulting in progressive conductive deafness. Fixation of stapes leads to failure of transmission of sound vibrations.

Surgical Pathology: Otosclerosis

Light Microscopy

- Stapedectomy specimen shows similar morphology resembling Paget's disease.
- Biopsy shows foci of woven bone rather than normal lamellar bone and numerous cement lines.

Electron Microscopy

Stapedectomy specimen shows structure resembling measles virus, and nucleocapsid antigen.

Paraganglioma (Glomus Jugulare Tumor)

Paraganglioma is the commonest slow-growing vascular tumor of the middle ear that bleeds freely on surgical excision. Tumor may invade the petrous temporal bone reaching the intracranial region in some cases.

- Paraganglioma arises from paraganglionic tissue in the wall of the jugular bulb in majority of cases, which tends to invade the petrous bone. Paraganglioma arising from paraganglion located near the middle ear surface of the promontory and remains localized to the middle ear.
- Patient presents with red mass either behind tympanic membrane or protruding out in the auditory canal. Metastases are very rare. Recurrence is common after surgical resection.

Surgical Pathology: Paraganglioma

Light Microscopy

- Paraganglioma has similar morphology in all sites.
- Tumor is composed of well-defined nests of catecholamine-containing cells.
- The tumor cells are separated by highly vascular fibrous tissue septa.
- Tumor cells are surrounded by sustentacular cells.
- Predicting malignant behavior from histologic appearance is impossible.

Immunohistochemistry Technique

Tumor cells do not contain catecholamine but react with monoclonal antibodies against the nuclear marker S-100.

INNER EAR DISORDERS

Patient presents with hearing loss, tinnitus and/or vertigo.

Viral Labyrinthitis

Mumps is the most common cause of deafness in 80% of cases among the postnatal viral infections. On the contrary, prenatal infection of the labyrinth with rubella is usually bilateral, with permanent loss of cochlear and vestibular function.

- Numerus viruses are suspected to cause viral labyrinthitis, i.e. influenza and parainfluenza viruses, Epstein-Barr virus, herpesviruses, and adenoviruses.
- Temporal bone specimens of such cases reveal severe damage to the organ of Corti, with almost total loss of both inner and outer hair cells.

Presbycusis

Presbycusis is the commonest cause of sensorineural hearing loss with aging. There is loss of outer hair cells at the base of lower end of the cochlea.

Meniere's Disease

Meniere's disease is characterized by both hearing loss and balance disturbance. Patient presents with episodic deafness, tinnitus and vertigo.

Toxic Damage of Inner Ear

Inner ear damage may be caused by genticin, loop of diuretics, salicylates, quinine and cisplatin. Patient presents with sensorineural deafness.

Acoustic Neuroma

Acoustic neuroma arises from the Schwann cells of 8th cranial nerve. Most patients develop unilateral acoustic neuroma. Patient presents with sensorineural deafness. Bilateral acoustic neuromas are characteristic of neurofibromatosis type 2 and characterized by deletion of chromosome 22.

DISORDERS OF LARYNX

ANATOMY AND HISTOLOGY

Larynx is composed of several cartilaginous structures such as thyroid, cricoid and arytenoid. Epiglottis guards the opening of the larynx, which prevents food from entering the larynx. Glottis refers to the vocal cords from the edge of the ventricle to the free edge of the vocal cords. Larynx is lined by mixture of squamous and pseudostratified ciliated columnar epithelium. True cords are covered by squamous epithelium.

INFLAMMATORY DISORDERS

Laryngitis is caused by viruses or bacteria, irritants, or overuse of the voice resulting in inflammation and edema of the vocal cords, with resultant hoarseness. Acute epiglottitis is usually caused by *Haemophilus influenzae* that may be life-threatening in young children. Acute laryngotracheobronchitis is acute inflammation of the larynx, trachea, and epiglottis most often caused by viral infection. It is potentially life-threatening in infants. Infant presents with harsh cough and inspiratory stridor.

TUMORS AND TUMOR-LIKE DISORDERS

VOCAL CORD NODULE

Vocal cord nodule is also known as **singer's nodule**. It is small reactive benign laryngeal polypoidal lesion usually induced by chronic irritation and localized to the true vocal cords.

Pathogenesis

Vocal cord nodule has been associated with excessive use of the voice and heavy cigarette smoking. Chronic cigarette smoking and excessive voice abuse injures, disrupts basement membrane and superficial layer of lamina propria resulting in hemorrhage in the stroma. Healing occurs by fibrosis and fibrin deposition.

Surgical Pathology: Vocal Cord Nodule
Gross Morphology
Macroscopically, vocal cord nodule appears as gray or white broad-based nodule or polyp.
Light Microscopy
▪ Vocal cord nodule is lined by hyperplastic squamous epithelium surrounding fibrovascular core composed of pinkish fibrin deposits, plump proliferating fibroblasts and blood vessels. ▪ Stroma is infiltrated by chronic inflammatory cells. ▪ Telangiectatic variant of vocal cord nodule is chiefly composed of dilated blood vessels. ▪ Gelatinous variant of vocal cord nodule shows fibrin deposits and thin blood vessels (Fig. 17.21).

SQUAMOUS CELL PAPILLOMA

Squamous cell papilloma arises around the true vocal cords. It is most often associated with low-risk HPV (6 and 11) infections. Children and adolescents may develop multiple lesions, i.e. juvenile laryngeal papillomatosis, that may extend into the trachea and bronchi leading to life-threatening state. Recurrence after tumor resection is common. It may rarely undergo malignant change.

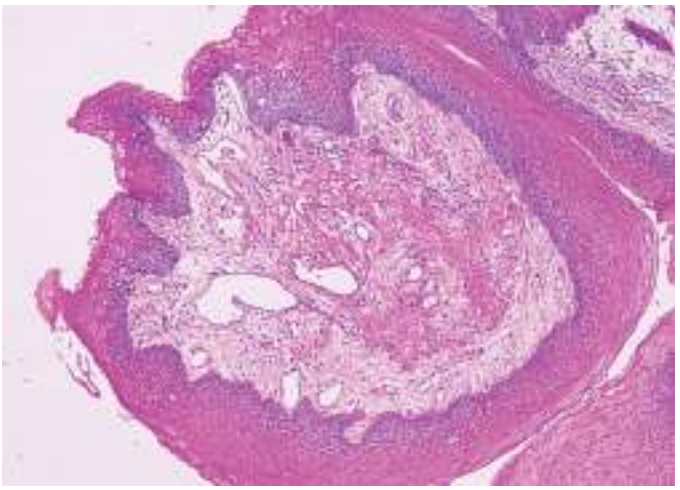


Fig. 17.21: Vocal cord nodule. Histologic examination shows hyperplastic squamous epithelium surrounding fibrovascular core composed of pinkish fibrin deposits, plump proliferating fibroblasts and blood vessels. Stroma is infiltrated by chronic inflammatory cells (100X).

Surgical Pathology: Squamous Papilloma
Gross Morphology
Tumor is exophytic, granular, friable, pink-red lesion.
Light Microscopy
▪ Tumor is composed of arborizing papillary fronds of squamous epithelium with some hyperplasia of the basal cells. ▪ Cells in the midlayer most often have clear cytoplasmic. ▪ Occasional suprabasal mitoses ciliated or columnar cells and koilocytes are common histologic findings in benign papilloma.

INTRAEPITHELIAL NEOPLASIA (CARCINOMA *IN SITU*)

Intraepithelial neoplasia is usually seen at the margins of invasive laryngeal carcinoma. It is characterized by variations in size and shape of dividing cells with mitotic activity arranged in disordered fashion with loss of cell maturation as cells progress to the surface, as a result of chronic irritation or inflammation.

Etiology

Tobacco smoking produces severe dysplasia (carcinoma *in situ*) in respiratory epithelium. It is potentially reversible if the patient stops tobacco smoking.

Diagnostic Criteria

Criterion for diagnosing intraepithelial neoplasia (carcinoma *in situ*) is the presence of atypia involving the epithelial covering of the vocal cord.

Clinical Features

Patient may present with hoarseness of voice as a result of slight reddening of vocal cords.

SQUAMOUS CELL CARCINOMA

Approximately 90% of malignant tumors of larynx are squamous cell carcinoma. Tumor most often arises from true vocal cords and affects 50–70 years of age. Men are more affected than women. Most cases develop squamous cell carcinoma of the glottic or supraglottic region. Patients are detected early. Squamous cell carcinoma of larynx arising from the vocal cord is shown in Fig. 17.22.

Risk Factors

Tobacco smoking appears to be main risk factor for development of laryngeal carcinoma. Excessive alcohol consumption is another risk factor.

Clinical Features

Patient presents with persistent hoarseness of voice, often associated with cervical lymphadenopathy.

Surgical Pathology: Squamous Cell Carcinoma of Larynx

Gross Morphology

Gross morphology of laryngeal squamous cell carcinoma is variable ranging from fungating, exophytic to endophytic, ulcerated tumors with raised edges (Fig. 17.23).

Light Microscopy

- **Keratinizing squamous cell carcinoma:** Tumor is composed of round to oval cells with abundant eosinophilic cytoplasm and prominent nucleoli arranged in nests and sheets and keratin pearls (Fig. 17.24).
- **Moderately differentiated carcinoma:** Tumor comprises pleomorphic cells with high nucleocytoplasmic ratio and moderate eosinophilic cytoplasm.
- **Poorly differentiated carcinoma:** Tumor is composed of small cells with less cytoplasm arranged singly or forming nests with marked mitotic activity.
- **Verrucous carcinoma:** Tumor is well differentiated nonmetastasizing variant of squamous cell carcinoma. Tumor appears as well circumscribed, warty exophytic growth with broad base white granular and friable mass. Tumor is composed of thick club-shaped papillae with broad pushing bases. There is absence of cytologic atypia and mitotic activity. Prognosis of this variant is excellent.
- **Spindle cell carcinoma:** Tumor is poorly differentiated carcinoma adopting spindle or sarcomatoid pattern. Patient develops polypoid mass with smooth ulcerated surface. Tumor is composed of sheets of spindle cells mimicking fibrosarcoma or malignant fibrous histiocytoma. There is presence of mitotic activity and necrosis.
- **Basaloid squamous cell carcinoma:** Tumor is a variant of squamous cell carcinoma. On gross examination, tumor shows central ulceration with thickening at edges. On histologic examination, tumor is composed of small basaloid cells.

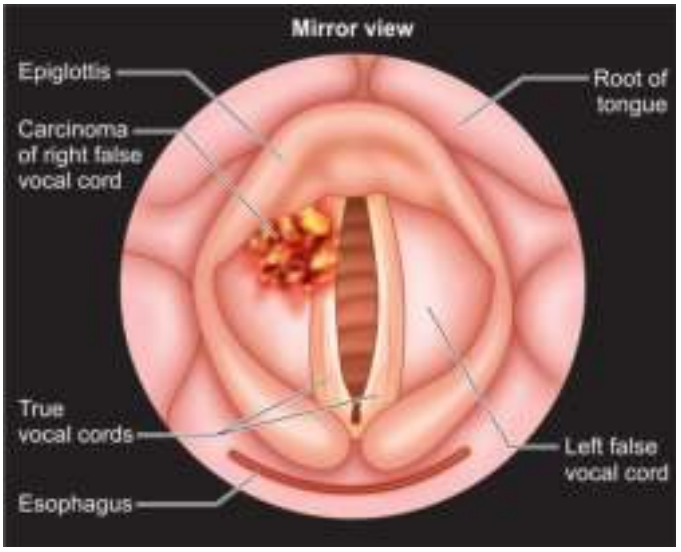


Fig. 17.22: Carcinoma of larynx. Tumor is arising from the vocal cord.

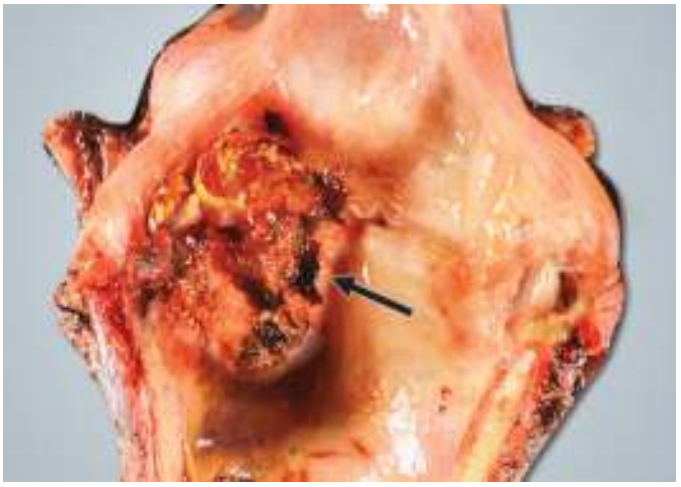


Fig. 17.23: Laryngeal carcinoma. Tumor shows fungating growth with ulceration (arrow).

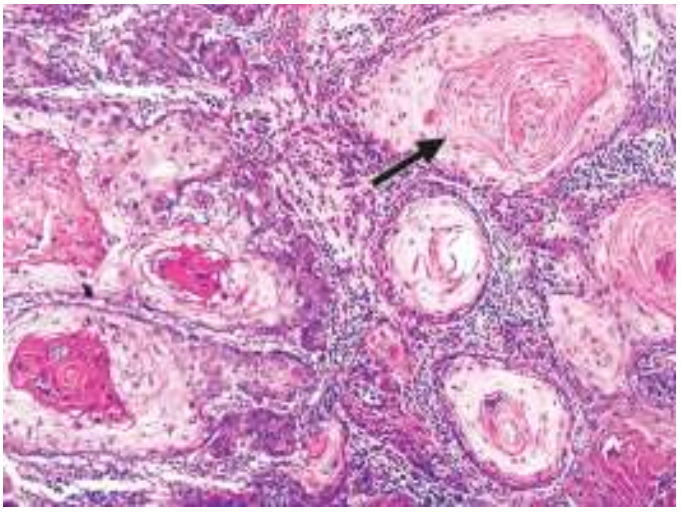


Fig. 17.24: Laryngeal keratinizing well differentiated squamous cell carcinoma. Tumor shows keratin pearls formation (arrow) (400X).

Table 17.5 Classification of laryngeal carcinoma based on location

Location	Frequency	Clinical Behavior	5-Year Survival Rate
Glottic region	60–65%	▪ Originating from true vocal cords (anterior portion) localized for long duration ▪ Cervical lymph node metastases late ▪ Tumor is treated by irradiation or surgical removal of vocal cords curative	80%
Supraglottic region	30–35%	▪ Tumor involving false vocal cord, laryngeal ventricle and epiglottis (33%) ▪ Surgical removal of tumor possible without permanent loss of voice ▪ Cervical lymph node metastases in 40% of cases	65%
Transglottic region	<5%	▪ Tumor crossing laryngeal ventricle ▪ Cervical lymph nodes frequently involved ▪ Total laryngectomy together with prophylactic cervical lymph node dissection curative	50%
Subglottic region	<5%	▪ Tumor arising from true vocal cord extending 1 cm or more into the subglottis region ▪ Thyroid gland and cricoid cartilage involved ▪ Cervical and paratracheal lymph nodes involved in 50% of cases	40%

Classification

Classification of laryngeal carcinoma is based on the location in decreased frequency: glottic, supraglottic, transglottic and subglottic regions (Table 17.5).

Prognosis

Prognosis is excellent in carcinoma arising from true vocal cord. Patient with carcinoma involving supraglottis and subglottis is less common but has poor prognosis.

DISORDERS OF NECK

NECK SWELLINGS: OVERVIEW

There are many causes of swellings in the neck region, which include lymphadenopathy, cysts, vascular masses, thyroid gland masses and salivary gland masses. The most common swellings are enlarged lymph nodes. Neck swelling may be congenital or acquired, solid or cystic, single or multiple, benign or malignant.

Primary and secondary tumors in the neck are shown in Fig. 17.25. Lateral neck swellings are given in Table 17.6. Midline neck swellings are given in Table 17.7.

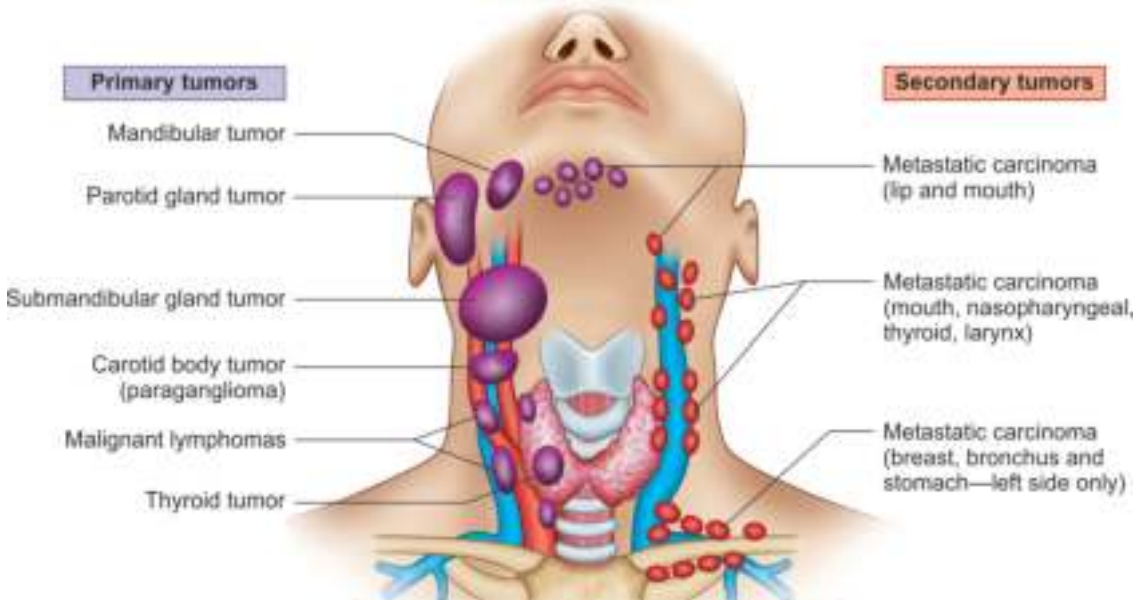


Fig. 17.25: Primary and secondary tumors in the neck.

Table 17.6 Lateral neck swellings

Nature of Swelling derived from Tissue and its Contents	Differential Diagnosis	
Solid swelling		
Glandular tissue	▪ Lymphadenitis ▪ Hodgkin disease ▪ Non-Hodgkin lymphoma ▪ Metastases in lymph nodes	▪ Thyroid neoplasms ▪ Multinodular goiter ▪ Thyroiditis ▪ Parathyroid adenoma
Blood vessels	▪ Carotid body tumor (paraganglioma) ▪ Glomus jugulare (tumor of the temporal bone involving middle and inner ear structures)	
Subcutaneous adipose tissue	Lipoma	
Nerves	▪ Schwannoma	▪ Neurofibroma
Sternocleidomastoid muscle	Organized hematoma of infants	
Bone	Cervical rib	
Cystic swelling		
Fluid in swellings	▪ Thyroglossal cyst ▪ Branchial cyst	▪ Epidermoid cyst ▪ Sebaceous cyst
Air in swellings	▪ Pharyngeal diverticulum ▪ Laryngocele	▪ Pneumatocele
Abscess (pus) in swellings	▪ Cold abscess (tubercular lymphadenitis) ▪ Parapharyngeal abscess	▪ Parotid abscess
Blood vessel swellings	▪ Hemangioma/malformation ▪ Carotid artery aneurysm ▪ Subclavian artery aneurysm	▪ Cystic hygroma (cystic lymphangioma in infants and children)

Table 17.7 Midline neck swellings

Nature of Swelling derived from Tissue and its Contents	Differential Diagnosis	
Solid swelling		
Glandular tissue	▪ Lymphadenitis (submental, prelaryngeal or pretracheal regions)	▪ Nodule in isthmus of thyroid gland ▪ Median ectopic thyroid tissue
Subcutaneous tissue	Lipoma in burns' space (suprasternal notch)	
Cystic swelling		
Glandular tissue	Lymphadenitis in submental region	
Fluid in swellings	▪ Thyroid gland cyst in the isthmus ▪ Thyroglossal cyst ▪ Dermoid cyst (sublingual or suprasternal regions)	▪ Sebaceous cyst ▪ Subhyoid bursa
Abscess (pus) in swellings	▪ Cold abscess (tubercular lymphadenitis)	▪ Pyogenic abscess in parapharyngeal and salivary glands
Blood vessel swellings	▪ Hemangioma/malformation ▪ Carotid artery aneurysm	▪ Subclavian artery aneurysm

AGE GROUP

Neck swellings are encountered in children ranging from infancy to adolescence, and adults with an extensive differential diagnosis ranging from congenital (cysts), and acquired (inflammatory/infective to neoplastic) diseases. Lymphadenopathy most often occurs in the neck region. The primary concern in adults with a persistent neck mass is malignancy.

HISTORY OF A NECK LUMP

History of duration of lump in neck region is most important. Seven days duration indicates infection, which results in reactive lymphadenitis. There is long history of seven weeks to months in patient with tumor. Congenital or developmental swellings occur within seven years of age. Past history of thyroid carcinoma, irradiation and previous scar must be enquired.

IMAGING TECHNIQUES

Neck swellings are assessed by imaging techniques, which include plain radiograph of neck (AP and lateral view), barium studies, sialography, laryngogram, carotid angiography and MRI studies. Endoscopic biopsy is oral cavity, nose and nasopharynx.

CERVICAL LYMPHADENOPATHY

The most common swellings in cervical region are enlarged lymph nodes. These are caused by bacterial or viral infections, lymphomas and secondary metastatic deposits. Inflammatory lymphadenopathy is most often painful, firm and mobile.

- Malignant lymph nodes are painless and hard, which may be located in the midline or lateral side of neck. Delphin lymph nodes located along thyrohyoid membrane are enlarged in thyroid carcinoma.
- Cervical lymph nodes showing draining areas and enlargement due to various disorders are shown in Fig. 17.26. Cervical lymph node swellings are given in Table 17.8.

BRANCHIAL CYST

Branchial cyst is also known as **lymphoepithelial cyst** present as solitary nodule anterior to sterno-cleidomastoid muscle. It is considered to arise from remnants of second branchial arch. It most often occurs in young adults. On clinical examination, branchial cyst measures 2–5 cm in diameter. Similar lesions may be encountered in parotid gland or oral cavity beneath tongue. It may rarely undergo malignant transformation.

Surgical Pathology: Branchial Cyst
Gross Morphology
▪ Cyst is well circumscribed measuring 2–5 cm in diameter. ▪ Cut section of cyst may contain clear, watery, mucoid or granular debris.
Light Microscopy
▪ Fibrous cyst wall is lined by stratified epithelium or pseudo-stratified columnar epithelium. ▪ Cyst wall contains lymphoid tissue forming germinal centers.

THYROGLOSSAL CYST

A thyroglossal cyst occurs due to persistence of thyroglossal duct. During normal development, thyroid gland descends from the base of the tongue to its final position in the neck. Thyroglossal cyst, a remnant of the thyroglossal duct is the most common thyroid anomaly. It may occur anywhere along the path of thyroid gland descent. It is midline cystic swelling that is close to or within the hyoid bone.

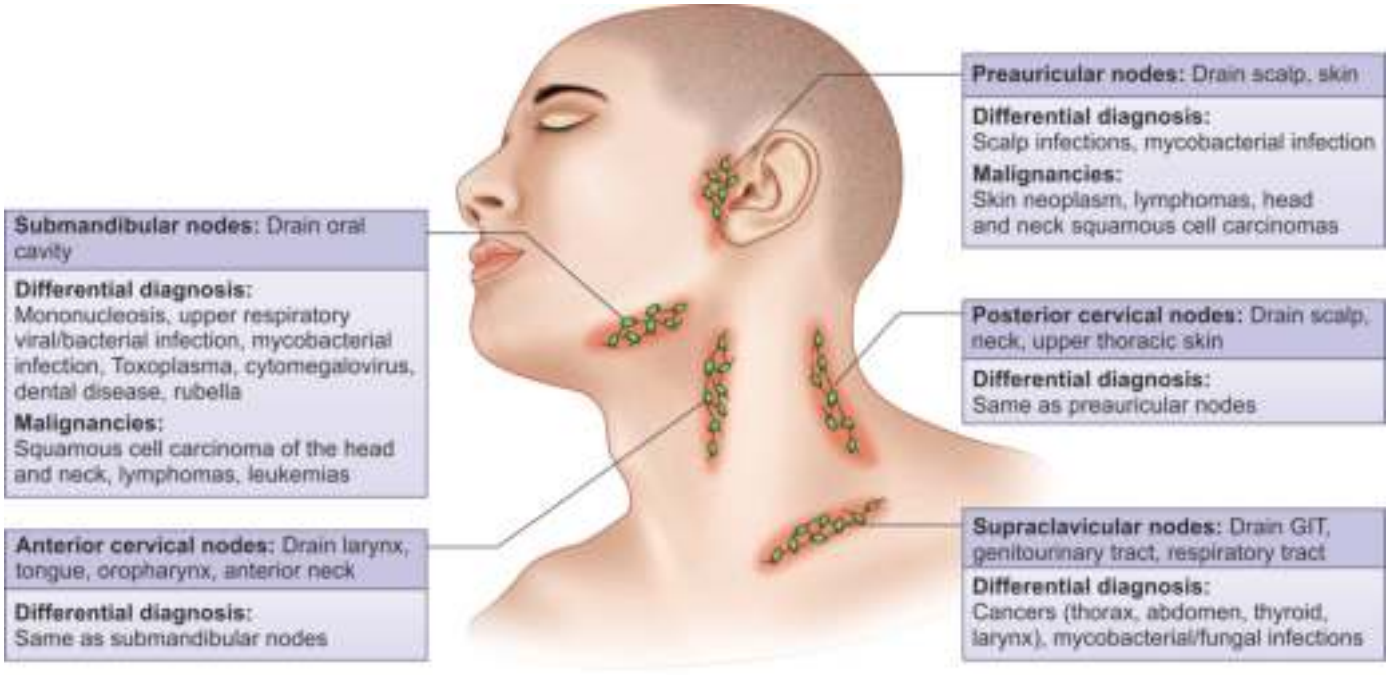


Fig. 17.26: Cervical lymph nodes showing draining areas and enlargement due to various disorders.

Table 17.8 Cervical lymph node swellings

Lymph Node Groups	Areas Drained by Lymph Node Group	Differential Diagnosis
Preauricular nodes	Scalp and skin	▪ Scalp infections, mycobacterial infections ▪ Skin neoplasms, Hodgkin's disease, NHL, SCC of head and neck
Posterior cervical nodes	Scalp, neck skin, thoracic skin	▪ Scalp infections, mycobacterial infections ▪ Skin neoplasms, Hodgkin's disease, NHL, SCC of head and neck
Supraclavicular nodes	Gastrointestinal tract, pulmonary and genitourinary systems	▪ Cancers of thorax, abdomen, thyroid and larynx ▪ Tubercular lymphadenitis ▪ Fungal infections
Submandibular nodes	Oral cavity	▪ Infectious mononucleosis, upper respiratory tract infections, tubercular lymphadenitis, Toxoplasma, cytomegalovirus, rubella, dental disease ▪ Squamous cell carcinoma of head and neck, leukemia, lymphomas
Anterior cervical nodes	Larynx, tongue, oropharynx, anterior neck	▪ Infectious mononucleosis, upper respiratory tract infections, tubercular lymphadenitis, Toxoplasma, cytomegalovirus, rubella, dental disease ▪ Squamous cell carcinoma of head and neck, leukemia, lymphomas

- Failure to surgical resection of hyoid bone often results in persistence of thyroglossal cyst.
- Patient presents with dysphonia, sore throat and midline swelling evident during adolescence and pregnancy. Patient does not develop endocrine complications. Surgery with removal of the proximal duct along with hyoid bone cures it.

Surgical Pathology: Thyroglossal Cyst

Light Microscopy

- Because thyroglossal cysts develop from a hollow tube, most of them do not show thyroid tissue on histopathologic examination.
- Thyroid tissue in surgical specimen is demonstrated in 5% of cases and 40% in serial sectioning. Otherwise, the lesion is lined by squamous or respiratory type of epithelium without a muscular wall.

PARAGANGLIOMA (CAROTID BODY TUMOR)

Paraganglia are clusters of neuroendocrine cells associated with sympathetic or parasympathetic nervous system.

- Cervical paraganglioma is a painless slow-growing tumor arising from the carotid body in the adventitia of the posteromedial aspect of the carotid artery bifurcation. There is higher incidence of paraganglioma in persons residing at higher altitude. It most often occurs in 50 years persons.
- Patient presents with firm, rubbery, mobile side to side and pulsatile tumor. It may decrease in size on carotid artery compression. Tumor recurs after incomplete surgical resection. Despite its benign nature, it may metastasize to regional lymph nodes and distant organs.
- Carotid angiography shows widening of carotid artery bifurcation. CT scan and MRI determine the extent of tumor.

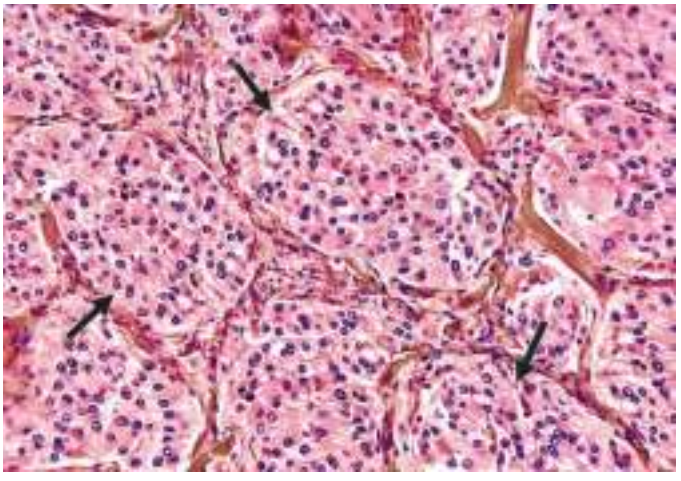


Fig. 17.27: Paraganglioma (carotid body tumor) shows clusters of large eosinophilic tumor cells separated by stroma containing elongated sustentacular cells (400X).

Surgical Pathology: Cervical Paraganglioma

Gross Morphology

- Tumor is well circumscribed mass with a fibrous pseudocapsule measuring 1–6 cm in diameter.
- Cut surface reveals gray-tan to pale brown color.

Light Microscopy

- Tumor is composed of uniform pale staining cytoplasm with finely granular nuclear chromatin surrounded by fibrovascular network.
- Compressed spindle-shaped cells are present at the periphery of nests of tumor cells (Fig. 17.27).

Immunohistochemistry Technique

Panel of markers is used to analyze expression in paraganglioma (chief cells) on histopathologic sections.

Markers	Expression
▪ Chromogranin	▪ Positive
▪ Synaptophysin	▪ Positive
▪ Neuron-specific enolase	▪ Positive
▪ CD56	▪ Positive
▪ CD57	▪ Positive

Compressed spindle-shaped cells at the periphery of nests of tumor cells show S-100 protein positivity.

CHAPTER

18

Respiratory System

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

- Respiratory system: overview
  - Anatomy
  - Physiology

PNEUMONIAS AND LUNG ABSCESS

- Pneumonias: overview
  - Anatomical classification
  - Etiologic classification
- Pneumonias
  - Lobar pneumonia
  - Bronchopneumonia
  - Interstitial pneumonia
  - Opportunistic pulmonary infections
- Lung abscess

TUBERCULOSIS

- Pulmonary and extrapulmonary tuberculosis
  - Mycobacterium tubercle bacillus
  - Risk factors
    - Tubercular infection and disease
  - Pathogenesis
- Tuberculosis: categories
  - Primary tuberculosis (primary complex)
  - Secondary tuberculosis
  - Tuberculosis in HIV cases

CHRONIC OBSTRUCTIVE PULMONARY DISEASES (COPDs)

- COPDs: overview and examples
  - Chronic bronchitis
  - Emphysema
    - Classification of emphysema
      - * Centriacinar emphysema
      - * Panacinar emphysema
      - * Distal acinar (paraseptal) emphysema
      - * Irregular emphysema
      - * Interstitial emphysema
  - Bronchiectasis
  - Bronchial asthma

RESTRICTIVE PULMONARY DISEASES

- Acute restrictive pulmonary diseases
  - Adult respiratory distress syndrome
  - Neonatal respiratory distress syndrome
  - Severe respiratory distress syndrome
  - Diffuse alveolar damage
- Chronic restrictive pulmonary diseases
  - Pneumoconiosis
    - Coal worker's pneumoconiosis
    - Asbestosis
    - Berylliosis
  - Drug-induced interstitial pulmonary fibrosis
  - Radiation-induced interstitial pulmonary fibrosis
  - Idiopathic pulmonary fibrosis
  - Sarcoidosis
  - Collagen vascular disease
  - Rheumatoid arthritis
  - Eosinophilic granuloma
  - Eosinophilic pneumonia
  - Hypersensitivity pneumonitis
  - Goodpasture syndrome
  - Byssinosis
  - Silo-filler's disease
  - Lymphangioleiomyomatosis
  - Pulmonary alveolar proteinosis

PNEUMOTHORAX AND ATELECTASIS LUNG

- Pneumothorax
  - Open pneumothorax
  - Closed pneumothorax
  - Tension pneumothorax
- Atelectasis of lung (pulmonary collapse)
  - Etiopathogenesis
    - Acquired atelectasis of lung
    - Atelectasis neonatorum of lung
  - Atelectasis: types
    - Obstructive atelectasis of lung
    - Compressive atelectasis of lung
    - Patchy atelectasis of lung
    - Contraction atelectasis of lung

PULMONARY VASCULAR DISORDERS

- Pulmonary thromboembolism
- Fat embolism syndrome
- Pulmonary hypertension
- Pulmonary edema
- Pulmonary vasculitis

NEOPLASTIC DISORDERS OF LUNG

- Benign tumors of lung
  - Pulmonary hamartoma
  - Bronchopulmonary carcinoids
- Primary malignant tumors of lung: overview
  - WHO classification of lung tumors
- Primary lung carcinomas
  - Small cell lung carcinoma
  - Squamous cell lung carcinoma
  - Lung adenocarcinoma
  - Large cell lung carcinoma
  - Adenosquamous lung carcinoma
  - Pleomorphic lung carcinoma
  - Carcinosarcoma of lung
- Tumors metastasizing to lungs
- Diagnostic approach of lung tumors
  - Imaging techniques
  - Other diagnostic modalitis
    - Fiberoptic bronchoscopy
    - Sputum examination
    - Bronchoalveolar lavage
    - Bronchopulmonary biopsy
    - Surgical pathology (gross morphology, light microscopy, histochemistry, immunohistochemistry technique)
    - Serum tumor markers in primary lung cancer
    - Serum tumor markers in metastatic lung cancer

PLEURAL DISEASES

- Non-neoplastic pleural disorders
  - Pleural effusion
  - Pleuritis
  - Pleural fibrosis
    - Pleural plaques
    - Diffuse fibrosis of pleural visceral layer
- Malignant pleural tumors
  - Malignant mesothelioma
  - Primary pleural effusion lymphoma
  - Pyothorax effusion lymphoma

RESPIRATORY SYSTEM: OVERVIEW

ANATOMY

Respiratory system comprises nose, pharynx, larynx, trachea, bronchi, bronchioles, terminal bronchioles and lungs. Major functions of the lungs are to supply oxygen (O_2) to the tissues and remove carbon dioxide (CO_2) from blood.

The respiratory epithelium is derived from endoderm. With the exception of the pharynx, epiglottis and vocal cords, the respiratory tract is lined by specialized ciliated mucus secreting epithelium. Structure of respiratory tract is shown in Fig. 18.1A and B.

Nose

Nose warms, moistens, and filters air and functions in olfaction and speech. Newborn with choanal atresia cannot breathe through the nose. Allergic polyps are most common polyps in the nasal cavity. In children with nasal polyps, sweat test must be done to rule out cystic fibrosis.

Pharynx

Pharynx comprises nasopharynx, oropharynx, and laryngopharynx lined by mucous membrane. The nasopharynx participates in respiration. The oropharynx and laryngopharynx function both in digestion and respiration.

Larynx

Larynx contains the thyroid cartilage (Adam's apple). The epiglottis guards the opening of the larynx, which prevents food from entering the larynx; the cricoid cartilage, which connects the larynx and trachea; and the paired arytenoids, corniculate, and cuneiform cartilages.

Trachea

Trachea measures 12 cm in length and 2.5 cm in diameter.

- Presence of incomplete (C-shaped) ring of hyaline cartilage prevents collapsing of trachea. It is lined with pseudostratified ciliated columnar epithelium. The carina is in located at approximately the T4 level.
- Two methods of bypassing obstructions in the airways are tracheostomy by making opening into trachea and intubation by putting tube into trachea.

Bronchi

The bronchial tree consists of the primary bronchi, secondary bronchi, tertiary bronchi, bronchioles, and terminal bronchioles.

- The walls of bronchi contain discontinuous foci of cartilage and seromucinous glands. Walls of bronchi contain plates of decreasing cartilage and increasing smooth muscles.
- Right bronchus is more vertical than left bronchus; hence foreign body enters more frequently in the right bronchus.

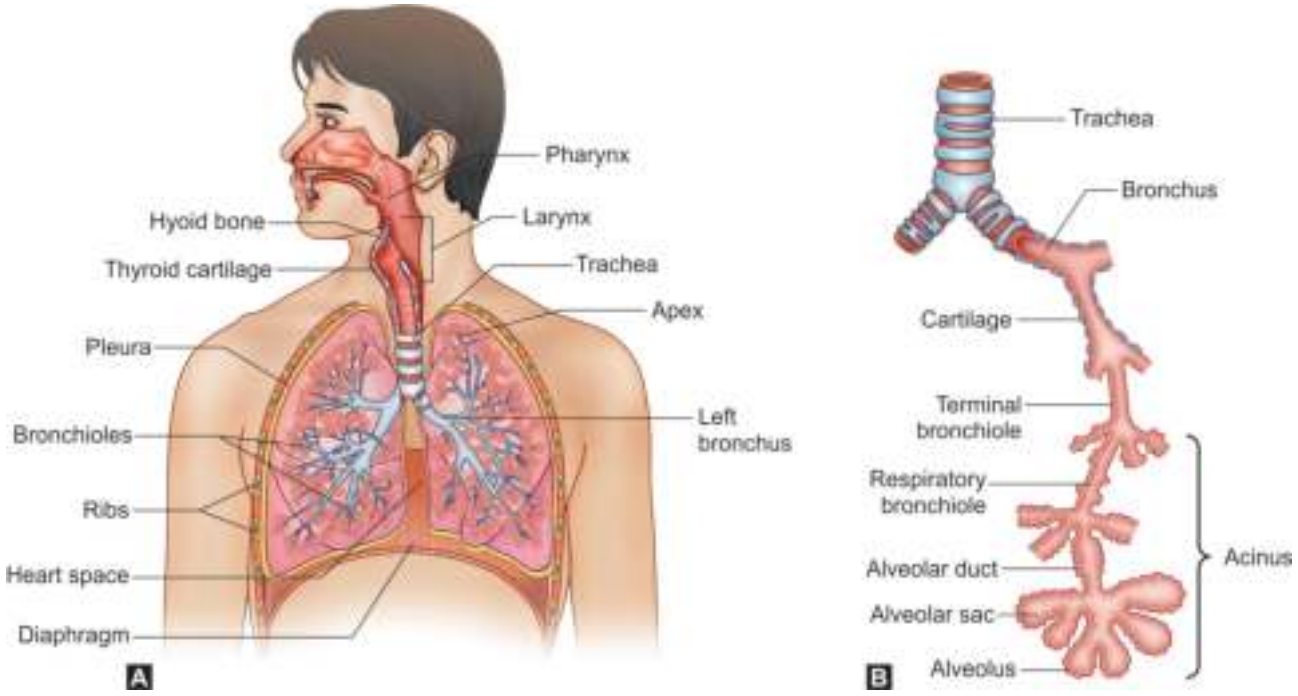


Fig. 18.1A and B: Structure of upper and lower respiratory tract.

- **Bronchogram** is a radiograph of the bronchial tree after introduction of an opaque contrast medium usually containing iodine.

Bronchioles

Bronchioles lack cartilage and submucous glands. The bronchioles are lined by ciliated columnar epithelium which contain Clara cells participating in synthesis of proteinaceous fluid. Terminal bronchiole divides to form respiratory bronchiole alveolar ducts and delicate alveoli.

Lungs

The lungs are a pair of organs surrounding mediastinum and in close proximity to the heart and major blood vessels. The lungs extend from above the clavicles to T1, T2 vertebral level with full inspiration. The right lung has 3 lobes, whereas the left lung has 2 lobes.

- **Pleura:** Pleural membrane covering lungs consists of outer parietal pleura and the deep visceral pleura derived from mesoderm. Pleural cavity contains 10–12 ml of pleural fluid low in protein content.
- **Root (hilum):** Root of lung consists of primary bronchus, pulmonary artery, two pulmonary veins and corresponding lymphatic channels. Each lung has a dual blood supply *via* the pulmonary and bronchial arteries. Bronchial arteries protect lungs from infarction. Dyspnea and tachypnea are common symptoms in a case of pulmonary infarction.
- **Bronchopulmonary segment:** It is the smallest functional unit of the lungs. Left lung consists of 8 bronchopulmonary segments and 10 bronchopulmonary segments in the right lung. A single bronchopulmonary segment can be resected when disease is confined to one segment. Each bronchopulmonary segment consists of lobules, which contain lymphatic channels, veins, arterioles, terminal bronchioles, respiratory bronchioles, alveolar ducts, and alveoli.
- **Alveoli:** Each lung consists of approximately 150 million alveoli. Alveolar walls consist of thin, delicate type 1 alveolar cells, i.e. pneumocytes lined by squamous cells, type 2 pneumocytes, and alveolar macrophages. Type 2 pneumocytes secrete surfactant dipalmitoylphosphatidylcholine, which decreases the surface tension exerted by alveolar fluid. Alveolar macrophages present in the lungs participate in phagocytosis. There are no lymphatic channels in the alveoli.
- **Pulmonary lymphatic drainage:** Each lung consists of superficial lymphatic plexus beneath visceral pleura and deep lymphatic plexuses located within the submucosa of the bronchi. The two plexuses communicate at the hilum, ending in bronchopulmonary lymph nodes which drain to tracheobronchial lymph nodes, which in turn drain to the left subclavian vein. Lung carcinoma generally spreads by the lymphatics to the hilar and mediastinal lymph nodes and then out of the thorax *via* the supraclavicular cervical lymph nodes. Axillary lymph nodes are generally not involved in lung cancer due to their lymphatic range.
- **Nerve supply:** The lungs are innervated by the sympathetic and parasympathetic supply.

Clinical Pearls: Applied Anatomy of Lungs

- **Compression of structures:** Direct extension of lung tumor itself or enlargement of adjacent lymph nodes may compress structures in close proximity to the lungs.
- **Hoarseness of voice:** Squamous cell lung carcinoma usually involves recurrent laryngeal nerve supplying left vocal cord leading to hoarseness of voice.
- **Horner's syndrome**: Pancoast tumor (usually squamous cell lung carcinoma) involves sympathetic chain/ganglion leading to Horner's syndrome. Horner's syndrome is characterized by drooping of upper eyelid, sunken eyes, constriction of pupil and loss of sweating on the affected side.
- **Diaphragm paresis:** Lung carcinoma involving phrenic nerve may lead to hemidiaphragm paresis.

PHYSIOLOGY

Exchange of Gases

Exchange of gases occurs across the alveolar–capillary (respiratory) membrane, which consists of 0.2 mm thick alveolar epithelium, epithelial basement membrane, thin interstitial space, capillary basement membrane and capillary endothelial cells.

- Alveoli are the primary sites of exchange of O_2 and CO_2 gases. Exchange of gases also occurs between blood and tissues by simple diffusion based on pressure/concentration gradient.
- Solubility of the gases and thickness of the respiratory membranes involved in diffusion are also important factors that can affect the rate of diffusion of gases. The solubility of CO_2 is 20–25 times higher than that of O_2 (Figs 18.2 to 18.4).

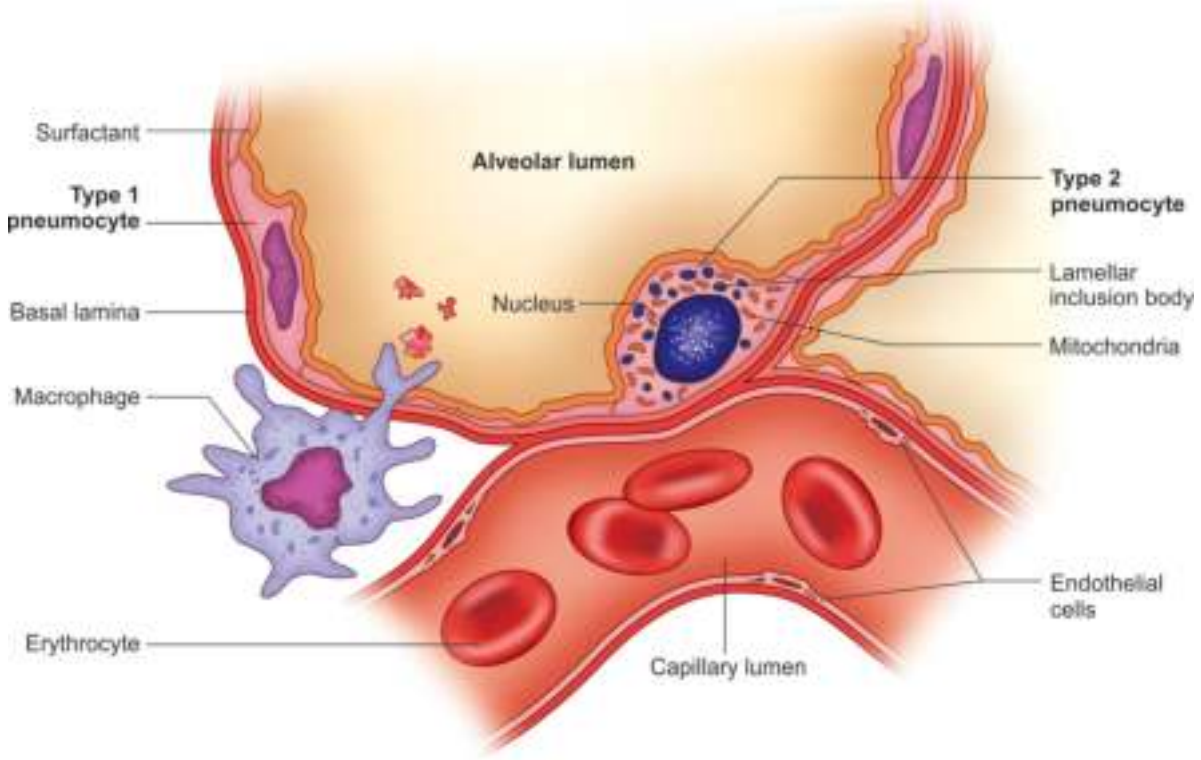


Fig. 18.2: Structure of alveolar–capillary (respiratory) membrane. Alveolus is lined by epithelial cells type 1 and type 2 pneumocytes. Alveolar basement membrane is thin on one side and widened where it is continuous with the interstitial space. Gas exchange occurs across the alveolar–capillary (respiratory) membranes. Type 2 pneumocytes secrete surfactant dipalmitoyl phosphatidylcholine, which decreases surface tension exerted by alveolar fluid.

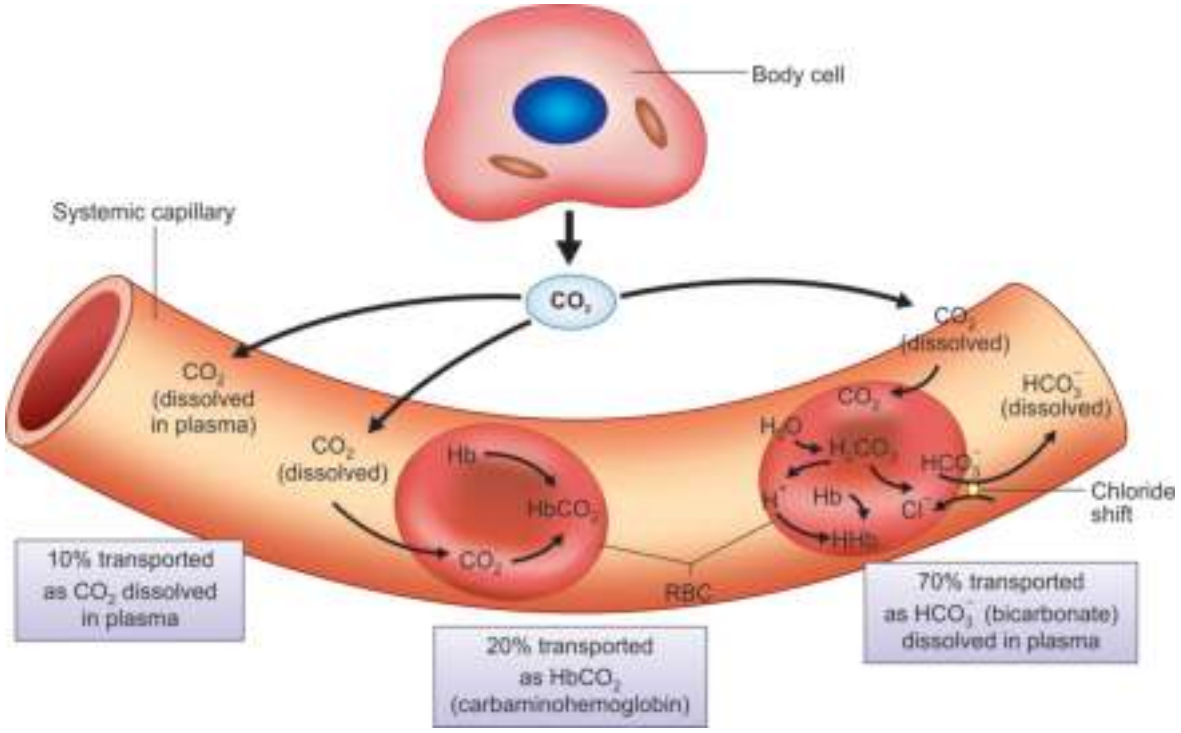


Fig. 18.3: Transport of CO_2 in blood. CO_2 circulates in blood vessel in three forms. Approximately 10% of CO_2 dissolved in plasma, 20% transported by red blood cells in the form of carbaminohemoglobin and 70% of bicarbonate dissolved in plasma.

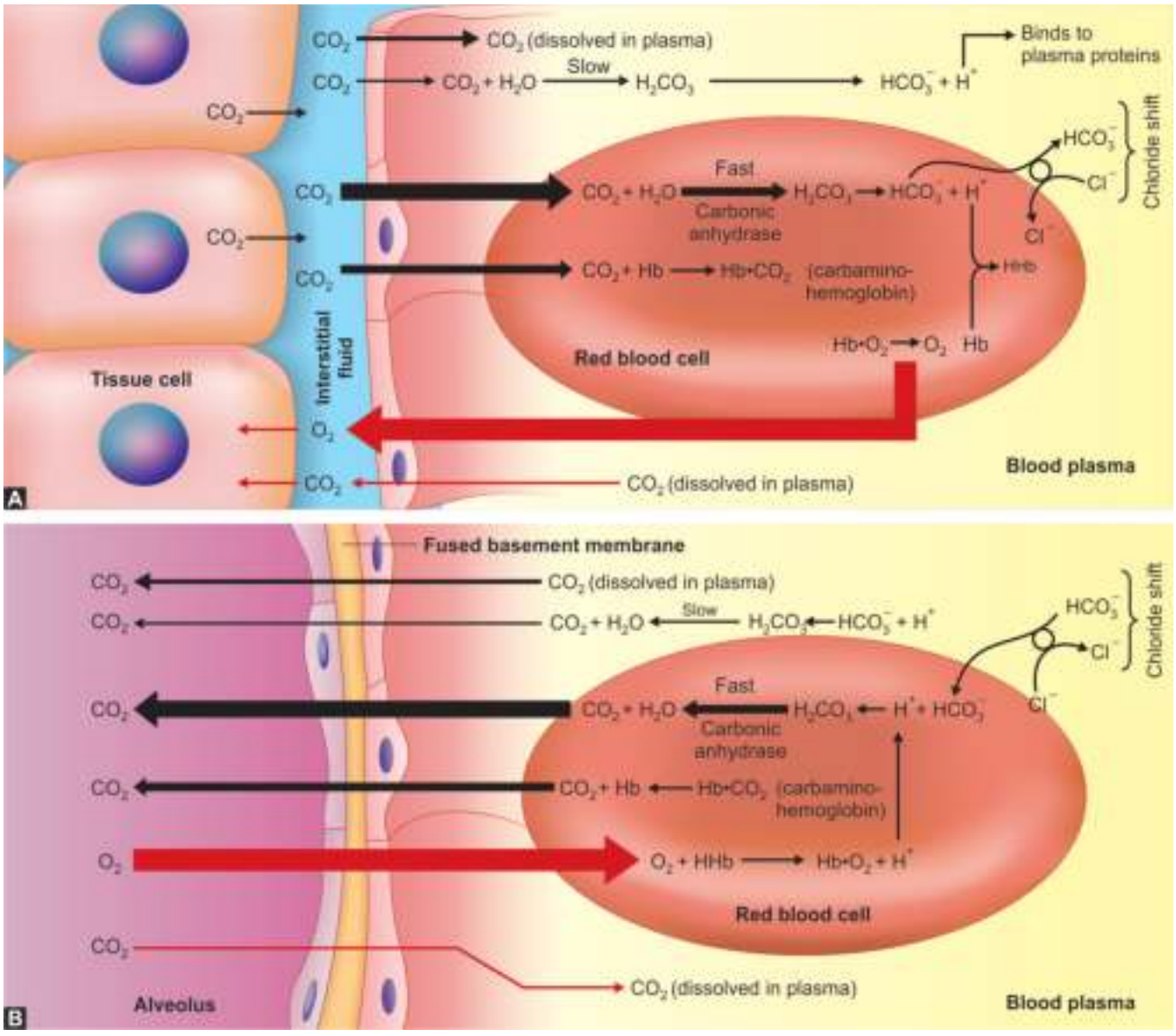


Fig. 18.4A and B: Exchange of gases in lung and tissue. It includes pulmonary ventilation, gas exchange in the lungs, transport of O_2 gas by the blood to tissues, gas exchange in the tissues. Transport of CO_2 gas by the blood to the heart and then to the lungs for oxygenation.

Partial Pressure of Gas

The partial pressure of gas is the pressure exerted by that gas in mixture of gases. It is represented as pO_2, pCO_2 and pN_2 for oxygen, carbon dioxide and nitrogen, respectively. Arterial blood gas, pO_2 and pCO_2, is equal to the alveolar pO_2 and pCO_2.

Pulmonary Volumes

Spirometer is an instrument used to measure volume of air in lungs. Alveolar ventilation is the volume of inspired air that reaches the alveoli.

Lung Capacities

Lung capacities are the sum of two or more volumes that include inspiratory, functional, residual, vital and total lung capacity. Lung volumes and capacities are given in Table 18.1.

Respiratory Disorders

Respiratory disorders affecting exchange of gases are caused by cystic fibrosis, α_1-antitrypsin deficiency, tobacco smoking, air pollution, pneumoconiosis and infectious agents. These disorders affect the respiratory airways, interstitial tissue and vasculature of lungs. Patient develops chronic obstructive diseases, acute lung injury, pulmonary infections, diffuse interstitial tissue infiltrative disorders, and lung tumors. General categories of pulmonary disease are given in Table 18.2.

Table 18.1 Lung volumes and capacities

Volume	Definition	Average Volumes (ml)
Tidal volume	Tidal volume is the amount of air moved in and out of the lungs in quite, relaxed breathing	500
Residual volume	The amount of air that remains in the lungs after maximum exhalation	1200
Inspiratory reserve volume	The additional amount of air that can be inhaled by force after a normal inhalation	2500–3000
Expiratory reserve volume	The additional amount of air that can be exhaled by force after a normal exhalation	1100–1200
Vital capacity	The volume of air that can be breathed out from the lungs by maximum exhalation after maximum inhalation	4000
Functional residual capacity	The amount of air remained in the lungs after normal exhalation	2500
Total lung capacity	The total volume of air that can be contained in the lungs after maximum inhalation	5200

Anatomical dead space refers to amount of air present in respiratory tract not participating in exchange of gases.

Table 18.2 General categories of pulmonary disease

Category	Subcategory
Infectious lung diseases	▪ Community acquired typical pneumonia ▪ Community acquired atypical pneumonia ▪ Nosocomial pneumonia ▪ Aspiration pneumonia ▪ Necrotizing pneumonia ▪ Pneumonia in immunocompromised persons
Obstructive lung diseases	▪ Bronchial asthma ▪ Chronic bronchitis ▪ Bronchiectasis ▪ Emphysema
Restrictive lung diseases	▪ Autoimmune (e.g. systemic lupus erythematosus, Wegener granulomatosis, rheumatoid arthritis) ▪ Idiopathic pneumonias, e.g. usual interstitial pneumonia (UIP), desquamative interstitial pneumonia (DIP) ▪ Occupational related, e.g. asbestosis, silica-induced lung disease, coal-induced lung disease ▪ Drug-related, e.g. bleomycin, busulfan, amiodarone, methotrexate
Neoplastic lung diseases	▪ Small cell lung carcinoma ▪ Squamous cell lung carcinoma ▪ Lung adenocarcinoma ▪ Large cell lung carcinoma ▪ Bronchopulmonary carcinoid tumors

PNEUMONIAS AND LUNG ABSCESS

PNEUMONIAS: OVERVIEW

Pneumonia is an inflammatory process of the lungs commonly caused by infectious agents (i.e. bacteria, viruses, fungi or *Pneumocystis jiroveci*), chemicals, and inhalation of secretions and aspiration of material.

Predisposing factors for pneumonias include loss of suppression of the cough reflex (i.e. cerebral stroke, dementia, Parkinson's disease), injury to mucociliary apparatus due to tobacco smoking, interference in the phagocytosis and bactericidal activity of alveolar macrophages, pulmonary congestion in heart diseases, chronic pulmonary obstructive disease), accumulation of secretions, frequent suction, immunosuppression, liver cirrhosis, diabetes mellitus and recent respiratory tract infections (Table 18.3).

Table 18.3 Type of immune deficiency determining the organisms responsible for pneumonia

Defects	Organisms
Neutrophil defects	▪ Klebsiella ▪ Aspergillus
Antibody defects	▪ *Haemophilus influenzae* ▪ Pneumococci
Cell-mediated immune defects	▪ Fungi ▪ *Pneumocystis carinii* ▪ Mycobacterium tubercle bacilli ▪ Measles virus ▪ Herpesvirus ▪ Cytomegalovirus

Pathology Pearls: Classification of Pneumonias

Pneumonias are classified as community acquired or nosocomial (hospital acquired pneumonias) or aspiration pneumonia.

- **Community acquired pneumonia:** It is the most common form of pneumonia caused by bacterial infection or a complication of flu.
- **Nosocomial or hospital acquired pneumonias:** Hospital acquired pneumonias are often fatal, in hospitalized patients suffering from serious, debilitating diseases. Patient develops infection within 48 hours being hospitalized or other health care settings. Respirator is most common source of *Pseudomonas aeruginosa* causing nosocomial pneumonia. *Escherichia coli* and *Staphylococcus aureus* may also be underlying cause. Endotoxins produced by these organisms play an important role in pulmonary infection.
- **Aspiration pneumonia:** This infection occurs, when a person accidentally inhales food, liquid or vomitus into the lungs. There is increased risk of aspiration pneumonia in persons with dysphagia or with a weakened cough reflex. Chest radiograph is gold standard for diagnosing pneumonia.

ANATOMICAL CLASSIFICATION

Pneumonias are classified according to etiology (i.e. bacteria, viruses, fungi) and involvement of the pulmonary lobes (i.e. lobar, bronchopneumonia and interstitial pneumonias). More than one type of pathogen may be present that makes diagnosis and treatment, a challenge. There are three morphologic and clinical patterns of pneumonias: lobar pneumonia, bronchopneumonia, and interstitial pneumonia (Fig. 18.5A to C). Clinical patterns of pneumonia and common causative organisms are given in Table 18.4.

- **Lobar pneumonia:** It is restricted to one lobe of the lungs. Eventually, the whole lobe may be affected.
- **Bronchopneumonia:** It starts as bronchial inflammation and extends to surrounding alveoli involving one or more lobules. It shows patchy and focal reddish-gray consolidation in relation to bronchi or bronchioles filled with pus (microabscesses) at the base of both lungs. Consolidation at the base of

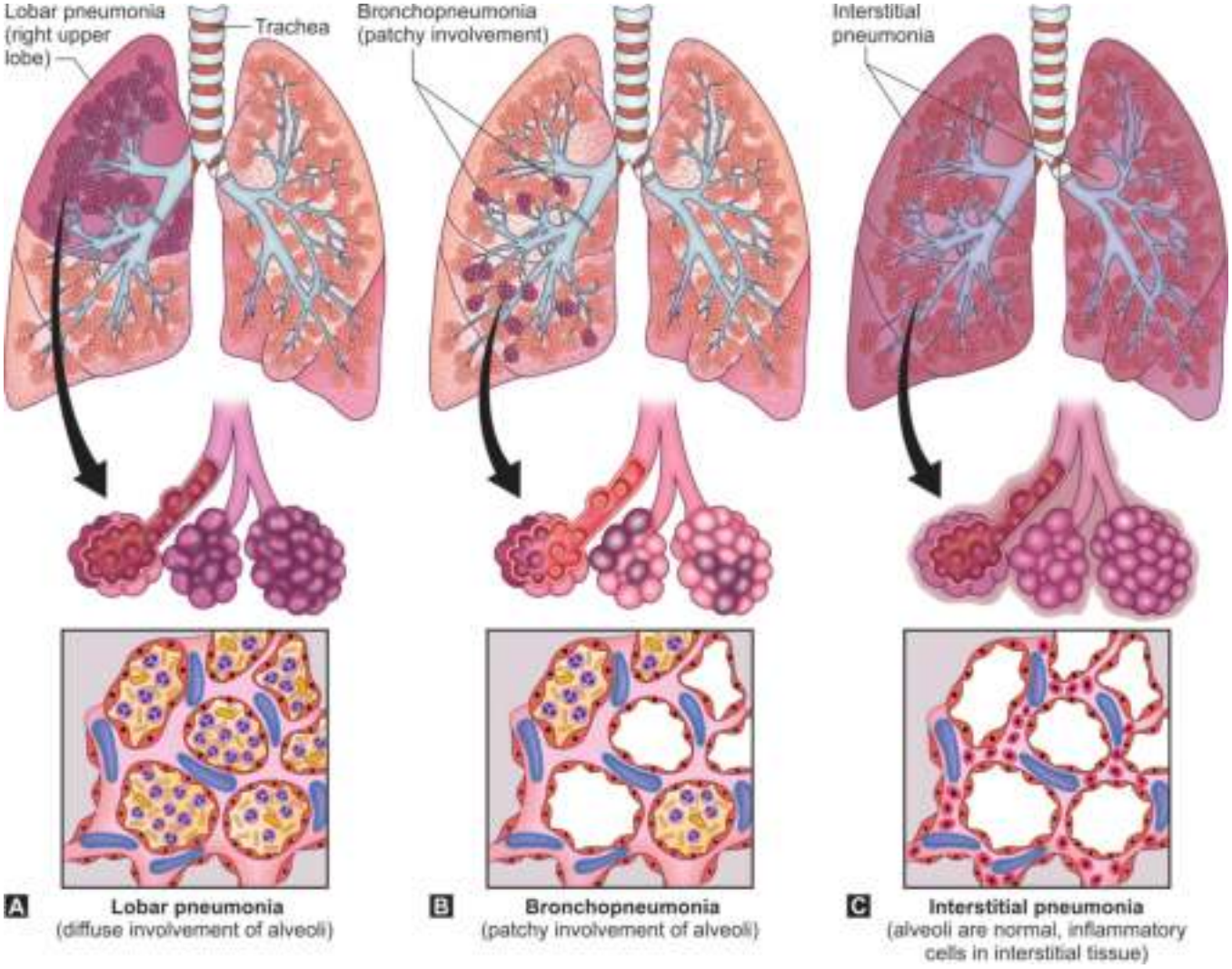


Fig. 18.5A to C: There are three morphologic and clinical patterns: Bronchopneumonia, lobar pneumonia, and interstitial pneumonia.

Table 18.4 Clinical patterns of pneumonia and common causative organisms

Pneumonia Variant	Clinical Pattern	Common Causative Pathogens
Bronchopneumonia	Patchy, multifocal consolidation	▪ *Streptococcus pneumoniae* ▪ *Staphylococcus aureus* ▪ *Haemophilus influenzae* ▪ *Escherichia coli* ▪ *Klebsiella pneumoniae* ▪ Enterococci ▪ *Pseudomonas aeruginosa* ▪ Anaerobes
Lobar pneumonia	Lobar consolidation	▪ *Streptococcus pneumoniae* (most common) ▪ *Klebsiella pneumoniae* (elderly, diabetics, alcoholic)
Interstitial pneumonia	Inflammation within alveolar interstitium	▪ Mycoplasma ▪ Chlamydia ▪ Legionella ▪ Viral (adenovirus, influenza, respiratory syncytial virus) ▪ Fungi (histoplasmosis, coccidioidomycosis, blastomycosis, cryptococci) ▪ *Pneumocystis jiroveci* in AIDS

lungs occurs because of the tendency of secretions to gravitate into the lower lobes or right middle lobe. It usually occurs in infancy and old age.

- **Interstitial pneumonia:** It is also called primary atypical pneumonia. Lung shows diffuse, patchy inflammation localized to interstitial areas of the alveolar walls. It involves one or more lobes.

Clinical Features

Patient presents with fever, chills, productive cough, blood-tinged or rusty sputum, pleuritic pain, hypoxia with shortness of breath, and sometimes cyanosis. If there is bacterial etiology, it is most characteristically associated with neutrophilic leukocytosis with an increase in band neutrophils with shift-to-the-left.

ETIOLOGIC CLASSIFICATION

Bacterial Pneumonia

- ***Streptococcus pneumoniae:*** *Streptococcus pneumoniae* is the most common cause of lobar pneumonia especially in immunocompromised persons. Capsule of *Streptococcus pneumoniae* prevents phagocytosis by inflammatory cells. This pathogen synthesizes copious mucoid secretion resulting in lobar pneumonia.
- ***Staphylococcus pneumoniae:*** *Staphylococcus pneumoniae* accounts for only 1% of bacterial pneumonias especially in intravenous drug abusers associated with bacterial endocarditis. These persons develop lung abscess and empyema. Hematogenous dissemination leads to abscess formation in distant organs such as brain and kidney.
- ***Klebsiella pneumoniae:*** *Klebsiella pneumoniae* is most common cause of bacterial pneumonia in debilitated hospitalized patients, diabetics or chronic alcoholic and elderly persons. The organism produces viscid capsular polysaccharide responsible for thick gelatinous sputum. Such individual may have difficulty in coughing up. The organism causes alveolar damage, necrosis and abscess formation.
- ***Pseudomonas aeruginosa:*** *Pseudomonas aeruginosa* causes pneumonia in patients with extensive burns, immunosuppression, and mechanical ventilation. The bacteria invade blood vessels with consequent extrapulmonary spread with fatal outcome within days. Histologic examination shows coagulative necrosis of lung parenchyma and vasculitis.
- ***Haemophilus influenzae:*** Capsule of *Haemophilus influenzae* prevents opsonization and phagocytosis by host cells. It can cause life-threatening pneumonias in infants and children. It may also affect debilitated adults with chronic obstructive pulmonary disease. Patient develops lobular or patchy pneumonia, but may involve entire lung.
- ***Legionella pneumophila:*** *Legionella pneumophila* is a gram-negative bacillus transmitted by inhalation of aerosol from contaminated water storage systems. It produces bilateral bronchopneumonia. Patient presents with cough, dyspnea, and chest pain with myalgia, headache, confusion, nausea, vomiting and diarrhea. On histopathologic examination, the alveoli are packed with fibrinous exudate, neutrophils and red blood cells. Alveolar capillaries are dilated and congested. The exudates may organize resulting in interstitial fibrosis with permanent loss of lung function.
- ***Moraxella catarrhalis:*** *Moraxella catarrhalis* constitutes one of the three most common causes of otitis media. The pathogen causes pneumonia in elderly persons.

Viral Pneumonia

Viral infections account for 50% of pneumonia cases. Influenza and respiratory syncytial viruses are the most common culprits. Other viruses include adenovirus, cytomegalovirus and herpes simplex. The incubation period is 1–3 days. Viral pneumonias most often affect children. Clinical course of viral pneumonia is short-lived and not serious in nature.

Fungal Pneumonia

Some endemic fungi are associated with pneumonia. These include Coccidioides, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Aspergillus fumigatus* and Phycomycetes.

Pneumonias in Immunocompromised Persons

Pneumocystis jiroveci is an infection almost exclusively associated with immunocompromised persons such as HIV/AIDS, patients receiving radiotherapy or chemotherapy for cancer, immunosuppressive drugs given in organ transplant persons or nonfunctional spleen.

Mycoplasma Atypical Pneumonia

Mycoplasma pneumoniae is smallest free-living bacteria responsible for Mycoplasma atypical pneumonia after incubation period of 1 to 4 weeks. It is less serious pneumonia than caused by bacteria or viruses. This type of pneumonia is also known as walking pneumonia or atypical pneumonia. Mycoplasma atypical pneumonia responds well to antibiotics.

PNEUMONIAS

LOBAR PNEUMONIA

Lobar pneumonia refers to diffuse consolidation of entire lung due to acute inflammatory exudate within the alveoli without bronchial involvement. There is abrupt demarcation at the interlobar fissure. Lobar pneumonia involves portion of a lung resulting in consolidation. Purulent exudate is observed in bacterial pneumonia.

Etiology

Streptococcus pneumoniae is the most common cause of lobar pneumonia in young healthy adults between 20 and 50 years of age especially in immunocompromised persons. Capsule of *Streptococcus pneumoniae* prevents phagocytosis by inflammatory cells. This pathogen synthesizes copious mucoid secretion resulting in lobar pneumonia. However, *Klebsiella pneumoniae* typically affects elderly, diabetics and alcoholics persons.

Risk Factors

Lobar pneumonia occurs due to impairment of normal defense mechanisms. Spleen plays important role in degradation of *Streptococcus pneumoniae*. Risk factors for lobar pneumonia are described as under:

- **Respiratory diseases:** These include atelectasis, bronchiectasis, or cystic fibrosis, lung carcinoma and tracheostomy patients.
- **Immunosuppression disorders:** These include malnutrition, immunosuppressive therapy, chronic illness, diabetes mellitus, chronic alcoholism and premature birth.
- **Inhalation of toxic gases and cigarette smoke:** These play important role in pathogenesis of lobar pneumonia.
- **Hematologic disorder:** Sickle cell disease is a predisposing risk factor for lobar pneumonia.

Pathogenesis

The *Streptococcus pneumoniae* reach the alveoli and cause inflammation by pouring of inflammatory exudate into the lung alveolar spaces. White blood cells migrate to alveoli. The alveoli are filled with exudates resulting in consolidation of lungs. It leads to occlusion of alveoli causing decrease in alveolar oxygen content. Venous blood reaching the affected pulmonary areas is not oxygenated and returned to systemic circulation. It leads to arterial hypoxemia and even death due to interference with ventilation. Risk factors for early deterioration in community-acquired pneumonia are given in Table 18.5.

Stages of Pneumonia

In this antibiotics era, full blown lobar consolidation is infrequent. Untreated patients may morphologically evolve through four stages: (a) congestion, (b) red hepatization, (c) gray hepatization, and (d) resolution (Fig. 18.6).

- **Stage of congestion (12–24 hours):** The first stage of congestion in lobar pneumonia lasts for about 24 hours. Alveolar congestion correlates with the onset of the illness described as typical pneumonias.

Table 18.5 Risk factors for early deterioration in community-acquired pneumonia

Multilobar lung involvement
Severe hypoxemia (arterial saturation >90%)
Severe acidosis (pH <7.30)
Severe tachypnea (>30 breaths/minute)
Neutropenia
Thrombocytopenia
Hypoglycemia
Hypoalbuminemia
Hyponatremia

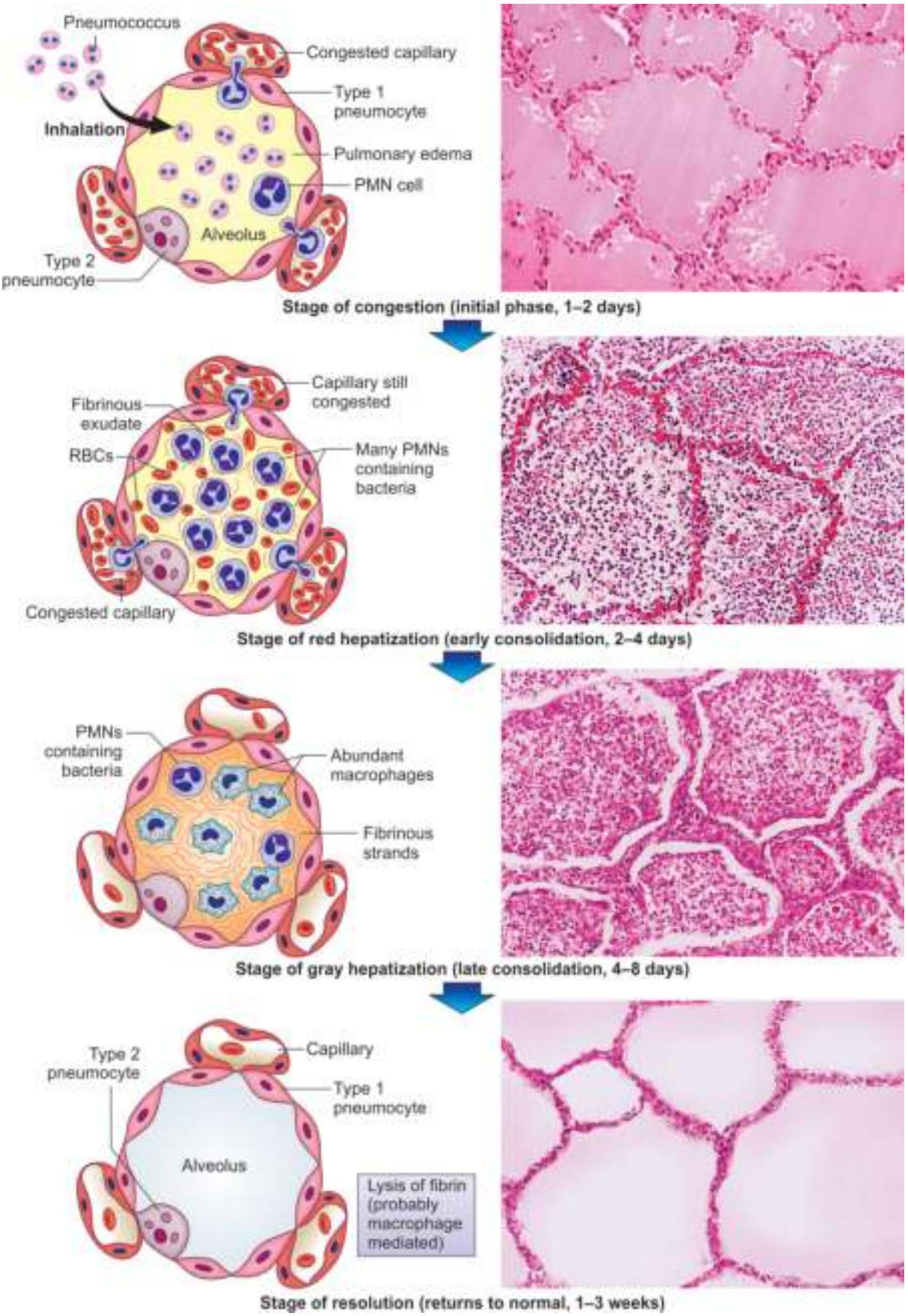


Fig. 18.6: Stages of lobar pneumonia: congestion, red hepatization, gray hepatization and resolution.

Surgical Pathology: Stage of Congestion in Lobar Pneumonia

Gross Morphology

Affected lung in early congestion stage of lobar pneumonia is heavy, edematous and red.

Light Microscopy

- Alveolar capillaries are congested.
- Increased alveolar capillaries permeability results to outpouring of protein-rich fluid with a few neutrophils (inflammatory exudate) and numerous bacteria into alveolar spaces.

Surgical Pathology: Stage of Red Hepatization in Lobar Pneumonia

Gross Morphology

The lung in stage of red hepatization of lobar pneumonia is brick red, solid and airless, with a consistency resembling fresh liver (Fig. 18.7).

Light Microscopy

- Alveolar capillaries are congested.
- The alveolar spaces are completely filled with fluid and polymorphonuclear cells, i.e. inflammatory exudates.
- Alveoli often show presence of many bacteria and RBCs.
- Overlying pleura bears fibrinous exudates (Fig. 18.8).

- **Stage of red hepatization (2–4 days):** Second stage of red hepatization in lobar pneumonia lasts for 2–4 days. There is progressive disintegration of red blood cells with persistent fibrinosuppurative exudate.
  - Clinical features in red hepatization stage in lobar pneumonia occur due to presence of inflammatory exudate. Onset of lobar pneumonia is usually abrupt. Patient presents with high grade fever 40°C with chills, productive 'rusty' sputum containing flecks of blood and occasionally patients may have hemoptysis.
  - Acute pleuritic chest pain on deep respiration reflects involvement of pleura. The clinical findings are dramatically modified by administration of antibiotics.
  - There is increased respiratory rate, guarding and lag on affected side. Children show sternal retraction and nasal flaring.
  - On palpation, chest expansion is decreased on affected side. Tactile fremitus is increased, if bronchus is patent, and decreased if bronchus is obstructed.
  - Percussion note is dull over lobar pneumonia, which recedes with resolution.

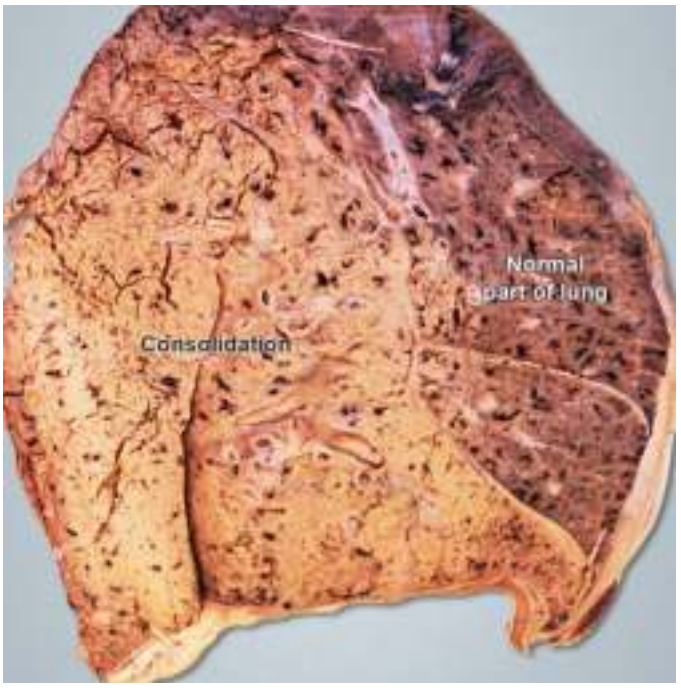


Fig. 18.7: Lobar pneumonia shows consolidation in major portion with small portion of normal lung.

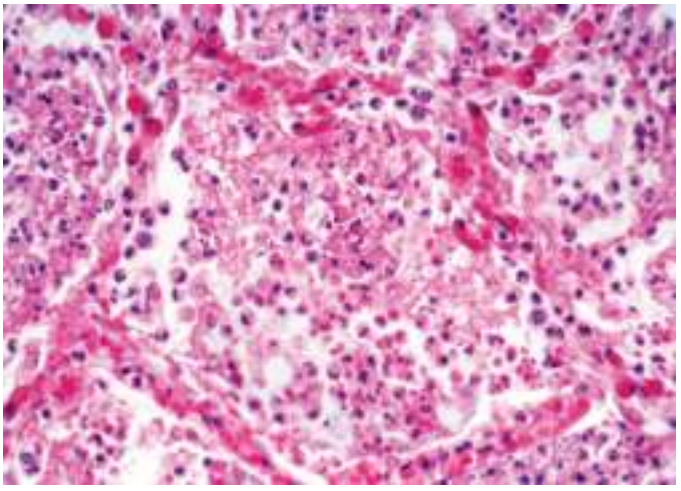


Fig. 18.8: Bacterial lobar pneumonia shows alveoli filled with exudate containing neutrophils. The surrounding alveolar walls having dilated capillaries filled with red blood cells. The exudate in alveoli gives rise to the productive cough of purulent yellow sputum (400X).

  - Breath sounds are louder with patent bronchus as if coming from larynx. Bronchophony, egophony and whispered pectoriloquy are present. Late inspiratory crackles of fine to medium size are heard.
- **Stage of gray hepatization (4–8 days):** Progression from red to gray hepatization in lobar pneumonia, there is progressive disintegration of red blood cells. There is persistent fibrinosuppurative exudate in alveoli resulting in consolidation of lung. Gray hepatization stage lasts for 4–8 days.

Surgical Pathology: Stage of Gray Hepatization in Lobar Pneumonia
Gross Morphology
▪ On cut section, lung in gray hepatization stage of lobar pneumonia appears gray brown and consolidated. ▪ Compression of alveolar capillaries by inflammatory exudates in alveoli reduces the pulmonary blood flow, thus giving this appearance.
Light Microscopy
▪ The alveoli are filled with fibrinopurulent exudates. ▪ The capillaries in the alveolar walls appear compressed and contain less blood than seen in red hepatization.

- **Stage of resolution (8–10 days):** It is characterized by granular, semi-fluid debris lobar pneumonia, which represents enzymatic digestion of inflammatory debris, ingested by macrophages with preservation of the underlying alveolar wall architecture.
- **Stage of organization:** Carnification of lung is also called organization of lobar pneumonia. When lobar pneumonia fails to resolve, it gets organized by formation of granulation tissue and finally fibrosis. Lung parenchyma becomes hard in consistency.

Complications of Lobar Pneumonia

- **Pleuritis:** Patient develops pleuritis as a result of extension of inflammation to the pleural surface.
- **Lung abscess:** Pulmonary abscess is a focal suppurative destructive necrosis within the lung parenchyma. It is most often caused by *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, Klebsiella, or Proteus, often in combination with anaerobic organisms.
- **Empyema:** It refers to collection of pus in pleural cavity. It follows spread of bacterial infection to the pleural space.
- **Bacterial dissemination (sepsis):** It results in endocarditis, pericarditis, meningitis, suppurative arthritis, abscesses in kidneys and spleen.

Laboratory Diagnosis

- **Imaging techniques:** Chest radiograph is gold standard diagnostic tool with 50–80% sensitivity to diagnose pneumonias. Bronchopneumonia shows patchy/focal opacities, whereas lobar pneumonia shows radiopaque infiltrate involving the entire lobe.
- **Sputum examination:** Gram stained sputum examination shows presence of neutrophils containing numerous lancet-shaped diplococci with tapered ends pointing to each other establishes evidence of pneumonia due to streptococcal pneumoniae. But it must be remembered that *Streptococcus pneumoniae* is a part of normal flora and may give false positive results.
- **Blood culture:** The identification of the causative microorganism and the subsequent determination of its antibiotic sensitivity are the key notes to appropriate therapy. During early phase of illness, blood culture is positive in 20–30% of persons with lobar pneumonia. Whenever possible, antibiotic sensitivity must be done.

BRONCHOPNEUMONIA

Bronchopneumonia is characterized by patchy distribution of inflammation that generally involves more than one pulmonary lobe.

- Bronchopneumonia results from extension of initial infection of bronchi or bronchioles extending into surrounding alveoli involving one or more lobules.
- Inflammatory pulmonary focus shows patchy reddish-gray consolidation and microabscesses in bronchi or bronchioles.
- The infection is often bilateral and basal because of the tendency of secretions to gravitate into the lower lobes or right middle lobe. It usually occurs during infancy and old age especially in immunosuppression state.

Etiopathogenesis

Bronchopneumonia is acute suppurative inflammation caused by *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Other predisposing factors include whooping cough, measles, chronic bronchitis, malnutrition and cancer.

Clinical Features

Patient often becomes septicemic and toxic and presents with fever, productive cough with mucus, shortness of breath, chest pain, rapid breathing, chills and headache.

Surgical Pathology: Bronchopneumonia
Gross Morphology
▪ Consolidated region of lungs shows acute suppurative inflammation. ▪ Cut surface shows dry, granular, and grey-red to yellow patchy consolidation with poorly defined margins. ▪ Pleural involvement is less common than lobar pneumonia (Fig. 18.9 and Table 18.6).
Light Microscopy
Histologic examination shows focal suppurative exudate filling the bronchioles and surrounding alveolar spaces (Fig. 18.10).



Fig. 18.9: Bronchopneumonia. It shows tan yellow patchy consolidation. Areas of consolidation are more firm than the surrounding lung (arrows).

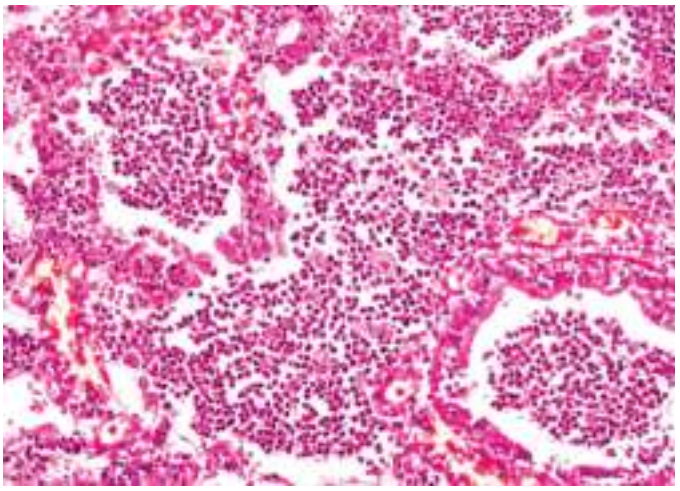


Fig. 18.10: Bronchopneumonia. It shows alveoli filled with neutrophilic exudate corresponding to the areas of consolidation seen on grow examination. Focal suppurative exudate filling the bronchioles involving surrounding alveolar spaces (100X).

INTERSTITIAL PNEUMONIA

Interstitial pneumonia is also called **primary atypical pneumonia**. Unlike typical acute pneumonias, sputum production is modest. There is absence of physical

Table 18.6 Differences between lobar pneumonia and bronchopneumonia

Parameters	Lobar Pneumonia	Bronchopneumonia
Definition	Acute respiratory tract infection involving alveoli	Acute respiratory tract infection involving bronchioles and extending to alveoli
Age group	Adults	Infants, old persons
Immunity status	Previously healthy persons	Immunocompromised persons
Organisms	Pneumococci, streptococccal pneumococci, *Haemophilus influenzae, Klebsiella pneumoniae*	Staphylococcus, streptococci, pneumococci, *Haemophilus influenzae, Pseudomonas aeruginosa* coliforms
Opportunistic infection	Absent	May be present
Pathology	Lung alveoli filled with exudate	Bronchitis/bronchiolitis involving lung alveoli
Consolidation lung	Diffuse consolidation	Patchy consolidation
Lobes involved	Part of lobe or entire lobe	Multiple lobes, often bilateral
Lung lobe involved	Upper or lower lobe or whole lung	Basal portion of lower lobe due to secretions
Stages of inflammation	Early congestion (1–2 days), red hepatization (2–4 days), gray hepatization (4–8 days) and resolution (7–21 days)/organization	Not clearly defined
Exudation seen on light microscopy	More exudate	Less exudate
Severity	More severe disease	Less severe disease
Sputum	Initially scanty, watery. Later on thick, purulent, hemorrhagic	Purulent
Complications	Uncommon, organization, pleural effusion, empyema, lung abscess	Uncommon, organization, pleural effusion, empyema, lung abscess and bronchiectasis

findings of consolidation. Bacteria and viruses are not isolated.

Surgical Pathology: Interstitial Pneumonia

Gross Morphology

- Lung in interstitial pneumonia shows diffuse, patchy inflammation localized to interstitial areas of the alveolar walls.
- Interstitial pneumonia involves one or more lobes. It occurs as a result of droplet infection.

Light Microscopy

- Inflammatory reaction in interstitial pneumonia is confined to the interstitium of lung.
- Alveolar spaces are usually free of exudates.
- The alveolar septa are widened and edematous, infiltrated by lymphocytes, macrophages and occasionally plasma cells.

Clinical Features

Patient has insidious onset of low-grade fever, non-productive cough, and chest pain, flu-like symptoms, i.e. pharyngitis, laryngitis, myalgia, and headache. Patient's lungs show no signs of consolidation.

Etiopathogenesis

- **Bacterial interstital pneumonia:** *Mycoplasma pneumoniae* is most common cause of primary atypical pneumonia, i.e. interstitial pneumonia with insidious onset. It usually occurs in children and young adults. It usually follows a mild self-limited course.
- **Viral interstitial pneumonia:** Viruses most often cause interstitial pneumonia in children. These include influenza virus, adenovirus, rhinovirus, and respiratory syncytial virus, rubeola (measles) or varicella (chickenpox). Cytomegalovirus produces a characteristic interstitial pneumonia with marked enlargement of infected cells containing intranuclear inclusions. Measles virus produces interstitial pneumonia and marked by numerous giant cells.
- **Lymphocytic interstitial pneumonia:** It is often associated with Sjögren's syndrome and HIV infection. Clinical course of the disease varies from an indolent condition to one of those progresses to end-stage lung disease and respiratory failure.
- **Hypersensitivity pneumonitis:** It occurs in response to repeated exposure to a variety of organic materials e.g. pigeon bird droppings, feathers, mushrooms, and tree bark. Inhalation to pigeon bird droppings is known as pigeon breeder lung. Hypersensitivity pneumonitis may be caused by *Thermoactinomyces sacchari* in sugarcane workers, *Cryptostroma corticale* fungus in maphe bark workers, fungus growing in airconditioners and swimming pools; and *Saccharopolyspora rectivirgula* in moldy hay seen in farmer's lung. Histologic examination of lung lesion shows poorly formed granulomas. Antigens associated with hypersensitivity pneumonias are given in Table 18.7.

Table 18.7 Antigens associated with hypersensitivity pneumonias

Disease	Antigen	Environmental Antigens Source
Farming/food processing		
Farmer's lung	Thermophilic actinomycetes (*Saccharopolyspora rectivirgula*)	Grain, moldy hay, silage
Bagassosis	Thermophilic actinomycetes	Sugarcane
Cheese washer's lung	*Penicillium casei*; *Aspergillus clavatus*	Cheese castings
Coffee worker's lung	Coffee bean dust	Coffee bean dust
Malt worker's lung	*Aspergillus* species	Barley
Miller's lung	*Sitophilus granaries* (wheat weevil)	Wheat flour
Mushroom worker's lung	Thermophilic actinomycetes; mushroom spores	Mushrooms
Potato riddler's lung	Thermophilic actinomycetes; *Aspergillus* species	Moldy hay around potatoes
Tobacco grower's lung	*Aspergillus* species	Tobacco
Wine maker's lung	*Botrytis cinerea*	Grapes
Birds and other animals		
Bird fancier's lung (also named by specific bird exposures)	Proteins derived by parakeets, pigeons, budgerigars	Bird features, droppings, serum proteins
Duck fever	Duck features, serum proteins	Ducks
Fish meal worker's lung	Fish meal dust	Fish meal

Contd...

Table 18.7 Antigens associated with hypersensitivity pneumonias (*Contd...*)

Disease	Antigen	Environmental Antigens Source
Furrier's lung	Dust from animal furs	Animal furs
Laboratory worker's lung	Rat urine, serum, fur	Laboratory rats
Pituitary snuff taker's lung	Animal proteins	Pituitary snuff from bovine and porcine sources
Poultry worker's lung	Chicken serum proteins	Chickens
Turkey handing disease	Turkey serum proteins	Turkeys
Other occupational and environmental exposures		
Chemical worker's lung	Isocyanates	Polyurethane foam, varnish
Detergent worker's lung	*Bacillus subtilis* enzymes	Detergent
Hot tub lung	*Cladosporium* species; *Mycobacterium avium* complex	Contaminated water, mold or ceiling
Humidifier fever and air conditioner lung	Several microorganisms including *Aureobasidium pollulans*; *Candida albicans*; thermophilic actinomycetes; *Mycobacterium* species; *Klebsiella oxytoca*; *Naegleria gruberi*	Humidifier (in water heaters) and air conditioners (contaminated water)
Machine operator's lung	*Pseudomonas* species' *Naegleria gruberi*	Metal working fluid
Sauna taker's lung	*Aureobasidium* species; other antigens	Sauna bath
Suberosis	*Penicillium glabrum*; *Chrysonilia sitophila*	Cork dust
Summer type pneumonitis	*Trichosporon cutaneum*	House dust mites, bird droppings
Wood worker's lung	*Alternaria* species; *Bacillus subtilis*	Oak, cedar, pine, mahogany dusts

- **Interstitial pneumonia of unknown etiology:** It is of unknown etiology in majority of cases. It occurs in various clinical settings, i.e. collagen vascular disease, chronic hypersensitivity pneumonitis, and drug toxicity.
- **Nonspecific interstitial pneumonia (NIP):** It is associated with collagen vascular disease, hypersensitivity pneumonitis, and drug reaction. Patient develops diffuse, temporarily uniform proliferative and fibrosing changes in the lungs. Prognosis is better than usual interstitial pneumonias with five-year survival in >80% of cases.
- **Desquamative interstitial pneumonia (DIP):** It is chronic fibrosing interstitial pneumonitis of unknown etiology. Lungs show minimal fibrosis with preservation of lung architecture. It primarily occurs in tobacco smokers. Prognosis is better than usual interstitial pneumonias with 10-year survival in 70–100% of cases. The pathologic changes can regress following tobacco smoking cessation.

OPPORTUNISTIC PULMONARY INFECTIONS

Immunocompromised patients may develop pneumonias due to opportunistic infections.

Pneumocystis Jiroveci

Pneumocystis jiroveci is most common pathogen causing pneumonia in acquired immune deficiency syndrome. This organism has been reclassified as a fungus.

- **Light microscopy:** Lung lesion shows interstitial infiltrate of plasma cells and lymphocytes, diffuse alveolar damage, and hyperplasia of type 2 pneumocytes. The alveoli contain foamy exudate. The organisms appear as small bubbles in a background of proteinaceous exudates.
- **Bronchoalveolar lavage:** Specimen obtained by bronchoalveolar lavage impregnated with silver shows a cluster of cysts. The cysts appear as round or indented (crescent moon) bodies measuring 2–3 μm in diameter.

Candida Albicans

In immunocompromised patients, invasing form of *Candida albicans* produces blood-borne dissemination, resulting in pulmonary, renal, and hepatic abscesses and vegetative endocarditis.

Cytomegalovirus

Cytomegalovirus (CMV) infection is now well recognized in immunocompromised persons. CMV causes

marked enlargement of infected cells, which contain typical intranuclear and often cytoplasmic inclusions. Diagnosis is confirmed by morphologic demonstration of cytomegalovirus in biopsy or bronchial washing specimens.

Nocardiosis

Nocardia are gram-positive aerobic, filamentous, weakly acid-fast bacteria closely related to *Actinomyces israelii*. Typically opportunistic infection may disseminate to lung, skin, brain and meninges.

Aspergillus Fumigatus

Invasion of blood vessels and tissue infarcts are common in *Aspergillus fumigatus* infection. *Aspergillus* species may also grow in pre-existing cavities caused by tuberculosis or bronchiectasis. The fungus proliferates to form fungus balls, which are also referred to as aspergillomas or mycetomas. Lung shows focal yellow areas of consolidation.

Invasive form of *Aspergillus fumigatus* has predilection for growth into vessels, with consequent widespread hematogenous dissemination.

Cryptococcus Neoformans

Pneumonia results from the inhalation of spores of *Cryptococcus neoformans*, a fungus frequently encountered in pigeon droppings. Most serious cases occur in immunocompromised persons. *Cryptococcus neoformans* stains positively with a mucicarmine stain or India ink for capsular polysaccharides.

Histoplasma Capsulatum

Histoplasma capsulatum results in multiple pulmonary lesions with late calcification. Disseminated form of disease is marked by multisystem involvement with infiltrates of macrophages filled with fungal yeast forms.

Coccidioidomycosis

Coccidioidomycosis occurs in primary and disseminated forms. Fungal spherules contain endospores found within granulomas of involved lung.

Actinomyces Israelii

Actinomyces israelii is gram-positive anaerobic filamentous bacteria no longer classified as a fungus. It is normal colonizer of the vagina, colon, and mouth. It is an opportunistic pathogen. Infection is established first by a breach of the mucosal barrier during various procedures (dental, gastrointestinal), aspiration, or diverticulitis. Patient develops lung abscess and sinus tract formation. Histologic examination shows exudates containing sulfur granules, yellow clumps of the organism in lungs.

LIPID PNEUMONIA

Lipid pneumonia is an uncommon noninfectious inflammatory pulmonary disease caused by the presence of lipids in the alveoli. It is classified into two groups, depending on whether the lipid/oil in the respiratory tract is from an exogenous or endogenous/idiopathic source. Pathologically, lipid pneumonia is a foreign body reaction characterized by lipid-laden macrophages.

- Exogenous lipid pneumonia is caused by various types of lipids such as mineral oils, animal oils, vegetable oils or use of nasal petroleum jelly, lip gloss.
- Endogenous lipid pneumonia results from breakdown of lung distal to obstructed airway either by tumor or bronchiolitis obliterans or following chemotherapy given for lung carcinoma and lymphoma or radiotherapy leading to release of cholesterol and other lipids in alveoli.
- Bronchoalveolar lavage fluid is initially turbid white. Lipid phagocytosis by alveolar macrophages is observed under a microscope with Sudan III staining. Physical examination reveals no abnormal findings, with the exception of inspiratory fine crackles in the lower fields on auscultation.
- Chest radiograph and CT scan reveal ground glass opacity and interseptal thickening in the bilateral lungs. Comparison of exogenous and endogenous lipid pneumonias is given in Table 18.8.

Table 18.8 Comparison of exogenous and endogenous lipid pneumonias

Features	Exogenous Lipid Pneumonia	Endogenous Lipid Pneumonia
Lipid	Usually mineral (saturated and therefore nonosmophilic)	Endogenous derived lipid (unsaturated and therefore osmophilic)
Obstructing lesion	Absent	Present
Foamy macrophages	Intra-alveolar space and interstitial tissue	Intra-alveolar space
Giant cells	Present around droplets	Absent (except around crystals)
Extracellular fat droplets	Fat droplets of varying size present	Fat droplets absent

Endogenous lipid pneumonia is caused by airway obstruction by tumor, amiodarone drug reaction and Niemann-Pick disease.

LUNG ABSCESS

Lung abscess is a focal suppurative process within the lung characterized by necrosis of lung parenchyma. It is most often caused by Staphylococcus, streptococci, Pseudomonas, Klebsiella, or Proteus, often in combination with anaerobic organisms.

- **Pathogenesis:** Lung abscess most often occurs due to aspiration of oropharyngeal and gastric material, superior segment, right lower lobe is most common site for aspiration. Other causes of lung abscess include bronchial obstruction (often by tumor), or complication of bacterial pneumonias, septicemias and penetrating lung injury. Microbial pathogens causing lung abscess are given in Table 18.9.

Table 18.9 Microbial pathogens causing lung abscess

Primary Lung Abscess (Uusually with Risk Factors for Aspiration)
▪ Anaerobes (e.g. *Streptococcus milleri*) ▪ Microaerophilic streptococci
Secondary Lung Abscess (Most Often with Underlying Immunocompromising State)
▪ *Staphylococcus aureus* ▪ *Pseudomonas aeruginosa* ▪ Enterobacteriaceae ▪ *Nocardia* species ▪ *Aspergillus* species ▪ *Pneumocystis jiroveci* ▪ *Cryptococcus* species ▪ Legionella
Infective Embolic Lesions in Lung
▪ *Staphylococcus aureus* (most often from infective endocarditis) ▪ *Fusobacterium necrophorum* (recurrent acute sore throats, peritonsillar abscess, meninigitis and thrombosis of cerebral veins and internal jugular veins)
Endemic Infections (with or without Underlying Immunocompromised State)
▪ *Mycobacterium tuberculosis* ▪ *Mycobacterium avium* ▪ *Mycobacterium kansasii* ▪ *Histoplasma capsulatum* ▪ Blastomyces (blastomycosis) ▪ *Entamoeba histolytica* ▪ *Strongyloides stercoralis*
Miscellaneous Conditions
Staphylococcus aureus after influenza or other viral infections

  - **Aspiration:** Person with decreased cough reflex due to alcohol or drug overdose, general anesthesia or neurological disorders may aspirate infected oropharyngeal material, tonsillitis, sinusitis or gingivodental sepsis, that localize in the lung depending on the posture of patient. Person in sitting or standing posture, aspirated material is located in the posterobasal segment of the right lower lobe. In the supine posture, superior segment of the right lower lobe is affected. In right-sided posture, it is localized in the right middle lobe or the posterior segment of the right upper lobe.
  - **Bronchial obstruction:** It occurs due to centrally located squamous cell lung carcinoma in 10–15% of cases, bronchopulmonary carcinoid tumor, and foreign body.
  - **Pneumonias:** Lung abscess occurs in patients suffering from pneumonias caused by streptococci, *Staphylococcus aureus*, pneumococci, *Klebsiella pneumoniae*, and anaerobic bacteria.
  - **Septicemia:** Septic emboli in case of infective endocarditis, thrombophlebitis and puerperal sepsis by pyogenic organisms seed the lung *via* hematogenous route.
  - **Direct penetrating lung injury:** It may cause lung abscess.
- **Clinical features:** Patient presents with productive cough (foul smelling sputum), chest pain and fever of spiking pattern. Chest radiograph shows cavitation with an air-filled level.
- **Complications:** Lung abscess may spread to pleura, mediastinum, meninges, and bones. Patient may develop amyloidosis and septicemia.

Surgical Pathology: Lung Abscess

Gross Morphology

- Lung abscess varies in size and location depending on underlying cause.
- Aspiration of infected oropharyngeal material is located in the superior segment of right lung due to vertical course of right bronchus.
- Distribution of lung abscess is shown in Fig. 18.11 and Table 18.10.

Light Microscopy

Necrotic material obtained from lung abscess shows inflammatory cells. Necrotic area is surrounded by granulation tissue and fibrosis (Fig. 18.12).



Fig. 18.11: Lung abscess present in apical region (arrow).

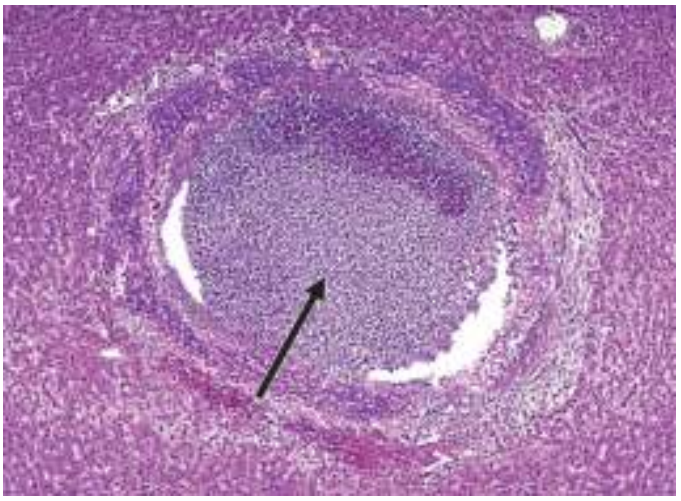


Fig. 18.12: Lung abscess shows collection of neutrophils (arrow) (100X).

Table 18.10 Distribution of lung abscess

Causes of Lung Abscess	Location of Abscess in Lungs
Aspiration sitting or standing posture	Posterobasal segment of the right lower lobe
Aspiration supine posture	Superior segment of the right lower lobe
Aspiration right-sided posture	Right middle lobe or the posterior segment of the right upper lobe
Pneumonia	Multiple lung abscesses
Septic emboli	Multiple lung abscesses
Bronchiectasis	Base of the lung

Treatment

Most acute lung abscesses resolve with appropriate antibiotic therapy (clindamycin) and postural drainage.

In a few cases, imaging guided, tube is inserted to drain the abscess. Bronchoscopy is done if lung abscess does not resolve with antibiotics and postural drainage.

TUBERCULOSIS

PULMONARY AND EXTRAPULMONARY TUBERCULOSIS

Tuberculosis is worldwide health problem caused by Mycobacterium tubercle bacilli (hominis strain). It is transmitted by inhalation of droplets (most common), ingestion of infected milk and skin inoculation (handling infected specimens). It may involve multiple systems.

MYCOBACTERIUM TUBERCLE BACILLUS

- **Acid-fast bacillus:** Mycobacterium tubercle bacillus is strict aerobe. It is acid-fast due to presence of mycolic acid in its cell wall and stained by Ziehl-Neelsen staining (decolorized by 20% sulfuric acid).
- **Composition:** Mycobacterium tubercle bacillus contains large quantity of hydrophobic waxes and fatty acids of high molecular weight, which prevents entry of Gram's stain.
- **Virulence:** Virulence of tubercle bacillus depends on presence of cord factor (a sulfated glycolipid), which prevents fusion of phagosomes with lysosomes and thus favors intracellular survival of AFB in macrophages. If cord factor is extracted, AFB becomes avirulent.
- **Viability:** Tubercle bacillus is viable for months in dry or wet sputum. Single AFB is potentially infective in immunocompromised persons (Table 18.11).

RISK FACTORS

There is increased risk of tuberculosis in person, who is in close contact with a newly diagnosed open

Table 18.11 Four groups of atypical mycobacteria

Group	Example	Pathologic Lesions
Group I: Photochromogens	*Mycobacterium kansasii*	▪ Koch's chest in adults ▪ Cervical lymphadenopathy in children
Group II: Scotochromogens	*Mycobacterium scrofulaceum*	Cervical lymphadenopathy in children, rarely involving lungs
Group III: Nonphotochromogens	▪ *Mycobacterium intracellulare* ▪ *Mycobacterium avium*	▪ Koch's chest in adults ▪ Cervical lymphadenopathy in children ▪ Osteomyelitis may affect any age group
Group IV: Rapid growers	*Mycobacterium fortuitum*	Koch's chest rare (organism present in fresh salty water and swimming pools)

case of pulmonary tuberculosis case (sputum smear positive), history of previous tuberculosis exposure and immunocompromised individuals (AIDS and corticosteroids therapy), history of silicosis, diabetes mellitus, malnutrition, cancer, Hodgkin's disease, or leukemia, gastrectomy, chronic renal failure and alcoholism.

Clinical Pearls: Tubercular Infection and Disease

Tubercular Infection

Tubercular infection refers to positive TB skin test, i.e. Mantoux test or tuberculin test or purified protein derivative (PPD) test with no evidence of active disease. PPD is injected by intradermal route on forearm. Area of induration is demonstrated after 48–72 hours in immunocompetent individuals on exposure to tubercle bacilli after 2–4 weeks. Positive tuberculin test does not distinguish tubercular infection, i.e. inactive disease from active disease.

Tubercular Disease

Tubercular disease refers to cases that have positive acid-fast smear or culture for *Mycobacterium tuberculosis* or radiographic and clinical presentation of tuberculosis. Children below 2 years of age are more susceptible. Patient with silicosis and immunosuppression is more susceptible to tuberculosis.

PATHOGENESIS

Mycobacterium tubercle bacillus antigens play important role in cell-mediated immunity and type IV hypersensitivity reaction. Virulence and bacterial factors in tubercle bacilli are given in Table 18.12.

Pathogenesis of Tuberculosis <3 Weeks Duration

Mycobacterium tubercle bacilli's mannose capped glycolipid (lipoarabinomannan) binds to mannose receptors expressed on alveolar macrophages, which engulf these organisms. Cord factor synthesized by these organisms prevent fusion of phagosomes with lysosomes. Hence, unchecked proliferation of tubercle bacilli continues inside alveolar macrophages.

Pathogenesis of Tuberculosis >3 Weeks Duration

Macrophages are antigen presenting cells, which transmit Mycobacterium tubercle bacilli antigen to CD4+ helper T cells. Macrophages synthesize IL-12, which takes part in differentiation of CD4+ helper T cells in lungs and lymph nodes.

- **Bactericidal activity of immune system:** CD4+ helper T cells synthesize interferon-γ (IFN-γ), which stimulates nitrogen synthase enzyme resulting in synthesis of nitrogen intermediates (NO, NO_2 and

Table 18.12 Virulence and bacterial factors in tubercle bacilli

Characteristics	Name	Functions
Virulence factors	▪ Cord factor composed of glycolipids, mycolic acid and trehalose dimycolate	▪ Cord factor prevents fusion of phagosomes with lysosomes and thus favors intracellular survival of AFB in macrophages. If cord factor is extracted, AFB becomes avirulent. Cord factor can elicit granulomas formation in the tissue
	▪ Catalase-peroxidase	▪ It resists the host cell oxidative response
	▪ Sulfatides and trehalose dimycolate cord factor	▪ These can trigger toxicity in animal models
	▪ Lipoarabinomannan (LAM)	▪ It can induce cytokines and resist host oxidative stress
Bacterial factors	▪ Nonreplicating persistent 1 (NRP1) state	▪ Under aerophilic conditions, it has increased glycine dehydrogenase activity
	▪ Nonreplicating persistent 2 (NRP2) state	▪ Under anaerobic conditions, it has decreased glycine dehydrogenase activity
	▪ A sigma factor	▪ Unknown
	▪ α-Crystallin (αc) like heat shock protein	▪ Unknown

HNO_3) and oxygen-derived free radicals causing oxidative destruction of AFB.

- **Formation of epithelioid cell granulomas:** Macrophages synthesize tumor necrosis factor and chemokines participate in recruitment of monocytes and T cells. This process leads to formation of epithelioid cell granulomas composed of macrophages and surrounded by lymphocytes. Epithelioid cell granulomas prevent spread of infection by confining bacteria within a compact collection of activated macrophages and lymphocytes.
- **Caseous necrosis:** CD8+ cytotoxic T cells cause destruction of macrophages by Fas-dependent mechanism resulting in caseous necrosis.

TISSUE SUSCEPTIBILITY AND DRUG RESISTANCE

Tissue Susceptibility

Most common organs involved in children with tuberculosis include lungs, lymph nodes, spleen and meninges. In adults, most common organs involved comprises lungs, adrenal glands, kidneys, liver, spleen, bone, meninges, serous membranes, fallopian tubes, epididymis and lymph nodes. Lungs are the most common sites of tuberculosis in children and adults. Tissues rarely involved include cardiac and skeletal muscle, stomach, thyroid gland, and pancreas.

Drug Resistance in TB Patients

Patient develops drug resistance due to mutations involving mycolic acid, catalase and peroxidase enzyme. Catalase enzyme is required to activate isoniazid in TB patient.

TUBERCULOSIS: CATEGORIES

Tuberculosis is divided into two categories—pulmonary tuberculosis (85% of cases) and extrapulmonary tuberculosis (15% of cases). Summary of patterns of tuberculous infection of the lungs is given in Table 18.13. Extrapulmonary tuberculosis in order of frequency is given in Table 18.14. Active disease develops in 5–15% of those infected with *Mycobacterium tuberculosis*.

- Pulmonary tuberculosis has two distinct phases, i.e. primary tuberculosis and secondary tuberculosis. Organs involved in primary, secondary and miliary tuberculosis are shown in Fig. 18.13A and B.
- Histologic examination of tubercular lesion shows epithelioid cell granulomas, caseous necrosis and Langhans giant cell (Fig. 18.14).

Table 18.13 Summary of patterns of tuberculous infection of the lungs

Primary Tuberculosis (Ghon's Focus + Lymphatics + Regional Lymph Nodes = Primary Complex)

- Reparative lesion (usually heals by fibrosis and calcification)
- Quiescent lesion (Mycobacterium tubercle bacilli dormant)
- Progressive lesion
  - Pleural involvement
  - Airway dissemination (tuberculous laryngitis, tuberculous bronchopneumonia)
  - Segmental tuberculosis
  - Miliary tuberculosis (hematogenous dissemination to multiple organs)

Post-primary Tuberculosis (Fibrocaseous Apical Cavitation (High Oxygen Tension)

- Reparative lesion (heals by fibrosis and calcification)
- Quiescent lesion (mycobacterium tubercle bacilli dormant)
- Progressive lesion
  - Local extension
  - Pleural involvement
  - Airway dissemination (tuberculous bronchopneumonia)
  - Miliary tuberculosis (hematogenous dissemination to multiple organs)

Nonreactive Tuberculosis (Immunocompromised Persons)

- Tubercular lesion lacks epithelioid cell granulomas
- Ziehl-Neelsen stain demonstrated numerous Mycobacterium tubercle bacilli

Table 18.14 Extrapulmonary tuberculosis in order of frequency

Extrapulmonary Tuberculosis	Mechanism of Involvement	Comments
Tuberculous lymphadenitis	Hematogenous and lymphatic spread	▪ Painless enlarged lymph nodes in cervical and supraclavicular regions ▪ Cervical lymph node may form fistulous tract in skin
Pleural tuberculosis	Pleural tuberculosis can be part of pulmonary tuberculosis	▪ Pleural effusion ▪ Empyema ▪ Pleural fibrosis ▪ Restrictive lung disease
Tuberculosis of the upper respiratory airways	Hematogenous spread	▪ Involvement of the larynx, pharynx and epiglottis in advanced secondary tuberculosis ▪ Symptoms such as hoarseness, productive cough and dysphagia

Contd...

Table 18.14 Extrapulmonary tuberculosis in order of frequency (*Contd...*)

Extrapulmonary Tuberculosis	Mechanism of Involvement	Comments
Genitourinary tuberculosis	Hematogenous spread	▪ Urinary tract infection ▪ Genital tuberculosis in females affects the fallopian tubes and endometrium causing infertility and menstrual abnormalities ▪ Genital tuberculosis in males affects epididymis producing a tender mass that may form a fistulous tract
Skeletal tuberculosis	Hematogenous or lymphatic spread	▪ Weight-bearing joints involved ▪ Spinal tuberculosis (Pott disease) may cause abscess formation that track along muscles and ligaments
Tuberculous meningitis and tuberculoma	Hematogenous spread or rupture of subependymal tubercle	▪ Tuberculous meningitis is more common in children than adults present with headache and altered sensorium and/or paresthesia ▪ Tuberculoma can cause seizures and focal symptoms
Gastrointestinal tuberculosis	Swelling of infected sputum or ingestion of infected milk with *Mycobacterium bovis*	▪ *Mycobacterium bovis* trapped in the lymphoid follicles of intestinal mucosa causing bowel inflammation and ulceration ▪ Ileocaecal mass
Tuberculous pericarditis	Progression of a pericardial focus or rupture of a nearby tubercular lymph node	▪ Pericarditis may progress to constrictive pericarditis ▪ Pericardial effusion
Miliary (disseminated) tuberculosis	Hematogenous spread to multiple organs	▪ Small yellow tubercles that may coalesce forming large areas of consolidation ▪ Nonspecific symptoms such as fever, night sweats and weight loss

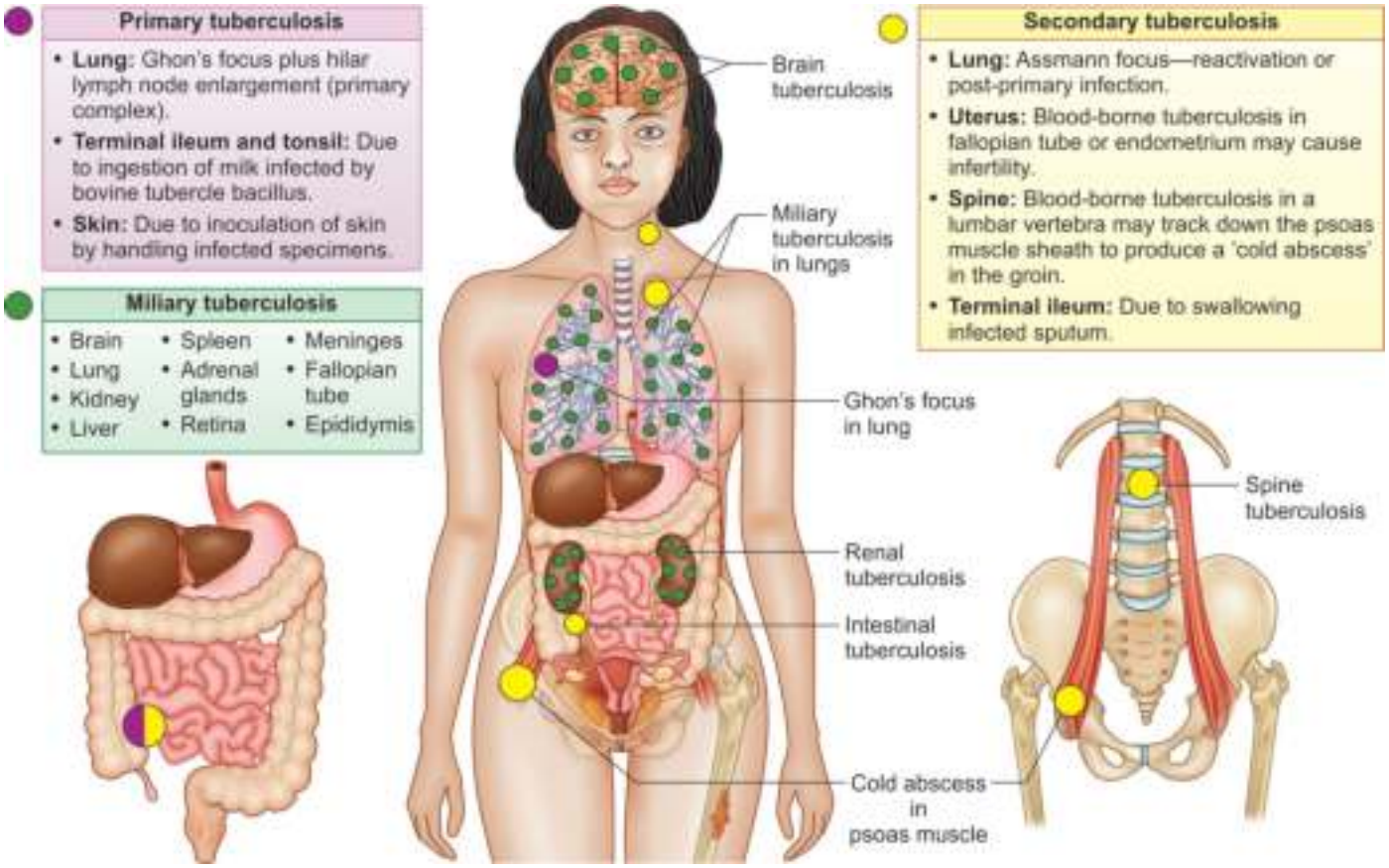


Fig. 18.13A: Organs involved in primary, secondary and miliary tuberculosis. Primary tuberculosis produces a subpleural mid-zone lesion in lung. In secondary tuberculosis, lesion is located in apical region of lung. Miliary tuberculosis shows tubercles in various organs.

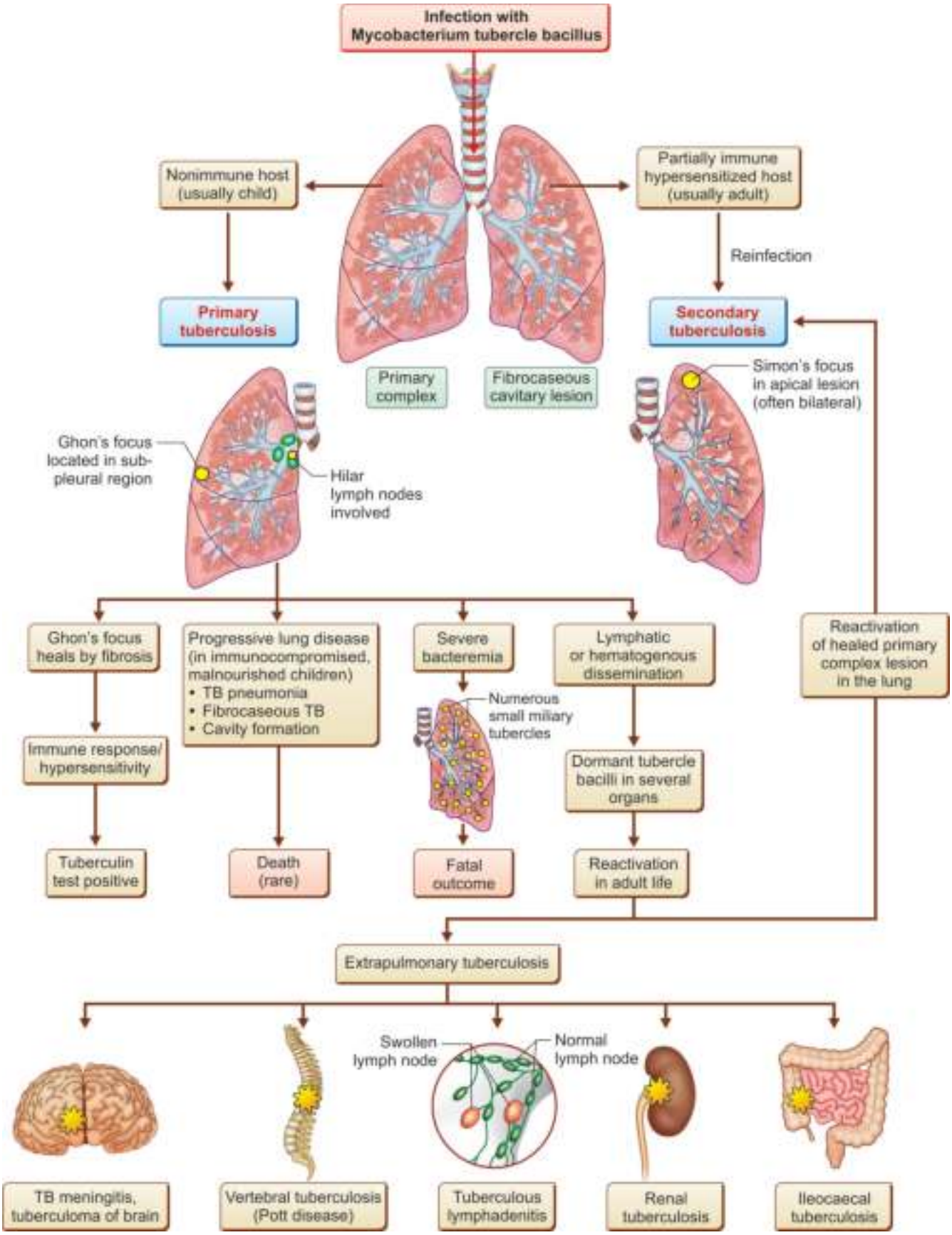


Fig. 18.13B: Pulmonary (primary and secondary) and extrapulmonary manifestations of tuberculosis.

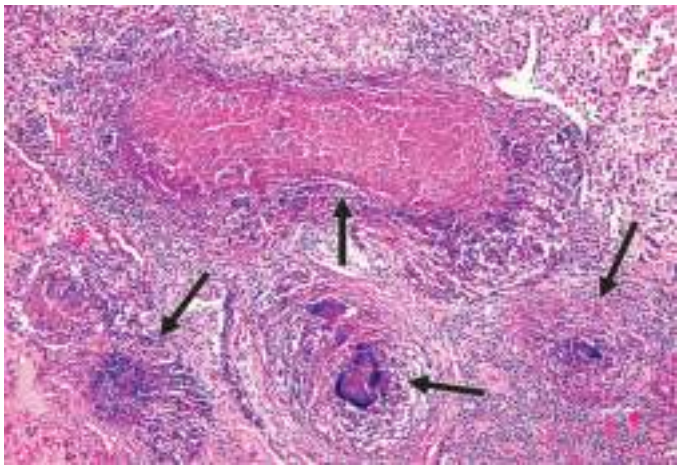


Fig. 18.14: Tuberculosis lung. It shows epithelioid granulomas, Langhans giant cells and caseous necrosis (arrows) (400X).

PRIMARY TUBERCULOSIS (PRIMARY COMPLEX)

Primary tuberculosis is also known as Ghon's focus caused by Mycobacterium tubercle bacillus. It occurs in lung, tonsil, ileocecal region and skin. It usually does not progress to clinically evident disease. Epithelioid cell granuloma in tuberculosis is known as tubercle.

Mode of Transmission

- **Lungs:** Tubercular infection is most common contracted by droplet infection from open case of pulmonary tuberculosis with cavities in lung. Initial exposure to tubercle bacilli produce clinically insignificant nonspecific alveolitis. Patient is asymptomatic at this stage.
- **Tonsils and intestine:** Ingestion of infected milk with Mycobacterium tubercle bacilli produces Ghon's focus in tonsils and ileocecal region. Tubercle bacilli reach the intestine either by ingestion of infected sputum or ingestion of unpasteurized milk and affects terminal ileum and most often ileocecal region. It produces circumferential ulcers perpendicular to long axis of small intestine. Fibrosis in this region is responsible for stricture formation. Histologic examination of intestinal lesion reveals caseating epithelioid cell granulomas with Langhans giant cells. Remember Crohn's disease also produces stricture formation, in which segmental involvement of intestine with skip areas is demonstrated. Thickened wall in Crohn's disease resembles pipe-like and show irregular linear ulcers in the mucosa.
- **Skin inoculation:** Primary cutaneous inoculation of AFB occurs in person handling infected specimens. Patient develops painless nonhealing papule (primary focus) in 2–4 weeks after inoculation, which forms cold abscess resulting in discharging sinuses after several weeks.
- **Vertical transmission:** Placental transmission of tuberculosis from mother to fetus shows lesions in liver and portal lymph nodes seen at birth.

Primary Tuberculosis (Ghon's Complex in Lung)

Ghon's complex consists of Ghon's focus in organs (lung, tonsil, ileocecal region and skin), lymphatic channel and draining lymph nodes involvement.

Ghon's Focus in Lung

- **Location:** Primary tuberculosis produces a small mid-zonal subpleural focus of consolidation near the interlobar fissure with involvement of lymphatic channels and hilar lymph nodes.
- **Dystrophic calcification:** Primary tuberculosis most often heals by fibrosis and dystrophic calcification in 95% of cases. Other lung lesion known as Ranke's complex is often visible on chest radiograph.
- **Lung lesions:** Approximately 5% of immunocompromised children develop symptomatic infection (pulmonary cavities, bronchopneumonia, pleural effusion or miliary tuberculosis).
- **Nidus for secondary tuberculosis:** Because, acid-fast bacilli persisting in dormant form in old necrotic calcified lesions are still capable of initiating lesion, and thus nidus for secondary tuberculosis.

Lymphatic Channels Involvement

Lymphatic channels are laden with Mycobacterium tubercle bacilli without producing lesion.

Draining Lymph Nodes

Lymph nodes draining Ghon's focus show caseous necrosis. Lymph nodes are enlarged in hilar region in pulmonary tuberculosis. Cervical lymphadenopathy occurs in tonsillar tuberculosis. Intestinal tuberculosis involves ileocecal lymph nodes. Cutaneous lymph nodes are involved in skin tuberculosis. Lymph node lesions of tuberculosis take longer time to regress, hence remain a potential source of reactivation (Fig. 18.15).

SECONDARY TUBERCULOSIS

Secondary tuberculosis is also known as post-primary pulmonary tuberculosis or reactivation disease. Lung lesion in secondary tuberculosis is known as **Simon's focus**. It develops during adult life especially in immunocompromised persons. It occurs either due to inhalation of Mycobacterium tubercle bacilli or reactivation of AFB present in dormant lymph node and old calcified healed parenchymal lesion. It causes extensive tissue destruction producing caseous necrosis by the action of cytokines synthesized by memory T cells.

Locations

- Reactivation of tubercular focus produces cavitary lesions in the apical regions or posterior segments

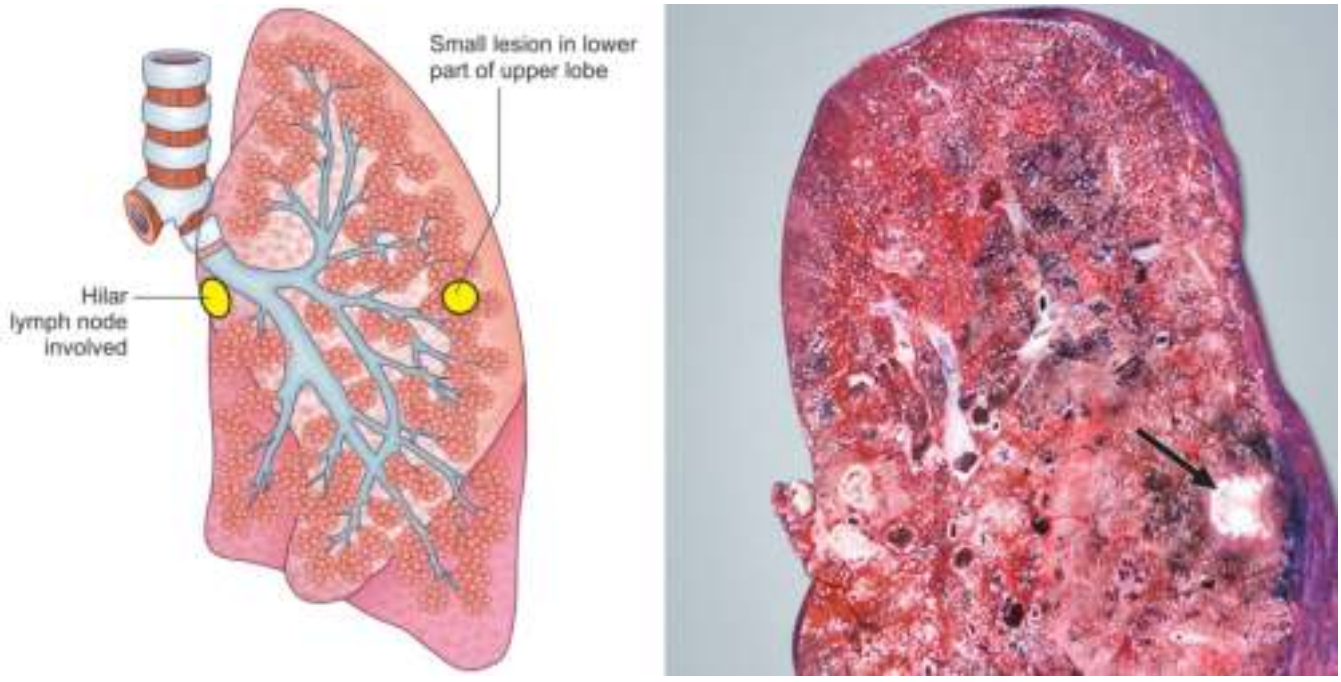


Fig. 18.15: Primary tuberculosis. It shows subpleural mid-zonal Ghon's focus and hilar lymph node enlargement. Gross examination of lung shows subpleural mid-zonal Ghon's focus (arrow).

of the upper lobes either unilateral or bilateral. Hilar lymph node involvement is also common. Apical lesions occur due to high oxygen tension and paucity of macrophages due to decreased blood supply.

- Growth of Mycobacterium tubercle bacilli is inhibited at pH <6.5. Large pulmonary cavities are associated with hemoptysis.
- *Aspergillus* species may also grow in pre-existing cavities caused by tuberculosis. They proliferate to form fungus balls, which are also referred as aspergillomas or mycetomas.

Morphologic Lung Lesions in Tuberculosis

Initially, pulmonary tubercular lesion is 2 cm, solid, gray-white to yellowish, with poorly defined margins and central areas of caseous necrosis. Some patients may develop tubercular cavities, which are highly contagious. The lesion frequently ruptures into the bronchi.

- **Tuberculous cavities:** Caseous material may liquefy resulting in pulmonary cavitary lesions in secondary tuberculosis, but not primary tuberculosis. The cavity is filled with yellow-grayish caseous material more or less surrounded by fibrous tissue. AFB can easily be demonstrated in open pulmonary cavities. Tubercular lesion may erode into airways. Patient may develop empyema and hemoptysis. The sputum becomes positive for AFB.
- **Lung consolidation:** Immunocompromised young children and elderly persons may develop tubercular bronchopneumonia or lobar pneumonia. These patients are very infectious. Patient presents with high-grade fever and productive cough.
- **Tubercular pleuritis and effusion:** These lesions usually develop soon after initial infection. Tuberculous focus located at the edge of the lung ruptures into the pleural space resulting in pleurisy and pleural effusion. Patient with pleurisy presents with dyspnea and sharp chest pain that worsens with a deep breath (pleurisy). Tubercular pleurisy generally resolves without treatment. Morphology of lung in tuberculosis is shown in Fig. 18.16A to F. Differences between primary and secondary tuberculosis are given in Table 18.15. Differences between tuberculosis and sarcoidosis are given in Table 18.16.

Complications of Pulmonary Tuberculosis

Complications of secondary tuberculosis are lung fibrosis, lung cavitation, miliary tuberculosis and other lesions described as under:

- **Lung fibrosis:** Healing of secondary pulmonary TB occurs by fibrosis in favorable conditions.
- **Lung cavitation:** In some patients, tubercular lesion continues to progress for months and years results in further pulmonary damage. It leads to cavity

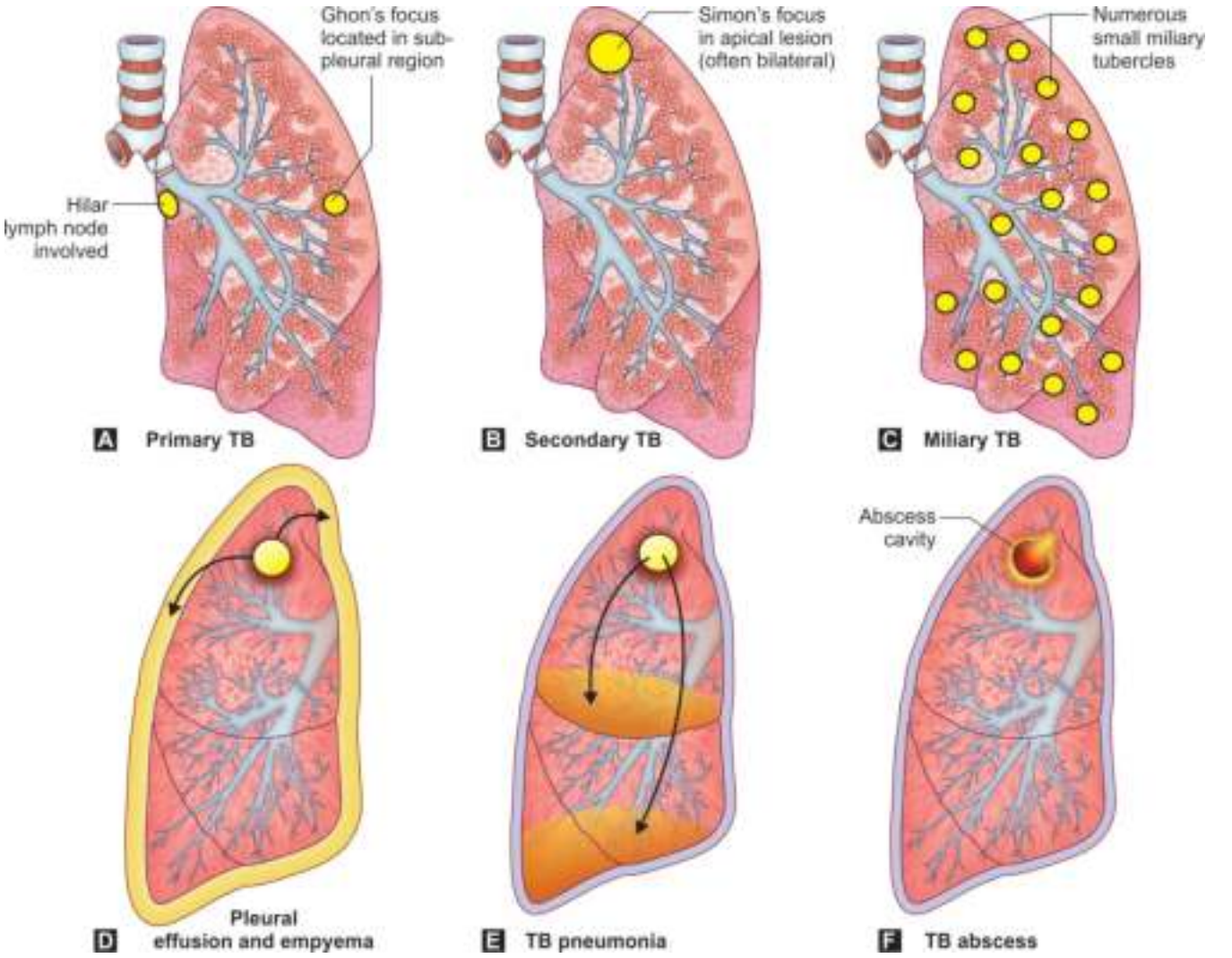


Fig. 18.16: Morphology of lung in tuberculosis. (A) Primary tuberculosis shows subpleural mid-zonal region and lesion hilar lymph node enlargement, (B) apical lesion is seen in secondary tuberculosis, (C) small miliary tubercles are demonstrated in lung, (D to F) pleural effusion, empyema, consolidation (pneumonia) and abscess are complications in tuberculosis.

Table 18.15 Differences between primary and secondary (post-primary) tuberculosis

Parameters	Primary Tuberculosis	Secondary Tuberculosis
Exposure to AFB	First time exposure	Reactivation or reinfection
Age group	Children/younger age group	Any age group
Consolidation	Solitary lesion	Multifocal lesions (poorly defined)
Location	Mid-zone (Ghon's focus in subpleural lesion), other sites (tonsils, ileocecal region, skin)	*Upper lobes (apical region), Simon's focus in lungs:* 2 cm gray-white to yellowish well circumscribed consolidation in apical regions of one or both lungs
Lymphadenopathy	Common	Rare
Severity of lesion	Less severe, healing by fibrosis and dystrophic calcification	More severe progressing to cavitation, lobar pneumonia, extension in lumen of bronchi, trachea, and larynx with vocal cord involvement. Sputum positive for AFB in these persons
Pleural effusion	Common	Empyema
Cavitation	Rare	Common
Miliary tuberculosis	Yes (lung, liver, bone marrow, spleen, adrenal glands, meninges, kidneys, fallopian tubes, epididymis)	Yes (lung, liver, bone marrow, spleen, adrenal glands, meninges, kidneys, fallopian tubes, epididymis)

Table 18.16 Differences between tuberculosis and sarcoidosis

Characteristics	Tuberculosis	Sarcoidosis
Etiological agent	Mycobacterium tubercle bacilli	Unknown etiology
Granuloma	Caseating granuloma	Noncaseating granulomas
Cytoplasmic inclusions in giant cells	Absence of Schaumann bodies, asteroid bodies and birefringent crystals	Presence of Schaumann bodies, asteroid bodies and birefringent crystals
Steroid therapy	Worsens the disease	Improves the patient
Diagnosis	Acid-fast bacilli demonstration	Excluding causes of granulomatous lesions

formation, lobar tubercular pneumonia, fibrocaseous tuberculosis and miliary tuberculosis (Fig. 18.17).

- **Airways involvement:** Endobronchial or endotracheal spread of tuberculosis may cause laryngeal tuberculosis with involvement of vocal cords. Erosion of airways reveals AFB in sputum.
- **Miliary tuberculosis:** Tuberculosis may disseminate via hematogenous route resulting in innumerable small millet seed-like tubercular granulomas known as miliary tuberculosis. Mycobacterium tubercle bacilli may disseminate via systemic circulation to seed distant organs such as bone marrow, liver, spleen, adrenal glands, retina, meninges, kidneys, fallopian tubes, and epididymis. Mycobacterium tubercle bacilli reach the lungs via pulmonary arteries. Chest radiograph reveals very small nodules throughout the lungs that look like millet seeds. Miliary tuberculosis occurs shortly after primary infection in immunocompromised persons (Fig. 18.18).
- **Other complications:** Patient may also develop tubercular laryngitis, bronchopleural fistula, bronchiectasis, vertebral tuberculosis, i.e. Pott's disease, amyloidosis, scar carcinoma of lung and granulomatous hepatitis.

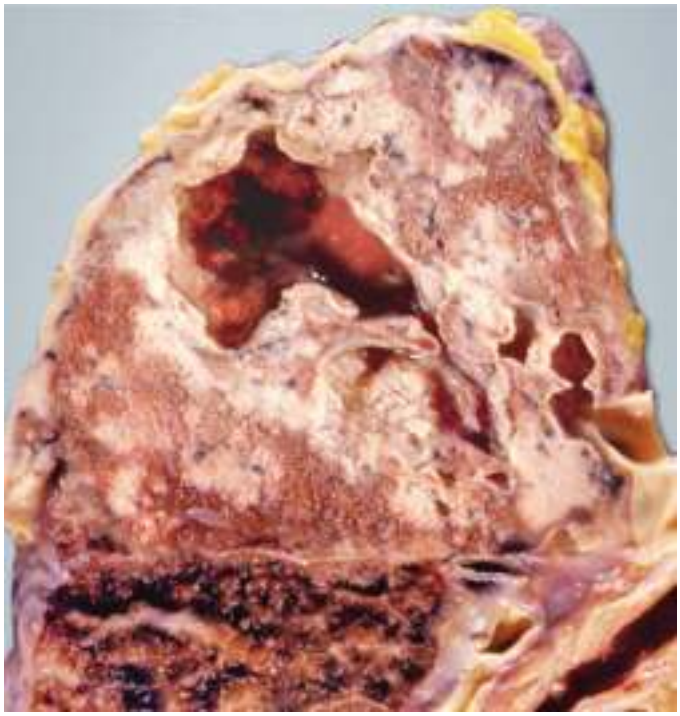


Fig. 18.17: Lung shows cavity in the upper lobe in a case of secondary tuberculosis.

Clinical Features

Patient presents with cough, expectoration, hemoptysis (blood-tinged sputum), night sweats, evening rise of temperature, anorexia, loss of weight, lassitude, and chest pain.

- Fever is probably caused by the absorption of toxic products from the site of infection and synthesis of cytokines (TNF-α and IL-1) by macrophages.
- On clinical examination, percussion note is dull over the affected area. On auscultation, one can hear bronchial breath sounds, crepitant crackles and wheeze. Sound is heard through stethoscope, when the patient whispers, it is known as **whispered pectoriloquy**.

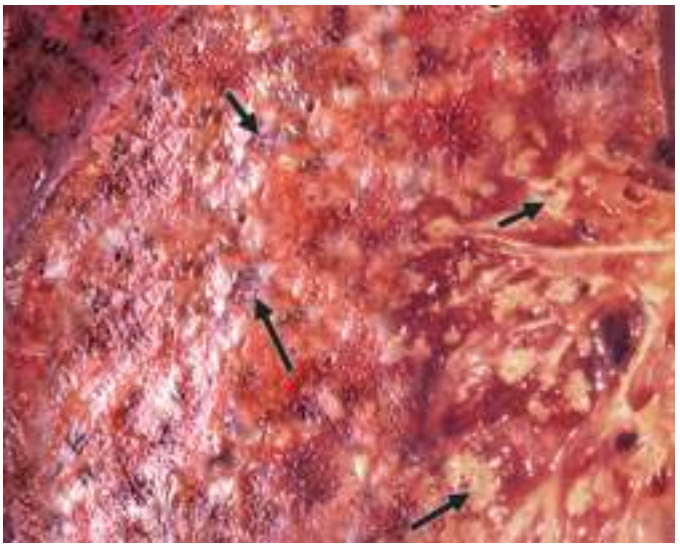


Fig. 18.18: Miliary tuberculosis. Lung shows millet-like seedlings of tuberculosis (arrows).

TUBERCULOSIS IN HIV CASES

Mycobacterium avium–intracellulare (MAI) infection is a progressive systemic disorder, often occurring in patients, who have AIDS. Approximately 33% of all patients develop overt disease due to depletion of CD4+ helper T cells.

CD4+ Helper T Cells Count <200/cu mm

- Immunosuppression is more severe. Consolidation occurs in lower and middle lobe of lungs. Lymphadenopathy occurs in 50% of cases. This pneumonia is characterized by an extensive infiltrate of macrophages.
- Tubercular lesions range from epithelioid granulomas containing a few organisms to loose aggregates of foamy macrophages.
- Clinical presentation associated with *Mycobacterium avium–intracellulare* resembles that of tuberculosis.

CD4+ Helper T Cells Count >300/cu mm

Immunosuppression is less severe. Apical regions of lungs are affected. Extrapulmonary organs involvement occurs in 15% of cases.

CHRONIC OBSTRUCTIVE PULMONARY DISEASES (COPDs)

COPDs: OVERVIEW AND EXAMPLES

Chronic obstructive pulmonary diseases (COPDs), or airway diseases are characterized by chronic obstruction of airflow (partial or complete obstruction). Examples of COPDs are chronic bronchitis, bronchiectasis, emphysema, and bronchial asthma. On the other hand, restrictive pulmonary diseases caused by interstitial or infiltrative lesion of lung and skeletal abnormalities like kyphoscoliosis is characterized by decrease in both FEV1 and FVC resulting in a normal FEV1:FVC ratio.

- **Obstruction of air respiratory passages:** There is increased resistance to airflow from the trachea and large bronchi to the terminal and respiratory bronchioles due to increased luminal secretions in cystic fibrosis or tobacco smokers, thickening of airway wall (edema or hypertrophy), or loss of the elastic recoil of the airway (parenchymal destruction).
- **Respiratory volumes:** Patients show a marked decrease in the forced expiratory volume 1 (FEV1) and an increased or normal forced vital capacity resulting in a decreased FEV1:FVC ratio.

Differences between obstructive and restrictive lung diseases are given in Table 18.17.

Chronic Obstructive Pulmonary Disease: Categories

- **Local obstructive pulmonary disease:** Obstruction due to tumor or inhalation of foreign body causes distal lung collapse. It may be complicated by lipid pneumonia or lobar pneumonia. Pulmonary function tests are normal.
- **Diffuse chronic obstructive pulmonary disease:** Obstruction occurs in bronchi and bronchioles. Pulmonary function tests are abnormal revealing obstructive pathology. Chronic obstructive pulmonary disease is caused by chronic bronchitis, emphysema, bronchiectasis, and bronchial asthma. Spectrum of chronic obstructive pulmonary diseases is given in Table 18.18.

CHRONIC BRONCHITIS

Chronic bronchitis is an inflammation of bronchi caused by prolonged inhalation of tobacco smoke (most common) and air pollutants. It is characterized by persistent cough with sputum production for at least 3 months for 2 consecutive years in the absence of identifiable cause. Productive cough occurs due to hyperplasia and hypersecretion of bronchial submucosal

Table 18.17 Differences between obstructive and restrictive lung diseases

Characteristics	Chronic Obstructive Pulmonary Diseases	Restrictive Pulmonary Diseases
Definition	Increased resistance to airway owing to partial or complete obstruction at any level from trachea, large bronchi to terminal bronchioles	Reduced lung capacity (expansion) of lung either due to chest wall or skeletal abnormality or infiltrative parenchymal diseases
FEV1	Decreased	Decreased
FVC	Increased	Decreased
FEV1:FVC ratio	Decreased	Normal
Associated with disorders	Chronic bronchitis, bronchiectasis, emphysema and bronchial asthma	Chest wall disorders (kyphoscoliosis) and acute respiratory distress syndrome

Table 18.18 Spectrum of chronic obstructive pulmonary diseases

Clinical Entity	Anatomic Site	Age Group	Pathologic Changes	Etiology	Clinical Manifestations
Chronic bronchitis	Bronchi	Adults	Mucous gland hyperplasia, hypersecretion and increased Reid index, bronchial wall thickened	Tobacco smoke, air pollutants causing impaired mucociliary apparatus	Cough, sputum production for at least 3 months for 2 consecutive years
Bronchiectasis	Bronchi	Adults	Abnormal permanent dilation of bronchi associated with scarring	Bronchial obstruction, persistent infections, α_1-antitrypsin (antiprotease) deficiency damaging bronchi	Cough, copious foul smelling sputum and fever
Bronchial asthma	Bronchi	Adults and children	Smooth muscle hyperplasia, increased mucus, inflammation, mucus plugs in bronchioles	Immunological mechanism (IgE-sensitized mast cells) and undefined etiology causing bronchospasm	Episodic wheezing, cough and dyspnea
Emphysema	Acinus	Adults	Enlargement of airspaces along with destruction of alveolar walls	Tobacco smoke and α_1-antitrypsin (antiprotease) deficiency	Severe progressive exertional dyspnea, crackles and wheezing
Bronchiolitis (small airways disease)	Bronchiole	Children	Decreased synthesis of surfactant, occluded bronchiole	Viral infections and pollutants	Cough and breathlessness

glands. These patients are called **blue bloaters** due to decreased O_2 saturation from hypoxemia.

Pathogenesis

Chronic bronchitis is caused by tobacco smoke (90%), pollen grains, cotton, silica dust, air pollution and infectious agents such as bacteria and viruses.

Surgical Pathology: Chronic Bronchitis

Gross Morphology

Bronchi are hyperemic and swollen. Bronchial lumen shows mucinous or mucopurulent secretions.

Light Microscopy

Pathological changes include hyperplasia of bronchial submucosal glands, leading to increased Reid index, i.e. ratio of the thickness of the gland layer to that of the bronchial wall.

- **Bronchi:** Bronchi show chronic inflammation with lymphocytic infiltrate), squamous metaplasia, goblet cell hyperplasia, fibrosis and loss of cilia resulting in narrowing of bronchi.
- **Bronchial submucosal glands:** These undergo hyperplasia and hypertrophy and secrete abundant mucus responsible for sputum overproduction and retention in air passages.
- **Reid index:** Reid index is increased in chronic bronchitis. Increase in size of the mucosal gland layer is assessed by Reid index. Ratio of thickness of mucous gland layer to the thickness of bronchial wall (epithelium to cartilage) is called Reid index. Normal Reid index is 0.4 (Fig. 18.19).

Consequences

- **Chronic obstructive airway disease:** Patient develops wheezing due to narrowing of the terminal bronchioles by mucus plugs. Resistance to airflow in the small airways leads to severe ventilation–perfusion imbalance and decreased arterial oxygenation.
- **Cor pulmonale and heart failure:** Patient develops exertional dyspnea, cyanosis and cor pulmonale. The combination of cyanosis and edema secondary to cor pulmonale has led to the label **blue bloaters** (cyanosis) for such patients. Hypoxia-induced erythropoiesis by erythropoietin increases hemoglobin.
- **Cyanosis:** Though the amount of hemoglobin is high, yet oxygenation low, therefore, cyanosis is evident. Patient may develop respiratory acidosis.
- **Dysplastic change:** Respiratory epithelium may undergo dysplasia or metaplasia.

Chest Radiograph

Chest radiograph shows prominent blood vessels with an enlarged horizontally oriented heart and increased bronchial markings.

Clinical Features

Patient presents with productive cough, dyspnea in late cases, cyanosis of skin and mucous membranes, hypoxemia due to decreased O_2 saturation, expiratory wheezing, sibilant rhonchi, and cor pulmonale. Comparison of chronic bronchitis and bronchial asthma is given in Table 18.19.

Complications

Complications of chronic bronchitis include cor pulmonale, bronchiectasis, bronchopneumonia and bronchogenic carcinoma.

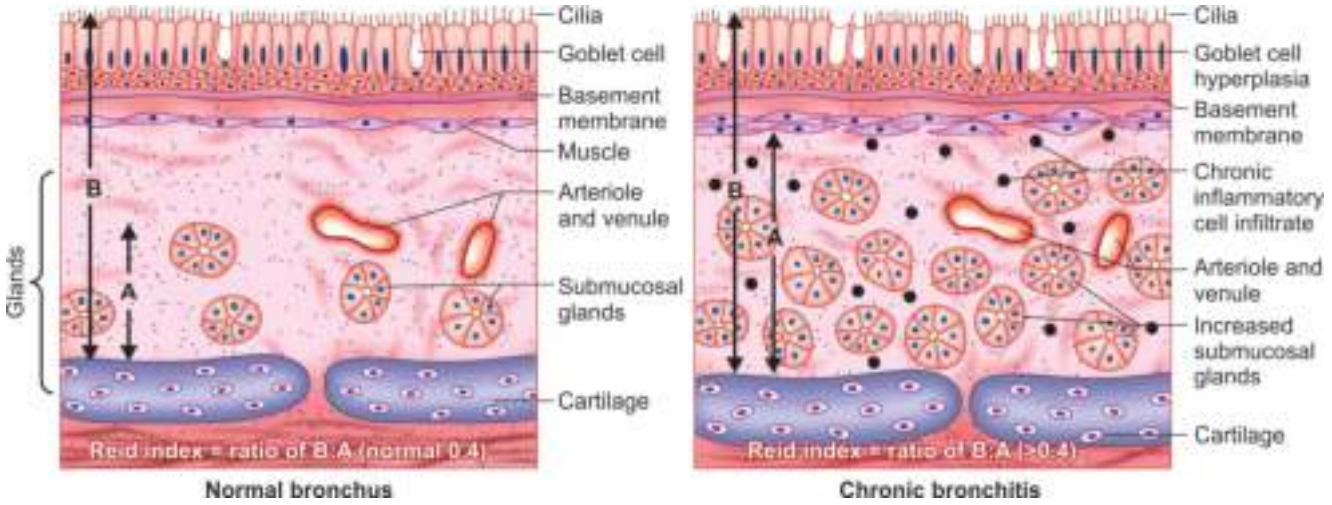


Fig. 18.19: Chronic bronchitis. The lumen of the bronchus shows marked thickening of the mucous glands approximately twice the normal. Squamous metaplasia of epithelium may also occur. Reid index is defined as ratio between the thickness of the submucosal mucus secreting glands and the thickness between the epithelium and cartilage that covers the bronchi under microscope (normal Reid index is 0.4).

Table 18.19 Comparison of chronic bronchitis and bronchial asthma

Features	Chronic Bronchitis	Bronchial Asthma
Clinical history		
Age group	<35 years	Children and adults
Tobacco smoking	Almost invariable	Possible
History of cough	Persistent and productive	Intermittent and nonproductive
History of breathlessness	Progressive and persistent	Intermittent and variable
Nocturnal symptoms	Uncommon	Common
Family history	Uncommon	Common
Concomitant allergic rhinitis or eczema	Uncommon	Common
Airflow obstruction	Fixed and irreversible	Variable and reversible
Sputum examination		
Sputum examination	Macrophages and neutrophils	Eosinophils, Charcot-Leyden crystals, creola bodies, Cruschmann's spirals
Airway muscles	Hypertrophy	Hypertrophy
Histologic features		
Airway congestion and edema	Present	Present
Airway inflammation	CD8+ cytotoxic T cells, neutrophils periodically	CD4+ helper T cells, eosinophils, mast cells
Airway epithelium	Intact, goblet cell hyperplasia and squamous metaplasia	Fragile with stripping, goblet cell hyperplasia and squamous cell metaplasia
Basement membrane reticular layer thickening	Mild-to-moderate	Marked
Bronchial glands	Marked enlargement of the mucous acini	Moderate enlargement of both mucous and serous acini
Histochemistry		
Shift from neutral mucin to acidic mucin; sialo-acidic mucin to sulpho-acidic mucin	Present	Unchanged
Major complications		
Examples	Cor pulmonale	Allergic bronchopulmonary aspergillosis

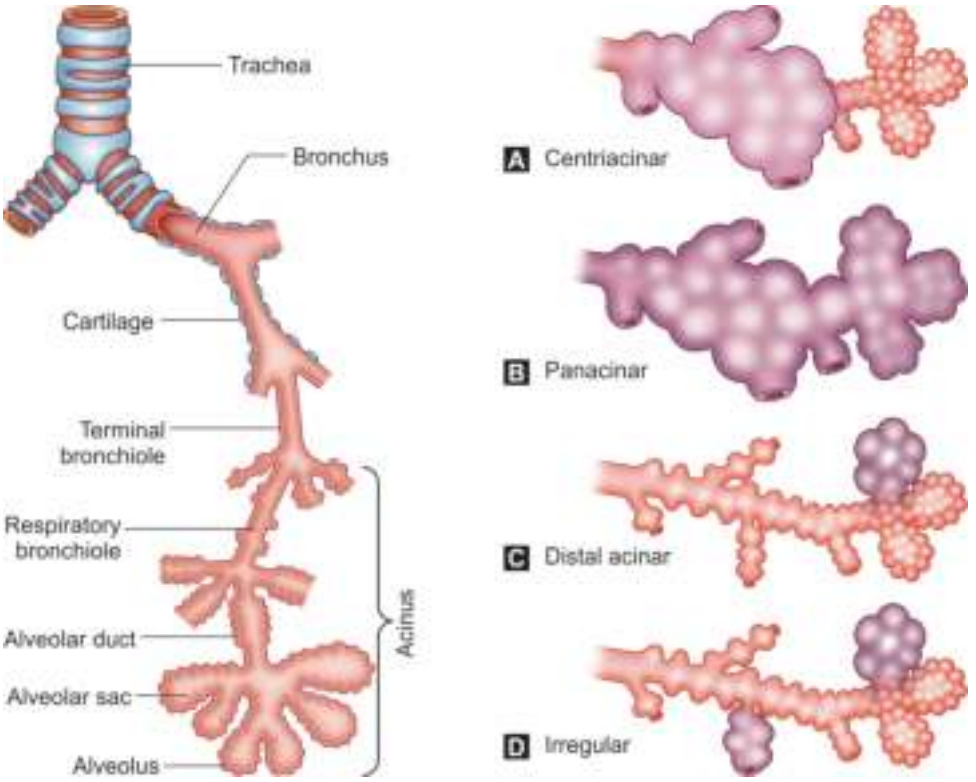


Fig. 18.20: Types of emphysema (centriacinar, panacinar, distal acinar, irregular).

EMPHYSEMA

Emphysema is a chronic lung disease characterized by abnormal permanent enlargement of the airspaces, i.e. respiratory bronchioles, alveolar ducts and alveoli distal to the terminal bronchiole associated with destruction of the alveolar walls without obvious fibrosis and inflammation. Patient develops dyspnea due to impaired oxygen–carbon dioxide gaseous exchange.

Emphysema: Types

Centriacinar and panacinar emphysemas are most common. Paraseptal emphysema may result in spontaneous pneumothorax. Irregular emphysema may be associated with fibrosis. Centriacinar and panacinar emphysemas cause clinically significant airflow obstruction (Fig. 18.20).

Etiopathogenesis

Emphysema occurs as a consequence of protease–antiprotease; and oxidant–antioxidant imbalances. Tobacco smoking is most common cause of emphysema followed by α_1-antitrypsin deficiency (Fig. 18.21).

- **α_1-Antitrypsin (antiprotease):** Normally, α_1-antitrypsin (antiprotease) inhibits elastases activity. The α_1-antitrypsin is a protein encoded on the protease inhibitor (Pi) locus on chromosome 14.
  - Normal amounts of α_1-antitrypsin are synthesized by liver in normal genotype persons (PiMM phenotype). It is a major inhibitor of variety of proteases including elastase, and responsible for 90% of antiprotease activity in the blood.
  - Homozygous PiZZ genotype has decreased synthesis of α_1-antitrypsin. Serum electrophoresis reveals absence of α_1-globulin peak. Most patients with clinically diagnosed emphysema, who are younger than 40 years of age have α_1-antitrypsin (PiZZ phenotype) deficiency.

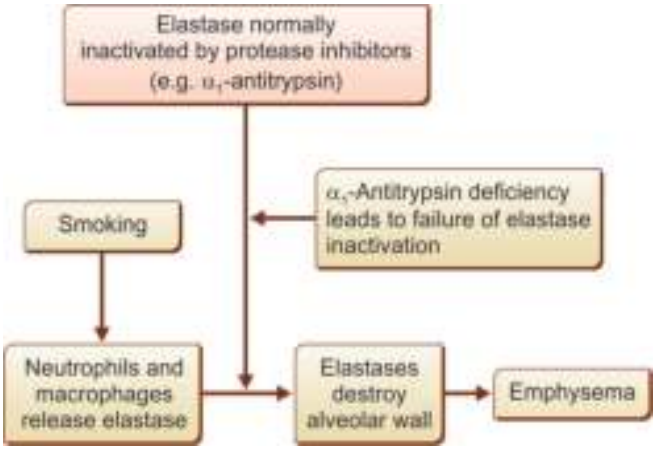


Fig. 18.21: Pathogenesis of emphysema. Under physiologic state, proteases synthesized by inflammatory cells are inactivated by extracellular proteases especially α_1-antitrypsin. If proteases are not inactivated, these cause destruction of lung parenchyma. Imbalance of activity of proteases and protease inhibitors is responsible for emphysema.

- Intravenous purified pooled human plasma is recommended to enhance α_1-antitrypsin levels in these patients.

- **Tobacco smoking:** Tobacco smoke inhibits synthesis of α_1-antitrypsin, and increases the activity of the macrophage's elastases resulting in destruction of alveolar walls. Tobacco smoke induces tissue damage as a result of synthesis of oxygen-derived free radicals, which antagonise antioxidant mechanisms.

Classification of Emphysema

Classification of emphysema includes centriacinar, panacinar, distal acinar (paraseptal), irregular and interstitial types. Differences between centriacinar emphysema and panacinar emphysema are given in Table 18.20.

Centriacinar Emphysema

- Centriacinar emphysema occurs predominantly in heavy tobacco smokers, often in association with chronic bronchitis. It initially affects the respiratory bronchioles and then involve central portion of acinus. The lesions are more common and severe in the upper lobes especially in apical segments (i.e. upper two-thirds of the lungs).
- Tobacco smoke induces an inflammatory reaction as a result of synthesis of serine elastase and oxygen-derived free radicals synthesized by neutrophils and macrophages.
- Elastases cause destruction of the walls of respiratory unit, by promoting splitting peptide bond hydrolysis.
- Oxygen-derived free radicals inactivate α_1-antitrypsin (antiprotease) and antioxidant glutathione leading to destruction of walls of respiratory unit.

Panacinar Emphysema

- Panacinar emphysema initially affects peripheral structures of acinus (i.e. the alveolus and alveolar duct), and then extends to involve the respiratory bronchioles. It tends to occur more commonly in the lower zones as well as anterior margins of the lung.
- The acini are uniformly distended from the level of the respiratory bronchiole to the terminal bronchiole. It is usually more severe at the base of the lungs. It affects equally both men and women.

Distal Acinar (Paraseptal) Emphysema

- In distal acinar (paraseptal) emphysema, the proximal portion of the acinus is normal, but the dilatation predominantly involves distal part of the respiratory unit, i.e. alveoli and alveolar ducts in subpleural region and interlobar septa.
- The morphologic findings include multiple, continuous enlarged airspaces measuring <0.5 cm to >2 cm in diameter sometimes forming cyst-like structures (bullae) in subpleural region due to focal destruction of alveolar walls with confluence of multiple alveoli.
- There is increased risk for spontaneous pneumothorax as a result of rupture of bullae.

Irregular Emphysema

- The acinus is irregularly involved. It is almost invariably associated with scars from healed inflammatory process.
- Irregular emphysema is most common form of emphysema. Patients are asymptomatic and clinically insignificant.

Interstitial Emphysema

Interstitial emphysema occurs as a result of entrance of air into the connective tissue of lung, subcutaneous tissue or mediastinum in settings of penetrating chest injury, chronic bronchitis, and obstruction to airflow. Children with whooping cough are prone to interstitial emphysema. Differences between emphysema and chronic bronchitis are given in Table 18.21.

Table 18.20 Differences between centriacinar emphysema and panacinar emphysema

Characteristics	Centriacinar (Centrilobular) Emphysema	Panacinar (Panlobular) Emphysema
Sites involved	Targeting destruction of the distal terminal bronchioles and respiratory bronchioles (proximal or central part of the acini of respiratory unit)	Targeting destruction of terminal bronchioles and the entire respiratory unit
Frequency	More common	Less common
Lobes affected	Apical segments of upper lobes	Basal parts of lower lobes
Risk factors	Tobacco smoking or coal workers pneumoconiosis	Patients with α_1-antitrypsin deficiency (an autosomal dominant disorder)
Mechanism	Destruction of the distal terminal bronchioles and respiratory bronchioles	Destruction of terminal bronchioles and the entire respiratory unit
Gross morphology	Not significant	Voluminous lungs overlapping heart and hiding it

Table 18.21 Differences between emphysema and chronic bronchitis

Parameters	Emphysema	Chronic Bronchitis
Age group	50–75 years	15–40 years
Onset and severity of dyspnea	Early onset and severe	Late onset but mild
Cough	Late but scanty sputum	Early and copious sputum
Infections	Occasional	Common
Respiratory insufficiency	Terminal event	Repeated events
Cor pulmonale	Rare and in terminal stage	Common
Airway resistance	Normal or slightly increased	Increased
Elastic recoil	Low	Normal
Chest radiograph	Hyperinflation of lungs with small heart	Prominent blood vessels and large heart
Clinical appearance	Pink puffer	Blue bloater

Surgical Pathology: Emphysema

Gross Morphology

- In centriacinar emphysema, upper two-thirds of the lungs are more severely affected than the lower lungs.
- In panacinar emphysema, lungs are pale and voluminous obscuring heart.
- Distal acinar emphysema shows bullae in subpleural region due to focal destruction of alveolar walls with confluence of multiple alveoli leading to spontaneous pneumothorax (Figs 18.22 to 18.24).

Light Microscopy

- In all types of emphysema, alveolar walls show thinning and destruction. Adjacent alveoli become confluent, creating large airspaces.
- There is loss of elastic tissue in the surrounding alveolar septate. Number of alveolar capillaries is diminished.
- Ischemia is probably a factor in rupture of alveoli. Special stains show loss of elastic tissue in lungs (Fig. 18.25).

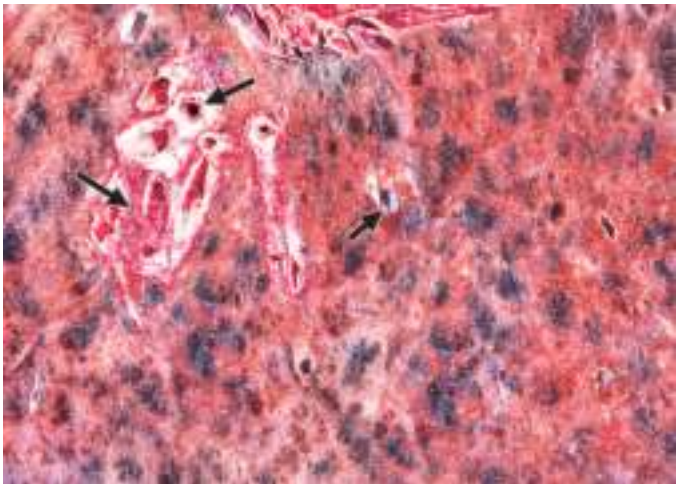


Fig. 18.22: Centriacinar emphysema. Central areas show marked emphysematous damage (arrows), surrounded by relatively spared alveolar spaces.

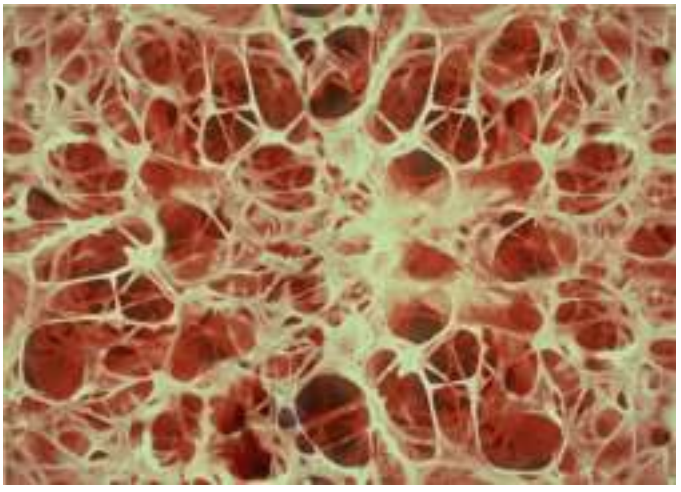


Fig. 18.23: Panacinar (panlobular) emphysematous lung. Cut surface shows dilated airspaces in all lung fields. Panacinar emphysema occurs with loss of all portions of the acinus from the respiratory bronchiole to the alveoli. This pattern is typical for α_1-antitrypsin deficiency. On the other hand, centrilobular (centriacinar) involves primarily the upper lobes and occurs with loss of the respiratory bronchioles in the proximal portion of the acinus, with sparing of distal alveoli especially in smokers.

Clinical Features

Classic presentation of emphysema (centriacinar or panacinar) is pink puffer who develops tachypnea, severe progressive exertional dyspnea and prolonged forced expiration, cough, crackles and wheezing on inspiration and barrel-shaped chest. Breath sounds are diminished due to hyperinflation.

- Chest expansion is decreased. Tactile fremitus is decreased. Centriacinar emphysema frequently coexists with chronic bronchitis.
- Cor pulmonale is uncommon. Pulmonary function tests reveal reduction of ratio of FEV1 to FCV.

Chest Radiograph

Chest radiograph in emphysema shows hyperlucent lung fields, increased anteroposterior diameter

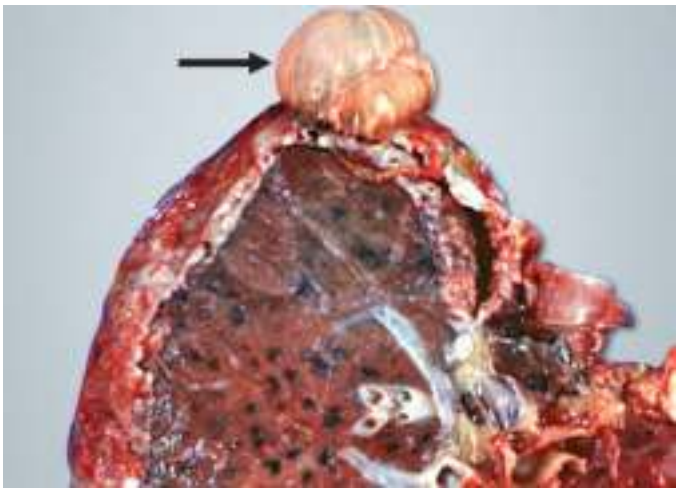


Fig. 18.24: Distal (paraseptal) emphysema. The characteristics of morphological findings include multiple, continuous enlarged airspaces measuring <0.5 cm to >2 cm in diameter sometimes forming cyst-like structures (bullae) in subpleural region due to focal destruction of alveolar walls with confluence of multiple alveoli. There is increased risk of spontaneous pneumothorax as a result of rupture of bullae. Large bullous is seen on subpleural region (arrow).

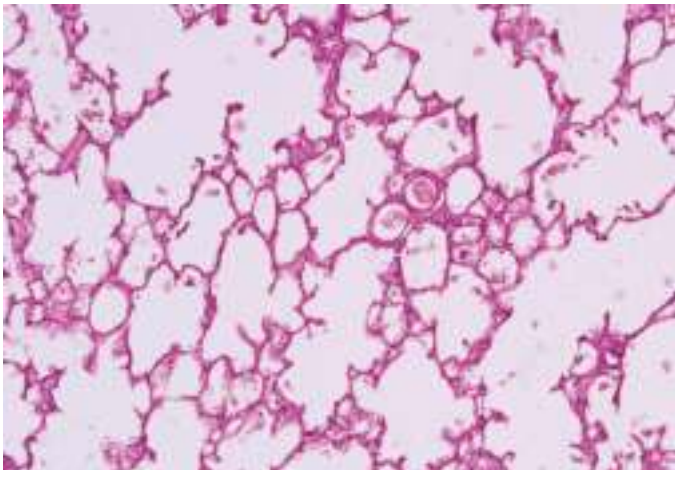


Fig. 18.25: Pulmonary emphysema. There is marked enlargement of airspaces with thinning and destruction of alveolar septa. Tobacco smoking and α_1-antitrypsin antiprotease are important predisposing risk factors (400X).

producing barrel-shaped chest, vertically oriented heart and depressed diaphragms due to hyperinflated lungs.

Complications

The complications of emphysema are interstitial emphysema, pneumothorax, cor pulmonale, respiratory acidosis, and coma. Expansion of emphysema into the interstitial tissues of the chest wall occurs as a result of acute overinflation of the lungs with rupture and perforation in multiple airspaces in interstitial fibrous septa.

Pathology Pearls: Functional Effects of Emphysema

These are categorized into two groups depending on tolerance to hypoxia or not.

- **Blue bloaters tolerate hypoxia:** Patient develops severe hypoxemia, hypercapnea, right ventricular hypertrophy, cor pulmonale with peripheral edema, and secondary polycythemia.
- **Pink puffers do not tolerate hypoxia:** Patient develops severe dyspnea, hyperventilation, and relatively normal blood gases.

BRONCHIECTASIS

Bronchiectasis is abnormal permanent (irreversible) dilation of bronchi and bronchioles caused by chronic necrotizing infections resulting in destruction of cartilage and elastic tissue. The bronchi and bronchioles are filled with mucus and neutrophils. Cough and expectoration of copious amounts of purulent sputum are characteristic findings. Obstruction and infection result in bronchiectasis. It more frequently affects young persons than adults.

Salient Features

- Normally, airways cannot be traced beyond 2–3 cm from pleural surface. Bronchiectasis is more common in vertical airways especially distal bronchi or bronchioles of lower lobes extending up to pleural surface. It is worth mentioning that upper lobes are affected in pulmonary tuberculosis.
- These airways undergo four times dilation than normal size, and lumen is filled with suppurative exudates.
- The dilatation of bronchi and bronchioles is permanent. It should not be confused with a transient mild cylindrical dilatation, which occurs in viral or bacterial pneumonia or collapsed lung; in which the changes become reversible within six weeks.
- *Aspergillus* species may also grow in pre-existing cavities caused by bronchiectasis. They proliferate to form fungus balls, which are also referred to aspergillomas or mycetomas.

Pathogenesis

Obstruction and chronic persistent infection cause damage to bronchial walls leading to weakening and permanent dilation of bronchial tree. Obstructive secretions and inflammation are present throughout the wall of bronchi. Major causes of bronchiectasis and proposed workup of patients are given in Table 18.22.

Genetic and Congenital Disorders

Genetic and congenital disorders play role in pathogenesis of bronchiectasis. These patients are asymptomatic and superadded infections appear late in life.

Table 18.22 Major causes of bronchiectasis and proposed workup of patients

Focal and Diffuse Lung Involvement Categories	Workup of Patients
Focal lung involvement	
Obstruction of bronchi (aspirated foreign body, tumor mass or compression of bronchi by adjacent lymphadenopathy)	Chest imaging (conventional chest radiograph, chest CT scan, bronchoscopy)
Diffuse lung involvement	
Infection (bacterial, nontubercular mycobacterium)	Sputum smear examination by Gram stain/sputum culture for acid-fast bacilli and fungi. If no pathogen is demonstrated, bronchoscopy and bronchoalveolar lavage should be considered
Immunodeficiency state (hypoglobulinemia, human immunodeficiency virus infection, bronchiolitis obliterans after lung transplantation)	Complete blood counts, differential blood counts, immunoglobulin analysis, HIV testing
Genetic causes (Kartagener's syndrome, cystic fibrosis, α_1-antitrypsin deficiency)	Analysis of nasal or respiratory tract brush/biopsy for dyskinesis/immotile cilia syndrome; measurement of chloride levels in sweat and CFTr genetic testing for cystic fibrosis; and α_1-antitrypsin level for α_1-antitrypsin deficiency. Genetic testing is performed to diagnose these disorders
William-Campbell syndrome	Absence of cartilage in the bronchi
Autoimmune disorders (rheumatoid arthritis, Sjögren's disease, inflammatory bowel disease) and immune-mediated disease (allergic bronchopulmonary aspergillosis)	Clinical examination with joint involvement and serologic testing (rheumatoid factor) is done for rheumatoid arthritis; complete workup for allergic bronchopulmonary aspergillosis especially in patients with refractory asthma
Recurrent aspiration in bronchi	Testing of swallowing function and general neuromuscular strength
Miscellaneous disorders (traction bronchiectasis from post-radiation fibrosis or idiopathic pulmonary fibrosis)	Guided by the clinician
Idiopathic	Exclusion of other causes

- **Kartagener's syndrome:** It is autosomal recessive disorder characterized by sinusitis, bronchiectasis and situs inversus, sometimes with male sterility and hearing loss. Structural defect occurs in the dynein arms known as primary ciliary dyskinesis. Impaired ciliary activity predisposes to infection in the sinuses and bronchi resulting in bronchiectasis. Male infertility is an important manifestation of ciliary dyskinesia.
- **Cystic fibrosis:** It is autosomal recessive disorder of exocrine glands characterized by synthesis of thick secretions in their ducts. It is also known as mucoviscidosis of pancreas linked to bronchiectasis. Normally, transmembrane conductance regulator (CFTr) gene located on long arm of chromosome 7 codes for a membrane protein that participates in the movement of chloride and other ions across membranes. Mutation of CFTr gene causes malfunction of exocrine glands and results in increased viscosity of mucus and chloride concentration in sweat and tears. Systemic manifestations of cystic fibrosis are given in Table 18.23.
- **Chronic granulomatous disease of childhood:** It is X-linked disorder with defect in NADPH oxidase activity in neutrophils. It leads to failure of synthesis of superoxide anion and hydrogen peroxide during phagocytosis. Phagocytic cells are unable to kill certain microorganisms and cause bronchiectasis.
- **α_1-Antitrypsin deficiency:** α_1-Antitrypsin synthesized by liver in normal genotype persons (PiMM phenotype) is a major inhibitor of a variety of proteases, including elastase. The α_1-antitrypsin is a protein encoded on the protease inhibitor (Pi) locus on chromosome 14. Its deficiency may be associated with bronchiectasis.
- **Williams-Campbell syndrome:** It is characterized by bronchial cartilage deficiency resulting in bronchiectasis.

Acquired Causes

Bronchial obstruction (partial or complete) and post-inflammation play an important role in pathogenesis of bronchiectasis.

Table 18.23 Systemic manifestations of cystic fibrosis

Organs	Secondary Effects
Skin	▪ Salty sweat results in hyponatremia ▪ Acrodermatitis ▪ Dilation of eccrine and apocrine glands
Gastrointestinal tract (distal region)	Meconium ileus resulting in obstruction, perforation, peritonitis, rectal prolapse
Pancreas	▪ Pancreatic duct obstruction causes pancreatic insufficiency (steatorrhea) ▪ Chronic pancreatitis ▪ Failure to thrive ▪ Diabetes mellitus
Liver	▪ Hepatic duct obstruction causes biliary cirrhosis resulting in esophageal varices ▪ Sclerosing cholangitis
Gall bladder	Cholecystitis
Kidney	▪ Nephrolithiasis ▪ Diabetic glomerulopathy ▪ Immune complex-mediated glomerulonephritis
Respiratory system	▪ Bronchiectasis ▪ Recurrent infections ▪ Atelectasis ▪ Allergic bronchopneumonia ▪ Aspergillosis
Heart	▪ Cor pulmonale ▪ Left ventricular fibrosis ▪ Carotid bodies enlarged
Male genital system	Male infertility (obstructive aspermia due to vas deferens atresia in 6% and male infertility in 1–2%)
Female genital system	▪ Multiple ovarian follicular cysts ▪ Cervicitis ▪ Reduced fertility
Musculoskeletal system	▪ Osteoporosis ▪ Muscle wasting ▪ Hypertrophic pulmonary arthropathy ▪ Clubbing
General manifestations	▪ Malnutrition ▪ Vitamin deficiencies (vitamin B_{12}, vitamin K*) ▪ Amyloidosis

Vitamin K deficiency at birth may lead to bleeding.

- **Bronchial obstruction:** It occurs due to neoplasm, foreign body, aspiration and compression by lymph node leading to bronchiectasis.
- **Post-inflammation (nonobstruction):** Bronchiectasis results from repeated episodes of pneumonia especially during first two decades of life. Patient with immunodeficiency states may develop superadded infection due to *Haemophilus influenzae* (most common), measles, whooping cough, tuberculosis and allergic bronchopulmonary aspergillosis. Traction bronchiectasis occurs from pulmonary fibrosis.

Surgical Pathology: Bronchiectasis

Gross Morphology

- Bronchiectasis most commonly affects pulmonary lower lobes.
- Bronchi and bronchioles reveal destruction of wall with replacement by scar tissue.
- Dilated airways may take various shapes, i.e. cylindrical, fusiform, varicose (numerous constriction and dilation) and saccular (bead-like bulge on one side).
- Dilated airways are easily visible up to <3 mm from the pleural surface. Normally, bronchi are traceable up to 2–3 cm from the pleural surface.
- Bronchi and bronchioles are dilated and filled with pus, which impairs normal inflow of O_2 in lungs and outflow of CO_2 (Fig. 18.26).

Light Microscopy

- **Intense inflammation:** The walls of bronchi and bronchioles are infiltrated by acute and chronic inflammatory cells, composed of neutrophils, lymphocytes, plasma cells and macrophages. Mature lymphoid follicles may also be present.
- **Desquamation of lining epithelium:** Mucosa of airways shows extensive areas of necrotizing ulceration. Remaining lining epithelium may show squamous metaplasia.
- **Granulation tissue:** In more advanced cases, there is presence of granulation tissue and fragmentation of cartilage. Mucous glands persist longer than other structures.
- **Fibrosis:** Fibrosis occurs in bronchi and bronchioles walls. Peribronchial fibrosis is also evident (Fig. 18.27).

Clinical Features

Patient presents with constant cough, foul smelling sputum, hemoptysis (in some cases), wheezing, shortness of breath, fever, digital clubbing and cor pulmonale. Some patients may develop pneumonia. On auscultation, moist crackling noises may be heard in the chest.

Complications

Patient with bronchiectasis may develop lung abscess, pneumonia, bronchopleural fistula, cor pulmonale, necrotising brain abscess, meningitis, amyloidosis and squamous cell lung carcinoma.

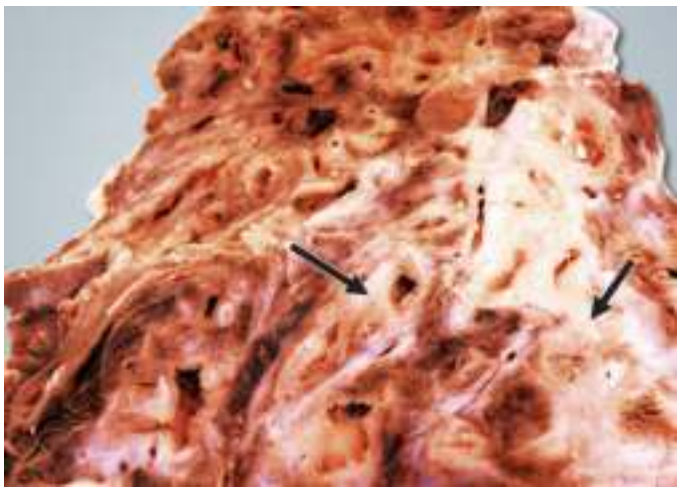


Fig. 18.26: Bronchiectasis. It shows abnormal permanent dilatation of bronchi of variable sizes filled with pus (arrows).

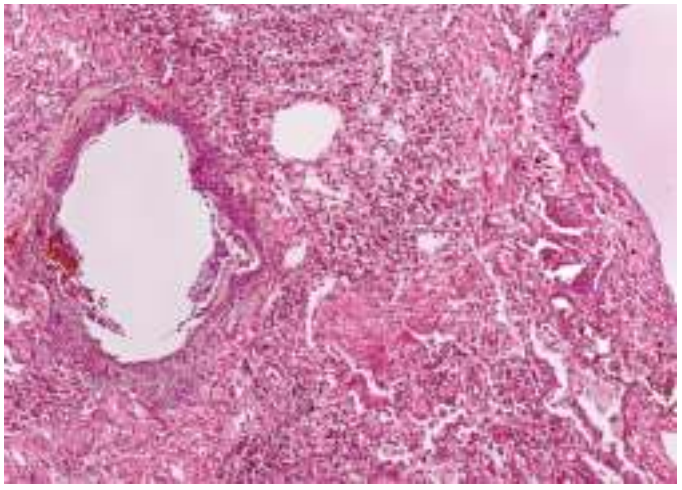


Fig. 18.27: Bronchiectasis. Bronchial wall is infiltrated by acute and chronic inflammatory cells (400X).

Diagnosis

A complete medical history and physical examination by a physician is necessary.

- **Imaging techniques:** Chest X-ray, bronchography, and CT may reveal evidence of bronchiectasis.
- **Sputum examination:** Sputum needs to be collected and cultured (grown in a laboratory dish), in order to examine it microscopically for the specific type of organism responsible for infection.
- **Pulmonary function tests:** These are performed to determine the presence and severity of abnormal airflow out of the lungs.
- **Diagnostic techniques:** A careful search for other underlying diseases is important, looking in particular for ciliary abnormalities, cystic fibrosis, or immunoglobulin deficiencies.

BRONCHIAL ASTHMA

Bronchial asthma is a chronic relapsing disorder resulting from bronchospasm of bronchi and terminal bronchioles. Airways are obstructed due to increased mucus secretion and mucosal edema. It is mediated by type 1 hypersensitivity reaction or undefined causes. Patient presents with episodic reversible wheezing, cough, dyspnea and respiratory alkalosis.

- Bronchial asthma can occur by intrinsic or extrinsic mechanisms.
- Atopy is the strongest risk factor. Atopy refers to the genetic tendency to develop allergic rhinitis and bronchial asthma associated with heightened immune response to allergens, drugs and food.

Etiology

Bronchial asthma occurs due to air-pollutants, viral-induced respiratory infections, i.e. rhinovirus, para-influenza virus, and respiratory syncytial virus, aspirin sensitivity, stress, cigarette smoke and fungal spores of *Aspergillus flavus.*

Pathogenesis

Bronchial asthma occurs by two mechanisms: extrinsic asthma is typically a childhood disease, whereas intrinsic asthma usually begins in adults.

- **Extrinsic (immune) asthma:** Bronchial asthma is mediated by type 1 hypersensitivity reaction involving IgE bound to mast cells. It occurs due to exposure to allergens. Disease starts in childhood. There is family history of allergy.
- **Intrinsic (nonimmune) asthma:** Bronchial asthma is associated with chronic bronchitis, exercise or cold-induced asthma. Disease most often begins in adult life. There is no family history of allergy.

Age Group

Bronchial asthma is most common chronic respiratory disease in children (<10 years) in 50% of cases than adults. Adult males and females are equally affected.

Pathologic Changes

Pathologic changes in bronchial asthma include bronchial smooth muscle hypertrophy, hyperplasia of bronchial submucosal glands and goblet cells, airways plugged by viscid mucus containing, Curschmann spirals, eosinophils, and Charcot-Leyden crystals in sputum.

Pathogenesis

There are early and late phase reactions in pathogenesis of bronchial asthma. Late phase reactions occur 4–8 hours after a symptom-free period.

Early Phase Reactions in Bronchial Asthma

- **First exposure to allergen:** Allergen may enter through nose by inhalation (most common) and ingestion. It is absorbed in the tissues and triggering immune response. CD4+ helper T cells release interleukins (IL-4 and IL-5). IL-4 stimulates B cells to synthesize IgE, which binds to the mast cells on the mucosal surfaces.
- **Second exposure to allergen:** Allergen re-enters nose and mouth. Inhaled antigens cross-link IgE antibodies on mast cells on mucosal surfaces. It triggers mast cells to release histamine and leukotrienes. These chemical mediators cause bronchospasm, increased mucus secretion, smooth muscle contraction, deposition of plasma proteins in the bronchial submucosa due to increased vascular permeability, and influx of leukocytes (LTB4).

Late Phase Reactions in Bronchial Asthma

- **Chemokine (eotaxin) synthesis:** Eotaxin synthesized by leukocytes participates in emigration of eosinophils in the tissues.
- **Leukotrienes synthesis:** Synthesis of leukotrienes (LTC_4, LTD_4, and LTE_4) by mast cells cause prolonged bronchoconstriction. Acetylcholine synthesis further causes bronchospasm (Fig. 18.28).

Surgical Pathology: Bronchial Asthma

Light Microscopy

- **Bronchi/bronchiole changes:** These include bronchial smooth muscle hypertrophy, hyperplasia of goblet cells, thickening and hyalinization of basement membranes.
- **Bronchial wall:** It is infiltrated by eosinophils, mast cells, macrophages and lymphocytes.
- **Bronchial lumen:** Bronchial mucosa shows edema, focal ulceration. Bronchial lumen is occluded with mucus plugs containing whorl-like accumulations of epithelial cells.
- **Curschmann spirals and crystalloids:** Eosinophils derived major basic protein and cationic proteins coalesce to form Charcot-Leyden crystals demonstrated in the sputum of bronchial asthma patients.

Clinical Features

Patient presents with widespread high-pitched wheeze rhonchi. Barrel-shaped chest occurs in severe chronic bronchial asthma. Widespread rhonchi are heard on auscultation. Disease can be controlled by administration of β-agonists, theophylline, corticosteroids and leukotriene receptor agonists. Acute severe bronchial asthma can be life-threatening. Patient requires immediate oxygen, nebulization, β-agonists, anticholinergic drugs and systemic corticosteroid therapy.

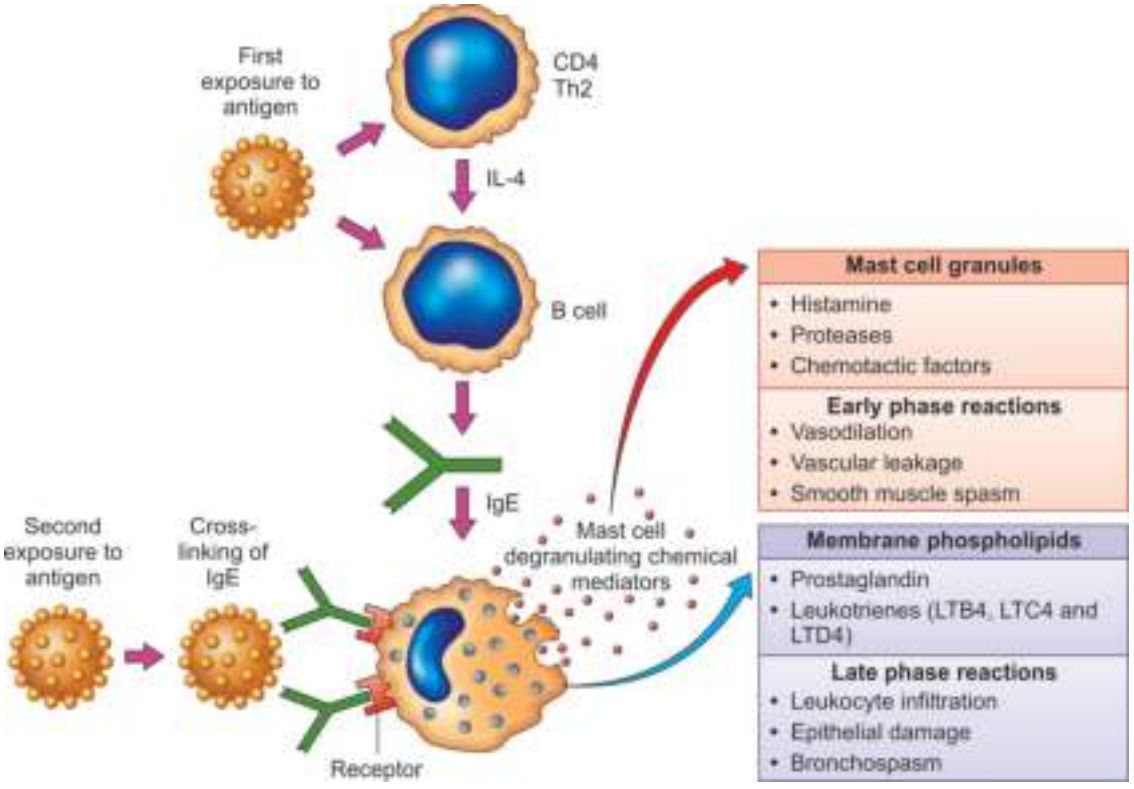


Fig. 18.28: Type 1 hypersensitivity reaction. The antigen stimulates B cells to produce IgE. IgE binds to mast cells or basophils *via* its Fc fragment, causing them to be sensitized. Further exposure to antigen causes cross-linking of bound IgE on mast cells, resulting in degranulation.

- **Breathlessness:** Bronchial asthma attack may last for hours or even days. Due to narrowing of airways, patient presents with sudden dyspnea, episodic expiratory wheezing, i.e. inspiratory in severe cases, tightness in the chest, nocturnal coughing, and tachypnea with use of accessory muscles for breathing.
- **Prolonged coughing:** After the bronchial asthmatic attack is over, there is prolonged coughing up of tenacious secretions. Anteroposterior diameter of chest is increased due to air trapping and increased residual volume. Initially, patient develops respiratory alkalosis. If bronchospasm is not relieved, patient may develop respiratory acidosis. Such patients need tracheal intubation and mechanical ventilation.

Complications

Patient may develop pneumothorax, pneumomediastinum, status asthmaticus and fatal outcome. When severe acute bronchial asthma is unresponsive to therapy, it is referred to as status asthmaticus. Light microscopy of lung in status asthmaticus often shows a bronchus containing luminal mucous plug, submucosal gland hyperplasia, smooth muscle hyperplasia, basement membrane thickening, and increased numbers of eosinophils (Table 18.24).

Table 18.24 Comparison of extrinsic and intrinsic bronchial asthma

Characteristics	Extrinsic Bronchial Asthma	Intrinsic Bronchial Asthma
Definition	Extrinsic asthma is caused by type 1 hypersensitivity reaction induced by exposure to an extrinsic antigen	Intrinsic asthma is caused by diverse non-immune mechanisms as a result of intrinsic stimuli
Age of clinical presentation	Childhood	Adult
Family history	Present	Absent
Preceding allergic reactions	Present in the form of rhinitis, urticaria, and eczema	Absent
Drug hypersensitivity	Absent	Present
Serum IgE level	Increased	Normal
Skin test	Positive skin test	Negative skin test
Emphysema	Unusual	Common
Associated chronic bronchitis	Absent	Present
Examples	▪ Atopic/allergic asthma ▪ Occupational asthma ▪ Allergic bronchopulmonary aspergillosis	Aspirin ingestion, pulmonary infection especially viral, cold, inhaled irritants, stress, exercise

RESTRICTIVE PULMONARY DISEASES

Restrictive pulmonary diseases comprise group of disorders characterized by reduced lung expansion and total lung capacity. There is decrease in both forced expiratory volume 1 (FEV1) and forced vital capacity (FVC) resulting in a normal FEV1:FVC ratio.

- Restrictive pulmonary diseases restrict lungs expansion and often interfere with respiratory gaseous exchange. Causes of restrictive pulmonary diseases are given in Table 18.25.
- Gas exchange barrier consists of alveolar epithelial cells, capillary endothelial cells, basement membrane and interstitial tissue.
- Initial lung injury occurs to capillary endothelial and alveolar epithelial cells leading to interstitial fibrosis and dyspnea. Damage to alveolar epithelium and capillaries leads to impaired ventilation–perfusion leading to hypoxia, cyanosis and cor pulmonale.

ACUTE RESTRICTIVE PULMONARY DISEASES

ADULT RESPIRATORY DISTRESS SYNDROME

Adult respiratory distress syndrome (ARDS) is characterized by pulmonary edema (noncardiogenic) resulting from acute alveolar–capillary damage. It is also known as shock lung. It can quickly lead to acute respiratory failure with fatal outcome within 48 hours in 50–70% of cases. Highest rate of mortality occurs due to aspiration of gastric contents, bacteremia and pneumonia.

- Normally, pulmonary surfactant reduces tension in alveoli and keeps alveoli open. Decrease in synthesis of surfactant by type 2 pneumocytes causes acute respiratory distress syndrome.
- Patient develops a clinical syndrome characterized by refractory hypoxemia not responsive to oxygen

Table 18.25 Causes of restrictive pulmonary diseases

Etiology	Disorders
Chest wall abnormality	▪ Deformities (kyphoscoliosis) ▪ Neuromuscular disorder
Acute primary lung disease	Acute respiratory distress syndrome
Chronic primary lung disease	▪ *Occupational lung diseases:* Asbestosis, silicosis, coal worker's pneumoconiosis ▪ Interstitial lung disease (interstitial pneumonia), idiopathic pulmonary fibrosis ▪ *Immune diseases:* Sarcoidosis, systemic lupus erythematosus, rheumatoid arthritis, Wegener's granulomatosis and Goodpasture syndrome ▪ *Physical injury:* Radiation-induced injury ▪ *Drugs:* Chemotherapeutic agents, methotrexate

therapy, reduction in lung compliance, normal oncotic pressure, normal capillary pulmonary wedge pressure and chest radiograph suggestive of bilateral pulmonary edema.

Etiopathogenesis

Adult respiratory distress syndrome may follow direct or indirect lung injury. Sepsis is most common cause of acute respiratory distress syndrome (ARDS). Other causes include pulmonary contusions, severe trauma and shock. Etiology of acute respiratory distress syndrome in adults is given in Table 18.26.

- **Direct lung injury:** Hantavirus, oxygen toxicity, inhalation of chemical irritants (chlorine), smoke inhalation, pneumonia, aspiration of gastric contents and post-lung transplant cause acute alveolar capillary damage in >30% of cases. Coronavirus destroys type 2 pneumocytes and causes diffuse alveolar damage.
- **Indirect lung injury:** Various systemic disorders cause indirect alveolar–capillary damage. Examples are septicemia (40% cases), severe trauma with shock (20% cases), acute hemorrhagic pancreatitis, therapeutic drug (bleomycin), overdose with street drug (heroin), multiple blood transfusions, cardiopulmonary bypass, disseminated intravascular coagulation, fat embolism, complicated abdominal surgery, and radiation injury.

Table 18.26 Etiology of acute respiratory distress syndrome in adults

Lung Damage	Disorders
Primary lung damage	▪ Aspiration of gastric contents (35%) ▪ Bacterial and viral pneumonias (12%) ▪ Inhalation of smoke and chlorine ▪ Oxygen toxicity ▪ Radiation-induced injury ▪ Drug-induced lung injury (salicylates, bleomycin) ▪ Paraquat poison
Secondary lung damage	▪ Disseminated intravascular coagulation (22%) ▪ Traumatic shock (5%) ▪ Multiple blood transfusions (5%) ▪ Extensive burns ▪ Septicemia (4%) ▪ Acute pancreatitis

Highest prevalence of acute respiratory distress syndrome in decreasing frequency occurs due to aspiration of gastric contents, disseminated intravascular coagulation, pneumonias, traumatic injury, and multiple blood transfusions.
Highest rates of mortality are aspiration of gastric contents, bacteremia and pneumonia.

Pathogenesis

Following alveolar–capillary damage, alveolar macrophages synthesize IL-1, IL-8, TNF-α and macrophage inhibiting factor (MIF).

- Chemical mediators IL-1, IL-8, and TNF-α cause sequestration of neutrophils into the alveolar spaces, and aggregation of platelets.
- Activation of the coagulation system cascade is suggested by the presence of microemboli containing aggregate of platelets in alveolar capillaries. Pathogenesis of acute respiratory distress syndrome in adults is shown in Fig. 18.29.

Exudative Phase of ARDS

Lung becomes dark, red and firm within 7 days. Histamine released by platelets increases the pulmonary capillary permeability resulting in pulmonary edema.

- Neutrophils synthesize chemical mediators like leukotrienes, oxidants, proteases and platelet-activating factor, resulting in damage to type 1 and type 2 pneumocytes.
- Damage to type 2 pneumocytes leads to inactivation of pulmonary surfactant (lecithin-rich surface-

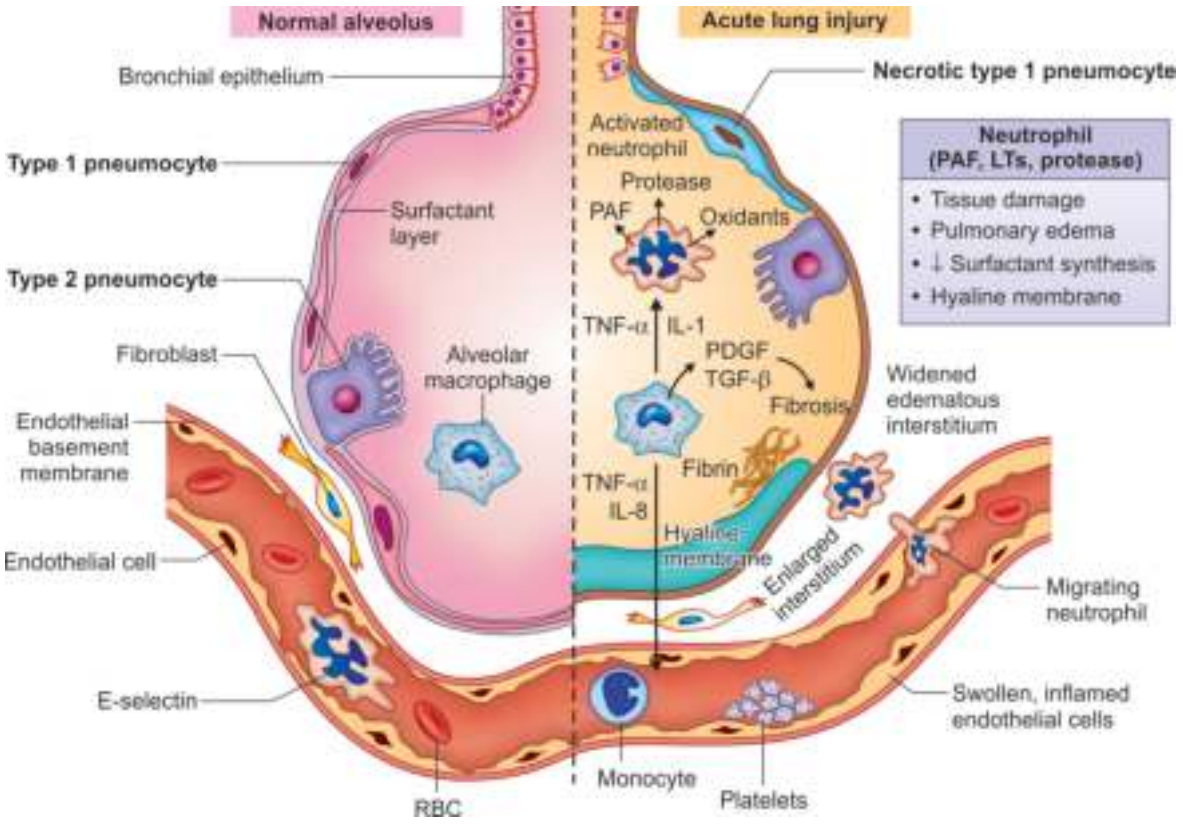


Fig. 18.29: Pathogenesis of acute respiratory distress syndrome. Normal alveolus is seen on left half of figure. Synthesis of IL-8 and TNF-α by macrophages and neutrophils occurs in the alveoli. Leukotrienes, oxidants, proteases, and platelet-activating factor (PAF) synthesized by neutrophils produce local tissue damage, pulmonary edema, surfactant inactivation, and hyaline membrane formation. Fibrogenic factors (PDGF and TGF-β) synthesized by macrophages stimulate fibroblast growth and collagen deposition associated with the healing phase of injury.

active lipid) resulting in atelectasis (collapse of alveoli).

- Exudative phase of ARDS leads to pulmonary edema and fibrinous exudation. Fibrinous exudates form hyaline membranes lining alveoli, which appear bright, pink in hematoxylin and eosin stained tissue sections.
- Macrophage inhibitory factor (MIF) released into the local milieu sustains the ongoing pro-inflammatory response. These pathologic changes result in impairment of respiratory gaseous exchange with consequent severe hypoxia.

Proliferative Phase of ARDS

Proliferative phase of ARDS begins about one week after the initial injury. It is marked by the proliferation of macrophage within alveolar walls.

- Alveolar macrophages digest the remnant of hyaline membranes and other cellular debris.
- TGF-β and PDGF synthesized by macrophages cause proliferation of fibroblasts leading to mild fibrosis that resolves in adult respiratory distress syndrome.

Fibrotic Phase of ARDS

In severe cases, it may progress to restructuring of the pulmonary parenchyma and cyst formation. Alveolar walls are thickened. Progressive pulmonary fibrosis may produce honeycomb appearance in rare cases.

Surgical Pathology: Adult Respiratory Distress Syndrome
Gross Morphology
Lungs are heavy, diffusely firm, solid, dark red and rubbery due to diffuse alveolar damage.
Light Microscopy
▪ **Acute exudative phase:** Lungs show congestion of capillaries, interstitial edema, and fibrinous exudates in alveoli. The alveolar walls are lined by hyaline membranes. ▪ **Proliferative/fibrotic phase:** Proliferative phase is marked by the proliferation of macrophage within alveolar walls. Fibrinous exudate undergoes organization leading to alveolar fibrosis and thickening of the alveolar septa. There may be superimposed bronchopneumonia in fatal cases. Histology of diffuse alveolar damage is shown in Fig. 18.30.

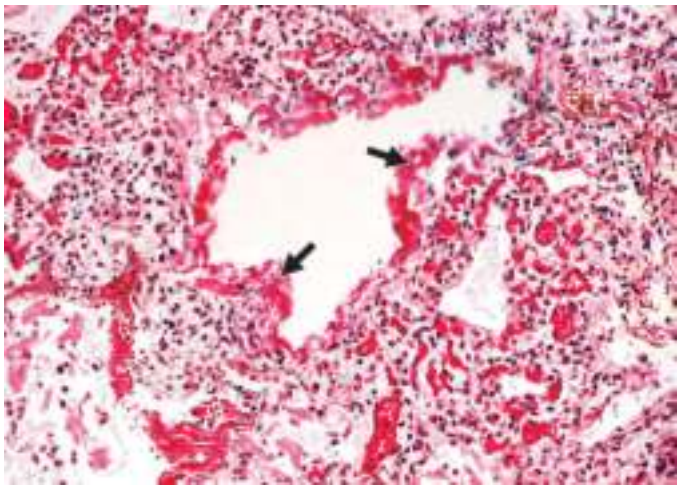


Fig. 18.30: Diffuse alveolar damage. Acute exudative phase shows protein-rich hyaline membranes, interstitial edema and inflammation (arrows).

Chest Radiograph

Initially, chest radiograph shows bilateral interstitial infiltrates followed by extensive pulmonary consolidation in 80% of cases.

Clinical Features

- **Respiratory system:** Patient develops rapid, shallow breathing, dyspnea, intercostal and suprasternal retraction crackles, rhonchi, respiratory and metabolic acidosis, and sometimes respiratory failure. Pulmonary edema in acute respiratory distress syndrome does not resolve, however, it resolves in cases of congestive heart failure.
- **Cardiovascular system:** Patient develops arterial hypoxemia refractory to oxygen therapy, cyanosis, tachycardia, and hypotension.
- **Central nervous system:** Patient develops restlessness, apprehension, mental sluggishness, and motor dysfunction.
- **Urinary system:** Patient has decreased urinary output (oliguria).

Prognosis

Patient with adult respiratory distress syndrome has frequently fatal outcome in 60% of cases. Approximately 30–40% of cases with good intensive care may survive. High concentration of oxygen therapy may further cause alveolar–capillary damage due to oxygen toxicity. Old age, multi-system failure and high levels of IL-1 indicate poor prognosis.

NEONATAL RESPIRATORY DISTRESS SYNDROME

The pathogenesis of neonatal respiratory distress syndrome is intimately linked to deficiency of surfactant. It leads to collapse of the alveoli followed by leakage of fibrin-rich fluid into the alveoli from injured vascular bed resulting in respiratory failure in newborn.

- Normally surfactant, lecithin-rich lipid synthesized by pneumocytes 2 prevents lung collapse (atelectasis) of the alveoli during expiration by lowering the surface tension of the alveoli at low lung volumes and keeps alveoli open.
- Cortisol increases the synthesis of surfactant, while insulin inhibits its synthesis. Pulmonary surfactant composition, surfactant-specific proteins (A, B, C, D apoproteins), their surface activity and functions; and surfactant dysfunction disorders are given in Tables 18.27 to 18.29.

Clinical Features

The first symptom of neonatal respiratory distress syndrome usually appearing within an hour of birth is cyanosis, increased respiratory effort, with forceful intercostal retraction and the use of accessory neck muscles in newborn. Despite advances in neonatal intensive care, the overall mortality of ARDS is about 15%, and 33% of infants born before 30 weeks of gestational age die of this disorder.

Predisposing Factors

Predisposing factors include prematurity, maternal diabetes mellitus, neonatal amniotic fluid aspiration and birth by cesarean section.

- **Premature newborn:** Premature newborn is unable to synthesize pulmonary surfactant. To reduce the potential for developing respiratory distress syndrome in newborn, woman is administered glucocorticoids to enhance fetal surfactant synthesis.
- **Maternal diabetes mellitus:** Fetal hyperglycemia increases the insulin release. Good blood sugar control of maternal diabetes mellitus by insulin

Table 18.27 Pulmonary surfactant composition

Composition	Constituents Percentage
Saturated phosphatidylcholine	50%
Unsaturated lipids	17%
Phosphatidylglycerol	7%
Phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, other phospholipids and lipids	16%
Serum proteins	8%
Surfactant-specific proteins (A, B, C, D apoproteins)	2%

Table 18.28 Surfactant-specific proteins (A, B, C, D apoproteins), their surface activity and functions

Features	Surfactant Apoprotein A	Surfactant Apoprotein B	Surfactant Apoprotein C	Surfactant Apoprotein D
Molecular weight	26–38 kDa	9 kDa	4 kDa	43 kDa
Hydrophilic or hydrophobic in nature	Hydrophilic nature	Hydrophobic nature	Hydrophobic nature	Hydrophilic nature
Lowering alveolar surface activity	None surface activity	High surface activity	Very high surface activity	None surface activity
Main functions	Acts as opsonin, promotes phagocytosis and regulates surfactant secretion by unwinding lamellar bodies	Optimizes alveolar surface activity	Optimizes alveolar surface activity	Acts as opsonin, promotes phagocytosis and regulates surfactant secretion by unwinding lamellar bodies

Hydrophilic surfactant apoproteins A and D act as opsonins and participate in phagocytosis. These are involved indirectly in lowering alveolar surface activity. Hydrophobic surfactant apoproteins B and C optimize alveolar surface activity in conjunction with surfactant phospholipids.

Table 18.29 Surfactant dysfunction disorders

Features	Surfactant Apoprotein B	Surfactant Apoprotein C	Surfactant Apoproteins A, B, C, A3
Genetic inheritance	Autosomal recessive	Autosomal dominant, sporadic	Autosomal recessive
Chromosomal abnormality	2p12–11.2	8p21	16p13.3
Age distribution	Neonates	Infants, children	Neonates, infants
Clinical features	Respiratory failure and death	Respiratory failure	Respiratory failure
Lung histologic features	Alveolar type 2 cell hyperplasia, alveolar proteinosis, interstitial inflammation and fibrosis	Desquamative pattern or nonspecific interstitial pneumonia	Alveolar type II cell hyperplasia, alveolar proteinosis, and accumulation of macrophages

Unexplained neonatal or infantile respiratory may occur. Lung histology shows endogenous lipid pneumonia, cholesterol pneumonitis, alveolar proteinosis, chronic pneumonitis of infancy, desquamative interstitial pneumonia or nonspecific interstitial pneumonia.

administration reduces the risk of respiratory distress syndrome in newborn.

- **Neonatal amniotic fluid aspiration:** Amniotic fluid aspiration in newborn causes severe damage to alveolar–capillary membrane resulting in impaired respiratory gaseous exchange.

Pathogenesis

There is decreased synthesis of pulmonary surfactant in the immature lungs of premature newborn infants. Immature lungs and decreased pulmonary surfactant cause decreased lung compliance, atelectasis and hypoxia.

- Hypoxia produces neonatal respiratory distress syndrome by causing vasoconstriction of pulmonary vasculature leading to pulmonary hypertension and decreased pulmonary perfusion.
- Hypoxia simultaneously increases pulmonary capillary permeability leading to alveolar exudation and formation of hyaline membrane. Pathogenesis of neonatal respiratory distress syndrome is shown in Fig. 18.31.

Surgical Pathology: Neonatal Respiratory Distress Syndrome

Gross Morphology

Lungs are dark red, airless heavier than usual, with areas of atelectasis alternating with occasional dilated alveoli or alveolar ducts.

Light Microscopy

- The alveolar ducts and alveoli are lined by conspicuous, eosinophilic, fibrin-rich, amorphous material, termed hyaline membranes.
- Pulmonary capillaries are congested.

Complications

Intraventricular hemorrhage in brain is a major complication of neonatal respiratory distress syndrome. Thin-walled veins in the periventricular germinal matrix are dilated as a result of anoxia resulting in rupture. Intraventricular hemorrhage reflects anoxic injury to the periventricular capillaries, dilation of thin-walled veins, thrombosis and impaired autoregulation.

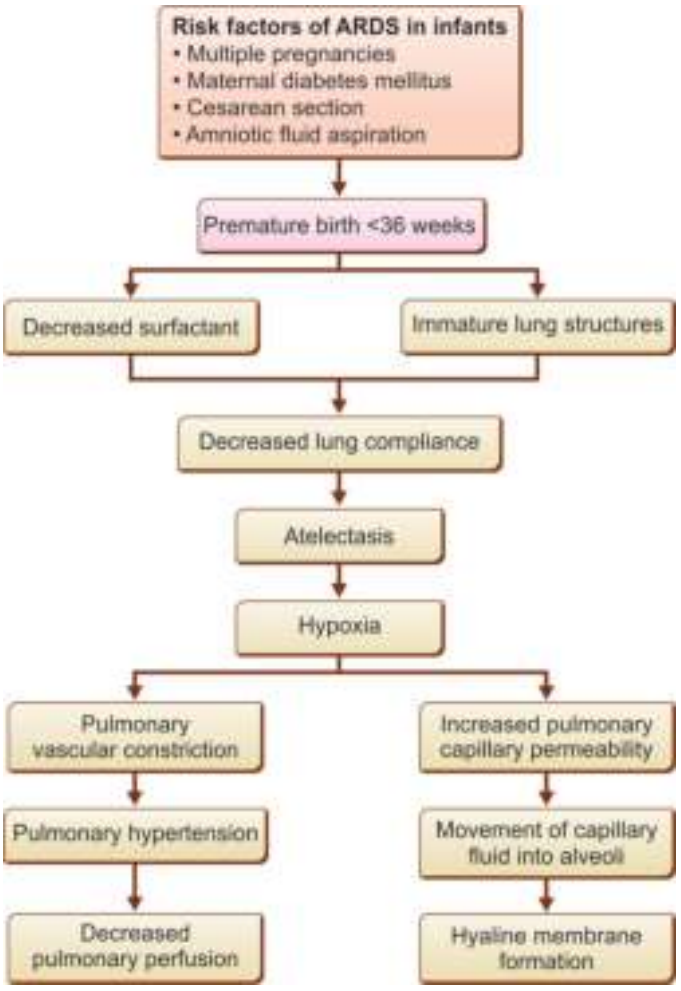


Fig. 18.31: Pathogenesis of neonatal respiratory distress syndrome.

Diagnostic Tools

Examination of amniotic fluid by flow cytometry and immunofluorescence polarizing microscopy is used to assess fetal pulmonary maturity.

- Previously, lecithin to sphingomyelin ratio greater than 2:1 in amniotic fluid analysis indicated fetal maturity.
- Currently, phosphatidylglycerol concentration is estimated in amniotic fluid and blood, because it is reliable diagnostic tool in spite of meconium contamination.

SEVERE RESPIRATORY DISTRESS SYNDROME

Severe respiratory distress syndrome is a viral respiratory tract infection caused by coronavirus that can progress to pneumonia and, eventually, death. Incubation of severe respiratory distress syndrome is 2–10 days.

Mode of Transmission

Coronavirus is transmitted by respiratory droplets produced when an infected person coughs or sneezes. Infected droplets are inhaled by the nearby person and get deposited on the mucous membranes of the mouth, nose, or eyes. It is also transmitted by using viral contaminated objects on mouth, nose, or eyes.

Risk Factors

Severe respiratory distress syndrome occurs due to close contact with an infected person, exhaled droplets and body secretions, and traveling to endemic areas.

Pathogenesis

Coronavirus attaches to receptors on the host cell membrane. It then releases enzymes and weakens the cell membrane. It then penetrates the cell membrane, and removes the protein coating that protects its genetic material resulting in replication and maturation. It then escapes from the cell by budding from the plasma membrane. The infection then can spread to other host cells (*refer* to Chapter 7 for SARS-CoV-2).

Clinical Features

Patient initially presents with fever, fatigue, headache, chills, myalgia, malaise, anorexia, and diarrhea. Later, patient develops dry cough, dyspnea, progressive and hypoxemia, and respiratory failure.

DIFFUSE ALVEOLAR DAMAGE

Diffuse alveolar damage (DAD) is nonspecific injury to alveolar epithelial and endothelial cells caused by variety of acute insults.

- The injurious agents include respiratory infections, sepsis, shock, aspiration of gastric contents, inhalation of toxic gases, near-drowning, radiation pneumonitis, and a large assortment of drugs and other chemical agents.
- The clinical counterpart of severe DAD is acute respiratory distress syndrome. In this disorder, a patient with apparently normal lungs sustains pulmonary damage and then develops rapid progressive respiratory failure (Table 18.30).

Table 18.30 Etiology of diffuse alveolar damage (DAD)

Category	Selected Agents
Infectious agents	Viruses, Mycoplasma, opportunistic infections in immunocompromised persons
Inhaled toxins	Oxygen toxicity, smoke
Therapeutic agents	Chemotherapeutic drugs, amiodarone, nitrofurantoin
Shock	Traumatic, cardiogenic shock
Sepsis	Any pathogen
Miscellaneous conditions	Radiation, burns, cardiopulmonary bypass, pancreatitis

CHRONIC RESTRICTIVE PULMONARY DISEASES

PNEUMOCONIOSIS

Pneumoconiosis refers to inhalation of mineral dust into the lungs. Dust particles are phagocytozed by alveolar macrophages, which then collect and drain into peribronchial lymphatic channels and hilar lymph nodes.

- Mineral dusts include coal dust, silica, asbestos and beryllium. These account for 25% of cases of chronic interstitial lung disease. Some dusts are apparently inert and cause a little or no damage. Certain dusts are antigenic and cause damage through immunological reactions. Exposure to asbestos may predispose to tuberculosis or neoplasia. Occupational causes of the pneumoconiosis are given in Table 18.31. Categories of occupational exposure and associated respiratory disorders are given in Table 18.32. Pulmonary reactions to mineral dust are given in Table 18.33.
- Radiologic findings in pneumoconiosis are related to the degree of associated pulmonary fibrosis. Highest atomic number (56) of the mineral dust gives a denser opacities on chest radiograph than least atomic number (12) of carbon, given the same amount of mineral particles inhaled.
- Predisposing factors which determine the extent of lung damage caused by an inhaled dust, its size, shape, solubility, concentration, and duration of exposure, effectiveness of mucociliary mechanism and co-existence of other lung diseases.

Table 18.31 Occupational causes of pneumoconiosis

Occupation	Agent	Disease
Mining	Coal dust	Pneumoconiosis
Quarrying (stone crushing)	Silica dust	Silicosis
Foundry work	Silica dust	Silicosis
Asbestos (mining, heating, building, demolition)	Asbestos fibers	Asbestosis—mesothelioma, lung carcinoma
Farming	Actinomycetes	Alveolitis
Paint spraying	Isocyanates	Bronchial asthma
Plastics manufacture	Isocyanates	Bronchial asthma
Soldering	Colonophony	Bronchial asthma

Table 18.32 Categories of occupational exposure and associated respiratory disorders

Occupational Exposures	Nature of Respiratory Responses	Comments
Inorganic dust		
Asbestos: Mining, processing, construction, ship repair	Asbestosis (pulmonary diffuse fibrosis), pleural plaques, mesothelioma, lung carcinoma	Virtually all new mining and construction with asbestos done in developing countries
Silica, mining, stone cutting, sandblasting, quarrying	Acute silicosis (alveolar lipoproteinosis), silicosis (nodular or massive pulmonary fibrosis), progressive massive fibrosis (PMF), chronic obstructive pulmonary disease (COPD), tuberculosis, lung carcinoma	Persistent risk of silicosis in developing countries
Coal dust: Mining	Coal worker pneumoconiosis (nodules formation), primary pulmonary fibrosis (PMF), COPD	Risk of coal worker pneumoconiosis persists in developing and developed countries
Beryllium: Processing alloys for high-tech industries	Chronic granulomatous disease of lung, lung carcinoma (high suspect), acute pneumonitis (rare)	Risk of berylliosis persists in high-tech industries
Other metals: Aluminum, chromium, cobalt, nickel, uranium, tungsten carbide, or hard metal (containing cobalt)	Acute pneumonitis, lung carcinoma, bronchial asthma, aluminum pneumoconiosis (nodular or massive fibrosis)	New pulmonary diseases appear with new process development

Contd...

Table 18.32 Categories of occupational exposure and associated respiratory disorders (*Contd...*)

Occupational Exposures	Nature of Respiratory Responses	Comments
Organic dust		
Cotton dust: Milling, processing	Byssinosis (an asthma-like syndrome), COPD, chronic bronchitis	Increasing risk of byssinosis in developing countries
Other agricultural dusts: Fungal spores, vegetable products, insect fragments, animal dander, bird and rodents faeces, endotoxins, microorganisms, pollens	Hypersensitivity pneumonitis (farmer's lung), bronchial asthma, COPD	Other agricultural dusts are important in migrant labor and also in-home exposure
Toxic chemicals: Wide variety of industries such as acid anhydrates, H_2SO_4 fumes, HNO_3 fumes, cadmium fumes, ammonia, formaldehyde, halides and acid salts (chloride, bromide, fluoride), nitrogen oxide, ozone, sulphur dioxide, hydrogen sulfide, acrolein and other aldehydes	Bronchial asthma, chronic bronchitis, COPD	Increased risk to exposure to toxic elements in developing countries
Other environmental agents		
Uranium, radon, passive tobacco smoke, biomass smoke, polycyclic aromatic hydrocarbons, welding fumes, diesel exhaust, wood finishing	Occupational exposures estimated to contribute to COPD, ≤10% of lung carcinomas, chronic bronchitis, COPD and pulmonary fibrosis	Occupational exposures to in-home environmental agents are increased in developing countries. Biomass smoke is a major risk factor for COPD among women

Table 18.33 Pulmonary reactions to mineral dust

Pulmonary Reaction to Mineral Dust	Mineral Dust
Macrophage accumulation with little deposition of reticulin fibers in lung	▪ Anthracosis (prolonged exposure to coal dust) ▪ Siderosis (prolonged exposure to iron) ▪ Stannosis (prolonged exposure to tin oxides) ▪ Baritosis (prolonged exposure to barium dust) ▪ Coal worker's pneumoconiosis (macules), coal dust (carbon and silica) ▪ Aluminum pneumoconiosis (granular aluminum)
Nodular or massive fibrosis of lung	▪ Silicosis (silica duct) glasswork, common material of masonry construction ▪ Coal worker's pneumoconiosis (nodules) ▪ Mixed dust pneumoconiosis
Diffuse fibrosis of lung	▪ Asbestosis (old insulation) ▪ Hard metal pneumoconiosis ▪ Aluminum pneumoconiosis
Epithelioid granulomas and giant cells	Chronic berylliosis
Alveolar lipoproteinosis	Acute silicosis
Small airway disease	Various dusts

- **Size of dust particle:** It determines site of deposition in lung alveoli. Dust particles measuring 1–5 micron are most dangerous, which reach alveoli and cause lung damage. Dust particles measuring <1.00 micron are suspended in respiratory tract and exhaled. Dust particles measuring >5 micron trapped in the mucus of nasopharynx, trachea, and bronchi are cleared by mucociliary apparatus.
- **Shape of dust particle:** Long and thin fibers of asbestos may reach the alveoli and cause lung damage.
- **Solubility of dust particle:** Finely divided dust may induce exudative reactions. Large dust particles resist dissolution, hence may persist within lung parenchyma for years together and produce fibrosis in lungs.

- **Concentration of dust particle:** Increased concentration of dust particles produces more lung damage.
- **Duration of exposure to dust particle:** Prolonged exposure to dust particles in mine workers increases risk of lung damage.
- **Mucociliary mechanism:** Normally, mucus secretion and mucociliary apparatus participate in clearance of dust particles. Damage to mucociliary apparatus by cigarette smoking predisposes to the accumulation of mineral dusts in lungs resulting in lung damage.

Coal Worker's Pneumoconiosis

Deposition of coal dust, i.e. anthracotic pigment derived from coal mines and tobacco smoke in the interstitial tissue of lungs and hilar nodes is known as coal worker's pneumoconiosis. Alveolar macrophages laden with anthracotic pigment are called dust cells. Pathologic lesions depend upon duration of exposure and magnitude of coal dust.

- **Forms of coal dust:** Coal dust exists in various forms—anthracite (most fibrogenic) and least fibrogenic bituminous, sub-bituminous. There is no study on lignite.
- **Pathogenesis:** Inhalation of massive amount of coal dust is engulfed by alveolar macrophages. These macrophages transmit the dust to interstitium of the lung and form aggregates around respiratory bronchioles. Coal worker's pneumoconiosis in lung is shown in Fig. 18.32.

Clinical Features

- **Asymptomatic anthracosis:** Coal dust is deposited in connective tissue along lymphatic channels and draining hilar lymph nodes without perceptible cellular reaction.

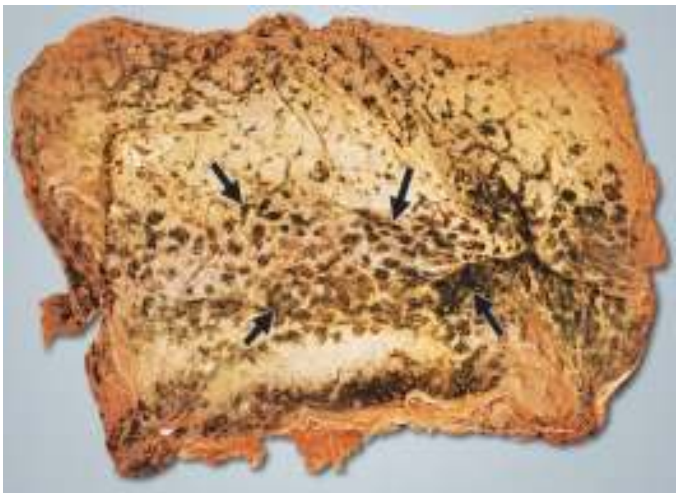


Fig. 18.32: Coal worker's pneumoconiosis. It shows deposition of coal dusts in lung (arrows).

- **Simple coal worker's pneumoconiosis:** Deposition of coal dust produces fibrotic opacities measuring <1.00 cm in upper lobes and upper region of lower lobes. Accumulation of coal dust near respiratory bronchioles produces centrilobular emphysema.
- **Complicated coal worker's pneumoconiosis:** It is also known as pulmonary progressive massive fibrosis (PMF) or black lung disease. Accumulation of coal dust produces fibrotic opacities measuring ≥1–2 cm with or without necrotic centers in posterior region of pulmonary upper lobes and upper segment of lower lobes. Patients may develop cor pulmonale. There is no increased risk of tuberculosis or lung cancer. Patient may develop Caplan syndrome characterized by coal worker's pneumoconiosis forming large cavities in lungs and rheumatoid arthritis (Table 18.34).

Table 18.34 Features of simple and complicated coal worker's pneumoconiosis

Lesions	Simple Coal Worker's Pneumoconiosis	Complicated Coal Worker's Pneumoconiosis
Fibrosis	Absent	Present
Size of lesion	<1.0 cm (macules 1–2 mm) and nodules <5 mm	Nodules >2.0 cm (usually 5–10 cm)
Region of lung	Upper region	▪ Upper lobe (posterior region) ▪ Lower lobe (upper segment)
Pulmonary functions	Normal	Impaired
Emphysema	Focal	Centriacinar or panacinar emphysema
Pulmonary vessels	Normal	Thickened
Chronic bronchitis	Absent	Present
Cor pulmonale	Absent	Present
Cavitation	Absent	Present (containing inky fluid)

Silicosis

Deposition of silica dust in lung parenchyma producing silicotic nodular opacities is known as silicosis.

- The greater the degree of exposure to silica and increasing length of exposure determine the amount of silicotic nodule formation and the degree of restrictive pulmonary disease.
- Silicosis is most common occupational disease across world. An occupational history is of importance in establishing the diagnosis. In silicosis, pulmonary opacities contain collagen and quartz.

Silica: Forms

Silica exists in three forms, i.e. crystalline, cristocrystalline and amorphous silica.

- **Crystalline silica:** Examples of crystalline silica are quartz, tridymite and cristobalite. Quartz is derived from foundries, i.e. casting material, sandblasting and mines. At higher temperature (800–1400°C), quartz gets converted into tridymite and cristobalite. Inhalation of tridymite and cristobalite is more potent than quartz in producing pulmonary fibrosis in upper lobes.
- **Cristocrystalline silica:** It contains minute crystals of flint, chert, opal, chalcedony. Higher temperature converts cristocrystalline silica into tridymite, which is potent mineral dust in producing lung fibrosis.
- **Amorphous silica:** It comprises glass, kaolin and diatomite. Repeated exposure to amorphous silica can cause lung fibrosis.

Pathogenesis

- **Pronged exposure to quartz:** It results in formation of silicotic nodule in lung composed of concentric layers of collagen with or without central cavitation. There is minimal inflammatory reaction. Quartz polarizes in the nodules. Egg shell dystrophic calcification is present in hilar nodes.
- **Prolonged exposure to tridymite and cristobalite:** It causes activation and cytolysis of alveolar macrophages, releasing cytokines, which stimulate pulmonary fibrosis. Chest radiograph in early phase reveals fine nodularity imparting snowstorm appearance; and later diffuse pulmonary fibrosis with irregular emphysema (Fig. 18.33A to D).

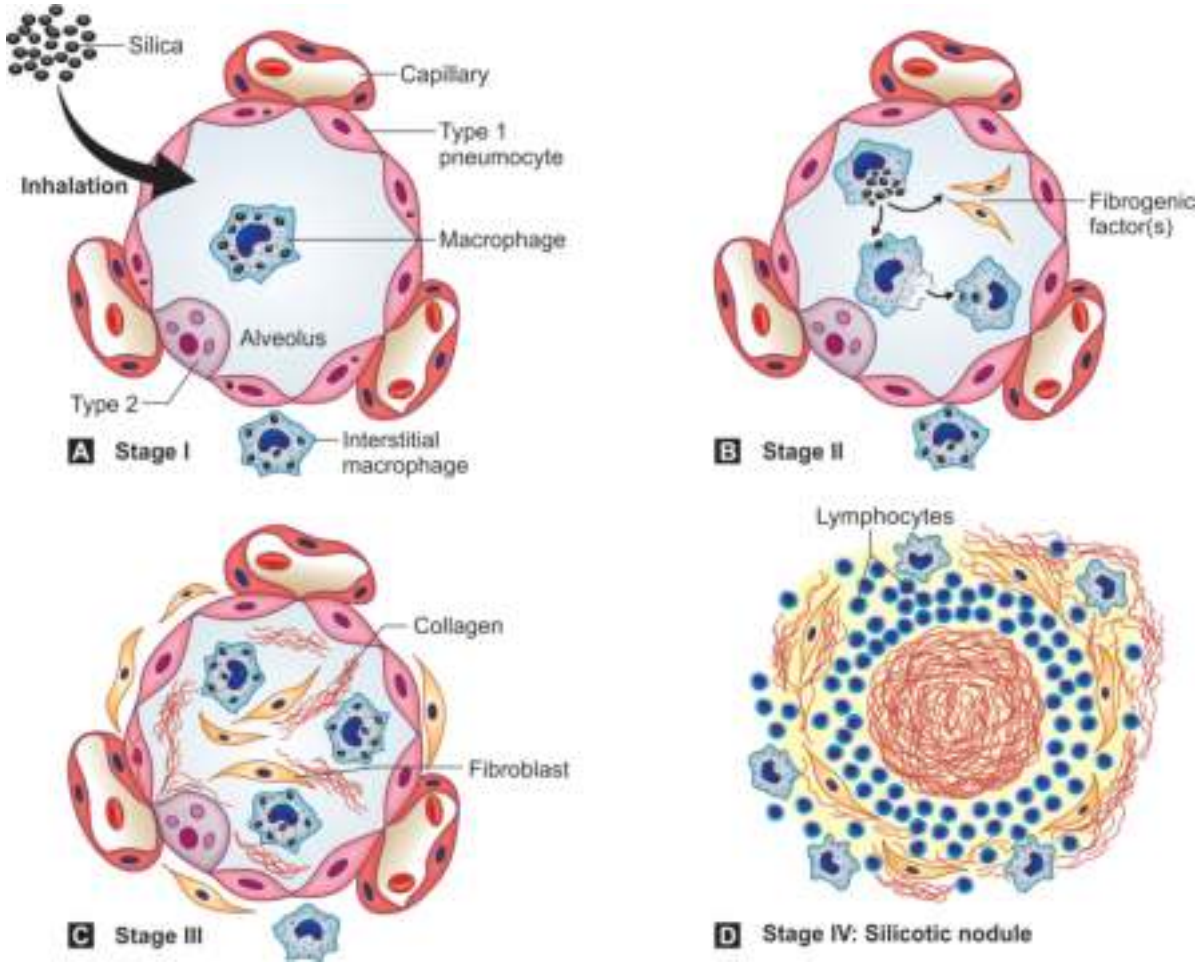


Fig. 18.33A to D: Pathogenesis of pneumoconiosis by silicosis. Inhaled silica particles are toxic to the alveolar macrophages, which die and release a fibrogenic factor. The released silica is again engulfed by alveolar macrophages. The process leads to formation of silicotic nodule.

Clinical Features

Clinical features depend on lesions present in simple or complicated silicosis. Patients may develop cor pulmonale and Caplan syndrome. There is increased risk for developing lung carcinoma and tuberculosis.

Asbestosis

Deposition of asbestos fibers in the respiratory unit, i.e. respiratory bronchioles, alveolar ducts and alveoli is known as asbestosis.

- Occupational hazards occur due to asbestos and its products after a prolonged exposure of 10 years. Lower lobes of lungs are severely affected.
- Asbestos occurs as silica compounds throughout earth. It is widely used in flooring, roofing materials, insulating conduits (i.e. sewer, water), brake linings, and clutch castings as well as a fire retardant. It is inconsumable air and water pollutant.

Asbestos Fibers: Types

Asbestos fibers measure 5–300 micron in length and 0.25–0.5 micron in width. There are two types of asbestos fibers, i.e. serpentine fibers and amphibole fibers (most dangerous). Types of asbestos fibers are given in Table 18.35 and grading of asbestosis is given in Table 18.36.

- **Serpentine asbestos fibers:** These are composed of **chrysolite fibers** (white asbestos). These are curly and flexible fibers, which reach the upper respiratory tract without fragmentation. These do not produce lung fibrosis.
- **Amphibole asbestos fibers:** These are composed of crosidolite fibers (blue asbestos) and amosite fibers (brown asbestos). These are straight and rigid fibers, which undergo fragmentation and reach the alveoli. These fibers are most dangerous responsible for pathologic lesions in lungs.

Pathogenesis

Straight and rigid amphibole fibers undergo fragmentation and reach the alveoli resulting in recurrent pleural effusions, pleural plaques, mesothelioma, bronchogenic carcinoma, other cancers (GIT, larynx), emphysema, bronchiectasis and pulmonary hypertension. Pathogenesis of asbestosis lung is shown in Fig. 18.34A to D.

Pathologic Lesions

Serpentine fibers of asbestos coated with ferritin and proteins are phagocytozed by macrophages and form golden brown beaded ferruginous bodies; demonstrated in sputum or in distal, small airways. Amphiboles asbestos fibers have been associated with cancerous and noncancerous lung diseases.

Table 18.35 Types of asbestos fibers

Characteristics	Serpentine Fibers	Amphibole Fibers
Composition	Chrysolite fibers (white asbestos)	▪ Crosidolite fibers (blue asbestos) ▪ Amosite fibers (brown asbestos)
Fibers shape	Curled fibers	Straight fibers
Fibers texture	Flexible textue	Stiff texture
Fragmentation	No fragmentation	Fragmentation occurs
Site of deposition	Upper respiratory tract (alveoli spared)	Respiratory unit (terminal bronchioles, acini and alveoli)
Lesions	These do not produce lung lesions	▪ Pleural effusion (recurrent) ▪ Pleural plaques ▪ Lung extensive fibrosis ▪ Mesotheliomas ▪ Lung cancer (adenocarcinoma and squamous cell lung carcinoma) ▪ Cancers of gastrointestinal tract and larynx ▪ Other lesions such as emphysema, bronchiectasis and pulmonary hypertension

Table 18.36 Grading of asbestosis

Criteria	Grade
Amount of lung parenchyma involved	▪ A: Lung parenchyma not involved ▪ B: <25% of lung parenchyma involved ▪ C: 25–50% of lung parenchyma involved
Severity of fibrosis and distortion of lung parenchyma	0–4: Zero (no fibrosis) and 4 (severe fibrosis)

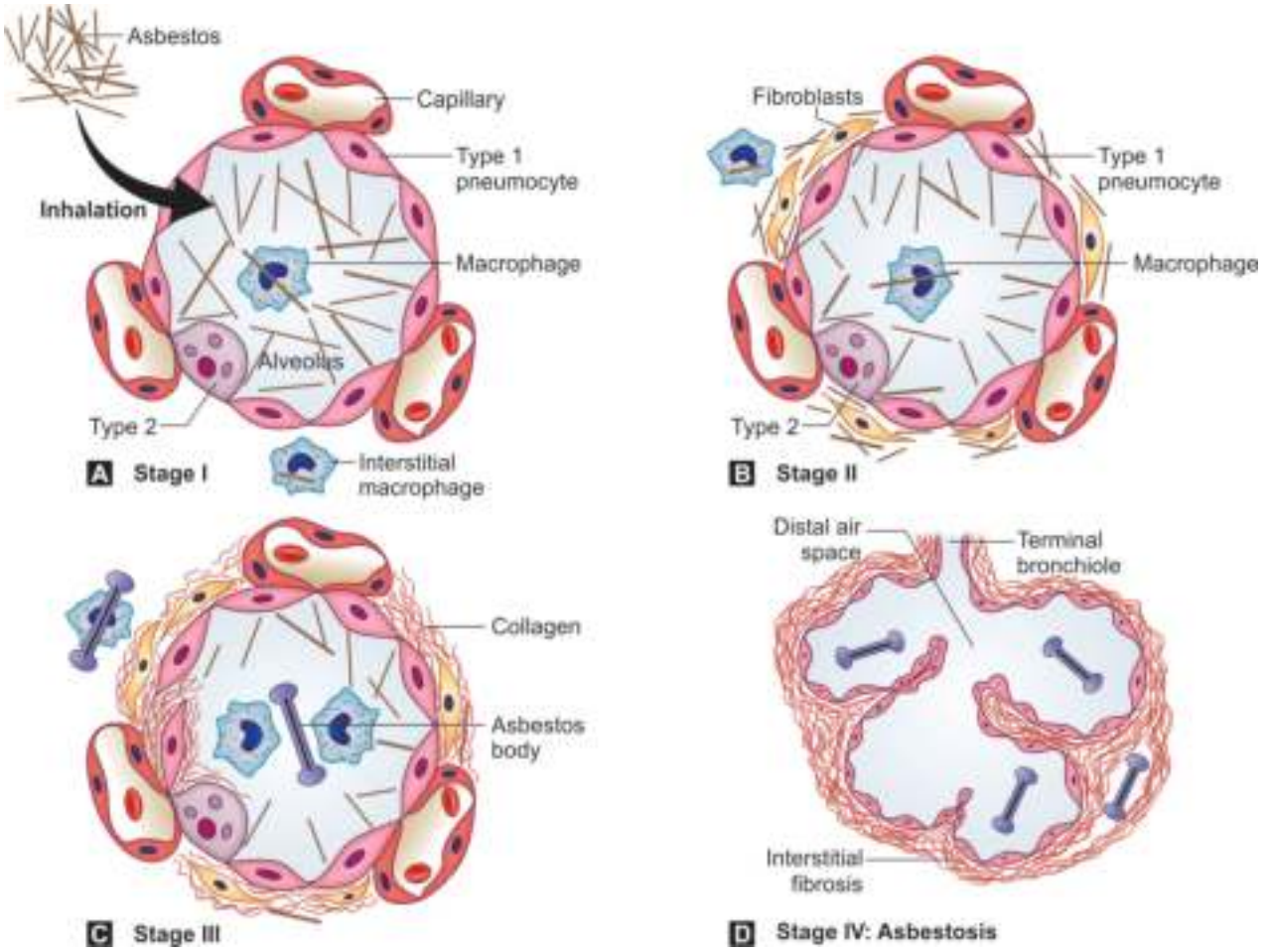


Fig. 18.34A to D: Pathogenesis of pneumoconiosis by asbestos fibers. Asbestosis is characterized by inhalation of small amount of dust producing extensive pulmonary fibrosis.

Asbestos-related Pulmonary and Extrapulmonary Lesions

- **Pleural effusion:** Patient exposed to asbestosis develops recurrent and bilateral pleural effusion.
- **Pleural plaques:** Benign pleural plaques are most common lesions in asbestosis. These are well circumscribed tan-white benign pleural plaques containing calcium over the domes of diaphragm and costophrenic angles. They do not contain asbestos bodies.
- **Mesothelioma:** Mesothelioma arises from visceral layer of pleura and peritoneum. It occurs in 5–10% of cases, 25–40 years after first exposure. Tumor is dense white bulky filling pleural cavity. Patient presents with dyspnea, pain and effusion. Malignant mesothelioma arises from serosa of pleura encasing the lung.
- **Lung cancer:** Bronchogenic carcinoma is most common asbestos-related lung cancer such as adenocarcinoma (most common) and squamous cell lung carcinoma. Cigarette smoking increases the risk for developing lung carcinoma manifold.
- **Other malignancies:** There is increased risk for cancer of gastrointestinal tract, larynx and lymph nodes.
- **Other respiratory tract lesions:** Patients may develop emphysema, bronchiectasis and pulmonary hypertension.

Berylliosis

Deposition of beryllium (mineral dust, fumes) in the lungs is known as berylliosis. There is increased risk of cor pulmonale and lung cancer.

Source of Beryllium

Grinding, heating, melting of beryllium metal and alloys produce dangerous dust particles in the form of beryllium oxide. These are inhaled and producing two types of lesions in respiratory tract.

Pathologic Lesions

On prolonged inhalation of beryllium oxide, patient develops acute nonspecific chemical tracheo-bronchopneumonia. Later, the patient develops diffuse interstitial pulmonary fibrosis and noncaseating epithelioid cell granuloma entrapping birefringent crystals. These may become 50 micrometer in size called **Schaumann bodies** similar demonstrated sarcoidosis. In biopsy, other causes of noncaseating epithelioid cell granulomatous disorders must be excluded.

DRUG-INDUCED INTERSTITIAL PULMONARY FIBROSIS

Prolonged administration of amiodarone, bleomycin, busulfan, cyclophosphamide, methotrexate, methysergide, nitrosourea and nitrofurantoin cause chronic interstitial pulmonary fibrosis. Pulmonary manifestations of drug reactions include diffuse interstitial pneumonia, diffuse alveolar damage, bronchiolitis obliterans, organizing pneumonia, eosinophilic pneumonia, pulmonary hemorrhage, pulmonary venoocclusive disease and large and small vessel vasculitis.

RADIATION-INDUCED INTERSTITIAL PULMONARY FIBROSIS

Acute pneumonitis may occur 1–6 months after radiotherapy. Patient presents with fever, dyspnea, pleural effusions. Some patients develop chronic radiation-induced pneumonitis, that can be demonstrated on chest radiograph.

IDIOPATHIC PULMONARY FIBROSIS

Idiopathic pulmonary fibrosis accounts for 15% of cases of chronic interstitial pulmonary diseases. Men are more affected than women in 40–70 years of age group.

Pathogenesis

Unknown agent triggers repeated cycles of alveolitis resulting in alveolar fibrosis and proximal dilation of the small airways. IL-8, FGF, TGF-β, PDGF synthesized by macrophages stimulate fibroblasts resulting in alveolar fibrosis. Lung exhibits '**honeycomb appearance**'.

Clinical Features

Patient presents with fever, breathlessness, chronic nonproductive cough, late inspiratory crackles, hypoxemia and cyanosis. Patient has poor prognosis with mean survival of 4–6 years.

Surgical Pathology: Idiopathic Pulmonary Fibrosis

Gross Morphology

- Normal architecture of lungs is impaired showing extensive fibrosis.
- Gross morphology of lung is shown in Fig. 18.35.

Light Microscopy

- Histologic examination reveals interstitial pulmonary fibrosis, interstitial pneumonia, desquamative pneumonia, bronchiolitis obliterans and organizing pneumonia.
- Alveolar septa are thickened by fibrosis.
- Capillaries are diminished. Interalveolar septa are infiltrated by chronic inflammatory cells (Fig. 18.36).

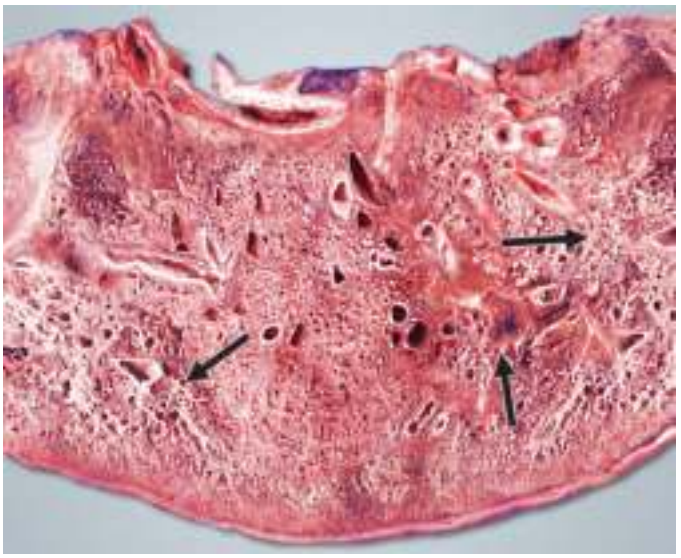


Fig. 18.35: Idiopathic pulmonary fibrosis. Lung shows extensive fibrosis in a patient with idiopathic pulmonary fibrosis (arrows).

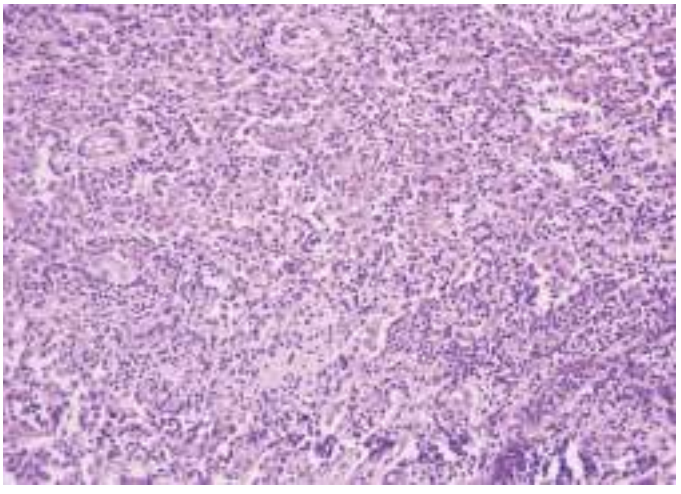


Fig. 18.36: Idiopathic pulmonary fibrosis. It shows desquamative pneumonia. Alveolar septa are thickened by fibrosis. Capillaries are diminished. Interalveolar septa are infiltrated by chronic inflammatory cells (100X).

SARCOIDOSIS

Sarcoidosis is a noncaseating granulomatous disease of unknown etiology. It becomes clinically apparent in teenagers and young adults. It is more common in women than men.

Pathology Pearls: Organs Involved in Sarcoidosis

Noncaseating granulomas are seen in lymph nodes, bone marrow, spleen and skin.

- **Lung lesion:** It shows interstitial pulmonary fibrosis and respiratory failure. Symptoms of pulmonary disease include dyspnea, cough, and wheezing.
- **Skin lesion:** Patient develops rashes on the nose and cheeks (lupus pernio), lateral painful nodules on extremities, i.e. erythema nodosum and inflammation of subcutaneous fat.

- **Anterior uveitis:** It results in blurred vision, glaucoma and corneal opacities. Angiotensin-converting enzyme (ACE) synthesized by epithelioid macrophages is elevated in the blood.
- **Liver lesion:** Patient develops granulomatous hepatitis.

Chest Radiograph

Chest radiograph reveals bilateral hilar lymphadenopathy and interstitial pulmonary disease manifesting as diffuse reticular densities.

Laboratory Diagnosis

Tuberculin test is negative. Blood γ-globulins and calcium levels are raised with hypercalciuria. Definitive diagnosis requires biopsy demonstrating noncaseating granulomas, laminated calcium concretions, i.e. Schaumann bodies and star-shaped inclusions called *asteroid bodies*.

COLLAGEN VASCULAR DISEASE

Collagen vascular diseases (i.e. scleroderma and systemic lupus erythematosus) account for 10% of chronic interstitial pulmonary fibrosis. Approximately 50% of patients with systemic lupus erythematosus produce chronic interstitial pulmonary fibrosis, pleuritis and pleural effusions.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis may produce interstitial pulmonary fibrosis, nodules undergoing cavitation, pleuritis and pleural effusion. Caplan syndrome comprises rheumatoid nodules and pneumoconiosis.

EOSINOPHILIC GRANULOMA

Eosinophilic granuloma of lung is characterized by localized proliferation of histiocytes closely related to the Langerhans' cells of the skin. These histiocytes contain Birbeck granules resembling tennis rackets. Histiocytes are admixed with lymphocytes and eosinophils. Patient develops pneumonitis. Eosinophilic granuloma most often occurs in the lung or ribs.

EOSINOPHILIC PNEUMONIA

Eosinophilic pneumonia refers to accumulation of eosinophils and macrophages in alveolar spaces due to allergens. The alveolar septa are thickened by the presence of numerous eosinophils and formation of hyaline membranes. Eosinophilic pneumonia is classified as either idiopathic or secondary to an underlying illness. Patient with acute eosinophilic pneumonia responds dramatically to corticosteroids than chronic eosinophilic pneumonia.

HYPERSENSITIVITY PNEUMONITIS

Extrinsic allergic alveolitis (hypersensitivity pneumonitis) is associated with exposure to a known inhaled thermophilic Actinomyces, i.e. *Saccharopolyspora rectivirgula* antigen in moldy hay. Patient develops farmer's lung.

Pathogenesis

On first exposure to thermophilic Actinomyces antigen, patient's blood IgG antibodies level is increased.

- On subsequent exposure to antigen, antigen–antibody complexes are formed resulting in inflammatory reaction in lung tissue due to type III hypersensitivity response.
- Later, patient develops granulomatous inflammation of lungs due to type IV hypersensitivity reaction.
- Persons handling moldy hay should use facial mask. These patients respond well to corticosteroid therapy.

GOODPASTURE SYNDROME

Goodpasture syndrome is an autoimmune disorder, i.e. *in situ* immune complex-mediated disorder involving kidneys and lungs.

- Hemorrhagic pneumonitis and glomerulonephritis are caused by antibodies (IgG) directed against glomerular basement membrane, i.e. noncollagenous domain of type IV collagen.
- Local complement activation results in the recruitment of neutrophils, tissue injury, pulmonary hemorrhage, and glomerulonephritis.
- Patient presents with hematuria due to glomerular involvement and hemoptysis as a result of pulmonary hemorrhage.

BYSSINOSIS

Exposure to bacterial endotoxin from gram-negative bacteria growing on the cotton, linen or hemp products in textile factories produces byssinosis in occupational workers in textile factories. Patient presents with breathlessness due to pulmonary involvement.

SILO-FILLER'S DISEASE

Inhalation of wheat weevil protein causes Silo-filler's disease due to immediate hypersensitivity reaction resulting in dyspnea. Patient responds to corticosteroid therapy.

LYMPHANGIOLEIOMYOMATOSIS

Lymphangioleiomyomatosis is a rare interstitial pulmonary disease that occurs in women of childbearing age.

- Patient develops widespread abnormal proliferation of smooth muscle in the lung, mediastinal and retroperitoneal lymph nodes, and the major lymphatic ducts.
- The lungs show bilateral, diffuse enlargement, with extensive cystic changes resembling those of emphysema.
- Hormonal ablation through oophorectomy and antiestrogen and progesterone therapy has shown some promise in the management of patients.

PULMONARY ALVEOLAR PROTEINOSIS

Pulmonary alveolar proteinosis (PAP) is also termed lipoproteinosis. It is an uncommon disease of unknown etiology, in which the alveoli are filled with a granular, proteinaceous and eosinophilic amorphous material, which are PAS positive-diastase resistant, and rich in lipids. This material sometimes appears to be pulmonary surfactant.

Acquired Pulmonary Alveolar Proteinosis

Recent studies have revealed that acquired pulmonary alveolar proteinosis is associated with immunocompromised state, leukemia and lymphoma, respiratory infections, and exposure to environmental inorganic dusts. Histologic examination shows homogenous, agranular precipitate within the alveoli producing focal to confluent consolidation with minimal inflammation. The patients are treated by repeated bronchoalveolar lavage that may halt progression of the disease.

Congenital Pulmonary Alveolar Proteinosis (PAP)

Congenital PAP is a rare cause of immediate-onset neonatal respiratory distress syndrome. It has a fatal outcome shortly after birth. Without lung transplantation, death occurs within 3–6 months.

PNEUMOTHORAX AND ATELECTASIS LUNG

PNEUMOTHORAX

Pneumothorax is the presence of air outside the lung, within the pleural space. It leads to partial or complete pulmonary collapse. It may be due to traumatic perforation of the pleura or may be spontaneous.

- Spontaneous pneumothorax occurs when the visceral pleura ruptures without an external traumatic injury or iatrogenic etiology.
- Pneumothorax causes pulmonary collapse of a previously expanded lung, a condition that is termed atelectasis. The most common types of pneumothorax are open, closed, and tension.

OPEN PNEUMOTHORAX

In open pneumothorax, there is loss of negative intrathoracic pressure. In spontaneous pneumothorax, trachea deviates to side of spontaneous pneumothorax (Fig. 18.37).

Etiopathogenesis

- Penetrating wounds or rib fracture results in open pneumothorax. Due to penetrating injury, atmospheric air flows directly into the pleural cavity due to negative intrapleural pressure. As the air pressure in the pleural cavity becomes positive, the lung collapses on the affected side resulting in

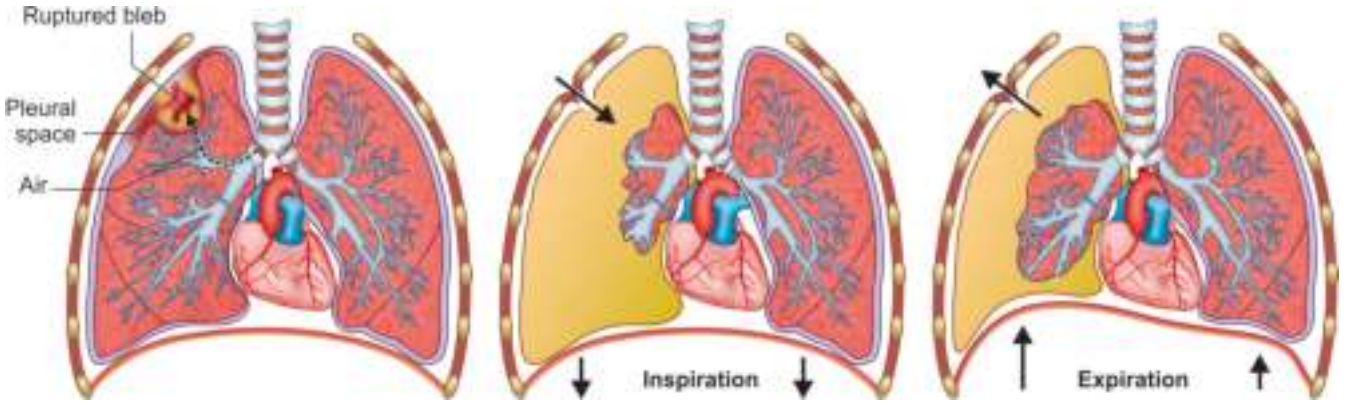


Fig. 18.37: Open pneumothorax. Air enters into the pleural cavity leading to lung collapse.

decreased total lung capacity, vital capacity, and lung compliance.
- Iatrogenic induced open pneumothorax occurs as a result of aspiration of pleural fluid (thoracocentesis) or pleural and transbronchial lung biopsies.

Clinical Features

Patient presents with chest pain (90%), dyspnea (80%), hypotension and tachycardia. On clinical examination, breath sounds are absent on affected side of pneumothorax. Chest rigidity is seen on affected side. Crackling lung sounds are observed beneath skin on palpation.

CLOSED PNEUMOTHORAX

Closed pneumothorax develops in a spontaneous manner especially in tobacco smokers in adults of 30–40 years of age. It is more common in men than women. It may recur in the opposite lung in 25% of cases within a span of two years.

Pneumothorax: Types

- **Primary pneumothorax:** It occurs in young age group. The condition runs in families. Rupture of paraseptal emphysema is the most common cause of closed pneumothorax. It may also occur due to rupture of apical subpleural bleb. Patient presents with sharp pleuritic pain and breathlessness. Clinical course is self-limited. Chest drain is not required, in patient not having respiratory distress.
- **Secondary pneumothorax:** It occurs as a result of visceral pleural leakage in tuberculosis, necrotizing malignant tumors and lung diseases with cavities.

Clinical Features

Patient presents with shortness of breath, respiratory distress, cyanosis and sudden onset severe pleuritic pain enhanced by coughing, breathing, and chest movement. On clinical examination, there is asymmetrical chest wall movement. Percussion note is hyperresonant. Trachea deviates to same side of spontaneous pneumothorax due to loss of negative intrathoracic pressure.

Chest Radiograph

Chest radiograph reveals white visceral pleural line and absence of vessel markings in the peripheral region of visceral pleural line.

TENSION PNEUMOTHORAX

There is an increase in pleural cavity pressure in tension pneumothorax. Trachea deviates to contralateral side of tension pneumothorax (Fig. 18.38).

Pathogenesis

Tension pneumothorax occurs as a result of trauma to pleura in mechanically ventilated patients. Each inspiration results in rushing out of air from alveoli and enters the pleural space through punctured site.
- Tension pneumothorax acts as a one-way check valve, but cannot escape as the rupture site closes on expiration. Each inspiration keeps on increasing intrapleural pressure resulting in pulmonary collapse. It forces mediastinal structures, i.e. heart, trachea, esophagus, and great vessels to opposite side.
- Heart and opposite lung are compressed resulting in impairment of venous return. Trachea deviates

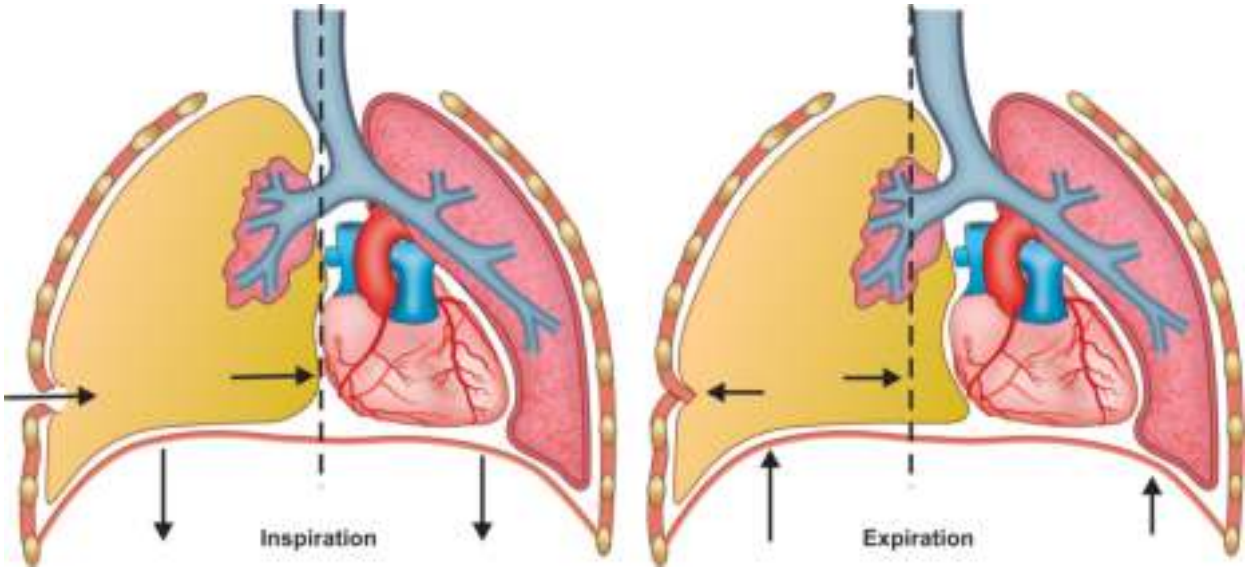


Fig. 18.38: Tension pneumothorax. Each inspiration results in rushing out of air from alveoli and enters the pleural space through punctured site. It acts as a one-way check valve, but cannot escape as the rupture site closes on expiration (arrows).

to opposite side of pneumothorax due to increase in pleural cavity pressure. The diaphragm is flattened.

Clinical Features

Patient presents with sudden onset of severe dyspnea, pleuritic chest pain, hypotension, decreased cardiac output and cardiac arrest. On auscultation, breath sounds are absent. Percussion note is resonant due to presence of air in the pleural cavity on the affected side of pneumothorax.

Treatment

Pressure of air in pleural cavity is relieved by inserting a needle into the second intercostal space on the mid-clavicular line or putting a chest tube.

ATELECTASIS OF LUNG (PULMONARY COLLAPSE)

Atelectasis refers to incomplete expansion of the lungs or the pulmonary collapse of previously inflated lung. Severe atelectasis significantly reduces oxygenation and predisposes to infection. Obstruction of airways causes resorption atelectasis, while air or fluid in pleural cavity under increased pressure results in compression atelectasis.

ETIOPATHOGENESIS

Acquired Atelectasis of Lung

Acquired atelectasis is generally encountered in adults. It may be divided into following categories: obstructive or resorption atelectasis, compression atelectasis, patchy atelectasis and contraction atelectasis. Atelectasis is a reversible disorder except that is caused by contraction.

Atelectasis Neonatorum of Lung

Atelectasis neonatorum is failure of alveolar spaces to expand adequately at birth. Primary atelectasis is failure of initial aeration of the lungs at birth. The alveoli remain collapsed and respiration is never fully established. It is associated with premature birth and intrauterine hypoxia.

- Secondary neonatal atelectasis is collapse of previously aerated lungs.
- Atelectasis neonatorum occurs due to deficient synthesis of pulmonary surfactant phosphatidylcholine (lecithin) synthesized by type 2 pneumocytes and stored in lamellar bodies. Surfactant reduces surface tension in the small airways. Synthesis of pulmonary surfactant begins in 28th week of gestation.
- Cortisol and thyroxine increase its synthesis while decreased by insulin.

ATELECTASIS: TYPES

Obstructive Atelectasis of Lung

Obstructive atelectasis involves regions of the lung distal to an obstruction in cases of aspiration of foreign bodies, centrally located bronchogenic carcinoma, and mucopurulent plugs after postoperative states, bronchial asthma, chronic bronchitis, and bronchiectasis. Airway obstruction by thick secretions prevents air from reaching the alveoli. It occurs due to obstruction of bronchi, segmental bronchi, or terminal bronchioles.

Compressive Atelectasis of Lung

Compressive atelectasis usually involves the entire lung. The pleural space is filled with fluid compressing the lung, which has retracted lung towards the hilus.

- Similar findings can be produced by pneumothorax, because of entry of air into the pleural space.
- Collapse of lung occurs due to lack of air and distal resorption of pre-existing air through the pores of Kohn in the alveolar walls, involving whole or part of a lung.
- Air or fluid in the pleural cavity under increased pressure induce collapse of small airways beneath the pleura. In tension pneumothorax, air compresses the lung. Accumulation of fluid in the pleural cavity compresses the lung.

Patchy Atelectasis of Lung

Patchy atelectasis is characterized by the presence of airless irregular patches in small segments of both lungs. It occurs in neonatal and adult acute respiratory distress syndrome.

Contraction Atelectasis of Lung

Contraction atelectasis occurs when local or generalized fibrotic changes in the lungs or pleura prevent full expansion of the lung parenchyma, especially in subpleural regions.

- Patient presents with fever and dyspnea, both usually occur within 24–36 hours of collapse. Percussion note is dull due to collapse of alveoli. Breath sounds are absent.
- There is ipsilateral elevation of the diaphragm and tracheal deviation. Collapsed lung does not expand on inspiration, i.e. inspiratory lag.

PULMONARY VASCULAR DISORDERS

Bronchial arteries originate from thoracic aorta and intercostal arteries; protect lungs from infarction. A pulmonary embolus produces a hemorrhagic infarction in 10% of cases.

Patient with decreased blood flow in bronchial artery as well as obstructive lung disease will likely to result in a hemorrhagic pulmonary infarct, hence increases the risk of morbidity and mortality.

PULMONARY THROMBOEMBOLISM

Pulmonary thromboembolism is a potentially fatal disorder. It is an obstruction of the pulmonary arterial bed by a dislodged thrombus. Patient presents with abrupt onset of pleuritic pain, shortness of breath and hypoxia. Bronchial arteries protect lungs from infarction. Dyspnea and tachypnea are common symptoms in cases of pulmonary infarction.

PATHOGENESIS

Most pulmonary emboli arise from the thrombi deep veins of the lower extremities in 50% of cases. It may originate in femoral and pelvic veins in many cases.

- Rarely, it can be due to nonthrombotic particulate material such as fat, amniotic fluid, clumps of tumor cells or bone marrow, or foreign matter, such as bullet fragments.
- Predisposing factors for developing pulmonary thromboembolism include stasis of venous blood flow, prolonged bed rest or immobilization, thrombophlebitis, varicose veins, hypercoagulable states, due to use of hormonal contraceptives, and pregnancy.

CONSEQUENCES

- The size of the embolus determines large-, medium- and small-sized pulmonary vessel that is occluded.

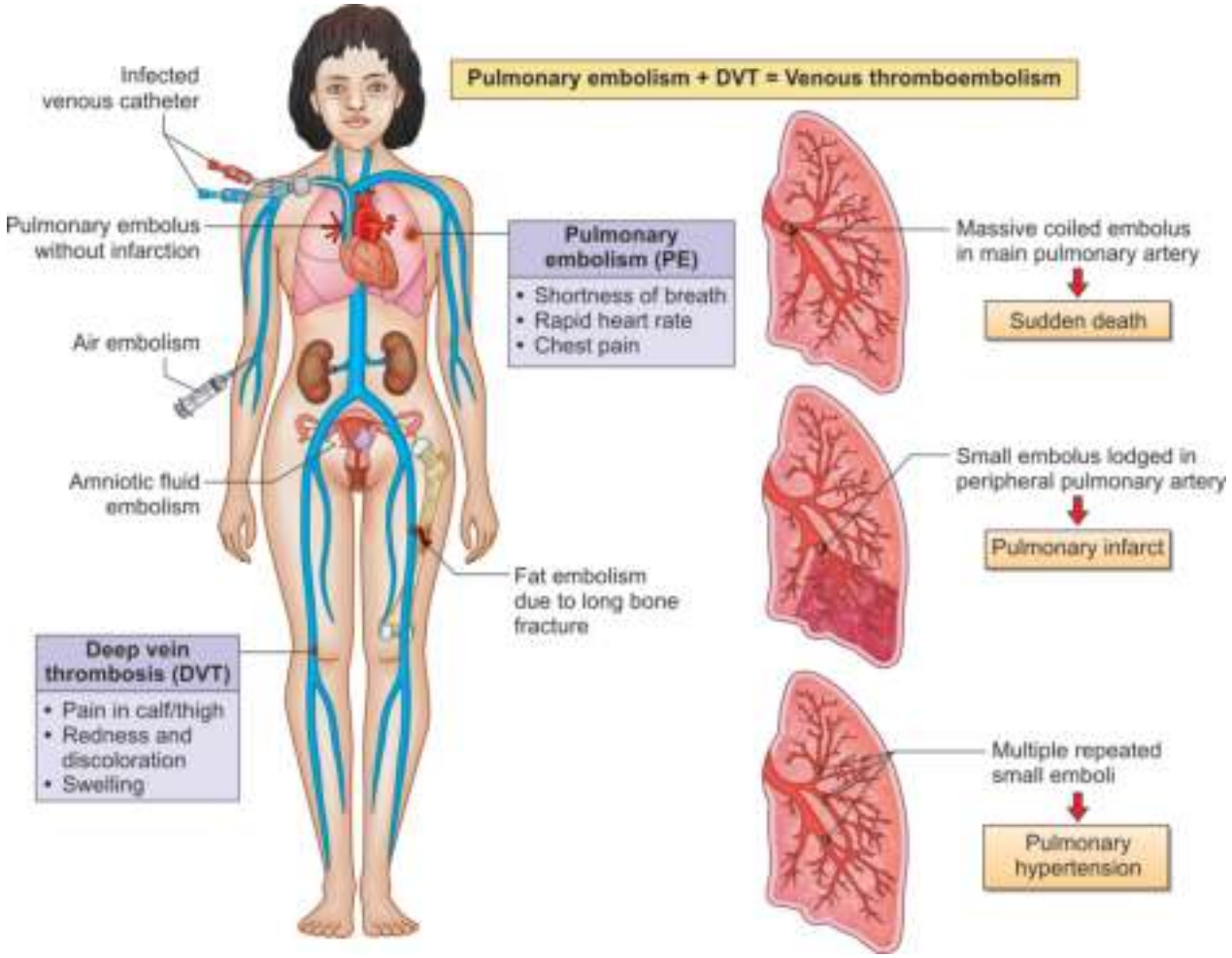


Fig. 18.39: Pathogenesis and consequences of pulmonary thromboembolism. Massive embolus in main pulmonary artery leading to sudden death. Small embolus in peripheral branches of pulmonary artery causes pulmonary hemorrhagic infarct. Multiple repeated small emboli lead to pulmonary hypertension.

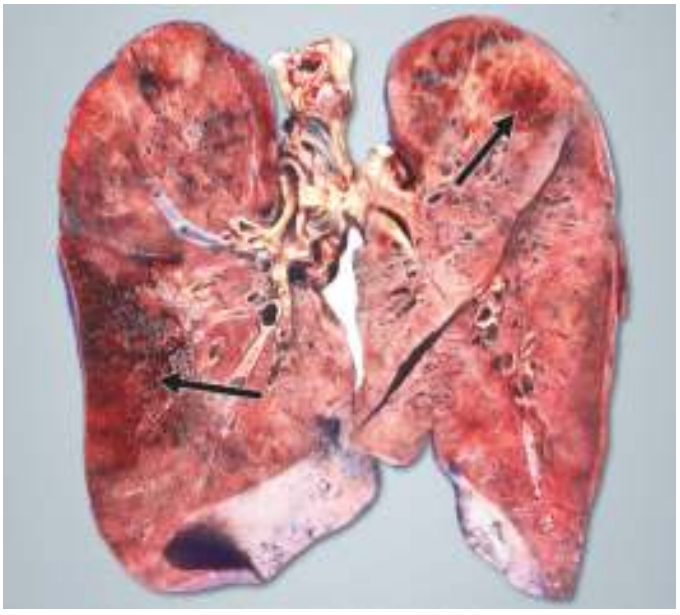


Fig. 18.40: Pulmonary hemorrhagic infarct (arrows).

- Large embolus occluding the major pulmonary vessels is known as '**saddle embolus**'.
- Small emboli occlude medium-sized and small-sized pulmonary arteries.
- Occlusion of pulmonary artery decreases the perfusion of lung parenchyma and resulting in hemorrhagic pulmonary infarction with fatal outcome (Fig. 18.39).
- Hemorrhagic pulmonary infarct is raised, wedge-shaped area with red blue discoloration that extends to the pleural surface. Majority of presons have hemorrhagic pulmonary infarct located in the lower lobes. Perfusion is greater than ventilation in the lower lobes (Fig. 18.40).

CLINICAL FEATURES

Approximately 50% of patients with mild pulmonary thromboembolism are asymptomatic. But in rest 50% of cases, massive pulmonary thromboembolism results in obstruction of pulmonary arterial circulation.

- Patient develops dyspnea or pleuritic chest pain on inspiration, expiratory wheezing, tachycardia, productive cough, blood-tinged sputum, low-grade fever and pleural effusion with fibrinous exudate.
- Overall survival rate in pulmonary thromboembolism patients is 80%. Hemorrhagic pulmonary infarct resolves without treatment in 60% of cases. In 90% of cases, the hemorrhagic pulmonary infarct resolves with treatment.

Imaging Diagnostic Techniques: Pulmonary Thromboembolism

- **Chest radiograph:** It shows pleural effusion; cut-off sign of one or more pulmonary arteries. Hypovascularity is demonstrated beyond the blocked blood vessel. Wedge-shaped area of lung consolidation seen is known as Hampton's hump.
- **Radionuclide scan:** It shows abnormal perfusion radionuclide scan. Ventilation scan is normal, but the perfusion scan is abnormal.
- **Pulmonary angiogram:** It is gold standard confirmatory test. It is expensive and not readily available in smaller hospitals.
- **Spiral (helical) CT:** It is excellent diagnostic technique if pre-existing lung disease present.
- **Positive D-dimer:** It is usually performed in conjunction with ventilation/perfusion scan or spiral CT.

FAT EMBOLISM SYNDROME

Fat embolism syndrome is a rare but potentially fatal problem. Pulmonary, cerebral, and cutaneous manifestations occur within 24–48 hours after a traumatic injury of long bone. Fat emboli comprise fat microglobules in the circulation. Grud and Wilson diagnostic criteria for fat embolism syndrome and clinical and laboratory findings are given in Table 18.37.

Table 18.37 Grud and Wilson diagnostic criteria for fat embolism syndrome and clinical and laboratory findings

Major Diagnostic Criteria
Skin (petechial hemorrhages)
Respiratory symptoms (tachypnea, dyspnea, bilateral inspiratory crepitations, hemoptysis, bilateral diffuse patchy shadowing on chest radiograph)
Neurological signs (confusion, drowsiness, coma)
Minor Diagnostic Criteria
Tachycardia (heart rate >120 per minute)
Pyrexia >38.5°C
Retinal petechial hemorrhages and fat emboli
Renal changes (anuria or oliguria)
Jaundice
Unexplained thrombocytopenia and low hemoglobin level
Erythrocyte sedimentation rate increased
Fat macroglobulinemia
Fat globules in urine
Fat globules in sputum

Etiopathogenesis

Fat embolism syndrome most often occurs as a consequence of severe trauma to long bone fractures such as femur with abundant fatty marrow. It can also be seen with extensive trauma to fat laden tissues, burns, and very rarely with orthopedic procedures.

- Following long bone fracture, bone marrow fatty globules enter the circulation, lodge in small blood vessels of the lung, skin, kidneys, brain and other organs producing ischemia and hemorrhage leading to clinical manifestations.
- Fat microglobules are converted into fatty acids, which damage vascular endothelium resulting in formation of platelet thrombi in areas of injury.
- Capillary permeability is increased allowing protein-rich fluid to leak into the alveoli, resulting in pulmonary edema.

CLINICAL FEATURES

Patient develops pulmonary fat embolism syndrome within 24–72 hours. It is characterized by pulmonary distress, cutaneous petechial hemorrhages, and various neurologic manifestations associated with fatal outcome.

- **Pulmonary manifestations:** Patient develops dyspnea and tachypnea due to presence of fat microglobules in pulmonary capillaries causing hypoxemia.
- **Petechial hemorrhage:** Patient develops petechial hemorrhage over the chest and upper extremities resulting from thrombocytopenia due to platelets adhesion to microglobules of fat.
- **Neurologic manifestations:** Numerous petechial hemorrhages are produced by fat emboli in the brain, particularly in white matter known as '**brain purpura**'. Patient develops neurologic manifestations such as loss of consciousness, cerebral edema and herniation within a week in <10% of cases with fatal outcome (Fig. 18.41).

LABORATORY DIAGNOSIS

Fatty acids released from fat emboli are taken up by alveolar macrophages. Bronchoalveolar lavage is performed in suspected cases of fat embolism syndrome. Smears are stained with fat stains (Sudan III, Sudan IV, Sudan black and Oil red O). Positivity of smears with fat stains is suggestive of fat embolism syndrome.

PULMONARY HYPERTENSION

Primary pulmonary hypertension is a rare disorder of unknown etiology that arises in the absence of heart or lung disease associated with poor prognosis. It is characterized by thickening of the media of pulmonary muscular arteries. As pulmonary hypertension becomes more severe, there is extensive intimal fibrosis and muscle thickening within arteries and arterioles, which may be occlusive.

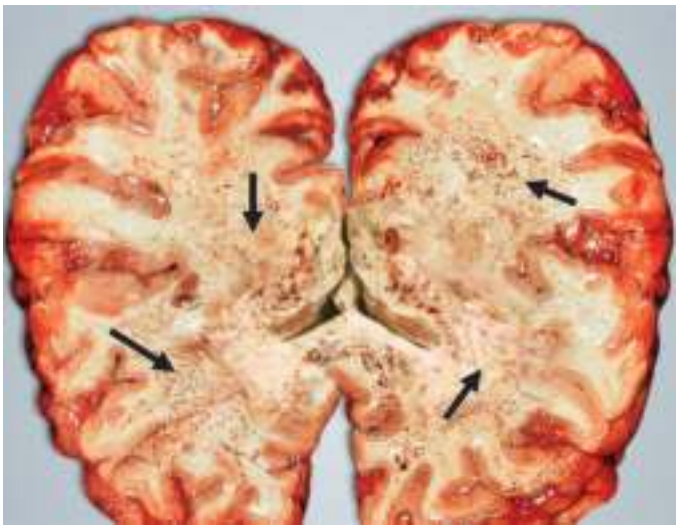


Fig. 18.41: Fat embolus. The patient was in a motorcycle accident and sustained multiple fractures of both legs. The fat emboli caused petechial hemorrhages in the white matter throughout the brain, resulting in coma and death. The macroscopic appearance of cerebral malaria is identical to this (arrows).

ETIOPATHOGENESIS

- **Primary pulmonary hypertension:** It is characterized by mean pulmonary artery pressure >25 mm Hg at rest and >30 mm Hg with exercise. It most common affects in young women. Gene mutation is associated with increased synthesis of transforming growth factor (TGF) which causes proliferation of smooth muscle cells of pulmonary artery.
- **Secondary pulmonary hypertension:** It is more common than primary pulmonary hypertension. Hypoxemia and respiratory acidosis stimulate vasoconstriction of pulmonary arteries. Hypoxemia occurs in chronic obstructive pulmonary disease (COPD), congenital left-to-right shunt, mitral stenosis, recurrent pulmonary thromboembolism and increased blood viscosity from polycythemia. Vasoconstriction is caused by increased synthesis of endothelin vasoconstrictor and loss of nitric oxide vasodilator.

PATHOLOGY

Pulmonary hypertension imposes an increased overload on the right ventricle resulting in right ventricular hypertrophy. Histologic changes in muscular arteries in pulmonary hypertension may show media hypertrophy, cellular intimal proliferation, plexiform, angiomatous and necrotizing lesions. Changes seen in muscular arteries in the lung in pulmonary hypertension are

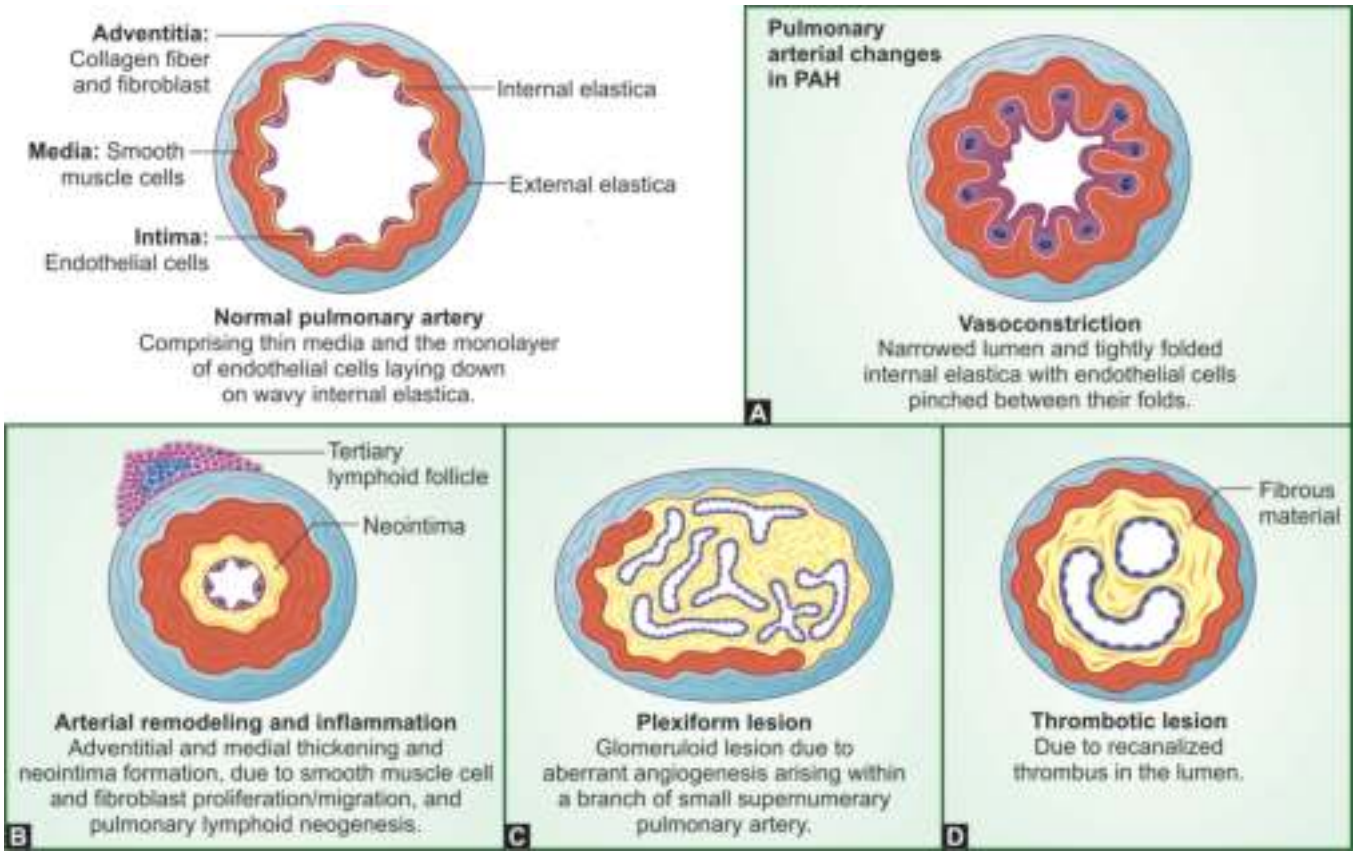


Fig. 18.42A to D: Changes seen in muscular arteries of the lung in pulmonary hypertension.

shown in Fig. 18.42A to D. Grading of pulmonary vascular lesions is given in Table 18.38.

CLINICAL FEATURES

Patient with pulmonary hypertension presents with dyspnea on exertion, chest pain and left parasternal heave, sign of right ventricular hypertrophy.

- On auscultation, there is accentuation of P2 as a result of pulmonary hypertension. Features of right-sided heart failure occur due to cor pulmonale.
- Chest radiograph shows enlargement of main pulmonary arteries and tapering of the distal vessels.
- Catheterization is done to measure mean pulmonary artery pressures at rest and after exercise.

Table 18.38 Grading of pulmonary vascular lesions

Grade	Pathologic Lesions
Grade 1	Vascular media hypertrophy
Grade 2	Grade 1 + intimal cell proliferation
Grade 3	Grade 2 + intimal fibrosis
Grade 4	Grade 3 + plexiform vascular lesion
Grade 5	Grade 4 + vascular dilatation (angiomatous) lesions
Grade 6	Grade 5 + necrotizing vascular lesions

PULMONARY EDEMA

Pulmonary capillary blood and alveolar gases are separated by the alveolar–capillary membrane, which consists of three different layers: (a) the capillary endothelium, (b) the interstitial space, which may contain connective tissue, fibroblasts, and macrophages; and (c) the alveolar epithelium.

- Exchange of fluid normally occurs between the vascular bed and the interstitium.
- Pulmonary edema is fluid accumulation in the interstitial tissue and air spaces. It leads to impaired gaseous exchange and may cause respiratory failure.
- Depending on the cause, pulmonary symptoms may appear suddenly or develop over time.
- Patient can have mild to extreme breathing difficulty, cough, chest pain and fatigue and other symptoms.

PATHOPHYSIOLOGY

Pulmonary edema can be caused by the following major pathophysiologic mechanisms: (a) imbalance of Starling forces, i.e. increased pulmonary capillary pressure, decreased plasma oncotic pressure, increased

negative interstitial pressure; (b) damaged to the alveolar–capillary barrier; and (c) impaired lymphatic drainage.

- The lymphatics play an important role in maintaining adequate fluid balance in the lungs by removing solutes, colloid and liquid from the interstitial space at a rate of 10–20 ml/hour. An acute elevation of pulmonary arterial capillary pressure to >18 mm Hg can increase filtration of the fluid into the pulmonary interstitium, but the removal of fluid by lymphatic channels does not increase correspondingly. In contrast, in the presence of sustained elevated left atrial pressure, the rate of removal of fluid by lymphatic channels can be as high as 200 ml/hour, which protects the lungs from pulmonary edema.
- Acute pulmonary edema can lead to fatal respiratory distress or cardiac arrest due to hypoxia. It is a cardinal feature of congestive heart failure. The most common cause of pulmonary edema is congestive heart failure (CHF). Heart failure happens when the heart can no longer pump out blood properly throughout the body. This creates a backup of pressure in the small blood vessels of the lungs, which causes the small blood vessels to leak fluid in the interstitial tissue and air spaces (alveoli). The alveoli are filled with smooth or floccular pink material. Alveolar capillaries are congested and containing red blood cells. Clinical manifestations of congestive heart failure are shown in Fig. 18.43. Histologic examination of pulmonary edema is shown in Fig. 18.44.

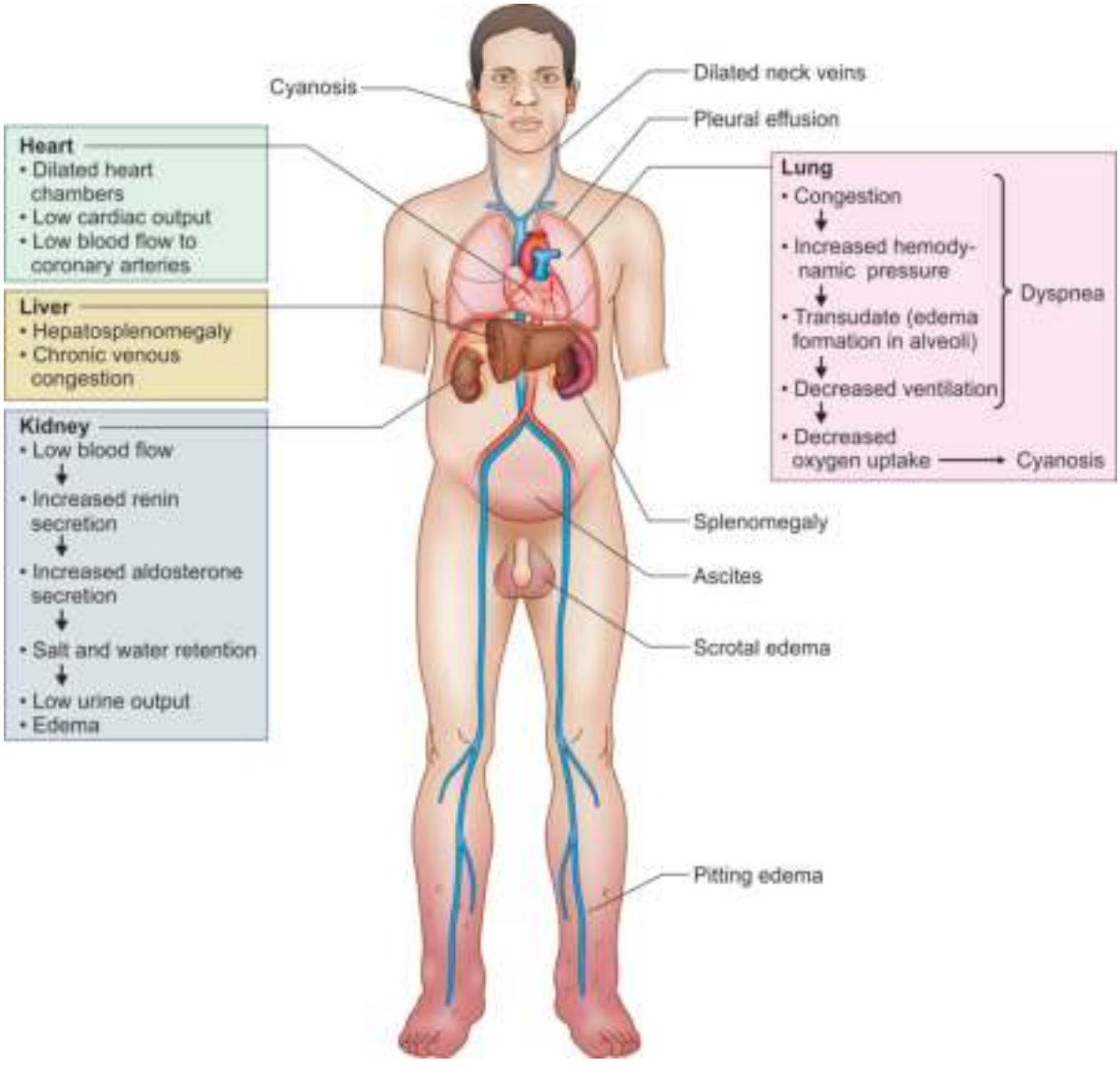


Fig. 18.43: Clinical manifestations in heart failure. Left heart failure is associated with retention of salt and water, pulmonary congestion, dyspnea, cyanosis, generalized edema, ascites, hydrothorax and scrotal edema. Right heart failure is associated with systemic venous congestion, peripheral pitting edema over ankles and hepatosplenomegaly.

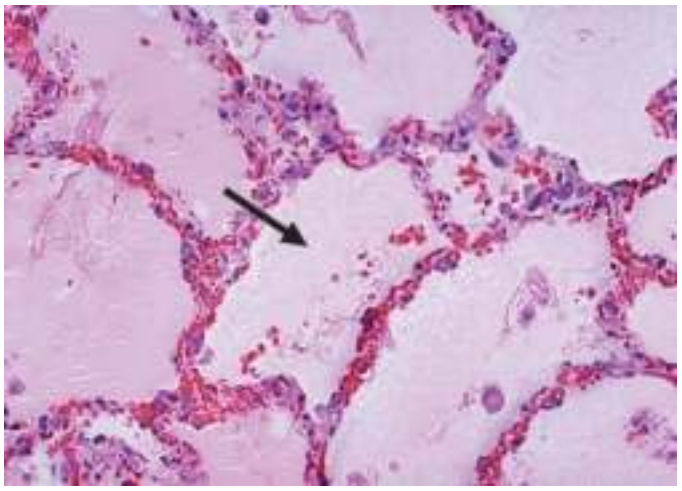


Fig. 18.44: Pulmonary edema. The alveoli in this lung are filled with a smooth to slightly floccular pink material. Alveolar capillaries are congested (arrow) (400X).

Cardiogenic Pulmonary Edema

Cardiogenic pulmonary edema is most common and potentially fatal cause of acute respiratory distress. It usually occurs due to acute decompensated heart failure, i.e. left ventricular systolic or diastolic dysfunction.

- Elevated left atrial pressure increases pulmonary venous pressure and hydrostatic pressure in the lung microvasculature resulting in fluid accumulation in interstitial tissue and alveolar spaces. Decreased breath sounds, dull percussion note, crackles on auscultation and orthopnea are clinical findings.
- Left atrial outflow obstruction can occur due to mitral stenosis, atrial myxoma and thrombosis of a prosthetic valve. Left ventricle systolic dysfunction is a common cause of cardiogenic pulmonary edema resulting from decreased myocardial contractility, that reduces cardiac output. The fall in the cardiac output stimulates sympathetic activity and blood volume expansion by activating the renin-angiotensin system resulting in retention of sodium and water, which further causes deterioration by decreasing left ventricular filling time and increasing capillary hydrostatic pressure.
- Causes of acute exacerbations of cardiogenic pulmonary edema include acute myocardial infarction, myocarditis, cardiomyopathies, aortic stenosis, aortic valve regurgitation and mitral valve regurgitation.

Noncardiogenic Pulmonary Edema

Noncardiogenic pulmonary edema is a distinct clinical syndrome associated with diffuse filling of the alveolar spaces in the absence of elevated pulmonary capillary wedge pressure.

- Adult respiratory distress syndrome is characterized by noncardiogenic pulmonary edema resulting from acute alveolar–capillary damage induced by toxins. There is leakage of fibrin-rich fluid into the alveoli resulting from injured vascular bed. It is also known as shock lung.
- Patient develops a clinical syndrome characterized by refractory hypoxemia not responsive to oxygen therapy and reduction in lung compliance.
- In infants, noncardiogenic pulmonary edema is linked to a deficiency of surfactant (a lecithin-rich lipid synthesized by pneumocytes II), that normally prevents alveolar collapse during expiration by lowering surface tension. Deficient pulmonary surfactant results in leakage of fibrin-rich fluid into the alveoli, that is not responsive to oxygen therapy. Clinical history, physical examination, electrocardiogram, echocardiography, laboratory investigations and direct measurement of pulmonary capillary wedge pressure can be done to distinguish cardiogenic from noncardiogenic pulmonary edema causing respiratory distress.

CLINICAL FEATURES

Patient presents with frothy pink or white sputum and breathlessness especially on lying down position. Clinical examination reveals weak pulse and cool and moist extremities. On auscultation, crackles and wheeze breath sounds are heard.

PULMONARY VASCULITIS

Patient with pulmonary vasculitis and related lesions often presents with alveolar hemorrhages resulting from Goodpasture syndrome, idiopathic rapidly progressive glomerulonephritis, Wegener's granulomatosis, microscopic polyarteritis, systemic lupus erythematosus and toxins. Patient may develop pulmonary hemorrhagic syndromes as given in Table 18.39.

Table 18.39 Pulmonary hemorrhagic syndromes

Syndrome	Inflammation of Capillaries	Immunofluorescence Study
Goodpasture syndrome	+/–	Linear staining
Idiopathic rapidly progressive glomerulonephritis	+	Granular staining
Wegener's granulomatosis	+	–
Microscopic polyarteritis	+	–
Collagen vascular disease, e.g. SLE	+	Granular staining
Toxins	–	–

NEOPLASTIC DISORDERS OF LUNG

Many benign and malignant tumors originate in the lungs. Approximately 90–95% are malignant tumors, 5% carcinoid tumors and 2–5% mesenchymal and tumor-like disorders.

- Cigarette smoking remains the most important risk factor for the development of lung cancer across world. Other risk factors for lung cancer include asbestos exposure, radiation, chemicals, heavy metals, fibrosing lung disorders, immunosuppression and genetic syndromes.
- Most common types of lung cancer in clinical practice include small cell lung carcinoma, squamous cell lung carcinoma, adenocarcinoma, bronchioloalveolar carcinoma and large cell lung carcinoma.
- Pulmonary Langerhans cell histocytosis is considered a malignant disorder. Tumor is composed of cells with nuclear groove and slightly eosinophilic cytoplasm. Tumor cells are positive for CD1a. Electron microscopy shows granules with a pentalaminar configuration and bulbous ends.

BENIGN TUMORS OF LUNG

Benign tumors of lung include bronchial adenoma and hamartomas (most common). These are relatively uncommon. Size of tumor remains constant for >2 years, but may exhibit dystrophic calcification on chest radiograph. Patient with tumor in peripheral region is usually asymptomatic, until it becomes large in size. But centrally located benign tumor may present with hemoptysis and signs of bronchial obstruction.

PULMONARY HAMARTOMA

Pulmonary hamartoma is disorganized benign mature tissue with altered architecture found in their normal anatomic location.

Age and Sex Predilection

Pulmonary hamartoma affects adults over 40 years of age, with a peak in the sixth decade of life.

Molecular Genetic Alterations

Chromosomal anomalies such as rearrangements of 12q14–15 or 6p21 suggest that pulmonary hamartoma is a neoplastic process.

- Pulmonary hamartoma has a high frequency of chromosomal translocation t(3; 12)(q27–28; q14–15), resulting in gene fusion of the high mobility group protein gene HMGA2 and the LPP gene.
- The HMGA2–LPP fusion gene usually consists of exons 1–3 of HMGA2 and exons 9–11 of LPP and seems to be expressed in pulmonary hamartoma with this chromosomal translocation.

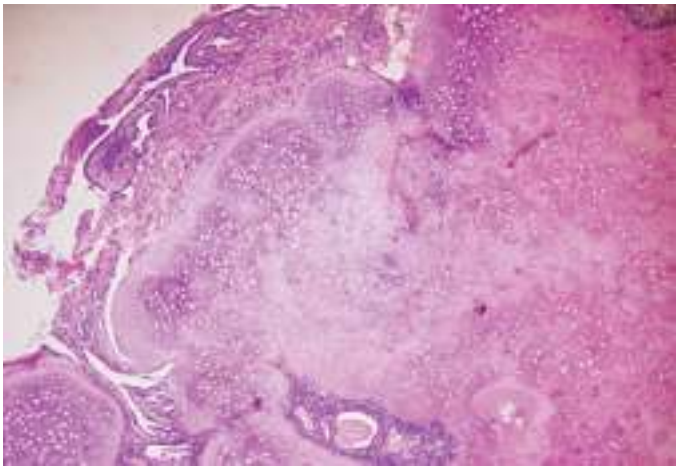


Fig. 18.45: Pulmonary hamartoma is composed of cartilage, bronchial epithelium, and smooth muscle cells (100X).

Imaging Technique

Pulmonary hamartoma accounts for 10% of coin lesion discovered incidentally on chest radiograph. A characteristic popcorn pattern of calcification is often seen on chest radiograph.

Surgical Pathology: Pulmonary Hamartoma
Light Microscopy
▪ Pulmonary hamartoma is composed of cartilage, bronchial epithelium, and smooth muscle cells. ▪ Myxoid components with spindle and stellate cells are often present at the periphery of pulmonary hamartoma (Fig. 18.45).
Immunohistochemistry Technique
Smooth muscle actin and S-100 are expressed in spindle cells. In males, the cells express ER, PR and androgen receptors.

BRONCHOPULMONARY CARCINOIDS

Bronchopulmonary carcinoid tumors of lung are derived from pluripotent neuroendocrine cells of major bronchial epithelium constituting up to 2% of primary lung tumors. These are not associated with cigarette smoking.

- Carcinoid tumors contain neuroendocrine granules, but the tumor cells are arranged in a distinctive pattern.
- Cushing's syndrome is often encountered in patients with small cell lung carcinoma, but not in bronchopulmonary carcinoids.

Clinical Behavior

Bronchopulmonary carcinoid tumors are slow growing highly vascular tumors. About 85% tumors are benign in nature. These tumors do not metastasize but may spread by direct extension. In approximately 15% of cases, metastasis may occur in distant organs.

Age Group

Bronchopulmonary carcinoid tumor is most common solitary primary tumor in children but may affect elderly persons.

Location of Tumor

Bronchopulmonary carcinoid tumor may be located in central, peripheral and midportion of lung. Centrally located carcinoid tumor appears as fleshy, smooth polypoid growth protruding into bronchial lumen, which obstructs bronchial lumen resulting in pneumonia.

Clinical Features

Patient with bronchopulmonary carcinoid tumor confined to lung presents with hemoptysis (most common), cough, and carcinoid syndrome associated with synthesis of neuropeptide in <1% in cases. Regional lymph node metastases occur in 20% of patients.

Surgical Pathology: Bronchopulmonary Carcinoids

Light Microscopy

Histologic variants of bronchopulmonary carcinoid tumors include classic and atypical variants. Comparison of typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma and small cell lung carcinoma is given in Table 18.40. Comparison of atypical carcinoid tumor and neuroendocrine large cell lung carcinoma is given in Table 18.41.

- **Classic carcinoid tumor:** It is slow-growing tumor that does not metastasize and has excellent prognosis with appropriate surgery (5-year survival in 90%). Tumor is composed of monomorphic round to oval cells with **salt and pepper nuclei** and eosinophilic granular cytoplasm. The tumor cells are arranged in small groups or nests, cords or tubular structures separated by thin fibrous septa (Fig. 18.46).
- **Atypical carcinoid tumor:** Presently atypical carcinoid tumor is reclassified alongside small cell lung carcinoma as part of the spectrum of neuroendocrine carcinoma. On histologic examination, tumor shows 5–10 mitotic figures per 10 high power fields, pleomorphism with hyperchromatic nuclei and tumor necrosis.

Histochemistry

Silver stain shows black granules in the cytoplasm, which may be argentaphilic or argyrophilic in nature.

- **Argentaffin cells:** These cells reduce silver salts to metallic silver (black color) without the help of reducing agent.
- **Argyrophil cells:** These cells reduce silver salts to metallic silver (black color) only after addition of a reducing agent.

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in bronchopulmonary carcinoid on histologic sections.

Markers	Expression
▪ Neuron-specific enolase (NSE)	▪ Positive
▪ Chromogranin (neural marker)	▪ Positive
▪ Synaptophysin (neural marker)	▪ Positive
▪ Cytokeratin	▪ Positive
▪ CDX2*	▪ Negative

**CDX2 is positive in GIT carcinoids*

Table 18.40 Comparison of bronchopulmonary typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma and small cell lung carcinoma

Feature	Typical Carcinoid	Atypical Carcinoid	Large Cell Neuroendocrine Carcinoma	Small Cell Lung Carcinoma
Etiology				
Role of tobacco smoking	None	Weak association	Strong association	Strong association
Male:female ratio	0.8:1	2:1	2.5:1	Declining
Mean age	50 years	56 years	63 years	62 years
Histologic features				
Architecture	Well organized	Focal loss	Moderate loss	Poor
Pleomorphism	Usually absent	Moderate	Marked	Mild
Mitoses	<2 per 10 high power field	2–10 per 10 high power fields	>10 per 10 high power field	>10 per 10 high power field
Necrosis	None	Focal necrosis	Abundant necrosis	Abundant necrosis
Argyrophilia	Common	Variable	Absent	Rare
DNA deposition on blood vessels (Azzopardi effect)	None	None	None	Frequent
Immunohistochemistry				
Neuroendocrine markers	Common, diffuse ++	Common, diffuse ++	Common often patchy +	Common often patchy +
Electron microscopy				
Granule numbers	Many	Moderate number	Nil	Scanty in number

Contd...

Table 18.40 Comparison of bronchopulmonary typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma and small cell lung carcinoma (*Contd...*)

Feature	Typical Carcinoid	Atypical Carcinoid	Large Cell Neuroendocrine Carcinoma	Small Cell Lung Carcinoma
Granule size	15–250 nm	80–140 nm	Nil	80–140 nm
Clinical behavior				
Local invasion	Present	Present	Present	Present
Lymphatic route metastasis	Occasional	Not frequent	Frequent	Usual
Hematogenous route distant metastasis	Rare	45–70%	>50%	90–100%
Five-year survival	95%	60%	27–35%	2%

Neuroendocrine markers are synaptophysin (SYN) cytoplasmic marker, chromogranin A (CHG-A) cytoplasmic marker, neuron-specific enolase (NSE) cytoplasmic marker and CD56 (neuronal cell adhesion molecule) membranous marker.

Table 18.41 Comparison of atypical carcinoid tumor and neuroendocrine large cell lung carcinoma

Feature	Atypical Carcinoid Tumor	Neuroendocrine Large cell lung carcinoma
Cell size	Medium size cell	Large cell
Cells arrangement	Small cells arranged in groups in a trabecular or mosaic pattern	Large cells arranged in groups in well circumscribed manner with peripheral palisading
Nuclear/cytoplasmic ratio	Moderate nucleocytoplasmic (N/C) ratio	Low nucleocytoplasmic (N/C) ratio
Nuclear chromatin	Fine chromatin	Coarse chromatin
Nucleoli	Indistinct nucleoli	Prominent nucleoli
Nuclear atypia	Moderate atypia	Prominent atypia
Mitoses	≥2 per high power fields	≥10 per high power fields
Necrosis	Necrosis present usually confined to the centers of the cell groups	Necrosis present in large areas of tumor
Neuroendocrine stains	Optional (positive)	Essential (positive)

Neuroendocrine markers are synaptophysin (SYN) cytoplasmic marker, chromogranin A (CHG-A) cytoplasmic marker, neuron-specific enolase (NSE) cytoplasmic marker and CD56 (neuronal cell adhesion molecule) membranous marker.

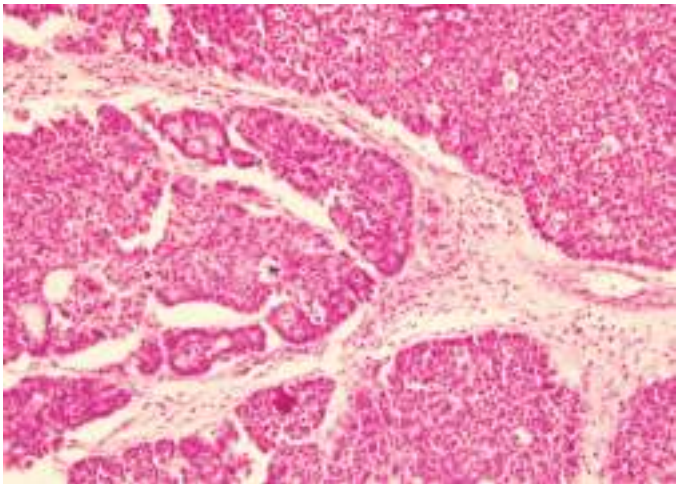


Fig.18.46: Bronchopulmonary carcinoid is composed of monomorphic round to oval cells with salt and pepper nuclei and abundant eosinophilic granular cytoplasm arranged in small groups or nests, cords or tubular structures and separated by thin fibrous septa (100X).

PRIMARY MALIGNANT TUMORS OF LUNG: OVERVIEW

Lung cancer is the most common fatal malignancy in both men and women in the age group above 50 years. Lung cancers are rare under age of 30 years. Surgical excision has limited role in management, as majority of cases report to hospital very late with locally advanced disease or wide dissemination (Fig. 18.47).

RISK FACTORS

Occupational exposure to various agents and their risk in development of lung cancers is given in Table 18.42.

- **Tobacco smoking:** Tobacco smoking accounts for 80–90% of lung cancers. Lung cancer correlates with duration and cumulative amount of exposure and depth of inhalation of tobacco smoke. Neutral fraction of tobacco smoke contains polycyclic hydrocarbons,

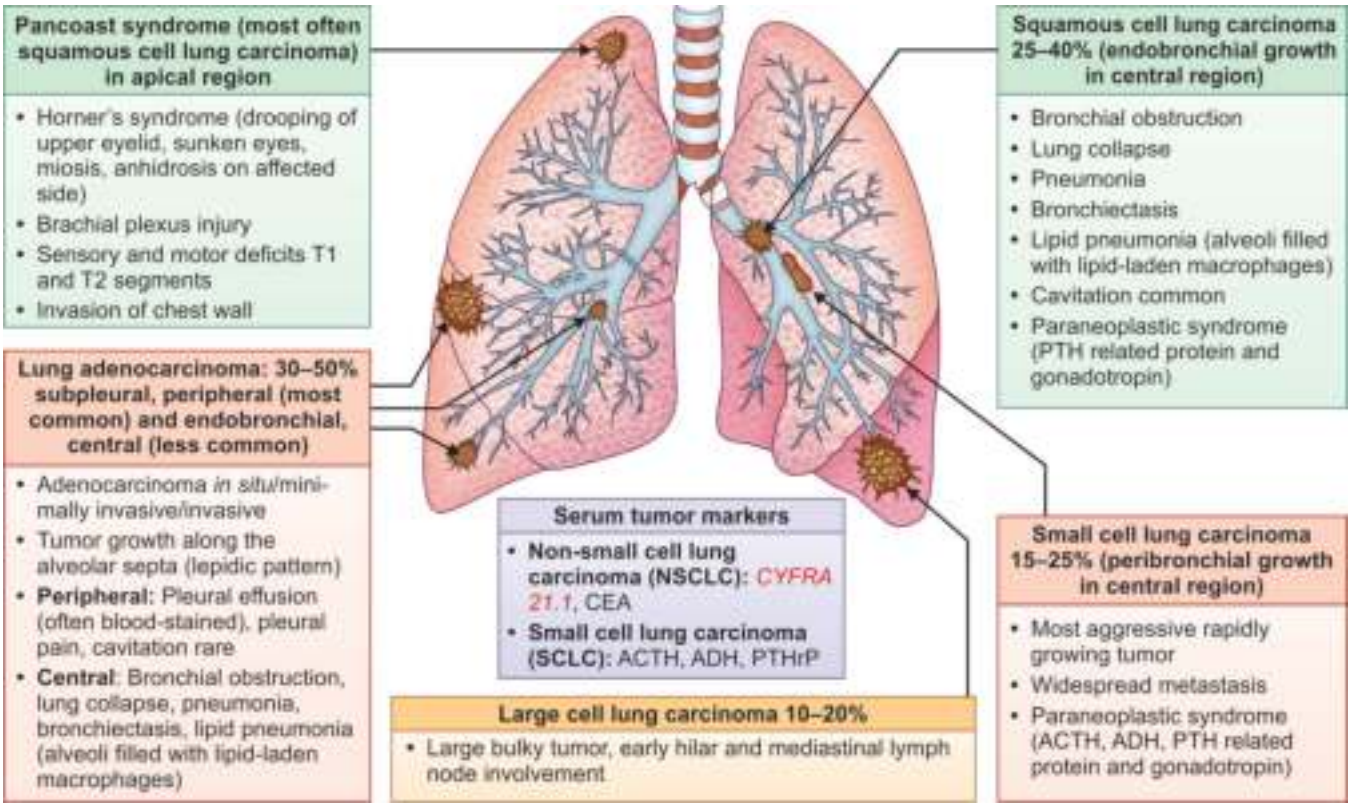


Fig. 18.47: Histologic variants of primary lung cancers. Clinical features of lung cancer depend on site of lesion, invasion of neighboring structures and extent of metastases.

Table 18.42 Occupational exposure to various agents and their risk in development of lung cancers

Occupational Agents	Main Industrial use
Asbestos	Insulation, filters, textiles
Arsenic and arsenic compounds	Glasses, metals, pesticides
Beryllium and beryllium compounds	Aerospace industry
Bis (chloromethyl) ether and chloromethyl methyl ether	Chemical intermediates
Cadmium and cadmium compounds	Dyes/paints
Chromium hexavalent compounds	Pigments (for textile dyes, paints, plastics), metal industry, chrome plating
Coal-indoor emissions from household combustion	Fuel for heating, cooking
Coal tars	Fuel
Coal tar-pitch	Construction, electrodes
Dioxin (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin)	Chemical industry
Engine exhaust, diesel	Fuel
MOPP chemotherapy (a mixture of vincristine, prednisone, nitrogen mustard and procainamide)	Treatment fo Hodgkin disease
Nickel compounds	Metallurgy, alloys, catalyst
Outdoor air pollution	Pollutants in atmosphere
Particulate matter in outdoor air pollution	Particulate matter in atmosphere
Lutonium-239	Nuclear plants
Polycyclic aromatic compounds	Petrochemical industries

Contd...

Table 18.42 Occupational exposure to various agents and their risk in development of lung cancers (*Contd...*)

Occupational Agents	Main Industrial use
Radon-222 and its decay products	Mining
Silica, crystalline	Stone cutting, mining, glass, paper
Soot	Pigments
Sulphur mustard	Chemical warfare
Talc containing asbestiform fibers	Paper, paints
Tobacco smoke, second hand	Exposure to passive tobacco smoke
Tobacco smoking	Exposure to active tobacco smoke
X-rays and γ-radiation	Medical, nuclear

Exposure circumstances include aluminum production, coal gasification, coal-tar pitch, coke production, hematite mining (underground) with exposure to radon, iron and steal founding, painter (occupational exposure) and occupational exposures in the rubber production industry.

which bind to nuclear DNA and exert mutagenic effect. Acidic fraction of tobacco smoke contains tumor promoting benzopyrene compound. Well differentiated squamous cell lung carcinoma, small cell lung carcinoma, large cell lung carcinoma and to some extent lung adenocarcinoma all demonstrate an increased incidence with increasing tobacco smoke inhalation. Approximately 25% of lung cancers in nonsmokers have been attributed to passive tobacco smoke inhalation. Many genetic alterations are caused by tobacco smoking. Major carcinogens of tobacco smoke are given in Table 18.43.

- **Occupational exposure to organ dust:** Occupational exposure to pollutants during metal refining and smelting processes such as arsenic, nickel, iron oxides, chromium, asbestos, beryllium, cadmium, mustard gas, pesticides, and uranium increases the risk of lung cancer in 10% of cases. Asbestos exposure is the best recognized occupational risk factor of lung cancer. Tobacco smoking is synergistic risk factor in asbestos-exposed persons to manyfolds in the development of lung cancers such as lung adenocarcinoma (most common) and squamous cell lung carcinoma. Asbestos fibers act as promoter and cigarette smoke as initiator as well as promoter of carcinogenesis. Incidence of lung cancer is increased in uranium workers. Uranium exposure emits particulate products and radon gas. Patient develops lung cancer after 17 years of exposure to uranium.
- **Exposure to ionizing radiation:** It can cause DNA mutations and chromosomal abnormalities resulting in lung cancer. Japanese are at increased risk for lung cancers, i.e. squamous cell lung carcinoma, lung adenocarcinoma and small cell lung carcinoma after atom bomb explosion in Hiroshima and Nagasaki.
- **Exposure to chemical agents:** Chloromethyl ether is a potent carcinogen that causes small cell lung carcinoma. Combustion of coal and other fossils releases polycyclic aromatic hydrocarbon benzopyrene, which increases risk for lung cancer.
- **Diffuse pulmonary fibrosis:** Diffuse pulmonary fibrosis in tuberculosis has been associated with increased risk of lung cancer.
- **Chronic obstructive pulmonary disease:** Risk for lung cancer increases in chronic bronchitis and diffuse pulmonary fibrosis. Proposed mechanisms of increased risk include decreased clearance of inhaled carcinogens and epithelial metaplasia.
- **Genetic predisposition:** In general, relatives of persons with lung cancer have higher risk for development of lung cancer. Defects in specific oncogenes, specific HLA antigens, and tumor suppressor genes increase the risk for development of lung cancer.

Table 18.43 Major carcinogens of tobacco smoke

Particulate Phase	Vapor Phase
Neutral fraction (e.g. benzpyrene, dibenzanthracene, benzofluoranthene)	Nickel carbonyl
Basic fraction (e.g. nitrosamine)	Hydrazine
Residual fraction (e.g. nickel, cadmium, polonium-210)	▪ Vinyl chloride ▪ Nitrogen oxides ▪ Nitrosodimethylamine

Mutation of TP53 tumor suppressor gene is common in both small cell lung carcinoma (70% of cases) and non-small cell lung carcinoma (30% of cases).

PRECURSOR LUNG LESIONS

The 'term' precursor lesion does not imply that it definitely progresses to lung carcinoma. Four types of epithelial precursor lesions are squamous dysplasia and carcinoma *in situ*, atypical adenomatous hyperplasia, adenocarcinoma *in situ* and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia.

WHO CLASSIFICATION OF LUNG TUMORS

Lung carcinoma is categorized into two main histologic groups: small cell lung carcinomas (SCLCs) and non-small cell lung carcinomas (NSCLCs) constituting 15% and 85% of lung carcinomas respectively. NSCLCs are usually categorized into squamous cell lung carcinoma (25–40%), adenocarcinoma (30–50%) and large cell lung carcinoma (10–20%). Ratio of occurrence SCLCs and NSCLCs is 1 : 4. The 2024 WHO classification of tumors of the lung is given in Table 18.44.

Small Cell Lung Carcinoma

Small cell lung carcinoma (SCLC) is most aggressive tumor having peribronchial growth pattern associated with paraneoplastic syndromes. It usually metastasizes to distant organs before the tumor mass attains large size and resulting in bronchial obstruction and lung collapse. It is usually treated by chemotherapy rather with radiotherapy and surgical resection. SCLC is associated with poor prognosis.

Non-small Cell Lung Carcinoma

Non-small cell lung carcinomas (NSCLCs) (e.g. squamous cell lung carcinoma, lung adenocarcinoma and anaplastic lung carcinoma) invade stroma and surrounding structures. Histopathologic characteristics of NSCLCs are less significant, but pathologic staging is critical for treatment and prognostic outcome. NSCLCs confined to lung are treated by surgical excision. Bronchoalveolar carcinoma and histologic variant of adenocarcinoma characterized by spreading of tumor along the alveolar septa in 'lepidic pattern' without invading stroma and pre-existing structures.

Table 18.44 The 2024 WHO classification of tumors of the lung

Epithelial Tumors

Adenocarcinoma
- Lepidic adenocarcinoma
- Acinar adenocarcinoma
- Papillary adenocarcinoma
- Micropapillary adenocarcinoma
- Solid adenocarcinoma
- Invasive mucinous adenocarcinoma
  - Mixed invasive mucinous adenocarcinoma
  - Nonmucinous adenocarcinoma
- Colloid adenocarcinoma
- Fetal adenocarcinoma
- Enteric adenocarcinoma
- Minimally invasive adenocarcinoma
  - Mucinous adenocarcinoma
  - Nonmucinous adenocarcinoma
- Preinvasive lesions
  - Atypical adenomatous hyperplasia
  - Adenocarcinoma *in situ*
    - Mucinous adenocarcinoma *in situ*
    - Nonmucinous adenocarcinoma *in situ*

Squamous cell lung carcinoma
- Keratinizing squamous cell lung carcinoma
- Nonkeratinizing squamous cell lung carcinoma
- Basaloid squamous cell lung carcinoma
- Preinvasive lesion
  - Squamous cell lung carcinoma *in situ*

Contd...

Neuroendocrine tumors
- Small cell lung carcinoma
  - Combined small cell lung carcinoma
- Large cell neuroendocrine carcinoma

Combined large cell neuroendocrine carcinoma
- Carcinoid tumors
  - Typical carcinoid
  - Atypical carcinoid
- Preinvasive lesion
  - Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

Large cell lung carcinoma

Adenosquamous carcinoma

Pleomorphic carcinoma

Spindle cell carcinoma

Giant cell carcinoma

Carcinosarcoma

Primary blastoma

Other and unclassified carcinomas
- Lymphoepithelioma-like carcinoma
- NUT carcinoma

Salivary gland-type tumors
- Mucoepidermoid carcinoma
- Adenoid cystic carcinoma
- Epithelial–myoepithelial carcinoma
- Pleomorphic adenoma

Contd...

Table 18.44 The 2024 WHO classification of tumors of the lung (*Contd...*)

Papillomas
▪ Squamous cell papilloma
• Exophytic squamous cell papilloma
• Inverted squamous cell papilloma
▪ Glandular papilloma
▪ Mixed squamous cell and glandular papilloma
Adenomas
▪ Sclerosing pneumocytoma
▪ Alveolar adenoma
▪ Papillary adenoma
▪ Mucinous cystadenoma
▪ Mucous gland adenoma
Mesenchymal Tumors
Pulmonary hamartoma
Chondroma
PEComatous tumors
▪ Lymhangioleiomymatosis
▪ PEComa benign clear cell tumor
▪ PEComa malignant tumor
Congenital peribronchial myofibroblastic tumor
Diffuse pulmonary lymphangiomatosis
Pluropulmonary blastoma
Synovial sarcoma
Pulmonary artery intimal sarcoma
Pulmonary myxoid sarcoma with EWSR1-CREB1 translocation
Myoepithelial tumors
▪ Myoepithelioma
▪ Myoepithelial carcinoma
Lymphohistiocytic Tumors
▪ Extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT) lymphoma
▪ Diffuse large B cell lymphoma
▪ Lymphomatoid granulomatosis
▪ Intravascular large B cell lymphoma
▪ Pulmonary Langerhans cell histiocytosis
▪ Erdheim-Chester disease
Tumors of Ectopic Origin
▪ Germ cell tumors
▪ Mature teratoma
▪ Immature teratoma
▪ Intrapulmonary thymoma
▪ Melanoma
▪ Meningioma, not otherwise specified
Metastatic Tumors
▪ Breast carcinoma
▪ Renal cell carcinoma
▪ Colon carcinoma
▪ Thyroid carcinoma
▪ Prostatic carcinoma
▪ Choriocarcinoma
▪ Osteosarcoma
▪ Ewing's sarcoma
▪ Non-Hodgkin lymphoma

Contd...

Uncommon Malignant Tumors

Uncommon primary malignant lung tumors include bronchopulmonary carcinoids, mucoepidermoid carcinoma, adenoid cystic carcinoma, angiosarcoma, hemangioendothelioma and lymphomas.

LUNG CARCINOMAS: ORIGIN

Lung carcinomas arise from cells present in the lower respiratory tract, that are capable of cell division, e.g. neuroendocrine cells in bronchoalveolar region (small cell lung carcinoma), basal cells in major bronchi (squamous cell lung carcinoma), mucinous cells in terminal bronchioles (adenocarcinoma) and clara cells (bronchoalveolar carcinoma replaced by adenocarcinoma *in situ*). Cell of origin and histologic variants of lung carcinomas are given in Table 18.45.

Four types of precursor lesions of lung include squamous dysplasia and carcinoma *in situ*, atypical adenomatous hyperplasia and adenocarcinoma *in situ*; and idiopathic neuroendocrine hyperplasia. It is not essential that precursor lesions progress to development of lung carcinoma in all cases.

MOLECULAR CARCINOGENESIS

Lung cancer is the leading cause of cancer-related mortality due to late diagnosis and limited treatment interventions. Malignant tumors of lung are

Table 18.45 Cell of origin and histologic variants of lung carcinomas

Cell of Origin of Lung Carcinoma	Histologic Variants of Lung Carcinoma
Neuroendocrine cells (also known as Kulchitsky cells) in broncho-pulmonary region	Small cell lung carcinoma
Basal cells in major bronchi (lobar or segmental)	Squamous cell lung carcinoma
Mucous cells in terminal bronchioles	Lung adenocarcinoma
Clara cell in terminal bronchoalveolar region	Bronchoalveolar carcinoma, histologic variant of adenocarcinoma

categorized into two main histologic groups: small cell lung carcinomas (SCLCs) and non-small cell lung carcinomas (NSCLCs) constituting 15% and 85% of lung cancers respectively. NSCLCs are usually categorized into squamous cell lung carcinoma (25–40%), lung adenocarcinoma (30–50%) and large cell lung carcinoma (10–20%). Ratio of occurrence of SCLCs and NSCLCs is 1:4. Lung tumors are clinically and genetically distinct; and varying in primary site, invasion, mode of spread and biological behavior. Oncogenes and tumor suppressor gene mutations associated with lung cancers and other cancers (Table 18.46).

- Recently, comprehensive molecular profiles of lung carcinoma and different subtypes at the levels of DNA alterations, DNA methylation, mRNA expression, microRNA expression and protein expression have been identified. These novel characteristics have increased the understanding of the molecular carcinogenesis of lung carcinoma and molecular targeted therapies. Major genetic changes in small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma are given in Table 18.47. Gene mutations in small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma are given in Table 18.48.
- In addition to complete molecular profile, an increasing number of tumor-related cytokines, which affect blood vessel formation, tumor growth, invasion and metastasis have been identified. Vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (β-FGF) are among the most important angiogenic factors. Based on available literature, mechanisms of angiogenesis and its prognostic significance in NSCLCs are estimated by microvessel density (MVD) and β-FGF in the tumor from NSCLC patients.

Table 18.46 Oncogenes and tumor suppressor gene mutations associated with lung cancers and other cancers

Category	Proto-oncogenes	Mode of Action	Associated Cancers
Growth factor receptors			
EGFR receptor	ERBB1 (EGFR) Erb-B2 (avian erythroblastosis)	Amplification	*Lung adenocarcinoma*
ALK receptor	ALK	Point mutation	*Lung adenocarcinoma*
Signal-transduction proteins			
GTP-binding proteins	K-RAS	Point mutation	▪ *Lung carcinoma* ▪ Colon carcinoma ▪ Pancreatic carcinoma
Cell cycle progression inhibitors (tumor suppressor gene)			
RB gene (locus 13p14)	RB gene codes for pRB protein inhibiting G1 to S of cell cycle and nuclear transcription factor (mutation by deletion and nonsense)	Familial cancers ▪ Retinoblastoma ▪ Osteosarcoma	Associated sporadic cancers ▪ *Lung carcinoma* ▪ Retinoblastoma and osteosarcoma ▪ Breast carcinoma ▪ Colon carcinoma ▪ Prostatic carcinoma ▪ Urinary bladder carcinoma

Table 18.47 Major genetic changes in small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma

Genetic Alterations	Small Cell Lung Carcinoma	Squamous Cell Lung Carcinoma	Lung adenocarcinoma
Gene Mutations			
▪ TP53	▪ >90%	▪ 50–80%	▪ 30–40%
▪ RB	▪ >90%	▪ 5–15%	▪ 5–15%
▪ BRAF	▪ Nil	▪ Nil	▪ <5%
▪ EGFR Caucasian	▪ <1%	▪ <1%	▪ 10–20%
▪ EGFR Asian	▪ <5%	▪ <5%	▪ 35–45%
▪ ERBB2/HER2/neu	▪ Nil	▪ Nil	▪ <5%
▪ K-RAS Caucasian	▪ <1%	▪ <5%	▪ 15–35%
▪ K-RAS Asian	▪ <1%	▪ <5%	▪ 5–10%
▪ PIK3CA	▪ <5%	▪ 5–15%	▪ <5%

Contd...

Table 18.47 Major genetic changes in small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma (*Contd...*)

Genetic Alterations	Small Cell Lung Carcinoma	Squamous Cell Lung Carcinoma	Lung adenocarcinoma
Gene amplification			
▪ Myc	▪ 20–30%	▪ 5–10%	▪ 5–10%
▪ FGFR1	▪ <1%	▪ 15–25%	▪ <5%
▪ EGFR	▪ <1%	▪ 10%	▪ 5–10%
▪ ERBB2/HER2/neu	▪ <1%	▪ <1%	▪ <5%
▪ MET	▪ <1%	▪ <5%	▪ <5%
Gene rearrangement			
▪ ALK	▪ Nil	▪ <1%	▪ <5%
▪ RET	▪ Nil	▪ Nil	▪ 1%
▪ ROS1	▪ Nil	▪ Nil	▪ 1–2%
▪ NTRK1	▪ Nil	▪ Nil	▪ <1%
▪ NRG1	▪ Nil	▪ Nil	▪ <1%

Table 18.48 Gene mutations in small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma

Lung Carcinoma and Genes Involved	Comments
Small cell lung carcinoma	
TP53 tumor suppressor gene	TP53 is located on short arm of chromosome 17(17p13.1). In human, a common polymorphism involves the substitution of an arginine for proline at codon position 72. It is linked with small cell lung carcinoma susceptibility.
RB1 tumor suppressor gene	RB1 gene is located on the long arm of chromosome 13(13q14.1-q14.2). It provides instructions for making a protein called pRB. RB1 gene mutation is linked with SCLC susceptibility
TP73 tumor suppressor gene	TP73 is located on long arm of chromosome 1(1p73). It induces apoptosis of cancer stem cells and may act as a tumor suppressor gene overlapping its functions with TP53. Loss of heterozygosity of TP73 contributes to human SCLC.
NOTCH family of genes	NOTCH signaling plays an essential role in development and homeostasis of multiple organs. Dysregulation of NOTCH signaling has been implicated in various diseases including small cell lung carcinoma.
Gastrin releasing peptide (GRP)	Human small cell lung carcinomas synthesize regulatory gastrin releasing peptide (GRP), that selectively stimulates small cell lung carcinoma clonal growth.
Squamous cell lung carcinoma	
TP53 gene (90%)	TP53 is located on short arm of chromosome 17(17p13.1). In human, a common polymorphism involves the substitution of an arginine for proline at codon position 72. TP53 gene mutation is linked with squamous cell lung carcinoma susceptibility
CDKN2A tumor suppressor gene	▪ Cyclin-dependent kinase inhibitor 2A (CDKN-2A) gene resides on chromosome 9 at the band 9p21 and contains 8 exons. The gene codes for two proteins including the NK4 family member p16 (INK4A) and p14 (ARF) proteins with different amino acid sequences. Both function as tumor suppressors by regulating the cell cycle. ▪ The p16 (INK4A) inhibits cyclin-dependent kinases 4 and 9 (CDK 4 and 9) and thereby activates the retinoblastoma (RB) family of proteins. CDKN-2A gene is the second most commonly inactivated gene in cancer after TP53. ▪ Germline CDKN-2A mutation is also associated with lung carcinoma, familial melanoma, glioblastoma and pancreatic cancer. ▪ The CDKN-2A gene also contains one of 27 SNPs associated with increased risk of coronary artery disease.
PTEN tumor suppressor gene	PTEN gene resides on long arm of chromosome 10q23. It acts as tumor suppressor gene, that codes for phosphatase and tensin homolog protein (PTEN) involved in the regulation of the cell cycle, preventing the cells from growing and dividing too rapidly. Somatic deletions of PTEN gene affect phosphatase domain of the protein and linked to squamous cell lung carcinoma.

Contd...

Table 18.48 Gene mutations in small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma (*Contd...*)

Lung Carcinoma and Genes Involved	Comments
PIK3CA gene	PIK3CA (phosphatidylinositol-4,5-bisphosphatase 3-kinase catalytic subunit α) gene is integral part of P13 pathway, that has been described as an oncogene. PIK3CA gene amplification and/or point mutations have been demonstrated in several human cancers.
KEAP1 tumor suppressor gene	KEAP1 gene codes for a protein containing Kelch-like ECH-associated protein 1. Under quiescent conditions, NRF1 is anchored in the cytoplasm through binding to KEAP1. KEAP1 interacts with NRF2, a master regulator of the antioxidant response. KEAP1 is not only a tumor suppressor gene but also a metastasis suppressor gene.
NFE2L2 gene	NFE2L2 (nuclear factor, erythroid 2 like 2) gene encodes a transcription factor which is member of a small family of basic leucine zipper (pZIP) proteins, that binds to antioxidant response (ARE). Recurrent loss of NFE2L2 exon 2 is a mechanism for NRF pathway activation in lung carcinoma and head and neck cancers.
MLL2 gene	MLL2 (myeloid/lymphoid or mixed-lineage leukemia) gene is located on chromosome 11q23 that has partial synteny with chromosome 19q. It encodes a regulator of homeotic gene expression. Mutation of cancer driver MLL2 results in transcription stress and genome stability.
NOTCH1 gene	NOTCH signaling plays an essential role in development and homeostasis of multiple organs. Dysregulation of NOTCH signaling has been implicated in various diseases including small cell lung carcinoma.
RB-1 tumor suppressor gene	RB1 gene is located on the long arm of chromosome 13 (13q14.1-q14.2). It provides instructions for making a protein called pRB. RB1 gene mutation is linked with cancer susceptibility.
SOX2 gene	▪ SOX2 gene resides on 3q26. 3-q27 and codes for transcriptional factor. It is a regulator of foregut development and an embryonic stem cell factor cooperating during induction of pluripotency in a terminally differentiated somatic cells. ▪ SOX2 gene regulates the transcriptional network of oncogenes and affects tumorigenesis of human lung cancer stem cells. ▪ SOX2 amplification and upregulation are frequent events in squamous cell lung carcinomas and are associated with indicators of good prognosis.
TP63 gene	▪ TP63 is a member of the TP53 gene family. In contrast to TP53 gene, TP63 gene is frequently not inactivated. ▪ TP63 gene codes for p63 protein, that regulates differentiation and morphogenesis in epithelial and mesenchymal tissues. ▪ Amplification of TP63 is demonstrated in 25% of squamous cell lung carcinomas.
Vascular endothelial growth factor (VEGF)	VEGF is a tumor-related angiogenic cytokine, that affects vessel formation, tumor growth, invasion and metastasis in NSCLCs.
Basic fibroblast growth factor (β-FGF)	Basic fibroblastic growth factor is a tumor-related angiogenic cytokine, that affects vessel formation, tumor growth, invasion and metastasis in NSCLCs.
Insulin-like growth factors I and II (IGF-1, IGF-II)	Insulin-like growth factors I and II (IGF-1, IGF-II) stimulate the proliferation of a variety of cancer cells including those from lung cancer.
Endothelin 1 (ET-1)	Endothelin, a potent vasoconstrictor enhances proliferation of cancer stem cells in lung carcinoma by increasing intracellular free calcium.
Lung adenocarcinoma	
K-RAS oncogene	▪ Oncogenic K-RAS decouples glucose and glutamine metabolism by enhanced glycolytic activity and increased utilization of glutathione for anabolic synthesis to support cancer cell growth in some non-small cell lung carcinomas (NSCLCs). ▪ Recent studies indicate that patients with mutant K-RAS tumors fail to benefit from adjuvant chemotherapy and associated with short survival.
EGFR	▪ Some non-small cell lung carcinomas (NSCLCs) harbor mutated oncogene in lung carcinoma encodes for epidermal growth factor receptor. ▪ EGFR mutations are highly specific for lung adenocarcinoma especially in cases with lepidic and papillary growth and associated with TTF-1 positivity. ▪ Families with germline mutations of EGFR T790M mutations have high risk for development of lung adenocarcinoma.

Contd...

Table 18.48 Gene mutations in small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma (*Contd...*)

Lung Carcinoma and Genes Involved	Comments
BRAF gene	Oncogenic BRAF gene codes for BRAF protein serine/threonine kinase, that induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. Analysis of BRAF sequence revealed mutations in adenocarcinomas, one in exon 11 (G465V), and a second in exon 15 (L596R)
ALK rearrangement	▪ EML4-ALK fusion accounts for >90% ALK rearrangements in lung adenocarcinomas ▪ Other less commonly ALK fusion partners include KIF5B-ALK, LKC1-ALK and TFG-ALK
MET exon 14 skipping	Recently, MET exon 14 skipping has been identified as a potential driver mutation in lung and colon cancers. Strikingly, NSCLC patients have shown remarkable responses to MET inhibitor (crizotinib or cabozatinib)
ERBB2 kinase (HER2/neu)	Gene codes transmembrane protein tyrosine kinase ERRB2 (also known as HER2/neu). Functional analysis of transmembrane receptor tyrosine kinase mutations in lung adenocarcinoma identifies oncogenic extracellular domain mutations of EBBR2. The patients harboring EBBR2 mutations could benefit from EBBR2 directed therapy
ROS1 fusion gene	Chromosomal rearrangement of the gene encoding ROS1 proto-oncogene receptor tyrosine kinase defines a distinct molecular subgroup of non-small cell lung carcinomas (NSCLCs) that may be susceptible to therapeutic ROS1 kinase inhibition
MAK2K1 gene	Dysregulation of miRNAs-449a contributes to carcinogenesis of the human non-small cell lung carcinoma (NSCLC). The miRNAs-449a suppresses cell invasion by inhibiting NSCLC
RET fusion gene	Rearrangement of RET gene generates fusion transcript, that encodes the tyrosine kinase domain. RET fusion gene is a new targetable driver gene in lung adenocarcinoma. Patients are treated by inhibitors of RET tyrosine kinase
MET gene	MET gene alteration is linked to non-small cell lung carcinoma
NF1 gene	NF1 gene resides on chromosome 17p11 and codes for neurofibronin 1. Reduced NF1 expression confers resistance to EGFR inhibition in lung adenocarcinoma
RIT1 gene	▪ RIT1 is a driver oncogene in a subset of lung carcinomas, that suggests P13K and MEK inhibition as a potent therapeutic strategy in RIT1-mutated tumors ▪ Somatic mutation in the small GTPase gene RIT1 is demonstrated in 2% of lung adenocarcinomas
Vascular endothelial growth factor (VEGF)	VEGF is a tumor-related angiogenic cytokine, that affects vessel formation, tumor growth, invasion and metastasis in NSCLCs
Basic fibroblast growth factor (β-FGF)	Basic fibroblastic growth factor is a tumor-related angiogenic cytokine, that affects vessel formation, tumor growth, invasion and metastasis in NSCLCs
Epidermal growth factor receptor (EGFR)	▪ Recently, it has been reported that mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene occur in a subset of patients with NSCLC especially in well to moderately differentiated adenocarcinoma ▪ Approximately 10% of patients with adenocarcinoma have a rapid response to the tyrosine kinase inhibitor gefitinib, which targets the epidermal growth factor receptor (EGFR)
Insulin-like growth factors I and II (IGF-1, IGF-II)	Insulin-like growth factors I and II (IGF-1, IGF-II) stimulate the proliferation of a variety of cancer cells including those from lung cancer
Endothelin 1 (ET-1)	Endothelin 1, a potent vasoconstrictor enhances proliferation of neoplastic cells in lung carcinoma by increasing intracellular free calcium

- Recently, it has been reported that mutations in receptor tyrosine kinase (RTK) domain of the epidermal growth factor receptor (EGFR) gene occur in a subset of patients with NSCLC especially in well-to-moderately differentiated adenocarcinoma. Approximately 10% of patients with lung adenocarcinoma have a rapid response to receptor tyrosine kinase inhibitor gefitinib, which targets the EGFR. Insulin-like growth factors I and II (IGF-I, IGF-II) stimulate the proliferation of cancer stem cells leading to lung cancer growth. Endothelin 1, a potent vasoconstrictor enhances proliferation of cancer stem cells in lung carcinoma by increasing intracellular free calcium. Human small cell lung carcinomas synthesize regulatory gastrin releasing peptide (GRP), that selectively stimulates small cell lung carcinoma clonal growth. Growth factors involved in pulmonary carcinogenesis are given in Table 18.49. Molecular pathogenesis of bronchial carcinoma is shown in Fig. 18.48.

Table 18.49 Growth factors involved in pulmonary carcinogenesis

Epidermal growth factor receptor (EGFR)
Basic transforming growth factor (β-TGF)
Vascular endothelium growth factor (VEGF)
Gastrin-releasing peptide (GRP)
Transferrin
Insulin-like growth factor
Endothelin 1

Small Cell Lung Carcinoma

Small cell lung carcinoma is characterized by highly complex genomic alterations such as biallelic inactivation of TP53 and RB1 tumor suppressor genes. Other tumor suppressor gene alterations have been observed in TP73 as a result of rearrangement of TP73. In small cell lung carcinoma, inactivating mutations of NOTCH family of genes have also been demonstrated; that suppress neuroendocrine differentiation via regulation of ASCL1 expression in 25% of cases.

- **Messenger RNA profiling:** Based on messenger RNA (mRNA) profiling, small cell lung carcinomas are divided into two groups: Group 2 and group 1. Group 2 is characterized by higher expressions of CHGA, GRP, ASCL1, and DLK1 genes. Group 1 shows lower expressions of CHGA, GRP, ASCL1, and DLK1 genes.
- **MicroRNAs and SCLC:** Small cell lung carcinoma is a neuroendocrine tumor. It demonstrates ASCL1 transcription factor that promotes neuroendocrine differentiation. ASCL1 also promotes miR-375 in small cell lung carcinoma. The miR-375 may reduce the YAP1-associated proliferative arrest by inhibiting YAP1. Serum miR-92a-2 analysis is a potential non-invasive method for SCLC diagnosis. High expression of miR-34a may be associated with atypical carcinoids.

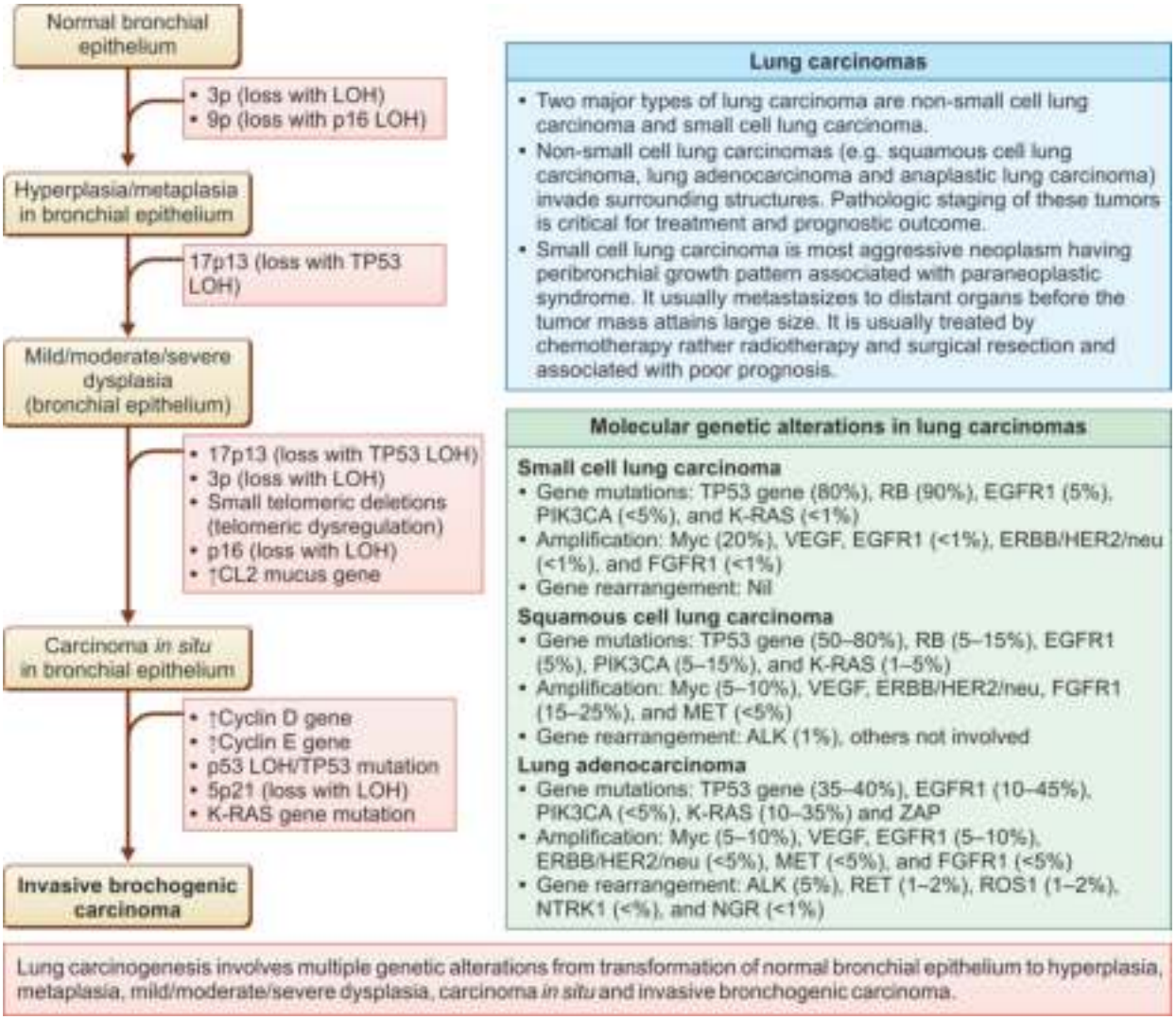


Fig. 18.48: Molecular pathogenesis of bronchogenic carcinoma.

Squamous Cell Lung Carcinoma

In the 2024 WHO classification of lung tumors, squamous cell lung carcinomas are classified into keratinizing, non-keratinizing and basaloid variants. However, basaloid squamous cell lung carcinoma immunohistochemically demonstrates positivity for p40, CK5, CK6, and p53, hence categorized under squamous cell lung carcinoma.

- **Gene mutations and affected signaling pathways:** The complete molecular profiling of squamous cell lung carcinoma has demonstrated statistically significant genomic alterations: TP53, CDKN2A, PTEN, PIK3CA, KEAP1, NFE2L2, MLL2, HLA-1, NOTCH1, RB1, and PDYN (promoter of the human prodynorphin gene).
- **TP53 gene mutation:** It has been demonstrated in 90% of squamous cell lung carcinomas. Oxidative damage causes mutations of KEAP1 and NFE2L2 in 35% of cases. Squamous cell differentiation pathway including overexpression of SOX2 and TP63; and P13K/AKT pathway occur in 45% of cases. CDKN2A gene mutation is demonstrated in 70% of cases.
- **Messenger RNA (mRNA) profiling:** Based on the messenger RNA profiling, squamous cell lung carcinomas have been divided into four subtypes: classical, basal, secretory and primitive subtypes. Classical subtype of squamous cell lung carcinoma demonstrates hypermethylation, chromosomal instability and genetic alterations of PTEN, KEAP1 and NFE2L2. The basal subtype of squamous cell lung carcinoma shows NF1 gene alterations. The primitive subtype of squamous cell lung carcinoma demonstrates RB1 and PTEN gene alterations.
- **MicroRNA profiling:** Based on the microRNA (miRNA) profiling, squamous cell lung carcinomas have been categorized into four subtypes. DNA methylation profiling also divided squamous cell lung carcinoma into four subtypes (methylation cluster 1–4). Methylation cluster 4 demonstrates little DNA hypermethylation and includes most of the primitive mRNA subtype. Cluster 3 is predominantly composed of the classical mRNA subtype and enriched for NFE2L2 mutations. Clutter 3 and cluster 2 demonstrate the highest levels of DNA. Cluster 1 demonstrates intermediate DNA hypermethylation levels.

Lung Adenocarcinoma

Significant molecular genetic alterations in lung adenocarcinoma reported in the recently published literature include TP53, K-RAS, KEAP1, STK11, EGFR, NF1, BRAF, SETD2, RBM10. MGA, MET, ARID1A, PIK3, SMARCA4, RB1, CDKN2A, U2AF1 and RIT1, in decreasing frequency. Characteristics of ALK gene fusions and EGFR mutations in lung adenocarcinoma are given in Tables 18.50 and 18.51. RAS family of genes associated lung adenocarcinoma is given in Table 18.52.

- **RTK/RAS/RAF aberrant signaling pathway:** About 75% of the lung adenocarcinomas harboring genetic alterations promote RTK/RAS/RAF signaling pathway include K-RAS, EGFR, BRAF. Other genetic alterations that promote RTK/RAS/RAF signaling

Table 18.50 Characteristics of ALK gene fusions in lung adenocarcinoma

Although ALK fusions are common in anaplastic large cell lymphoma and inflammatory myoblastic tumor, yet the specific EML4-ALK fusion is almost demonstrated in lung adenocarcinomas
ALK rearrangement is strongly associated with lung adenocarcinoma histology, in particular with acinar and/or solid growth pattern, or cellular features of signet cell carcinoma
EML4-ALK fusion accounts for >90% ALK rearrangements in lung adenocarcinomas. Other less commonly ALK fusion partners include KIF5B-ALK, LKC1-ALK and TFG-ALK
ALK rearrangement is frequent in lung adenocarcinoma in non-smokers, but less associated with female sex
ALK positive lung adenocarcinoma constitutes 4–5% on NSCLCs and ethnic differences have not been reported, unlike EGFR mutations
ALK rearrangement in lung adenocarcinoma is predictive of response to ALK inhibitor, but it is not a prognostic factor

Table 18.51 Characteristics of EGFR mutations in lung adenocarcinoma

EGFR mutations occur in the receptor tyrosine kinase domain and lead to constitutive activation of downstream signaling without ligand
Two most common EGFR mutations: Point mutation at codon 858 (L858) and the in-frame deletions in exon-19 accounts for >90% of cases, although other mutations at codon G719 and in-frame insertions in exon 20 in lung adenocarcinoma
EGFR mutations are highly specific for lung adenocarcinoma especially in cases with lepidic and papillary growth and associated with TTF-1 positivity
Families with germline mutations of EGFR T790M mutations have high risk for development of lung adenocarcinoma
EGFR mutations in lung adenocarcinoma demonstrate ethinin differences with prevalence ranges of 10–15% in Caucasians and 30–40% in Asians
Patients with EGFR mutations are treated by EGFR tyrosine kinase inhibitor treatment
Mutations in EGFR exon 20, in-frame insertions and rarely EGFR T790M mutations in lung adenocarcinoma are associated with primary resistance to EGFR tyrosine kinase inhibitors. Acquisition of an additional TT/790M mutation is the most common cause of secondary resistance to EGHR tyrosine kinase inhibitor

Table 18.52 RAS family of genes associated with lung adenocarcinoma

RAS gene family is a part of the RAS superfamily including RAS (K-RAS, H-RAS, N-RAS), RAL, RAC, RHO, RAP and RAB gene families. RAS gene mutation is linked to lung adenocarcinoma
K-RAS (Kirsten rat sarcoma 2 viral) oncogene resides on 12p12.1. The oncogene encodes a small GTPase transducer protein called K-RAS protein, that acts as an on/off switch in cell signaling. When K-RAS gene mutated, aberrant p21 proteins produced by mutated gene have the potential to transform normal cells to cancer stem cells and increased resistance to chemotherapy and biological therapies targeting EGFR in lung adenocarcinoma
RAS gene mutations have been demonstrated in 20–25% of human cancers. Frequency of K-RAS mutations in various cancers include pancreas (80–90%), colon and rectum (25–60%), lung (25–60%), prostate (0–25%), skin (0–25%), thyroid (0–60%), liver (50%), ovary (0–50%), endometrium (10–40%), kidney (0–15%), brain (0–15%), seminoma testis (10–45%), acute nonlymphocytic leukemia and myelodysplasia (5–15%), urinary bladder (5%), head and neck (10%); and breast (10%)

pathway include MET exon 14 splice skipping, ERBB2 (HER2/neu), ROS fusion, MAK2K1 mutation, RET fusion, N-RAS and H-RAS mutations. Analysis of the DNA copy number without driver genetic alterations promotes RTK/RAS/RAF signaling pathway include amplification of ERBB2 and MET. Other genetic alterations that activate RTK/RAS/RAF signaling pathway include NF1 and RIT1 mutations.

- **EGFR mutation:** EGFR mutation is one of the common driver mutations in lung adenocarcinoma. Pathologically, EGFR-mutated lung adenocarcinoma typically demonstrates nuclear TTF-1 (NKX21) immunostaining. Lung adenocarcinoma with a micropapillary pattern demonstrates high frequency of EGFR mutations than adenocarcinoma without micropapillary pattern. Fusion genes have been recently identified as oncogene driver genes. Lung adenocarcinoma demonstrates rearranged genes such as ALK, ROS1, RET, NTRK1 and NRG1. Lung adenocarcinomas with these rearranged genes show a good clinical response to molecular-targeted therapeutic drugs.
- **ALK (anaplastic lymphoma kinase) rearrangement:** It is characterized by a TTF-1 cell lineage, an acinar structure with mucin/signet-ring pattern without or light tobacco smoking affecting young persons. Adenocarcinomas with rearranged ROS1 and RET genes have similar morphologic features demonstrated in ALK-rearranged adenocarcinoma such as mucinous cribriform pattern or solid signet-ring cell pattern.
- **NTRK1 rearrangement:** It responds to molecular TRKA kinase inhibitors. Five oncogenic fusions demonstrated in lung adenocarcinoma include CD74-NRG1, SLC3A2-NRG1, EZR-ERBB4, TRIM24-BRAF, and KIAA1468-RET. These are potential molecular targets in clinical practice.
- **K-RAS mutation:** It is usually demonstrated in lung adenocarcinomas in tobacco smokers. On the other hand, EGFR, ALK, ROS1 and RET are frequently demonstrated in non-tobacco smokers. Asians have high frequency of EGFR mutations in 50% cases of lung adenocarcinoma and low frequency of K-RAS mutations.

LUNG CARCINOMAS: LOCATION

- **Central region:** Squamous cell lung carcinoma and small cell lung carcinoma are centrally located. Squamous cell lung carcinoma shows endobronchial growth. Small cell lung carcinoma shows peribronchial growth.
- **Peripheral region:** Lung adenocarcinoma, bronchioloalveolar carcinoma and large cell lung carcinoma are located in peripheral region.
- **Central or peripheral region:** Some patients with lung adenocarcinoma may develop tumor in the central portion of lung that grows as endobronchial growth invading bronchial cartilage. Local and systemic manifestations of lung carcinoma are given in Table 18.53.

Table 18.53 Underlying cause and clinicopathological features of lung carcinoma

Clinicopathological Features	Underlying Cause and Mechanism
Pulmonary involvement by lung carcinoma	
Cough (75%)	Infection distal to airway blocked by lung carcinoma
Hemoptysis (<50%)	Ulceration of squamous cell lung carcinoma
Chest pain (40%)	Involvement of pleura or chest wall by lung carcinoma
Wheeze (15%)	Narrowing of airways by lung carcinoma
Systemic features (anorexia, loss of weight, malaise, fever)	General systemic manifestations in lung carcinoma

Contd...

Table 18.53 Underlying cause and clinicopathological features of lung carcinoma (*Contd...*)

Clinicopathological Features	Underlying Cause and Mechanism
Obstructive pneumonia (with or without cavitation)	Obstruction of respiratory tract by lung carcinoma
Pleural effusion	Involvement of pleura by lung carcinoma
Paraneoplastic syndrome	Synthesis of hormones by primary lung carcinoma
Extension of lung cancer to mediastinal structures	
Hoarseness of voice	Direct invasion of recurrent laryngeal nerve hooking below arch of aorta especially by squamous cell lung carcinoma
Paralysis of diaphragm and dyspnea	Invasion of phrenic nerve by lung carcinoma
Horner's syndrome	Direct invasion of cervical sympathetic ganglion by lung carcinoma and characterized by ptosis, sunken eyes, miosis and loss of sweating on the affected side
Brachial plexopathy	Direct invasion of brachial plexus by lung carcinoma arising in superior sulcus
Superior vena cava syndrome	Compression/obstruction of superior vena cava by lung carcinoma
Pain in T1 dermatome and wasting of intrinsic hand muscles	Direct invasion of brachial plexus by apical pancoast lung carcinoma
Pericarditis/pericardial hemorrhagic effusion	Direct invasion in pericardium by lung carcinoma may result in cardiac tamponade
Pleural effusion	Direct invasion in pleura by lung carcinoma
Rib destruction	Direct invasion and destruction of rib by lung carcinoma
Dysphagia	Compression of esophagus by lung carcinoma
Pneumonia, lung abscess, lung collapse	Obstruction of airways by lung carcinoma
Lipid pneumonia	Accumulation of cellular lipid in foamy macrophages due to airway obstruction by lung carcinoma
Metastatic spread of lung carcinoma	
Pathologic fracture	Lung carcinoma metastasis to bone
Hepatomegaly and/or jaundice	Lung carcinoma metastasis to liver
Central nervous system symptoms	Lung carcinoma metastasis to brain
Non-metastatic extrapulmonary syndromes	
Endocrinal manifestations	
Hyponatremia, high osmolality in serum and urine, confusion, weakness and seizures	Inappropriate antidiuretic hormone synthesis by small cell lung carcinoma
Hypercalcemia	Parathormone-like peptide synthesis by squamous cell lung carcinoma
Atypical Cushing syndrome	Adrenocorticotropic hormone (ACTH) synthesis by small cell lung carcinoma
Gynecomastia and testicular atrophy	Gonadotropin-like peptide synthesis by squamous cell lung carcinoma, small cell lung carcinoma, lung adenocarcinoma and large cell lung carcinoma
Skin pigmentation	Melanocyte-stimulating hormone synthesis by lung carcinoma
Carcinoid syndrome	Serotonin synthesis by lung carcinoma
Serum growth hormone level high	Growth hormone synthesis by lung carcinoma
Serum prolactin level high	Prolactin hormone synthesis by metastatic non-small cell lung carcinoma
Diarrhea	Vasoactive intestinal polypeptide by lung carcinoma

Contd...

Table 18.53 Underlying cause and clinicopathological features of lung carcinoma (*Contd...*)

Clinicopathological Features	Underlying Cause and Mechanism
Neurological syndromes	
Eaton-Lambert syndrome (weakness of pelvic girdles, proximal limbs and trunk)	Production of IgG autoantibodies against calcium channels in motor nerve terminals resulting in non-release of acetylcholine in small cell lung carcinoma
Peripheral neuropathy (motor, sensory or mixed)	Production of IgG autoantibodies against anterior and lateral horns of spinal cord in small cell lung carcinoma
Gastrointestinal tract and urinary bladder disturbances; and postural hypotension	Invasion of autonomic nervous system by lung carcinoma
Cerebral degeneration (ataxia, vertigo and dysarthria, i.e. difficulty in speech)	Production of IgG autoantibodies synthesis against cerebellum in small cell lung carcinoma
Weakness of proximal region of body associated with pain tenderness, purple facial rash, arthralgia, dysphagia and Raynaud phenomenon	Polymyositis and dermatomyositis occur in lung carcinoma
Flaccid paraparesis (partial paralysis of lower limbs) associated with sensory loss and sphincters malfunction	Acute transverse myelopathy occurs in lung carcinoma
Hypertrophic osteoarthropathy	
Hypertrophic osteoarthropathy (clubbing of fingers, swelling of wrists and ankles with periosteal new bone formation)	Hypertrophic osteoarthropathy occurs in 2–3% of squamous cell lung carcinoma and lung adenocarcinoma
Skin manifestations	
Acanthosis nigricans, dermatomyositis and tylosis (marked hyperkeratosis of palms and soles)	Skin manifestations in lung carcinoma (unknown mechanism)
Hematological/coagulation and vascular manifestations	
Disseminated intravascular coagulation (DIC)	Thrombotic occlusion and ischemia in non-small cell lung carcinoma
Thrombophlebitis migrans (migratory thrombophlebitis with tender clot beneath skin)	Inflammation, pain, redness and swelling in lung carcinoma
Nonbacterial thrombotic endocarditis	Nonbacterial thrombotic endocarditis in lung carcinoma
Venous thromboembolism	Venous thromboembolism phenomenon in lung carcinoma
Anemia, dysproteinemia, granulocytosis, eosinophilia, leukoerythroblastosis, bone marrow plasmacytosis and thrombocytopenia	Hematological abnormalities in patient with lung carcinoma
Miscellaneous (very rare) manifestations	
Henoch-Schönlein purpura, glomerulonephritis (nephrotic syndrome), systemic lupus erythematosus, lactic acidosis, hyperuricemia, amyloidosis	Miscellaneous manifestations rarely occur in patient with lung carcinoma

IMAGING TECHNIQUES

- Primary lung carcinomas are poorly circumscribed, solitary undergoing cavitation and dystrophic calcification.
- Tumors metastasizing to lung are sharply circumscribed and multiple. These usually lack cavitation and dystrophic calcification. Presence of calcification in the tumor indicates metastases from osteosarcoma and chondrosarcoma.

THERAPEUTIC RESPONSE

Small cell lung carcinoma (SCLC) initially responds to chemotherapy. Non-small cell lung carcinoma (NSCLC) confined to lung is curable by surgery. Small cell lung carcinoma is most often metastatic than non-small cell lung carcinoma.

PARANEOPLASTIC SYNDROME

Paraneoplastic syndromes occur in small cell lung carcinoma, squamous cell lung carcinoma, lung adenocarcinoma and large cell lung carcinoma. Paraneoplastic syndromes in lung carcinoma are given in Table 18.54.

Neuromuscular Manifestations

Nonmetastatic neuromuscular abnormalities associated with lung carcinoma are given in Table 18.55.

Intrathoracic Manifestations

Intrathoracic manifestations of bronchial carcinoma are given in Table 18.56.

Table 18.54 Paraneoplastic syndromes in lung carcinoma

System	Clinical Manifestations
Neuroendocrine manifestations	
General symptoms	Weight loss, fever and anorexia
Skin manifestations	Acanthosis nigricans, dermatomyositis, tylosis (marked hyperkeratosis of palms and soles)
Vascular manifestations	Thrombophlebitis migrans, noninfective thrombotic endocarditis, disseminated intravascular coagulation
Skeletal system	Hypertrophic osteodystrophy, clubbing of fingers
Nervous system	Myasthenia-like syndrome, peripheral neuropathies, cerebellar degeneration, myositis, and encephalopathy
Endocrine manifestations	
ACTH-like synthesis in small cell lung carcinoma	Atypical Cushing's syndrome (facial edema, cachexia, low serum potassium and alkalosis)
ADH-like synthesis in small cell lung carcinoma	Inappropriate secretion of antidiuretic syndrome (high urine osmolality and low serum sodium, confusion, weakness and seizures (if extreme)
Parathormone-like effects in squamous cell lung carcinoma	Serum calcium high, weakness, polyuria, polydipsia, constipation, abdominal pain and coma (if extreme)
Gonadotropin-like effects due to estrogens in squamous cell lung carcinoma, adenocarcinoma, small cell lung carcinoma and large cell lung carcinoma	Gynecomastia and testicular atrophy

Endocrine and neuroendocrine products of lung cancers: These include ACTH, calcitonin, parathormone-related peptide, melanocyte-stimulating hormone, growth hormone, chorionic gonadotropin, insulin, gastrin, renin, glucagon, estradiol, erythropoietin, histaminase, DOPA-decarboxylase, carcinoembryonic antigen, α-fetoprotein, bombesin and neuron-specific enolase.

Table 18.55 Nonmetastatic neuromuscular abnormalities associated with lung carcinoma

Neuromuscular Abnormalities	Clinical Manifestations
Peripheral neuropathy (motor, sensory or mixed)	Muscle weakness, muscle wasting, loss of tendon reflexes, glove and stocking type of loss of sensation
Autonomic neuropathy	Gastrointestinal and urinary bladder disturbances, and or postural hypotension
Cerebellar ataxia	Nystagmus, dysarthria, impaired coordination
Eaton-Lambert syndrome	Differs from myasthenia gravis in muscle function with improvement due to repeated effort monitored by electromyography
Polymyositis–dermatomyositis	Weakness of proximal region with pain and tenderness, purple facial rash, arthralgia, dysphagia and Raynaud's phenomenon
Acute transverse myelopathy	Flaccid paraparesis with sensory loss, sphincter functional loss

Table 18.56 Intrathoracic manifestations of bronchial carcinoma

Location	Clinical Manifestations	
Apical region	▪ Pancoast syndrome ▪ Horner's syndrome	▪ Sensory and motor deficits of T1 and T2 segments
Peripheral region	Pleural effusion (often blood stained), pleural pain	
Central region	▪ Bronchial obstruction ▪ Lung collapse ▪ Pneumonia	▪ Bronchiectasis ▪ Lipid pneumonia (alveoli filled with lipid-laden macrophages)

PRIMARY LUNG CARCINOMAS

SMALL CELL LUNG CARCINOMA

Small cell lung carcinoma (previously referred to as oat cell carcinoma) is rapidly growing highly malignant epithelial tumor of lung that exhibits neuroendocrine features. It constitutes 15–25% of lung cancers.

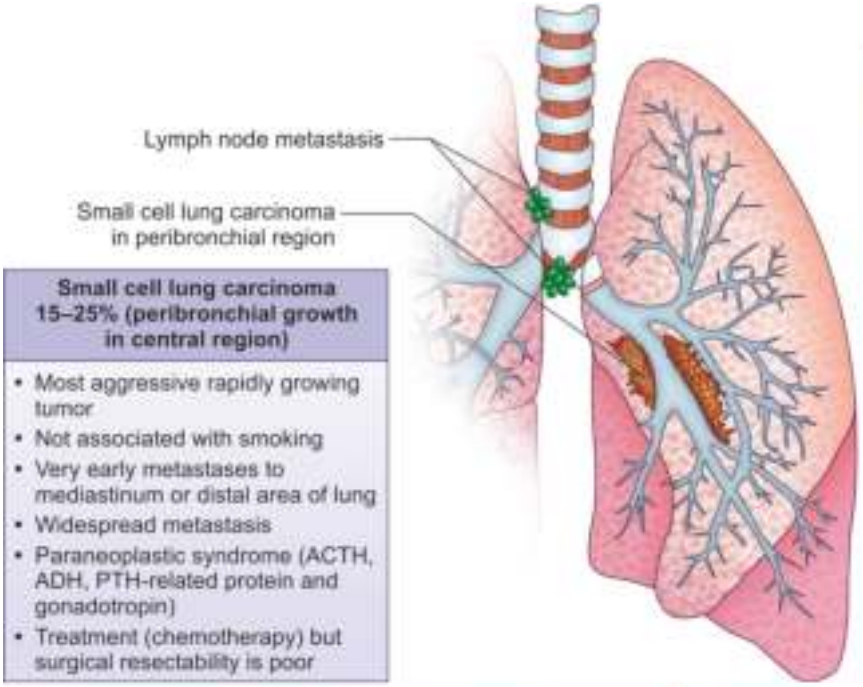
- Small cell lung carcinoma (SCLC) is strongly associated with cigarette smoking. It is more common in men than women. It is commonly associated with paraneoplastic syndrome.
- Patient develops SCLC in hilar or parahilar region. Extensive peribronchial growth causes bronchial narrowing. Lymph node metastasis is demonstrated in >90% of cases at diagnosis.
- Tumor causes extensive peribronchial invasion in the hilar or parahilar region.
- Bronchial narrowing, marked mediastinal lymphadenopathy and early widespread metastases are demonstrated in >90% of cases at diagnosis. It is a common cause of superior vena cava syndrome. Prognosis of SCLC is very poor.
- The tumor is responsive to chemotherapy. The median survival is measured in months. Small cell lung carcinoma shows risk factors, gene mutations, clinical manifestations, immunohistochemistry and metastases (Fig. 18.49).
- Desmoplastic small round cell tumor is a rare variant of small cell lung carcinoma. Patient presents with a solitary mass arising from pulmonary parenchyma. It is indolent neoplasm associated with good prognosis. The tumor cells are positive for cytokeratin (AE1/AE3) and desmin.

SCLC: Origin

Small cell lung carcinoma arises from neuroendocrine argentaffin cells (Kulchitsky cells) near central portion of chest. It accounts for 20% of all lung cancers.

Molecular Genetic Alterations

Small cell lung carcinoma exhibits mutations of both c-Myc oncogene and tumor suppressor gene, i.e. RB gene (80–100%), p53 gene (50–80%) and 3p deletion. There is increased expression of anti-apoptotic BCL-2 gene in 90% of cases. There is low expression of pro-apoptotic BAX gene in small cell lung carcinoma.



Small cell lung carcinoma 15–25% (peribronchial growth in central region)

- Most aggressive rapidly growing tumor
- Not associated with smoking
- Very early metastases to mediastinum or distal area of lung
- Widespread metastasis
- Paraneoplastic syndrome (ACTH, ADH, PTH-related protein and gonadotropin)
- Treatment (chemotherapy) but surgical resectability is poor

Paraneoplastic syndrome in small cell lung carcinoma

- **Lambert-Eaton myasthenic syndrome:** IgG autoantibodies against calcium channel in motor nerve terminals, weakness of pelvic girdles, proximal limbs and trunk (acetylcholine not released)
- **Peripheral neuropathy:** IgG autoantibodies against anterior and lateral horns of CNS
- **Cerebellar degeneration:** IgG autoantibodies against cerebellum leading to ataxia, vertigo and dysarthria
- **Corticotropic-like effects:** Facial edema, decreased serum potassium and alkalosis
- **ADH-like effects:** Increased urine osmolality, low serum potassium and weakness
- **Parathormone-like effects:** Increased serum calcium
- **Gonadotropic-like effects:** Gynecomastia and testicular atrophy

Gene mutations in small cell lung carcinoma

Oncogene	c-Myc oncogene
Tumor suppressor gene	RB gene (80–100%)
	p53 gene (50–80%)
Anti-apoptotic gene	BCL-2 gene (90%)

Immunohistochemistry of small cell lung carcinoma

Markers	Expression
Chromogranin	Positive
Synaptophysin	Positive
Leu7 (CD57)	Positive
Nonspecific esterase (specific marker)	Positive
CD56	Positive

Fig. 18.49: Small cell lung carcinoma shows risk factors, gene mutations, clinical manifestations, immunohistochemistry and metastases.

Small Cell Lung Carcinoma: Paraneoplastic Syndrome

Paraneoplastic syndrome most often occurs in small cell lung carcinoma (SCLC). The tumor synthesizes polypeptide hormones such as ACTH, ADH, parathormone-like peptide, gonadotropins, insulin-like growth factor and calcitonin.

- **Eaton-Lambert syndrome:** It is characterized by muscular weakness of pelvic girdles, skeletal muscle wasting, and fatigability of the proximal limbs and trunk. It is usually associated with small cell lung carcinoma in 65% of cases. Under physiologic state, calcium channels are necessary for release of acetylcholine. The pathogenic IgG autoantibodies are formed against voltage-sensitive calcium channels in motor nerve terminals. The calcium channels are greatly reduced in the presynaptic membrane in these patients, thereby interfering with neuromuscular transmission.
- **Peripheral neuropathy:** IgG autoantibodies against anterior and lateral horns of central nervous system cause motor and sensory loss resulting in peripheral neuropathy.
- **Cerebellar degeneration:** Autoantibodies cause subacute cerebellar degeneration, which presents with severe ataxia, vertigo and dysarthria.
- **Corticotropic effects:** ACTH synthesized by SCLC stimulates adrenal gland to produce corticosteroid hormones. Patient presents with atypical Cushing's syndrome, facial edema and cachexia. Serum calcium is decreased.
- **ADH effects:** Small cell lung carcinoma synthesizes ADH, which acts on renal collecting ducts and produces high urine osmolarity and low serum osmolarity resulting in decreased serum sodium level. Patient becomes irritable and develops convulsions and seizures.
- **PTH-related protein:** SCLC ectopically secretes PTH-related protein resulting in hypercalcemia. Patient presents with constipation, abdominal pain, polyuria and polydipsia.
- **Gonadotropic substances:** Small cell lung carcinoma ectopically synthesizes estrogens which cause development of gynecomastia and testicular atrophy.

Surgical Pathology: Small Cell Lung Carcinoma

Gross Morphology

Tumor is lobulated and soft in consistency. Cut surface of this tumor has a white to tan appearance (Fig. 18.50).

Light Microscopy

- **Tumor cell morphology:** Tumor is composed of small anaplastic oval to spindle cells with scanty cytoplasm and ill-defined borders, nuclear moulding and inconspicuous nuclei. Nuclear chromatin is finely granular exhibiting salt and pepper pattern. It shows 6–7 mitoses per high power fields.
- **Tumor cells arrangement:** Tumor cells are arranged in sheets, cords, or small aggregates that exhibit neither squamous nor glandular differentiation.
- **Tumor necrosis:** Extensive necrosis is often seen in small cell lung carcinoma.
- **Azzopardi effect of tumor:** It refers to the presence of cellular DNA in necrotic venules in small cell lung carcinoma. Necosis of venules results in release of cellular DNA, which adheres in patches in the walls of venules showing an intensely basophilic material on hematoxylin and eosin stain (Fig. 18.51A to C). Comparison of small cell lung carcinoma and large cell lung carcinoma is given in Table 18.57.

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in small cell lung carcinoma on histologic sections. These include chromogranin, synaptophysin, CD56, CD57 (HNK-1/Leu-7), neuron-specific enolase, cytokeratins (AE1/AE3) and TTF-1.

Markers	Expression
Chromogranin	Positive
Synaptophysin	Positive
CD56	Positive
CD57 (HNK-1/Leu-7)	Positive
Neuron-specific enolase (NSE)	Positive
Cytokeratins (AE1/AE3)	Positive
TTF-1	Positive

Electron Microscopy

- Dense-core neurosecretory granules (100 nm) are present in tumor cells in 66% of SCLC cases. The granules are similar to those found in the neuroendocrine Kulchitsky cells scattered along the bronchial epithelium particularly in fetus and neonates. These neurosecretory granules synthesize ectopic polypeptide hormones.
- Low-grade neuroendocrine tumor, e.g. typical carcinoid tumor has prominent neurosecretory dense-core granules on electron microscopy.
- High-grade neuroendocrine tumor, e.g. small cell lung carcinoma has fewer dense-core granules on electron microscopy.

Mode of Spread

- **Direct spread:** SCLC appears as a perihilar mass that spreads along the bronchi in the circumferential manner. Hence, it does not obstruct the bronchus lumen. On reaching a large size, it may cause bronchial obstruction and lung collapse.
- **Lymphatic route:** SCLC spreads to hilar tracheobronchial lymph nodes with extension to mediastinal lymph nodes in >50% of cases. It may also involve cervical lymph nodes.
- **Hematogenous spread:** Approximately 66% of SCLC cases have metastasized to extranodal location at time of diagnosis. It metastasizes to adrenal gland, liver, brain, bone, spinal cord, kidneys, and pancreas. Adrenal gland involvement is usually asymptomatic (Table 18.58).



Fig. 18.50: Small cell lung carcinoma. This most aggressive tumor arises centrally, spreads extensively along the bronchi. It often metastasizes widely before the primary tumor mass in the lung reaches a large size leading to bronchial obstruction and lung collapse. The cut surface of this tumor has a soft, lobulated, and white to tan appearance. Black rounded areas represent hilar lymph nodes with metastatic carcinoma. It is usually treated by chemotherapy rather than radiotherapy or surgery. Prognosis is very poor.

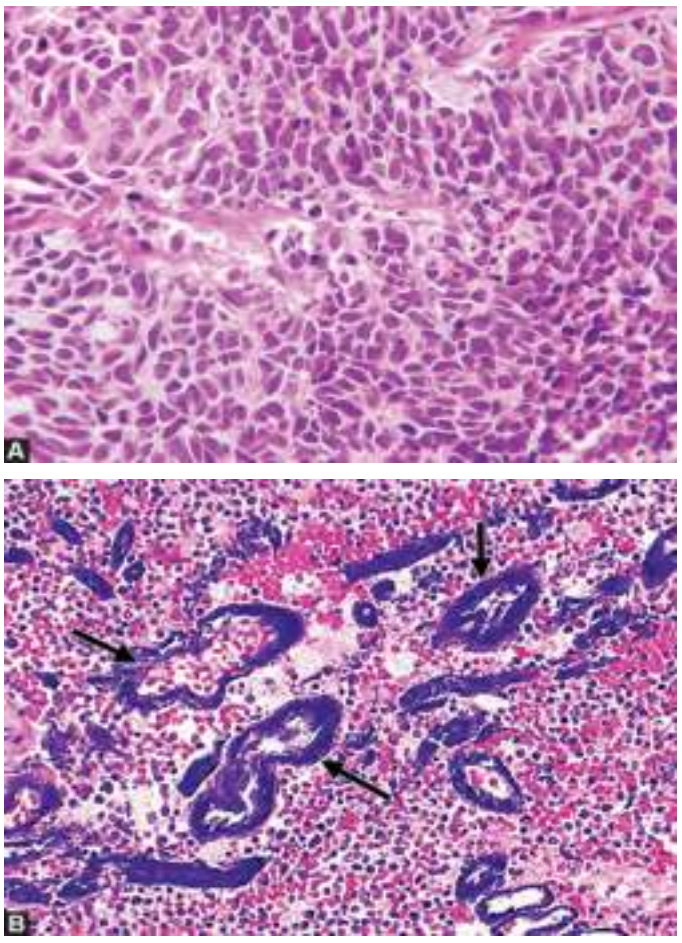


Fig. 18.51: Small cell lung carcinoma. (A) Tumor is composed of small dark blue cells with minimal cytoplasm, (B) Azzopardi effect encrusting of nuclear material on blood vessel wall is known as Azzopardi effect (arrows) (400X).

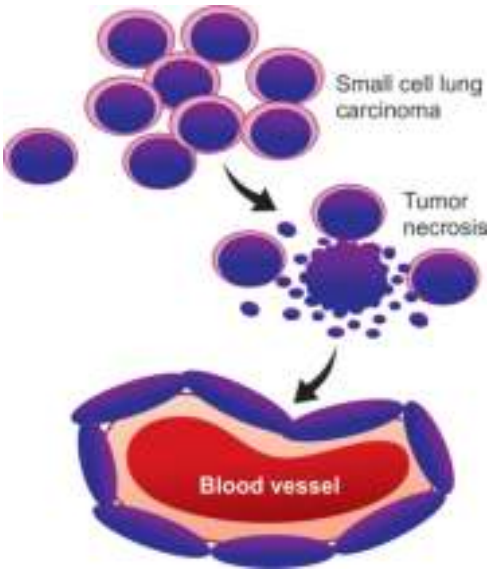


Fig. 18.51C: Azzopardi effect in small cell lung carcinoma. Encrusting of nuclear material on blood vessel wall is known as Azzopardi effect.

Therapeutic Response and Prognosis

Small cell lung carcinoma grows rapidly and metastasizes. It is very sensitive to chemotherapy and radiotherapy. Radiotherapy alone is rarely curative. Surgical excision of the tumor is not effective treatment.

- Combination of chemotherapy involves intravenous administration of cyclophosphamide, doxorubicin and vincristine in cycles lasting for three weeks each of these chemotherapeutic drugs.
- Disease is usually extensive at diagnosis, hence has poor prognosis associated with fatal outcome without treatment within 12 weeks.
- Treatment relieves symptoms, e.g. pain and superior vena cava syndrome due to invasion of chest wall by the tumor.

SQUAMOUS CELL LUNG CARCINOMA

Squamous cell lung carcinoma is the most common variant of lung cancer also known as epidermoid carcinoma affecting 55–75 years of age group. It accounts for 25–40% of cases. It is strongly associated with tobacco smoking. Squamous dysplasia is precursor lesion for squamous cell lung carcinoma.

- **Origin:** Tumor arises from basal cell of major bronchi in majority of cases in hilar region of chest; and bronchioles in a few patients.
- **Growth invasion:** Endobronchial growth causes bronchial obstruction and then invades bronchial wall and adjacent lung parenchyma or blood vessels. Atelectasis, bronchiectasis and consolidation are common in these cases. Tumor appears as solitary hilar mass. Central necrosis and cavitation in lungs are more common in SCLC than other cell types.

Table 18.57 Comparison of small cell lung carcinoma and large cell lung carcinoma

Histologic Features	Small Cell Lung Carcinoma	Large Cell Lung Carcinoma
Cell size	Small cell	Large cell
Cell shape	Fusiform	Polygonal
Nuclear/cytoplasmic ratio	High	Low
Nuclear chromatin	Fine chromatin	Coarse chromatin
Nucleoli	Indistinct	Prominent
DNA staining of blood vessels (Azzopardi phenomenon)	Frequent Azzopardi phenomenon	Rare

Table 18.58 Sites of metastases from lung cancers

Location	Frequency
Hilar tracheobronchial lymph nodes	80%
Extrathoracic lymph nodes (mediastinum and cervical region)	66%
Liver	50%
Adrenal glands (asymptomatic)	50%
Bone	30%
Kidney	25%
Brain	20%
Spinal cord	Uncommon
Pancreas	Uncommon

- **Signs and symptoms:** Endobronchial growth of tumor causes early symptoms. Tumor is detected on sputum cytological examination before being radiographically visible. It metastasizes late, hence has relatively good prognosis. Risk factors, gene mutations, clinical manifestations, immunohistochemistry and metastases of squamous cell lung carcinoma are shown in Fig. 18.52.

Risk Factors

- **Cigarette smoking:** Cigarette smoking is important cause of lung cancers, i.e. small cell lung carcinoma and squamous cell lung carcinoma.
- **Asbestosis:** Lung adenocarcinoma (most common) and squamous cell lung carcinoma are asbestos-related lung cancers in 34% of cigarette smokers. Asbestos fibers act as promoter in pulmonary carcinogenesis.

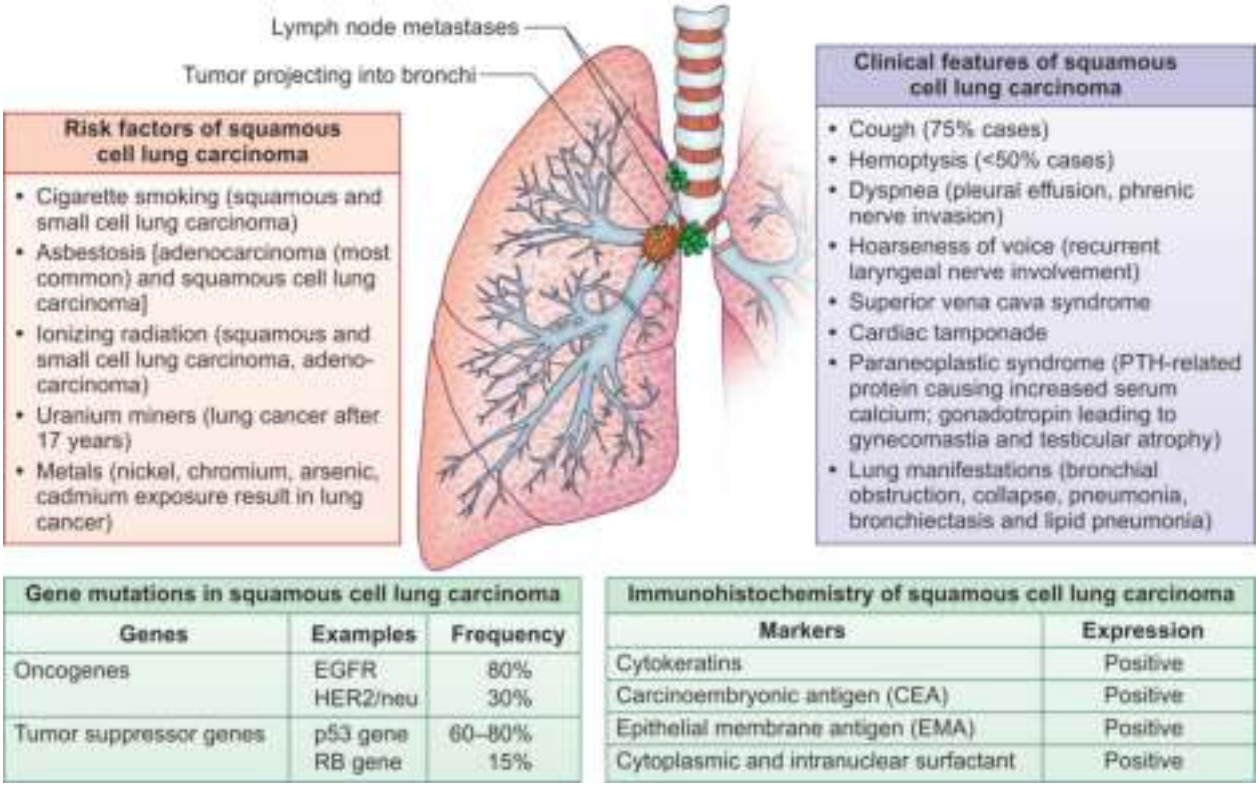


Fig. 18.52: Squamous cell lung carcinoma shows risk factors, gene mutations, clinical manifestations, immunohistochemistry and metastases.

- **Ionizing radiation:** Japanese are at higher risk for lung cancers, i.e. squamous cell lung carcinoma, lung adenocarcinoma and small cell lung carcinoma after atom bomb explosion in Hiroshima and Nagasaki.
- **Uranium miners:** Uranium exposure emits particulate products and radon gas. Inhalation of uranium particulate products may cause lung carcinoma after 17 years of exposure.
- **Metals exposure:** Metal refining and smelting processes of nickel, chromium, arsenic and cadmium exposure can result in lung carcinoma.

Molecular Genetic Alterations

Proto-oncogenes participate in normal cellular growth and development. Tumor suppressor genes inhibit this process.

- When proto-oncogenes undergo mutations, these are referred as oncogenes.
- Tobacco smoking harbors genetic abnormalities, i.e. chromosomal deletions involving tumor suppressor loci of 3p, 9p and 17p, which cause SCLC.
- Chromosome 3p and 9p deletions involving site of CDKN2A gene is demonstrated in 65% cases of squmous cell lung carcinoma.
- Chromosome 17p deletion involves TP53 gene. TP53 gene mutation is demonstrated in squamous dysplasia in 10–50% of cases and squamous cell lung carcinoma in 60–80% of cases.
- FGFR1 gene encoding fibroblast growth factor receptor tyrosine kinase is overexpressed in squamous cell lung carcinoma.
- HER2/neu is overexpressed in 30% of squamous cell lung carcinoma cases. Gene mutations in squamous cell lung carcinoma (*see* Fig. 18.52).

Surgical Pathology: Squamous Cell Lung Carcinoma

Gross Morphology

- Tumor is solid with cut surface pale white to tan, firm in consistency. Large tumor is necrotic, granular giving cheesy appearance as seen in tuberculosis.
- Hilar tumor is solid and located in central region of lungs.
- Peripheral tumor is small, far away from pleura and showing cavitation.

Light Microscopy

- Well-differentiated squamous cell lung carcinoma variant is composed of polygonal cells forming nests with hyperchromatic angular nuclei, pink cytoplasm with distinct cell borders.
- Keratinizing squamous cell lung carcinoma variant displays intercellular bridging and keratin pearls, which appear as a small round nest of brightly eosinophilic aggregates of keratin surrounded by concentric (onion skin) layers of squamous cells (Figs 18.53 and 18.54).
- Basaloid variant of squamous cell lung carcinoma has been associated with poor prognosis.



Fig. 18.53: Squamous cell lung carcinoma. It is arising centrally and obstructing the left main bronchus. The neoplasm is very firm and has a pale white to tan cut surface (arrows).

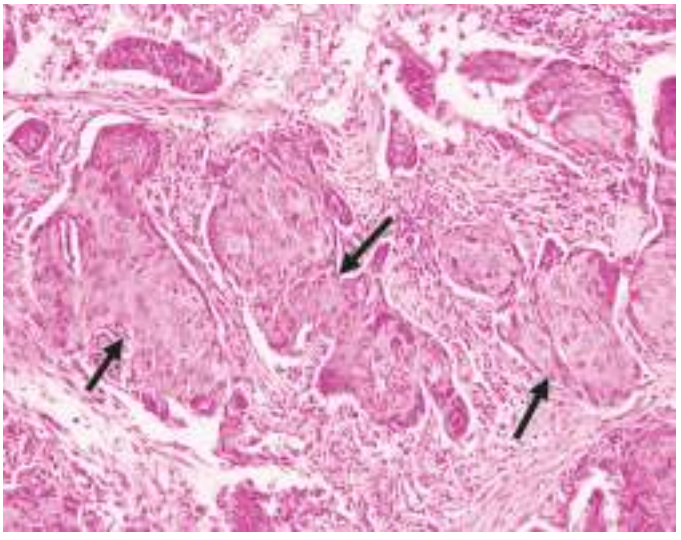


Fig. 18.54: Squamous cell lung carcinoma shows well differentiated polygonal tumor cells with pink cytoplasm with distinct cell borders and intercellular bridges. The nuclei are hyperchromatic and angular with many mitoses (arrows) (400X).

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in squamous cell lung carcinoma on histopathologic sections (*see* Fig. 18.52).

Markers	Expression
p53	Positive
p40	Positive
Cytokeratin (5, 6)	Positive
Epithelial membrane antigen (EMA)	Positive
Carcinoembryonic antigen (CEA)	Positive
Cytoplasmic and intranuclear surfactant	Positive

Table 18.59 Causes of hemoptysis

Etiology	Disorders
Inflammatory lesions	▪ Tuberculosis* ▪ Bronchiectasis* ▪ Pneumonias*
Neoplastic lesions	▪ Primary lung cancers* ▪ Metastatic lung cancers* ▪ Bronchopulmonary adenoma
Other lesions	▪ Pulmonary embolism and infarction* ▪ Mitral stenosis ▪ Left ventricular failure ▪ Anticoagulant therapy ▪ Bronchial adenoma ▪ Idiopathic pulmonary hemosiderosis

**Most common causes of hemoptysis are tuberculosis, bronchiectasis, pneumonias, bronchogenic carcinoma, pulmonary embolism and infarction.*

Clinical Features

Patient with squamous cell lung carcinoma presents with history of cough (75%), hemoptysis (<50%), severe chest pain (40%), dyspnea (20%) wheeze, stridor, hoarseness of voice, weight loss, paraneoplastic syndrome (ectopically secretes PTH-related protein resulting in hypercalcemia).

- **Cough:** It is common presentation in 75% cases of squamous cell lung carcinoma involving central airways.
- **Hemoptysis:** It occurs in <50% patients of squamous cell lung carcinoma at initial presentation (Table 18.59). It occurs due to hemorrhage from a tumor located in central airways.
- **Severe chest pain:** It suggests invasion of chest wall with intercostal nerves by tumor. Brachial plexus is involved in pancoast tumor located in apical portion.
- **Dyspnea:** Patient develops dyspnea due to loss of functioning lung parenchyma, invasion of lymphatics and pleura (massive pleural effusion). Hemorrhagic pleural effusion indicates invasion of pleura by tumor.
- **Digital clubbing:** It occurs due to reactive periosteal changes in the underlying bones in non-small cell lung cancers (e.g. squamous cell carcinoma, lung adenocarcinoma, and large cell lung carcinoma). Mechanism of digital clubbing is unknown. It may resolve with excision of the primary lesion.
- **Invasion of mediastinum:** Patient may develop hoarseness of voice due to involvement of recurrent laryngeal nerve supplying vocal cords, dysphagia due to invasion of esophagus or extrinsic pressure on esophagus and superior vena cava syndrome. Obstruction of the superior vena cava by tumor leads to venous congestion in head and neck.
- **Difficulty in respiration:** Phrenic nerve invasion causes paralysis of diaphragm.
- **Cardiac tamponade:** Tumor may cause erosion of major blood vessels and produce massive bleeding in pericardial cavity.
- **Lung lesions:** Patient with squamous cell lung carcinoma obstructing the bronchial lumen becomes symptomatic earlier. Complete bronchial obstruction by tumor may lead to pneumonia, bronchiectasis, lung collapse and lung abscess. Partial bronchial obstruction results in emphysema. Lipid pneumonia occurs due to accumulation of cellular lipid in foamy macrophages especially obstruction caused by lung carcinoma.
- **Paraneoplastic syndrome:** Tumor ectopically secretes PTH-related protein resulting to hypercalcemia. Patient presents with constipation, abdominal pain, polyuria and polydipsia. Serum calcium levels are increased. Gonadotropin synthesized by tumor leads to development of gynecomastia and testicular atrophy in male patients.

Clinical Manifestations: Pancoast Lung Tumor

Apical primary lung cancer (usually squamous cell lung carcinoma) is known as **Pancoast tumor** can involve structures of the neck. It may invade cervical sympathetic chain (Horner's syndrome), brachial plexus and ribs.

- **Horner's syndrome:** Involvement of the cervical sympathetic chain causes Horner's syndrome is characterized by ptosis-drooping of upper eyelid, enophthalmos (sunken eyes), miosis (constriction of pupil), and anhidrosis (loss of sweating) on the affected side.
- **Brachial plexus injury:** Apical tumor in superior sulcus may destroy brachial plexus.
- **Chest wall invasion:** Apical tumor in superior sulcus may destroy neighboring ribs.

Mode of Spread

- **Direct spread:** Tumor appears as a perihilar mass that spreads along the bronchi in the circumferential manner. Hence, it does not obstruct the bronchus lumen. On reaching a large size, it may cause bronchial obstruction and lung collapse.
- **Lymphatic route:** It spreads to hilar tracheobronchial lymph nodes with extension to mediastinal lymph nodes in >50% of cases. It may also involve cervical lymph nodes.
- **Hematogenous spread:** Approximately 66% of cases have metastasis in extranodal location at time of diagnosis. Tumor metastasizes to adrenal gland,

liver, brain, bone, spinal cord, kidneys, and pancreas. Adrenal gland involvement is usually asymptomatic.

Therapeutic Response

Most often, squamous cell lung carcinoma is treated surgically. It responds to radiotherapy.

LUNG ADENOCARCINOMA

Lung adenocarcinoma is the most common tumor in elderly women. Its association with tobacco smoking is relatively weak. It accounts for 30–50% of all lung cancers. It is most common lung cancer in the United States. Early metastases are common. Tumor is present in peripheral region of lung most often in upper lobe in 75% of cases. Speculated appearance of the tumor is demonstrated on radiograph.

- **Origin:** Lung adenocarcinoma is thought to arise from bronchial or alveolar epithelium and characterized by glandular differentiation with presence of mucin in the tumor cells.
- **Cell population:** Cell population of tumor may comprise columnar cells, goblet cells, Clara cells or mucous glands. Tumor shows glandular differentiation with presence of mucin in the tumor cells.
- **Invasion:** Invasion of lung parenchyma by tumor is most often present.
- **Metastases:** Early metastasis lung adenocarcinoma is more common than squamous cell lung carcinoma especially to central nervous system and adrenal glands.

Revised 2024 WHO Classification of Lung Adenocarcinoma

Based on revised 2024 WHO classification of lung tumors, the previously designated bronchoalveolar carcinoma has now been replaced with adenocarcinoma *in situ*.

Preinvasive Lesions of Lung

- Atypical adenomatous hyperplasia
- Adenocarcinoma *in situ* replacing bronchoalveolar carcinoma

Invasive Lung Adenocarcinomas

- Minimally invasive adenocarcinoma
- Lepidic predominant adenocarcinoma
- Invasive mucinous adenocarcinoma
- Invasive nonmucinous adenocarcinoma
- Solid predominant adenocarcinoma
- Acinar adenocarcinoma
- Papillary adenocarcinoma
- Micropapillary adenocarcinoma
- Colloid adenocarcinoma
- Fetal adenocarcinoma
- Enteric adenocarcinoma

Table 18.60 Molecular genetic abnormalities in lung adenocarcinoma

Molecular Genetic Abnormalities	Frequency
K-RAS	32%
EFGR	22%
BRAF	6%
ALK	3.9%
MET	2%
ERBB1	1.5%
ROS-1	1.5%
RET	1%
Other genes	30%

Location

Lung adenocarcinoma is a slow-growing irregular tumor measuring <4.0 cm in diameter in peripheral or central regions of lung. Large tumor may completely replace the entire lobe of the lung. It is often associated with pulmonary fibrosis. It may arise in relation to preexisting pulmonary fibrosis.

- **Peripheral subpleural mass:** Approximately 75% of patients present with peripheral subpleural solitary nodule in peripheral region. It is often associated with pleural fibrosis and subpleural scars, now recognized as desmoplastic response to tumor.
- **Central endobronchial growth:** In a few cases, lung adenocarcinoma originates within major bronchi in the central region of upper lobes. Tumor grows as endobronchial growth invading bronchial cartilage.

Risk Factors

Ionizing radiation may cause lung cancer. Japanese are at higher risk for lung cancers (squamous cell lung carcinoma, lung adenocarcinoma and small cell lung carcinoma) after atom bomb explosion in Hiroshima and Nagasaki.

Molecular Genetic Alterations

Molecular genetic abnormalities in lung adenocarcinoma are given in Table 18.60. Estimation of gene mutation prevalence is based on targeted next generation sequencing of lung adenocarcinoma.

Surgical Pathology: Lung Adenocarcinoma

Gross Morphology

Tumor measures 2–5 cm in diameter. Cut surface of tumor is gray white and glistening depending on amount of mucin production (Fig. 18.55).

Light Microscopy

Atypical adenomatous hyperplasia of lung

It refers to ≤5 mm lesion due to atypical proliferation of pneumocytes type II lining centriacinar alveoli with minimal widening of septa.

Lung adenocarcinoma *in situ*

- It replaces former designated bronchoalveolar carcinoma.
- It is characterized by tumor growth ≥5 mm to ≤3 cm.
- Tumor cells are growing along existing alveolar walls in a lepidic growth pattern.
- Alveolar septae show widening.
- There is no evidence of lymphovascular invasion, lung parenchyma and pleura.
- Patient has 5 years disease-free interval. Tumor cells express p53.

Minimal invasive lung adenocarcinoma

- It refers to formation of tumor growth measuring <3 cm with lepidic pattern with more than one foci of invasion in pulmonary parenchyma measuring <5 mm.
- There is no evidence of invasion of lymphovascular and pleura.
- Tumor necrosis is absent.
- On histologic examination, tumor is composed of angulated tumor cells arranged in nests and acini with desmoplasic stroma.
- Patient has 5 years disease-free survival.

Invasive mucinous lung adenocarcinoma

- It replaces former bronchoalveolar carcinoma.
- Tumor cells express mutation of K-RAS and EML-4 ALK translocation.
- Tumor is mistaken for metastatic disease.
- Most tumors are well differentiated composed of goblet cells along the framework of alveolar septa and projecting like papillary formation into the alveolar spaces.
- This pattern resembles butterfly sitting on fence, i.e. lepidic pattern.
- Tumor cells show mucin in cytoplasm and minimal atypia (Fig. 18.56).

Invasive nonmucinous lung adenocarcinoma

- This histologic variant constitutes 66% cases of adenocarcinoma.

Lepidic predominance lung adenocarcinoma

- It refers to ≥3 cm tumor growth invading pulmonary parenchyma, lymphovascular and pleura.
- Tumor shows areas of necrosis.
- About 90% of patients have 5-year survival with stage 1 disease (Fig. 18.57).
- Tumor is composed of clara cells and pneumocytes type II growing along the alveolar walls.

Solid predominance lung adenocarcinoma

- Tumor is composed of cells without glandular differentiation arranged in nests and cords.
- It should be differentiated from nonkeratinizing squamous cell lung carcinoma and large cell lung carcinoma.
- Histochemistry and immunohistochemistry help in establishing diagnosis.
- Tumor cells show presence of mucin.

Acinar lung adenocarcinoma

- It is an invasive tumor composed of cells forming glandular structures with visible lumina.
- Tumor cells are closely packed or back to back arrangement. These cells may invade desmoplastic stroma.

Papillary lung adenocarcinoma

It is an invasive tumor composed of papillary structure and fibrovascular cores. Its prognosis is similar to acinar adenocarcinoma.

Micropapillary lung adenocarcinoma

This histologic variant is composed of intra-alveolar detached papillae and tufts of tumor cells lacking fibrovascular core lymphovascular by tumor is often widespread at the time of diagnosis.

Colloid lung adenocarcinoma

- This histologic variant is composed of extracellular mucin lakes that expand and destroy alveolar spaces.
- The alveolar spaces are lined by mucinous epithelial goblet or columar cells.

Fetal lung adenocarcinoma

- This histologic variant is composed of columnar cells forming tubular and cribriform structures.
- It occurs in young age group.
- It is associated with mutation of β-catenin. There may be presence of morula metaplasia and endometrioid carcinoma.

Enteric lung adenocarcinoma

This histologic variant is characterized by the presence of enteric differentiation with acinar architecture and intracinar necrosis in at least 50% of the tumor.

Histochemistry

Mucin production presents in tumor cells may be demonstrated by mucicarmine, Alcian blue and periodic Schiff stain.

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in lung adenocarcinoma on histopathologic sections.

Markers	Expression
Cytokeratin 7 (CK 7)	Positive
Epithelial membrane antigen	Positive
Napsin A	Positive
TTF-1 (thyroid transcription factor 1)	Positive
Carcinoembryonic antigen (CEA)	Positive
B72.3 monoclonal antibody reacts with tumor-associated glycoprotein 72 (TAG-72)	Positive
Ber-EP4 monoclonal antibody reacts with glycoproteins of tumor cells	Positive
CD15 (Leu-M1) monoclonal antibody reacts with tumor cells	Positive

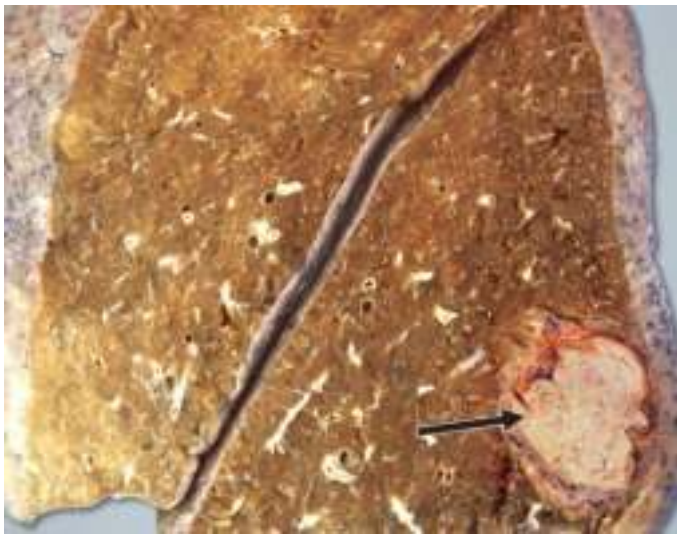


Fig. 18.55: Peripheral lung adenocarcinoma. It tends to occur more peripherally in lung. It occurs more often in nonsmokers and in smokers who have quit. Resection would have a greater chance for cure. The solitary appearance of this neoplasm suggests that the tumor is primary rather than metastatic (arrow).

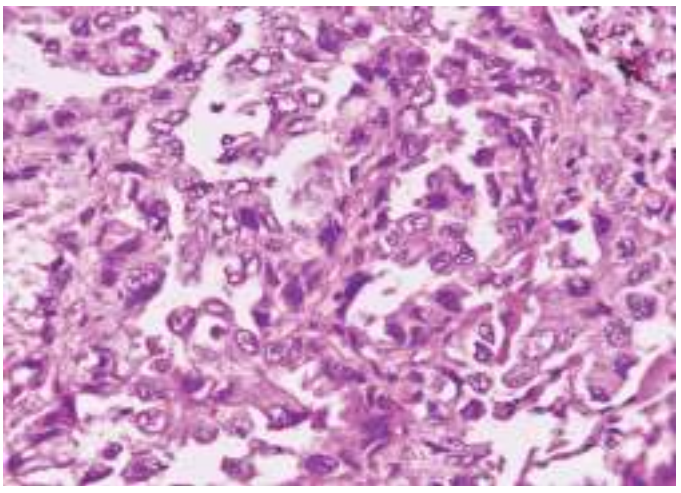


Fig. 18.56: Lung adenocarcinoma is showing well differentiated adenocarcinoma (400X).

Mode of Spread

Early metastasis of lung adenocarcinoma is more common than squamous cell lung carcinoma histologic variant. The metastatic deposits are especially demonstrated in nervous system and adrenal glands.

- **Lymphatic route:** Tumor spreads by lymphatic route to hilar tracheobronchial lymph nodes and extrathoracic lymph nodes of mediastinum and cervical region.
- **Hematogenous route:** Approximately 66% of cases metastases via hematogenous route are demonstrated in adrenal glands, liver, spinal cord, kidneys and pancreas at the time of diagnosis.

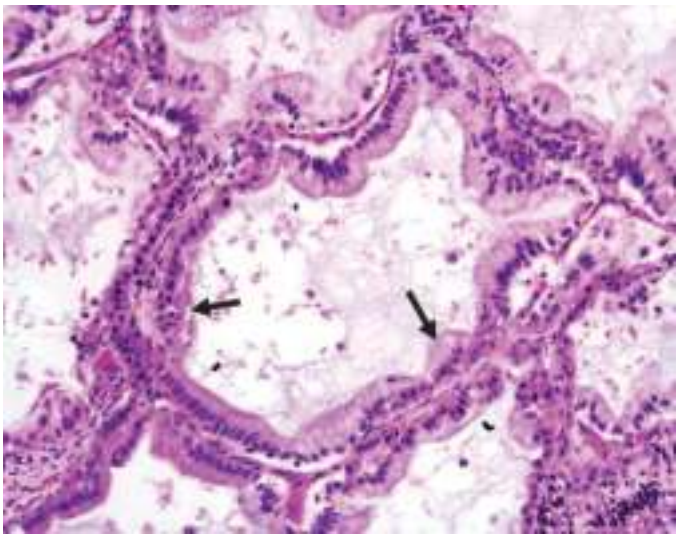


Fig. 18.57: Lepidic predominance adenocarcinoma. It is composed of columnar cells that proliferate along the framework of alveolar septa. The cells are well differentiated. It has a better prognosis than most other primary lung cancers (arrows) (400X).

Tumor Growth and Therapeutic Correlation

Patient with stage I adenocarcinoma confined to lung, who undergoes complete surgical excision has a 5-year survival in 50–80% of cases. Differences between squamous cell lung carcinoma and lung adenocarcinoma are given in Table 18.61.

There is no relationship to tobacco smoking. It affects patients of both sexes in all ages from the 20s to the advanced years of life. Noninvasive tumor characterized by lepidic growth has good prognosis. Diffuse or patchy ill-defined tumor showing consolidation has poor prognosis.

- **Noninvasive tumor growth:** Tumor tends to occur in peripheral region of lung. Tumor spreads as a thin layer of cells using the alveoli or bronchial walls as a framework or scaffold in lepidic pattern. This pattern of growth does not invade lung parenchyma, pleura or blood vessels. It has excellent prognosis when localized associated with 5-year survival rate approaching 100%.
- **Invasive tumor growth:** The growth most often invades and destroys lung parenchyma. Prognosis is poor. Differences between invasive mucinous adenocarcinoma and nonmucinous adenocarcinoma *in situ* (AIS)/minimally invasive adenocarcinoma (MIA)/lepidic adenocarcinoma are given in Table 18.62.

Comparison of lepidic-predominant lung adenocarcinoma and atypical alveolar hyperplasia is given in Table 18.63.

Table 18.61 Differences between squamous cell lung carcinoma and lung adenocarcinoma

Characteristics	Squamous Cell Lung Carcinoma	Lung Adenocarcinoma
Sex	Male	Female
History of tobacco smoking	Associated with tobacco smoking	Not associated with tobacco smoking
Location	Larger tumor, more centrally located	Tend to be more peripherally located
Growth rate	Rapid growth	Slow growth
Local spread	Predominant	Uncommon
Molecular genetics	FGFR 1 (80%), ERBB2 (*HER2/neu*) (30%), TP53 (60–80%), RB gene (15%), p16 and CDKN2K gene	K-RAS, FGFR 1, ALK, ROS, MET and RET genes
Distant metastases	Later stage	Early stage
Light microscopy	Well-differentiated carcinoma showing keratin pearls and intercellular bridges. May be poorly differentiated	Bronchial derived and bronchioloalveolar carcinoma
Immunohisto-chemistry	Cytokeratins, carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA) and cytoplasmic and intranuclear surfactant	Cytokeratins, carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA) and cytoplasmic, intranuclear surfactant, TTF-1 and CD15 (Leu-M1)
Size	Large tumor	Small tumor
Scarring	Absent	May be present

Table 18.62 Differences between invasive mucinous adenocarcinoma and nonmucinous adenocarcinoma *in situ* (AIS)/minimally invasive adenocarcinoma (MIA)/ lepidic adenocarcinoma

Characteristics	Invasive Mucinous Adenocarcinoma (Formerly Mucinous Bronchioloalveolar Carcinoma)	Nonmucinous Adenocarcinoma *in situ* (AIS)/Minimally Invasive Adenocarcinoma (MIA)/Lepidic Adenocarcinoma
General considerations		
Frequency	40%	60%
Female gender	60%	70%
Tobacco smokers	45%	45%
Invasion of lung, pleura and blood vessels	Present	Absent
Prognosis	Poor	Excellent
Radiological features		
Conventional radiographs	Majority of tumors demonstrate consolidation, air bronchogram, frequently multifocal and multilobar presentation	Majority of tumors with ground glass attenuation
Histologic features		
Cell type	Mucin-filled, columnar, and/or goblet cells	Type II pneumocytes and/or Clara cells
Immunophenotype		
CK7	90%	95%
CK20	50%	5%
TTF-1	15%	65%
Genotype		
K-RAS mutation	75%	15%
EGFR mutation	<5%	45%

Table 18.63 Comparison of lepidic-predominant lung adenocarcinoma and atypical alveolar hyperplasia

Parameters	Lepidic-predominant Lung Adenocarcinoma	Atypical Alveolar Hyperplasia
Size of lesion	>5 mm to ≤3 cm	Small usually <5 mm
Clinical features	Gross lesion demonstrated on radiographs	Often unsuspected. Atypical hyperplasia may be found in histologic sections of grossly normal lungs
Morphology of cells	Monomorphic cells with mild atypical features along alveolar walls in lepidic pattern	Polymorphic cells with mild atypical features

Clinical Features

Patient presents with copious watery sputum termed **bronchorrhea** as a result of extensive mucin production, cough, and pain. Atelectasis and emphysema are infrequent.

Imaging Technique

Lung adenocarcinoma is discovered as a single nodule or multiple nodules or pneumonia-like diffuse infiltrate (ground glass opacities) on the radiograph. Diffuse tumor may involve several lobes of lung or even bilateral associated with poor prognosis. After resection, tumor can appear in another lobe or opposite side lung.

Prognosis

Patient with solitary tumor (i.e. lung adenocarcinoma) in stage I has excellent prognosis with 5-year survival after surgical excision. Patient with tumor exhibiting diffuse pattern has poor outcome.

LARGE CELL LUNG CARCINOMA

Large cell lung carcinoma is peripherally located and accounts for about 10–20% of all lung cancers. It is characteristically aggressive and metastasizes early with fatal outcome. It is strongly associated with cigarette smoking. Patient presents with a mass >4 cm in diameter in peripheral region of lung in 60% of cases with early metastasis and poor prognosis.

Imaging Techniques

Radiologic appearance of large cell lung carcinoma is similar to adenocarcinoma except for its large size.

Surgical Pathology: Large Cell Lung Carcinoma

Gross Morphology

- Large cell lung carcinoma is usually peripheral and unifocal tumor measuring >4 cm in diameter with well-defined borders.
- Cut surface shows bulging lobulated homogenous gray-white 'fleshy' tumor mass with areas of hemorrhage and necrosis.

Light Microscopy

- **Classic variant of large cell lung carcinoma:** Tumor is composed of large cells with hyperchromatic nuclei and prominent nucleoli. There is minimal squamous or glandular differentiation. It does not express cytokeratin.
- **Tumor with neuroendocrine differentiation of large cell lung carcinoma:** Tumor is composed of tumor cells arranged in nests, trabecular, forming rosettes and palisading pattern. It suggests neuroendocrine differentiation on histochemical staining and electron microscopy.
- **Adenosquamous variant of large cell lung carcinoma:** Tumor has mixed histologic features of both lung adenocarcinoma and squamous cell lung carcinoma in 33% of cases. Patient presents with mass in the lung periphery and indistinguishable from adenocarcinoma or large cell lung carcinoma. Metastases are common. It has aggressive behavior with poor prognosis.

Differences between various histopathologic variants of lung cancers are given in Table 18.64.

ADENOSQUAMOUS LUNG CARCINOMA

Adenosquamous lung carcinoma shows admixture of adenocarcinoma and squamous cell lung carcinoma components. Each component should be >10% in the tumor.

PLEOMORPHIC LUNG CARCINOMA

Pleomorphic lung carcinoma is composed of any type of non-small cell lung carcinoma containing ≥10% spindle and/or giant cells.

CARCINOSARCOMA OF LUNG

Carcinosarcoma of lung consists of a mixture of non-small cell lung carcinoma and sarcoma containing heterologous elements, i.e. chondrosarcoma, osteosarcoma and rhabdomyosarcoma.

TUMORS METASTASIZING TO LUNGS

Pulmonary metastases are more common than primary lung carcinoma. In one-third of all fatal cancers, pulmonary metastases are evident at autopsy (Figs 18.58 and 18.59). Patterns of secondary tumor growth in the lungs are given in Table 18.65.

Table 18.64 Differences between various histopathologic variants of lung cancers

Parameters	Small Cell Lung Carcinoma (Neuroendocrine Carcinoma)	Squamous Cell Lung Carcinoma	Lung Adenocarcinoma	Adenocarcinoma *in situ*	Large Cell Lung Carcinoma
Frequency	15–25%	25–40%	30–50%	10%	10–20%
Cell of origin	Neuroendocrine tumor (Kulchitsky cells)	Basal cells of major bronchi	Mucus producing glands near previous scar	Clara cells (nonciliated epithelium) of terminal bronchiole	Epithelial tumor with squamous and glandular differentiation on EM
Location in lung	Central region	Central region	Peripheral region (75%), central region (25%)	Peripheral region	Peripheral region (65%)
Tobacco smoking	Strong association	Strong association	Weak association	No association	Strong association
Growth rate	Very rapid	Slow	Slow to moderate	Slow	Rapid
Metastasis	Very early to mediastinum or distal area of lung and widespread metastases	▪ Localized metastasis not common or occurs late ▪ Usually to hilar lymph nodes, adrenals, liver	Early metastasis throughout lung and brain or to other organs	Late metastasis	Early and widespread metastasis to kidney, liver and adrenals
Molecular genetics	▪ c-Myc gene ▪ RB gene (80–100%) ▪ p53 gene (50–80%)	▪ EGFR gene (80%) ▪ ERBB2 (*HER2/neu*) gene (30%) ▪ TP53 (60–80%) ▪ RB gene (15%)	▪ K-RAS gene ▪ EGFR gene ▪ ERBB2 (*HER2/neu*) ▪ p53 gene ▪ RB gene ▪ p16 gene	▪ K-RAS gene ▪ EGFR gene ▪ ERBB2 (*HER2/neu*) ▪ p53 gene ▪ RB gene ▪ p16 gene	▪ RB gene ▪ p16 gene
Light microscopy	Small cells, scanty cytoplasm, hyperchromic nuclei (EM showing neurosecretory granules)	Well differentiated SCC with keratin	Tumor cells forming glandular pattern	Lepidic growth pattern or diffuse pattern	Anaplastic or neuroendocrine differentiation or adenosquamous variants
Immunohistochemistry	▪ Chromogranin ▪ Synaptophysin ▪ CD56 ▪ CD57 (HNK-1 Leu-7) ▪ Neuron-specific enolase (NSE) ▪ Cytokeratin (AE 1/AE 3) ▪ TTF-1	▪ p63 ▪ p40 ▪ Cytokeratins (5, 6) ▪ Epithelial membrane antigen (EMA) ▪ Carcinoembryonic antigen (CEA) ▪ Cytoplasmic and nuclear surfactant	▪ TTF-1 (70–75%) ▪ Napsin A (80%) ▪ CK 7 ▪ Carcinoembryonic antigen ▪ B72.3 monoclonal antibody reacts with tumor associated glycoproteins in tumor cells ▪ MOC 31 ▪ CD15 (Leu-M1) monoclonal antibody reacts with tumor cells ▪ Ber EP4	–	–
Paraneoplastic syndrome	ACTH, ADH parathormone-related protein, gonadotropin	Parathormone-related protein, gonadotropin	Gonadotropin	Absent	Gonadotropin

Contd...

Table 18.64 Differences between various histopathologic variants of lung cancers (*Contd...*)

Parameters	Small Cell Lung Carcinoma (Neuroendocrine Carcinoma)	Squamous Cell Lung Carcinoma	Lung Adenocarcinoma	Adenocarcinoma *in situ*	Large Cell Lung Carcinoma
Clinical behavior	Most aggressive wide distant metastasis at diagnosis	Rapidly growing tumor with cavitation with late metastasis, lung collapse, consolidation	Cavitation rare	Solitary coin lesion or diffuse mimicking lobar consolidation	Large bulky tumor <4 cm in diameter
Treatment	Combination chemotherapy, surgical resectability is poor	Surgical resectability is good, if stage I or II	Surgical resectability is good, if stage I or II; chemotherapy, radiation and surgery may be combined for stage III	Chemotherapy and surgical resection or lung	Radiotherapy is palliative but chemotherapy is of limited use

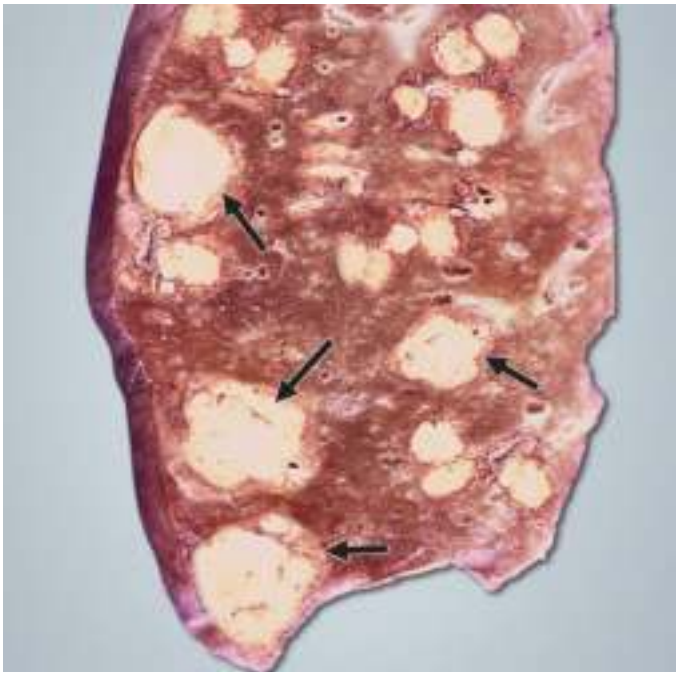


Fig. 18.58: Metastases in lung shows variable sized nodules (arrows).

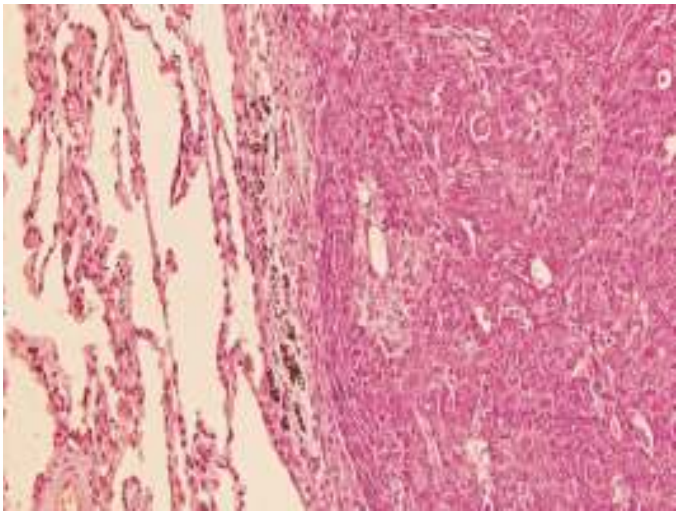


Fig. 18.59: Lung shows metastatic deposit from carcinoma (100X).

- Metastases are seen in lung parenchyma (most common), pleural cavity and lymphatics. Lymphatics may be diffusely involved, leading to the appearance of lymphangitis carcinomatosa.

Table 18.65 Patterns of secondary tumor growth in the lungs

Blood-borne Metastases in Lungs

- Solitary well-circumscribed nodule
- Multiple well-circumscribed nodules
- Massive tumor embolism (e.g. renal cell carcinoma, hepatocellular carcinoma invading systemic veins)
- Microscopic tumor microangiopathy (carcinomatous thrombotic microangiopathy) refers to presence of neoplastic cells occluding small muscular pulmonary arteries and arterioles. Breast carcinoma and gastric carcinoma produce microscopic tumor microangiopathy. This pattern may also be demonstrated in tumors of liver and placenta

Diffuse Lymphatic Permeation (Lymphangitis Carcinomatosa) in Lungs

- Entire lymphatic system of lungs demonstrates pale thread of tumor cells filling these vessels present on visceral pleura and broncho-arterial bundles
- Lymphangitis carcinomatosa may extend to regional lymph nodes

Contd...

Table 18.65 Patterns of secondary tumor growth in the lungs (*Contd...*)

Endobronchial Metastases in Lungs
▪ Tumor cells from intestine, uterine cervix and breast in lymphatic channels of the lung may occlude mucosal lymph channels thereby develop into secondary tumors in the main airways ▪ It is worth mentioning that primary tumors of bronchus with a propensity to grow in this way include carcinosarcomas, carcinoids and bronchial gland tumors
Intra-alveolar Growth Patterns in Lungs
▪ Diffuse air space involvement with compression of alveolar septa are demonstrated in primary lung tumors and some metastatic breast carcinoma and renal cell carcinoma. Prognosis in primary tumor involving diffuse air spaces have relatively favorable prognosis ▪ Lepidic growth refers to spread of neoplastic cells along the surface of alveolar epithelium. It is demonstrated in primary adenocarcinoma *in situ* (formerly known as bronchioloalveolar carcinoma)
Interstitial Metastases in Lungs
Extralymphatic route of tumor spread in the interstitial tissue of alveoli, which get thickened. Air spaces are obliterated.

Table 18.66 Immunohistochemistry of primary tumors metastasizing to lung in adults

Primary Tumors	Immunoprofiles
Breast carcinoma	ER, PR, *HER2/neu*, CK7, GCDFP-15 (gross cystic disease fluid protein 15), mammoglobulin, Ki-67, p53, CK5, NY-BR and E-cadherin
Renal cell carcinoma	Cytokeratin, vimentin
Colon carcinoma	CK20, CDX2
Thyroid carcinoma	TTF-1, thyroglobulin, CA-125 cell surface glycoprotein (monoclonal antibody OC-125)
Osteosarcoma	Vimentin
Ewing's sarcoma	CD99 (MIC2 specific marker), vimentin, nonspecific enolase (NSE), neurofilament and cytokeratin (low molecular weight)
Endometrium carcinoma type 1	p53, ER, PR, PTEN
Prostatic carcinoma	PSA (prostatic specific antigen), PSAP (prostatic specific acid phosphatase), PSMA (prostate specific membrane antigen), NXK3.1 and prostein
Non-Hodgkin's lymphoma B cell	LCA, CD10, CD19 and CD20
Non-Hodgkin's lymphoma T cell	CD3
Choriocarcinoma	Human chorionic gonadotropin (hCG)

Table 18.67 Immunohistochemistry of primary tumors metastasizing to lung in children

Primary Tumors	Immunoprofiles
Rhabdomyosarcoma	Desmin, vimentin, myoglobin and MyoD1

- Breast carcinoma is most common cause of lung metastases followed by colon carcinoma, and renal cell carcinoma. Other cancers metastasizing to lungs are derived from salivary glands, thyroid gland, uterus, ovaries, urinary bladder and prostate.
- Patterns of secondary tumor growths in the lungs are given in Table 18.65. Immunohistochemistry of primary tumors metastasizing to lung in adults and children, and differences between gross morphology of primary and secondary tumors of lungs are given in Tables 18.66 to 18.68.
- Lung metastases demonstrated by imaging techniques may be solitary, cannon balls, poorly differentiated, snow storm and diffuse alveolar patterns. Radiologic appearances of metastatic deposits in lungs are given in Table 18.69.
- Chest radiograph demonstrates solitary pulmonary nodule <5.0 cm or coin lesion in decreased frequency includes tuberculosis, histoplasmosis, primary lung cancer, bronchial hamartomas, amyloid deposits

Table 18.68 Differences between gross morphology of primary and secondary tumors of lungs

Feature	Primary Lung Tumors	Secondary Lung Tumors
Tumor	Usually solitary	Usually multiple
Circumscribed	Poorly circumscribed	Sharply circumscribed
Cavitation	Usual	Unusual
Calcification	Usual	Extremely unusual. If calcification present, then it is indicative of chondrosarcoma and osteosarcoma

Table 18.69 Radiologic appearances of metastatic deposits in lungs

Type of Lesions	Cancers
Solitary lesions	Breast carcinoma,* colon carcinoma, renal cell carcinoma
Cannonball multiple lesions	Breast carcinoma, colon carcinoma, renal cell carcinoma, testicular germ cell tumors, bone (osteosarcoma) and soft tissue sarcoma
Poorly differentiated multiple lesions	Breast carcinoma, choriocarcinoma
Snow storm pattern (multiple small nodules)	Breast carcinoma, thyroid carcinoma, urinary bladder carcinoma, prostatic carcinoma
Diffuse alveolar consolidation	Breast carcinoma, primary lymphomas (differential diagnosis pneumonias)

** On imaging study, breast carcinoma metastases in lung may show solitary multiple cannonballs, and multiple poorly differentiated nodules or diffuse alveolar consolidation. Multiple nodules are also demonstrated in Wegener's granulomatosis, rheumatoid lung disease, histoplasmosis and coccidioidomycosis.*

(pseudotumor), chondroma, lipoma and leiomyoma (*see* Figs 18.58 and 18.59).

DIAGNOSTIC APPROACH OF LUNG TUMORS

IMAGING TECHNIQUES

Imaging techniques are chest radiographs, CT scan, MRI, bone scan and positron emission tomography described as under:

- **Chest X-rays:** A chest radiograph detects most lung carcinomas.
- **Mediastinoscopy:** It is performed in case of involvement of mediastinum by lung carcinoma. It helps in evaluating the extent of the tumor.
- **CT scan:** It is most important imaging technique in detection of suspected lung carcinoma. It helps surgeon to know, whether the lung carcinoma is resectable or not.
  - Draining lymph nodes measuring >2 cm are likely to be involved due to metastases in 70% of cases.
  - Lymph nodes measuring <1 cm are unlikely to be involved.
  - The International Association for the Study of Lung Cancer (IASLC) lymph node map including the proposed grouping of lymph node stations into zones for the purpose of prognostic analysis is given in Table 18.70.
- **MRI technique:** It is essential to evaluate metastases in brain, spinal cord and other organs.
- **Bone scan:** It is done to evaluate bone metastases.
- **Positron emission tomography:** Presence or absence of metastatic deposits in draining lymph nodes and distant organs (liver, adrenal glands and elsewhere) is critical in management of lung cancer.
  - Positron emission tomography with radiolabeled fluorodeoxyglucose (FDG-PET) and biopsy are essential diagnostic tools. The dye is taken up by all cells, but more avidity with cancer stem cells.
  - Dye enters Krebs cycle but cannot complete and accumulates in the malignant cells.
  - A FDG-PET scan shows how well the lungs are functioning.

Table 18.70 The International Association for the Study of Lung Cancer (IASLC) lymph node map including the proposed grouping of lymph node stations into zones for the purpose of prognostic analysis

Study of Lung Cancer Lymph Nodes Map	Proposed Grouping of Lymph Node Stations into Zones	
Supraclavicular lymph nodes	▪ Low cervical lymph nodes ▪ Supraclavicular lymph nodes	▪ Sternal lymph nodes
Superior mediastinal lymph nodes	▪ Upper paratracheal lymph nodes on left side and right side ▪ Prevascular lymph nodes	▪ Retrotracheal lymph nodes ▪ Lower paratracheal lymph nodes on left side and right side
Aortic lymph nodes	▪ Subaortic lymph nodes ▪ Para-aortic lymph nodes	
Inferior mediastinal lymph nodes	▪ Subcarinal zone lymph nodes ▪ Paraesophageal lymph nodes below carina	▪ Pulmonary ligament lymph nodes
N1 nodes	▪ Hilar/interlobar zone of lung • Hilar lymph nodes • Interlobar lymph nodes	▪ Peripheral zones of lung • Lobar lymph nodes • Segmental lymph nodes • Subsegmental lymph nodes

OTHER DIAGNOSTIC MODALITIES

Fiberoptic Bronchoscopy

Fiberoptic bronchoscopy is used to take biopsy from representative area from centrally located lung cancers (squamous cell lung carcinoma and in some cases of small cell lung carcinoma).

Sputum Examination

Sputum cytology may reveal malignant cells in 85% cases of squamous cell lung carcinoma but the false-negative rate is high. Asbestos bodies may be demonstrated in sputum during prolonged exposure to asbestos.

Bronchoalveolar Lavage

Saline is injected into a lung segment down by bronchoscope. Saline is aspirated and examined for cells and organisms.

Bronchopulmonary Biopsy

- **Bronchial biopsy:** Bronchoscope is introduced to take biopsy to diagnose neoplasia, sarcoidosis and allergic aspergillosis.
- **Transbronchial biopsy:** Forceps is advanced to lung periphery down bronchoscope to take biopsy sample. It is done to diagnose infections, diffuse lung disease and transplant monitoring.
- **Ultrasound or CT-guided percutaneous needle lung biopsy:** It is performed to take biopsy from localized mass lesions (lung cancer) under ultrasound/CT-guidance.
- **Open lung biopsy:** It is done in cases of chronic diffuse lung diseases of uncertain origin.

Surgical Pathology: Lung Cancer

Gross Morphology and Light Microscopy

- Biopsy fixed in 10% formalin is processed in tissue processor. Sections are stained with hematoxylin and eosin.
- Diagnostic features to be reported with resected lung carcinomas include tumor margins, histologic type and grade, lymphatic and blood vessel involvement and staging.

Histochemistry

- Alcian blue, mucicarmine and PAS stains are used to demonstrate mucin in adenocarcinoma. Epithelial mucins are stained purple or dark blue color.
- Connective tissue mucins are stained as pale blue with Alcian blue, but it does not stain with PAS.
- Approximately, 75% of lung cancers show positivity with Kreberg's stain.

Immunohistochemistry Technique

Immunohistochemistry technique plays significant role in differential diagnosis of primary lung cancers described as follows:

- **Neuron-specific enolase:** It is specific for neural crest cells of the neuroendocrine series, i.e. small cell lung carcinoma.
- **Cytokeratin:** Squamous cell lung carcinoma shows positivity for cytokeratin.
- **Carcinoembryonic antigen (CEA):** CEA is a glycoprotein of heterogeneous composition normally detected in the glycocalyx of fetal epithelial cells, particularly those of mucin-secreting glandular nature. It is mainly used for the differential diagnosis between lung adenocarcinoma (usually positive) and mesothelioma (usually negative).
- **B72.3 monoclonal antibody:** It is a monoclonal antibody reacts with a high molecular weight glycoprotein that has been named tumor-associated glycoprotein 72 (TAG-72). One of its most common applications is in the differential diagnosis between mesothelioma (in which it is usually negative) and lung adenocarcinoma (usually positive).
- **Ber-EP4 monoclonal antibody:** It is mainly used for the differential diagnosis between lung adenocarcinoma and mesothelioma.
- **Calretinin:** It is a calcium-binding protein. Its main use is in the differential diagnosis between mesothelioma (almost always positive except for the desmoplastic variant) and lung adenocarcinoma (usually but not always negative).
- **Centromere protein H (CENP-H):** This is one of the fundamental components of the human active kinetochore and thought to be associated with tumorigenesis. High CENP-H protein expression has been related to poor outcome in patients with non-small cell lung carcinoma of lung.
- **CD15 (Leu-M1):** CD15 antibody has been found to react with lung adenocarcinoma. Its main use is in the differential diagnosis between lung adenocarcinoma (usually positive) and mesothelioma (usually negative). Immunohistochemistry of primary lung tumors is given in Table 18.71.

Serum Tumor Markers in Primary Lung Cancer

Certain tumors liberate products (e.g. cell surface antigens, cytoplasmic proteins, enzymes and hormones) that can be detected in blood samples, thereby acting as tumor markers. Their utility is to confirm the diagnosis.

Blood biochemistry analysis is done to evaluate biochemical alterations in primary and secondary lung cancers. Serum tumor markers analyzed in diagnosing primary lung cancers are given in Table 18.72.

- **Serum p53:** The TP53 gene alteration is most often demonstrated in human cancers. The p53 protein overexpression is detected by p53 antibodies in 60–70% of lung carcinoma. The p53 can be used as a precocious marker of TP53 gene alteration before clinical manifestations of lung carcinoma.
- **Serum carcinoembryonic antigen (CEA):** Elevated serum CEA level is demonstrated in patients with extrathoracic lung adenocarcinoma and anaplastic lung carcinoma in 23% of cases. Serum CEA is also elevated in carcinoma of colon, pancreas and breast.

Table 18.71 Immunohistochemistry of primary lung tumors

Markers	Expression
Squamous cell lung carcinoma	
p40	Positive
p63	Positive
CK5/6	Positive (diffuse staining)
CK903	Positive (diffuse staining)
CEA (carcinoembryonic antigen)	Positive
Surfactant protein	Positive
CK7	Positive/negative
CK20	Negative
TTF-1	Negative
Napsin A	Negative
Synaptophysin	Negative
Chromogranin A	Negative
NSE (neuron-specific enolase)	Negative
CD56 (neuronal cell adhesion molecule)	Negative
Small cell lung carcinoma	
TTF-1	Positive (90%)
Synaptophysin	Positive
Chromogranin A	Positive
NSE (neuron-specific enolase)	Positive
CD56 (neuronal cell adhesion molecule)	Positive
CAM5.2	Positive (punctate staining)
AE1/AE3	Positive (punctate staining)
CK7	Negative/positive
CK20	Negative
p63	Negative
CK5/6	Negative
CK903	Negative
Napsin A	Negative
Lung adenocarcinoma (mucinous variant)	
TTF-1	Positive (10–20%)
B72.3	Positive (75–100%)
CD15	Positive (60%)
MOC31	Positive
Ber-EP4	Positive
Napsin A	Positive/negative
p63	Positive/negative
CK20	Positive/negative
CK5/6	Positive/negative
CK903	Positive/negative
CDX2	Positive (focal staining)
Synaptophysin	Negative
Chromogranin A	Negative
NSE (neuron-specific enolase)	Negative
CD56 (neuronal cell adhesion molecule)	Negative
Lung adenocarcinoma (nonmucinous variant)	
TTF-1	Positive
Napsin A	Positive
CK7	Positive
p63	Positive/negative
Synaptophysin	Negative
Chromogranin A	Negative
NSE (neuron-specific enolase)	Negative
CD56 (neuronal cell adhesion molecule)	Negative
CK20	Negative
Bronchopulmonary carcinoids	
TTF-1	Positive (50%)
Synaptophysin	Positive
Chromogranin A	Positive
NSE (neuron-specific enolase)	Positive
CD56 (neuronal cell adhesion molecule)	Positive
Napsin A	Negative
CK7	Negative
CK20	Negative
p63	Negative

Contd...

- **Serum TTF-1:** Its concentration is increased in lung adenocarcinoma and thyroid carcinoma.
- **Serum combined NSE + ProGRP:** In the combined analysis of NSE + ProGRP, sensitivity and specificity in the diagnosis of small cell lung carcinoma is demonstrated in >80% of cases.
- **Serum combined TSGF + SCCAg + CYFRA21-1:** In the combined examination of serum levels of TSGF + SCCAg + CYFRA21-1 sensitivity and specificity in the diagnosis of squamous cell lung carcinoma (SCLC) is demonstrated in >80% of cases.

Serum Tumor Markers in Metastatic Lung Cancer

Serum tumor markers analyzed in metastatic lung cancers are given in Table 18.73.

Table 18.72 Serum tumor markers analyzed in diagnosing primary lung cancers

Categories	Serum Tumor Markers	Associated Malignancies
Oncofetal proteins	Carcinoembryonic antigen (CEA)	▪ *Lung carcinoma* (*adenocarcinoma and anaplastic carcinoma*) ▪ Nonseminomatous germ cell testicular tumor ▪ Colon carcinoma ▪ Stomach carcinoma ▪ Pancreatic carcinoma ▪ Breast carcinoma
Hormones	▪ Adrenocorticotropin hormone (ACTH)	▪ *Small cell lung carcinoma* ▪ Pancreatic carcinoma ▪ Neural tumors
	▪ Antidiuretic hormone (ADH) and atrial natriuretic hormone (ANH)	▪ *Small cell lung carcinoma* ▪ Intratracheal neoplasm
	▪ Parathyroid hormone-related protein (PTHrP)	▪ *Small cell lung carcinoma* ▪ Squamous cell lung carcinoma ▪ Breast carcinoma ▪ Ovarian carcinoma ▪ Renal cell carcinoma ▪ Adult T cell lymphoma
	▪ Gonadotropin	▪ *Small cell lung carcinoma* ▪ Squamous cell lung carcinoma ▪ Lung adenocarcinoma
	▪ Insulin ectopic hormone	▪ *Large cell lung carcinoma* ▪ Fibrosarcoma ▪ Mesenchymal sarcoma ▪ Hepatocellular carcinoma ▪ Cerebellar hemangioma ▪ Hepatocellular carcinoma ▪ Multiple myeloma ▪ Malignant lymphoma ▪ Liver carcinoma ▪ Prostatic carcinoma
Isoenzymes	Neuron-specific enolase (NSE)	▪ *Small cell lung carcinoma* ▪ Neuroblastoma
Mucins and glycoproteins	Cytokeratin fragment 21.1 (Cyfra 21.1)	Non-small cell lung carcinoma

Table 18.73 Serum tumor markers analyzed in metastatic lung cancers

Primary Cancers	Immunoprofiles
Breast carcinoma	CA 15.3, CA 27.29, CEA, ER & PR, PS2, HER2/neu. p53, DNA ploidy, S phase and EGFR, BRCA gene, CK7, GCDFP-15 (gross cystic disease fluid protein 15), and mammaglobin
Renal cell carcinoma	Erythropoietin
Colorectal carcinoma	CEA and p53
Thyroid carcinoma	TTF-1, thyroglobulin
Endometrial carcinoma type 1	ER, PR, PTEN
Prostatic carcinoma	PAP, PSA, DNA ploidy and IGFBP 2
Choriocarcinoma	Human chorionic gonadotropin

PLEURAL DISEASES

Serosal membranes are derived from the mesoderm. These serous membranes form the visceral and parietal surfaces of pleural, pericardial and peritoneal cavities including tunica vaginalis.

Parietal layer is perforated by lymphatic lacuna that connect with the lymphatic channels, which drain the enclosed cavities. Maximum quantity of fluid is absorbed into the lymphatic channels.

NON-NEOPLASTIC PLEURAL DISORDERS

PLEURAL EFFUSION

The pleural cavity contains small amount of pleural fluid <10 ml in a 70 kg man. Daily synthesis of pleural fluid is 17 ml. Maximum quantity of fluid is absorbed into the lymphatic channels.

Fluid moves from parietal pleura to pleural space to lungs. Movement depends upon the balance of Starling pressures. Mechanism of formation of pleural fluid is shown in Fig. 18.60.

Causes

Excessive accumulation of fluid in pleural cavity occurs in settings of congestive heart failure, cirrhosis, pneumonia, cancers and coronary artery bypass surgery. Leading causes of pleural effusions are given in Table 18.74. Differential diagnosis of pleural effusions is given in Table 18.75. Incidence of primary cancers causing pleural effusion is given in Table 18.76.

Pathogenesis

Pleuritis may result from the extension of any pulmonary infection to the visceral pleura. Causes of pleuritis include bacterial infections, viral infections, and pulmonary infarction involving the surface of the lung.

- **Pyothorax:** It refers to a turbid effusion containing many neutrophils. Empyema is a variant of pyothorax in which thick pus accumulates within the pleural cavity, often with loculation and fibrosis.
- **Transudative effusions:** Decreased oncotic pressure or increased hydrostatic pressure causes transudative pleural effusions. Transudate is ultrafiltrate of plasma involving disturbances in Starling pressures. Abnormal accumulation of fluid occurs due to

Table 18.74 Leading causes of pleural effusions

Etiology	Exudate	Transudate
Congestive heart failure	No	Yes
Cirrhosis	No	Yes
Pneumonia	Yes	No
Cancer	Yes	No
Viral disease	Yes	No
Coronary artery bypass surgery	Yes	No
Pulmonary embolism	Sometimes	Sometimes

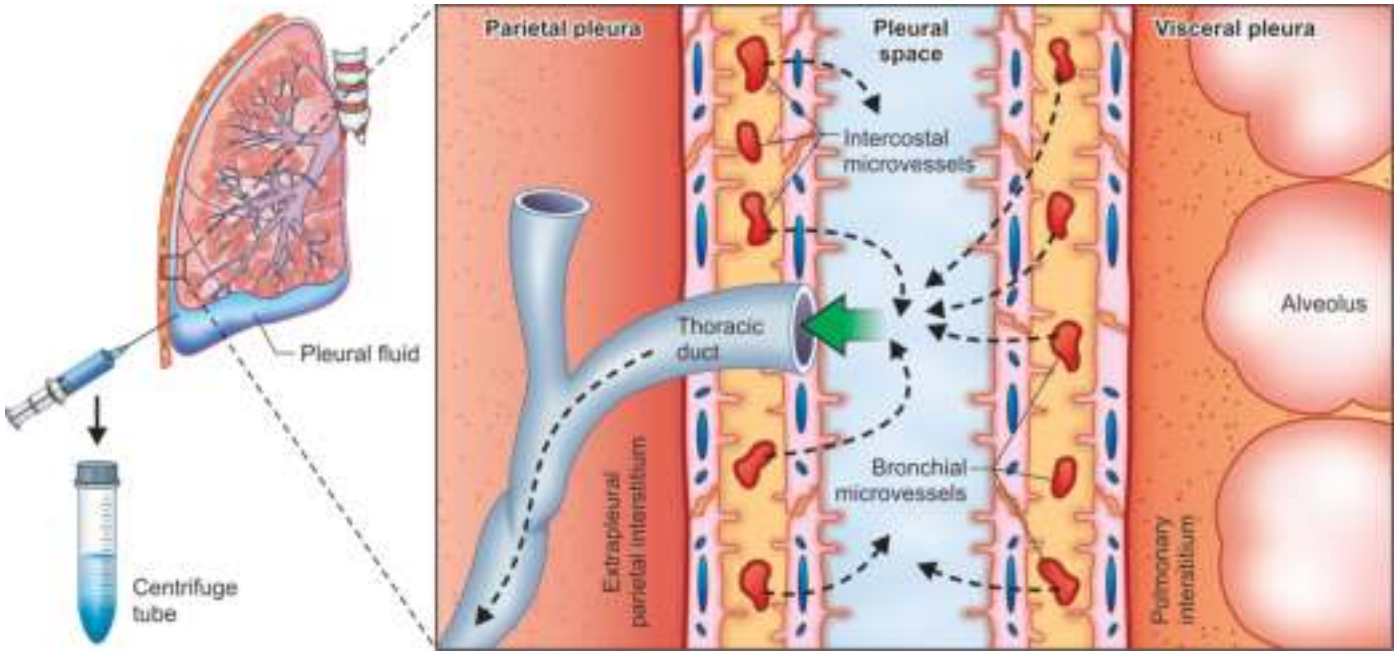


Fig. 18.60: Mechanism of formation of pleural fluid.

Table 18.75 Differential diagnosis of pleural effusions

Congestive heart failure
Nephrotic syndrome
Cirrhosis
Peritoneal dialysis
Superior vena cava syndrome
Myxedema
Urinothorax
Neoplastic diseases ▪ Metastatic disease (breast carcinoma, ovarian carcinoma, gastric carcinoma and lymphomas) ▪ Mesothelioma
Infectious diseases (bacteria including tuberculosis, viruses, fungi, parasites)
Pulmonary thromboembolism
Gastrointestinal disorders ▪ Esophageal perforation ▪ Endoscopic variceal sclerotherapy ▪ Pancreatic disease ▪ Intra-abdominal abscesses ▪ Diaphragmatic hernia ▪ Post-liver transplant cases ▪ Post-abdominal surgery cases
Collagen vascular disorders ▪ Systemic lupus erythematosus ▪ Rheumatoid arthritis ▪ Sjögren syndrome ▪ Wegener granulomatosis ▪ Chrug-Strauss syndrome
Post-coronary artery bypass surgery
Asbestos exposure
Sarcoidosis
Uremia
Meig's syndrome (ovarian fibroma associated with pleural effusion)
Yellow nail syndrome
Drug-induced pleural disease ▪ Nitrofurantoin ▪ Amiodarone ▪ Dantrolene bromocriptine ▪ Dasatinib
Trapped lung
Post-cardiac injury syndrome
Hemothorax
Iatrogenic injury
Ovarian hyperstimulation syndrome
Pericardial disease
Chylothorax

Contd...

Table 18.76 Incidence of primary cancers causing pleural effusion

Primary Neoplasms Causing Pleural Effusion	Frequency
Lung carcinoma	36%
Breast carcinoma	25%
Lymphomas	10%
Ovarian carcinoma	5%
Gastric carcinoma	2%
Unknown	7%

imbalance between hydrostatic pressure and/or reduction in plasma oncotic pressure. The pleura usually remains normal. This is termed transudative pleural effusion. Increased hydrostatic pressure in visceral pleura occurs in congestive heart failure. Nephrotic syndrome leads to decreased oncotic pressure resulting in transudative effusion.

- **Exudative effusions:** It most often results from pathological disorders of pleura resulting in increased vascular permeability and/or impaired fluid reabsorption (e.g. lymphatic obstruction). Increased vascular permeability of visceral pleural capillaries occurs due to pulmonary infarction, pneumonia, tuberculosis and metastatic cancers. Tuberculosis and malignancy are most common causes of pleural exudative effusion. Exudate is rich in proteins and inflammatory cells. Obstruction of lymphatic drainage from the visceral pleura by lung carcinoma also causes pleural effusion.
- **Chylothorax:** Pleural cavity contains milky, lipid-rich turbid lymph fluid as a result of thoracic duct (lymphatic) obstruction or rarely due to lymphomas in the posterior mediastinum. It can be iatrogenic tear during surgery or pathological process. Pleural fluid triglyceride >110 mg/dl is diagnostic of chylous effusion. Repeated thoracocentesis leads to malnutrition and compromised immune system. Chylothorax is treated by pleuroperitoneal shunt, surgical ligation of thoracic duct, radiation therapy and chemical pleurodesis.
- **Pseudochylous effusion:** In rheumatoid lung disease, there is excessive accumulation of milky and turbid fluid in pleural cavity due to inflammatory process and necrotic debris.

- **Hydrothorax:** It refers to transudation of edema fluid into the pleural cavity. The elevation of hydrostatic pressure in patients with congestive heart failure causes transudation of edema fluid into the pleural cavity (i.e. hydrothorax).
- **Hemothorax:** It refers to accumulation of blood in the pleural cavity due to chest trauma.
- **Urinothorax:** Unilateral pleural effusion is caused by ipsilateral obstructive uropathy.

Diagnostic Approach

Diagnostic approach of pleural effusion includes detailed clinical history, physical examination, imaging techniques, pleural fluid analysis and histologic examination.

- Indications of thoracocentesis include significant pleural effusion of unknown etiology and unilateral pleural effusion persisting for three days.
- Investigative parameters of pleural effusions are physical appearance, smell, total proteins content, LDH, microbial analysis, cytology, amylase, cholesterol triglyceride ratio, deaminase and PCR.

Physical parameters of pleural fluid are given in Table 18.77 and differences between pleural fluid exudate and transudate according to Light's criteria are given in Table 18.78.

Pleural Fluid Analysis: Pleural Diseases
Light Microscopy
Smears prepared from centrifuged pleural fluid are examined for inflammatory and malignant cells.

- **Neutrophil predominant exudates:** Exudate-rich in neutrophils is seen in pyothorax.
- **Lymphocyte predominant exudates:** Exudate-rich in predominant lymphocytes include tuberculosis, lymphomas, chylothorax, sarcoidosis, acute lung transplant rejection and coronary artery bypass surgery.
- **Eosinophil predominant pleural effusion:** Eosinophilia is characterized by presence of eosinophil count >10% of total nucleated pleural fluid cells. Etiology of eosinophil-rich pleural effusions is pneumothorax, benign asbestosis pleural disease, parasitic disease, fungal disease, drug-induced and Churg-Strauss syndrome.

PLEURITIS

Acute pleuritis is most often associated with bacterial pneumonia. Gram-positive bacteria are most often isolated. Spontaneous bacterial pleuritis is occasionally seen in a case of cirrhosis. Autoimmune pleuritis may mimic infectious pleuritis.

PLEURAL FIBROSIS

Reactive Pleural Fibrosis

Reactive pleural fibrosis most often occurs as a consequence of prior inflammation or surgery. Pleural fibrosis occurs due to adhesion between parietal and visceral layers of pleura.

Pleural Plaques

Pleural plaques are formed on parietal layer of pleura. Chronic pleural effusions and prolonged exposure to

Table 18.77 Physical parameters of pleural fluid

Parameters	Characteristic Findings	Probable Diagnosis
Color	▪ Pale yellow	▪ Classic paucicellular transudate
	▪ Red (bloody)	▪ Cancers, trauma, benign asbestos effusion, post-cardiac injury syndrome, pulmonary infarction
	▪ Milky white	▪ Chylothorax
	▪ Brown	▪ Amoebic liver abscess rupture, long-standing bloody effusion
	▪ Black	▪ Aspergillosis infection
	▪ Yellow green	▪ Rheumatoid pleurisy
	▪ Green	▪ Biliopleural fistula
Odor	Putrid	Anaerobic empyema

Table 18.78 Differences between pleural fluid exudate and transudate according to Light's criteria

Component	Pleural Exudate	Pleural Transudate
Pleural fluid/serum protein	>0.5	<0.5
Pleural fluid LDH/serum LDH	>0.6	<0.6
Pleural fluid LDH	>200 units/L	<200 units/L

asbestos produces raised, discrete, white to gray bilateral pleural plaques. Histologic examination of pleural plaque shows paucicellular dense collagenous tissue.

Diffuse Fibrosis of Pleural Visceral Layer

Diffuse fibrosis of pleural visceral layer occurs due to silicosis, advanced hypersensitivity reactions, connective tissue disorders and empyema.

Surgical Pathology: Diffuse Fibrosis of Pleural Visceral Layer
Gross Morphology
Diffuse fibrosis of pleural visceral layer is difficult to differentiate from desmoplastic mesothelioma.
Light Microscopy
Diffuse fibrosis of pleural visceral layer does not infiltrate the subjacent soft tissue. On the other hand, mesothelioma invades the subjacent soft tissue.

MALIGNANT PLEURAL TUMORS

MALIGNANT MESOTHELIOMA

Malignant mesothelioma arises from mesothelial cells of pleura. It occurs in occupational workers on prolonged exposure to asbestos for 30–40 years. Oncogenic SV40 virus has also been reported as etiological cause in some cases. Rare cases may be related to therapeutic radiation exposure.Widespread metastases of malignant mesothelioma can occur.

Pathology

Tumor begins as multiple small nodules on the parietal and visceral pleura encasing lung. It then invades the soft tissues of the chest wall and often extends into the mediastinum encasing of the pericardial sac. Disease is lethal and associated with 100% mortality.

Clinical Features

Patient presents with dyspnea, chest pain, and pleural effusion, constitutional symptoms such as weight loss, weakness and fatigue.

Surgical Pathology: Malignant Mesothelioma
Gross Morphology
Pleura exhibits multiple nodules to bulky dense white tumor.
Light Microscopy
▪ **Epithelioid mesothelioma:** Tumor is composed of bland cells with abundant eosinophilic cytoplasm arranged in sheet-like microglandular and tubulopapillary patterns. In some cases, tumor may show more anaplastic features. Psammoma bodies are occasionally seen.
▪ **Sarcomatoid mesothelioma:** Tumor is composed of spindle cells arranged in haphazard pattern. Immunohistochemical analysis shows positivity for cytokeratin 5/6. Tumor shows osteosarcoma and chondrosarcoma differentiation express actin, desmin, vimentin and/or S-100. Sarcomatoid mesothelioma has aggressive behavior.
▪ **Desmoplastic mesothelioma:** Tumor is most often misdiagnosed as organizing pleuritis in small biopsy specimens. Tumor is composed of scattered atypical cells arranged in storiform pattern in >50% of cases. There is presence of dense collagenous fibers in the background of tumor cells.
▪ **Biphasic mesothelioma:** Tumor comprises mixed epithelioid and sarcomatous elements. By definition, each component should comprise at least 10% of the tumor (Figs 18.61 and 18.62).
Immunohistochemistry Technique
Panel of immunomarkers is used to analyze expression in mesothelioma on histologic tissue sections. Immunohistochemistry analysis is done in differential diagnosis of lung adenocarcinoma and mesothelioma (Table 18.79).

Markers	Expression
▪ Calretinin	▪ Positive
▪ WT1	▪ Positive
▪ CK5/CK6	▪ Positive
▪ CK7	▪ Positive
▪ CK903	▪ Positive
▪ Thrombomodulin (CD141)	▪ Positive
▪ HBME-1	▪ Positive
▪ N-cadherin	▪ Positive
▪ D2–40	▪ Positive
▪ Mesothelin	▪ Positive/negative
▪ TTF-1	▪ Negative
▪ Napsin A	▪ Negative
▪ CK20	▪ Negative
▪ p63	▪ Negative
▪ Synaptophysin	▪ Negative
▪ Chromogranin A	▪ Negative
▪ NSE (neuron-specific enolase)	▪ Negative
▪ CD56 (neuronal cell adhesion molecule)	▪ Negative

TTF-1 (thyroid transcription factor 1) is positive in small cell lung carcinoma (90%), adenocarcinoma mucinous (20%) and adenocarcinoma nonmucinous variant, extrapulmonary small cell lung carcinoma (40%); NSE (neuron-specific enolase).

It is difficult to differentiate metastatic adenocarcinoma of pancreas and upper gastrointestinal tract from mucinous lung adenocarcinoma. Both metastatic tumors are CK7 positive, CK20 positive but TTF-1 negative. DPC4 is deleted in 55% cases of adenocarcinoma of pancreas but not lung origin.



Fig. 18.61: Mesothelioma shows bulky dense white tumor arising from visceral pleura most often associated with prolonged asbestos exposure. Asbestos exposure more commonly predisposes to bronchogenic carcinomas. Smoking increases the 10 times risk for lung cancer (arrows).

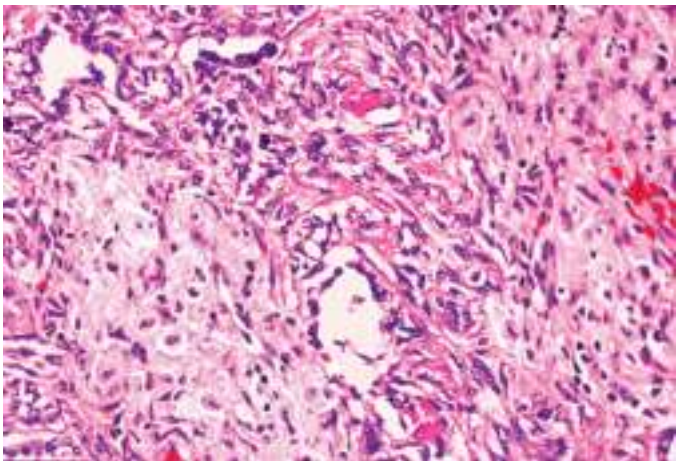


Fig. 18.62: Mesothelioma. Tumor shows plump rounded cells with abundant eosinophilic cytoplasm forming gland-like configurations (400X).

Differential Diagnosis

Mesothelioma should be differentiated from lung adenocarcinoma. Histochemical, electron microscopy and immunohistochemical staining characteristics of mesothelioma and lung adenocarcinoma are given in Table 18.80.

PRIMARY PLEURAL EFFUSION LYMPHOMA

Primary pleural effusion lymphoma presents as pleural effusion without an associated tumor mass.

Table 18.79 Histochemical, electron microscopy and immunohistochemical staining characteristics of malignant mesothelioma and lung adenocarcinoma

Features	Malignant Mesothelioma	Lung Adenocarcinoma
Histochemistry		
Glycogen content in cytoplasm	Present, but no diastase-resistant PAS (periodic acid–Schiff) positive material	Present but small, may demonstrate diastase-resistant PAS (periodic acid–Schiff) positive mucin
Acidophilic hyaluronic acids in glands or on neoplastic cell surface	Present	Absent
Electron microscopy		
Microvilli	Elongated, slender and bushy without glycalyceal bodies and may mingle with collagen fibers	Short and stubby with glycalyceal bodies, generally confined to the luminal surface and seldom mingle with collagen fibrils
Desmosomes	Long and well developed	Short and less developed
Filaments	Perinuclear filaments	Irregularly distributed intermediate filaments
Junctions	Apical tight junctions	Terminal bars near lumen
Mucin granules	Present	May be present
Glycogen	Abundant	Variable
Fat droplets	Scanty	Absent

Contd...

Table 18.79 Histochemical, electron microscopy and immunohistochemical staining characteristics of malignant mesothelioma and lung adenocarcinoma (*Contd...*)

Features	Malignant Mesothelioma	Lung Adenocarcinoma
Immunohistochemistry		
CEA (carcinoembryonic antigen)	Negative	Positive
CD15 (LeuM1)	Negative	Positive
Ber-Ep4	Negative	Positive
AUA1	Negative	Positive
EMA (epithelial membrane antigen)	Positive (membranous staining)	Positive in cytoplasm and at periphery of cells
CK5/CK6	Positive	Negative
Thrombomodulin (CD141)	Positive	Negative
Calretinin (calcium-binding protein)	Positive (except in desmoplastic variant)	Negative

Table 18.80 Panel of immunohistochemical markers applied in differential diagnosis of malignant mesothelioma and lung adenocarcinoma

Immunohistochemical Markers	Malignant Mesothelioma	Lung Adenocarcinoma
Mesothelial markers		
Calretinin	Positive (standard marker)	Negative
CK5/CK6	Positive (standard marker)	Negative
WT1	Positive	Negative
Thrombomodulin (CD141)	Positive	Negative
HBME-1	Positive	Negative
N-cadherin	Positive	Negative
D2–40	Positive	Negative
Mesothelin	Positive/negative	Negative/positive
Glandular differentiation markers		
Thyroid transcription factor 1 (TTF-1)	Negative (100%)	Positive/negative
Napsin A	Negative (100%)	Positive/negative
Ber-EP4	Negative	Positive/negative
Carcinoembryonic antigen monoclonal (mCEA)	Negative/positive	Positive/negative
CD15 (Leu-M1)	Negative	Positive
BG8	Negative/positive	Positive/negative
MOC-31	Negative/positive	Positive/negative
Epithelial markers		
Epithelial membrane antigen (EMA)	Positive/negative (membranous staining)	Positive/negative (membranous staining)
Cytokeratins (AE1/AE3, CAM 5.2)	Positive (perinuclear accentuation)	Positive (membranous accentuation)
Histochemical special stains		
Mucicarmine	Positive	Positive/negative
PAS	Positive	Positive
PAS/diastase	Negative	Positive
Alcian blue	Positive	Positive
Alcian blue/hyaluronidase	Negative	Positive
Electron microscopy		
Length of microvilli	Long (length to breadth ratio >10:1)	Short

Other markers for mesothelioma include p53, EMA, CD146 and GLUT1. Remember GLUT1 is positive in lung carcinoma.
Standard panel of markers for mesothelioma includes calretinin, CK5/CK6.
Standard panel of markers for adenocarcinoma includes TTF-1, napsin A, Ber-EP4.

Table 18.81 Comparison of primary effusion (body cavity-based) pleural lymphoma and pyothorax-associated pleural lymphoma

Features	Primary Effusion (Body Cavity-based) Pleural Lymphoma	Pyothorax-associated Pleural Lymphoma
Gross morphology	Pleural effusion	Pleural mass lesion
Etiology	Immunosuppression, e.g. HIV positive homosexual males	Chronic pleuritis following repeated artificial pneumothorax
HIV (human immunodeficiency virus) status	Positive	Negative
HHV (human herpesvirus)	Positive	Negative
EB virus (Epstein-Barr virus) status	Positive	Positive
Immunohistochemistry	▪ CD20 negative ▪ CD3 negative	▪ CD20 positive/negative ▪ CD3 positive/negative

It is associated with human herpesvirus 8 and acquired immunodeficiency syndrome. Immunohistochemical analysis reveals that primary pleural effusion lymphoma is of null phenotype, although occasional cases express B cell or T cell markers.

Comparison of primary effusion (body cavity-based) pleural lymphoma and pyothorax-associated pleural lymphoma is given in Table 18.81.

PYOTHORAX EFFUSION LYMPHOMA

Pyothorax effusion lymphoma is a diffuse large B cell lymphoma that arises from pleura in elderly persons, who have long-standing history of pyothorax in the settings of tuberculous pleuritis.

- It is strongly associated with EB virus infection.
- Immunohistochemical analysis of tumor shows positivity for B cell markers. CD20 is most often negative.

CHAPTER 19

Oral Cavity, Salivary Glands and Jaw

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

DISORDERS OF ORAL CAVITY

- Congenital and inherited focal disorders: overview
  - Congenital disorders
    - Facial clefts
    - Harelip (cleft lip)
    - Cleft palate
    - Hemifacial hypertrophy
  - Inherited focal disorders
    - Fordyce's granules
    - Congenital lip pits
    - Endogenous melanin pigmentation
    - Congenital epulis
    - White sponge nevus
- Inflammatory disorders
  - Acute necrotizing ulcerative gingivitis (Vincent angina)
  - Ludwig angina
  - Exudative pharyngitis
  - *Candida albicans* (oral thrush)
  - Herpes simplex virus 1 infection
  - Other viral infections
  - Aphthous stomatitis
- Tumors of oral cavity
  - Squamous cell papilloma
  - Squamous cell carcinoma
  - Kaposi sarcoma
  - Epithelial precursor oral lesions
    - Leukoplakia
    - Granuloma pyogenicum

DISORDERS OF SALIVARY GLANDS

- Anatomy of salivary glands
- Inflammatory disorders
  - Acute sialadenitis
  - Viral sialadenitis
  - Autoimmune sialadenitis
  - Chronic obstructive sialadenitis
  - Chronic recurrent parotitis
- Tumors of salivary glands
  - Pleomorphic adenoma
  - Warthin's tumor
  - Oncocytoma
  - Monomorphic adenoma
  - Mucoepidermoid carcinoma
  - Adenoid cystic carcinoma
  - Acinic cell carcinoma
  - Polymorphous low-grade adenocarcinoma
  - Carcinosarcoma
  - Squamous cell carcinoma

DISORDERS OF JAW

- Anatomy of jaw
- Dental and periodontal disorders of jaw
  - Dental caries
  - Periodontal disease
- Cystic lesions of jaw
  - Dentigerous cyst (follicular cyst)
  - Keratocystic odontogenic tumor (odontogenic keratocyst)
  - Lateral periodontal cyst
  - Gingival cyst
  - Calcifying odontogenic cyst
  - Glandular odontogenic cyst
  - Radicular (periapical cyst)
  - Nasopalatine duct cyst
  - Globulomaxillary cyst
  - Median palatal cyst
  - Simple bone cyst
  - Aneurysmal bone cyst
- Odontogenic tumors
  - Adenomatoid odontogenic tumor
  - Calcifying epithelial odontogenic tumor (Pindborg tumor)
  - Squamous odontogenic tumor
  - Ameloblastic fibroma
  - Odontoma
  - Cementoma
  - Odontogenic myxoma, myxofibroma and fibroma
  - Ameloblastoma (adamantinoma)
  - Ameloblastic carcinoma
  - Odontogenic carcinoma
  - Ameloblastic fibrosarcoma
- Nonodontogenic tumors
  - Fibrous dysplasia
  - Fibroma group lesions
  - Central giant cell granuloma
  - Cherubism
  - Brown tumor of hyperparathyroidism

DISORDERS OF ORAL CAVITY

CONGENITAL AND INHERITED FOCAL DISORDERS: OVERVIEW

Oral cavity is lined by stratified squamous epithelium similar to skin. Epithelium of oral cavity varies in respect of keratinization. There are variations in the presence and absence of rete ridges. Rete ridges are absent in the floor of mouth. Rete ridges are well marked in the palate and gums.

CONGENITAL DISORDERS

Facial Clefts

Facial clefts are most common congenital disorders in the oral cavity about 1 per 800 live births. These occur due

to failure of fusion of facial processes at 7th week of gestation. This gap is normally bridged by ectomesenchyme. According to recent studies, abnormalities of growth factors may be linked to formation of facial clefts.

Harelip (Cleft Lip)

Harelip, also known as cleft lip, is the most common congenital defect of upper lip. Normally, on the 35th day of gestation, the frontal prominence fuses with the maxillary process to form the upper lip. Harelip results from failure of fusion of the lower part of the median nasal process with the maxillary process.

- Disturbance in gene expression interferes with proper fusion resulting in cleft lip, with or without cleft palate.
- In addition to multifactorial inheritance, developmental anomalies may occur due to teratogenic effects of rubella infection and anticonvulsants administration involving chromosomes during pregnancy. Harelip is unilateral in 80% of cases. About two-thirds of patients have left-sided harelip.

Cleft Palate

Cleft palate is less common congenital defect than harelip. It occurs in about 1 per 2500 live births. Unlike harelip, isolated cleft palate is more common in females. It occurs due to failure of fusion of facial processes due to multifactorial inheritance in which multiple genes interact with various environmental factors to produce disease.

Cleft palate is more common in Pierre Robin syndrome characterized by small mandible. Backward displacement of chin and displaced small tongue posteriorly cause upper airway obstruction. Cleft palate may occur as part of trisomies 13 and 18.

Hemifacial Hypertrophy

Hemifacial hypertrophy is characterized by rapid growth of both hard and soft tissues on one side of the face. The teeth on the affected side and half of tongue with hypertrophied papillae may occur.

INHERITED FOCAL DISORDERS

Fordyce's Granules

Fordyce's granules are small yellowish-white granules located on the lateral aspect of the vermilion of the upper lip or surface of the buccal mucosa in normal adults without clinical significance. These granules represent ectopic development of sebaceous glands.

Congenital Lip Pits

Congenital lip pits are small fistulae communicating the surface epithelium with underlying mucous glands. These are most often bilateral located on the lower lip. There may be isolated lesions or associated with cleft palate, harelip and agenesis of second premolar teeth. Later defect may be inherited as autosomal disorder.

Endogenous Melanin Pigmentation

Endogenous melanin pigment may be deposited on lips, oral mucosa and circumoral skin in various disorders, which include neurofibromatosis, fibrous dysplasia of bone, Peutz-Jeghers syndrome, hemochromatosis and Addison's disease. Peutz-Jeghers syndrome is characterized by pigmentation of the vermilion of the lips and small intestinal polyps.

Congenital Epulis

Congenital epulis refers non-neoplastic benign lesion of the gingivae especially in females. It is most often a reparative growth rather than a true neoplasm. On histologic examination, lesion is composed of aggregates of large granular cells covered by well differentiated squamous epithelium. Electron microscopy suggests that lesion either originates from fibroblasts or perivascular pericytes. These granular cells are negative for S-100 protein.

White Sponge Nevus

White sponge nevus is an autosomal dominant disorder and characterized by shaggy, white patches on the oral and buccal mucosae. The thickened squamous epithelium becomes edematous that easily desquamate. Histologic examination of lesion shows hyperplastic squamous epithelium, in which there is marked intracellular and extracellular edema.

INFLAMMATORY DISORDERS

Inflammatory disorders of oral cavity are caused by bacteria, viruses or fungi. Oral lesions may occur in various systemic disorders (Table 19.1).

ACUTE NECROTIZING ULCERATIVE GINGIVITIS (VINCENT ANGINA)

Acute necrotizing ulcerative gingivitis also known as Vincent angina, which represents an infection by two symbiotic organisms; one is a fusiform bacillus, and the other is a spirochete (*Borrelia vincentii*) found in oral cavity in normal persons. These organisms multiply under anaerobic conditions and cause tissue breakdown. Malnutrition promotes the risk for development of this disorder.

Clinical Features

Patient develops acute ulcerative necrotizing gingivitis in persons with decreased resistance to infection

Table 19.1 Oral lesions in various systemic disorders

Systemic Disorders	Mechanism	Oral Lesions
Pemphigus vulgaris	*Autoimmune disorder:* IgG antibodies directed against the intercellular area between keratinocytes	Vesicular lesions
Erythema multiforme	Hypersensitivity to sulphonamide drug	Involves oral mucosa
Addison's disease	Adrenal gland disorder	Oral pigmentation
Amyloidosis	Amyloid deposition in tongue	Macroglossia
Lichen planus	Skin disorder	Fine, lacy white lines on the mucosal surface called Wickham's striae
Lead poisoning	Toxic effect	Lead line in oral mucosa

as a result of inadequate nutrition, immunodeficiency, or poor oral hygiene. The ulceration tends to spread and involves all gingival margins, which becomes covered by a necrotic pseudomembrane.

LUDWIG ANGINA

Ludwig angina is potentially life-threatening inflammatory process. It is rapidly spreading cellulitis (phlegmon) in submaxillary or sublingual space. Normal oral bacterial flora is responsible for infection. These microorganisms cause cellulitis following tooth extraction, hairline fractures of lingual cortex of the mandible. By involving the fascial planes, infection may dissect into the parapharyngeal space, and then, into the carotid sheath.

EXUDATIVE PHARYNGITIS

Patient with *Streptococcus pyogenes* develops exudative pharyngitis and tonsillitis in 20–30% of cases. Patient presents with painful exudative lesions in the pharynx and tonsils.

CANDIDA ALBICANS (ORAL THRUSH)

Candida albicans is a common surface inhabitant of the oral cavity, gastrointestinal tract, and vagina. Patient develops oral thrush including tongue with white pseudomembrane consisting of keratinous debris and yeasts. It most commonly occurs in debilitated infants, children, diabetes mellitus and AIDS in 40–90% of cases. About 5–10% of neonates are affected as a result of infection acquired during delivery. In more severe cases, extensive white pseudomembrane formation occurs.

HERPES SIMPLEX VIRUS 1 INFECTION

Herpes simplex virus 1 infection starts with painful inflammation of the affected mucosa, followed shortly by the formation of vesicles due to ballooning degeneration of the epithelial cells. Shallow ulcers are formed as a result of rupture of vesicles. The ulcers heal spontaneously without scar formation. It tends to recur following trauma, sunshine, febrile illnesses or menstruation.

Age Group

Disease due to human simplex virus 1 affects both children and young adults.

Clinical Features

Patient presents with fever, stomatitis involving lips and oral mucosa, blisters on vagina known as herpes viral vaginalis and cold sores due to herpesvirus type 1.

Surgical Pathology: Herpes Simplex Virus 1 Infection

Light Microscopy

- Lesion in herpes simplex virus 1 infection shows intranuclear inclusion bodies in some epithelial cells.
- There is presence of multinucleated epithelial cells with ground glass homogenized nuclei, often exhibiting nuclear molding at the edge of the ulcer.

OTHER VIRAL INFECTIONS

Patient with coxsackievirus A infection develops vesicular lesions in oral cavity, hand and feet. Patient with Epstein-Barr virus infection develops exudative pharyngitis, leukoplakia located on the lateral side of tongue in HIV positive patients.

APHTHOUS STOMATITIS

Patient presents with recurrent painful erosive, solitary or multiple ulcerations in the mouth. It may or may not be associated with systemic disease. Etiology is unknown. Histologic examination shows a shallow ulcer covered by a fibrinopurulent exudate.

TUMORS OF ORAL CAVITY

WHO classification of tumors of the oral cavity is given in Table 19.2.

Table 19.2 WHO classification of tumors of the oral cavity

Epithelial Tumors and Lesions

- Squamous cell carcinoma
- Oral epithelial dysplasia
  - Low-grade dysplasia
  - High-grade dysplasia
- Proliferative verrucous leukoplakia
- Squamous cell papilloma
- Condyloma accuminatum
- Verruca vulgaris
- Multifocal epithelial hyperplasia

Tumors of Uncertain Histogenesis

- Congenital granular cell epulis
- Ectomesenchymal chondromyxoid tumor

Soft Tissue and Neural Tumors

- Granular cell tumor
- Rhabdomyoma
- Lymphangioma
- Hemangioma
- Schwannoma
- Neurofibroma
- Kaposi sarcoma
- Myofibroblastic sarcoma

Oral Mucosal Melanoma Salivary Gland Tumors

- Pleomorphic adenoma
- Mucoepidermoid carcinoma

Hematolymphoid Tumors

- CD30 positive T cell lympho-proliferative disorder
- Plasmablastic lymphoma
- Langerhans cell histiocytosis
- Extramedullary myeloid sarcoma

SQUAMOUS CELL PAPILLOMA

Squamous cell papilloma is the most common benign tumor of oral mucosa, most often seen on the tongue, lips, gingivae, or buccal mucosa.

SQUAMOUS CELL CARCINOMA

Approximately 95% of the cancers of the head and neck are squamous cell carcinomas. These most often occur in the oral cavity in older age group. Biologically, squamous cell carcinoma in oral cavity is similar to elsewhere in head and neck.

Locations

- Squamous cell carcinoma occurs in lower lip, floor of the mouth, tongue, the palatine and lingual tonsils. Ventrolateral surface of tongue and floor of the mouth are the most frequent sites involved accounting for >50% of cases. Least affected sites are dorsum of the tongue and hard palate.
- Oral mucosa with thin nonkeratinized squamous epithelium and poorly developed or absent rete ridges and narrow lamina propria are at higher risk for development of oral cancer. Locations of oral squamous cell carcinoma are shown in Fig. 19.1.

Predisposing Factors

Combined abuse of oral tobacco, snuff-dipping or betel nut chewing and alcohol predispose to oral cancer. Leukoplakia may predispose to oral cancer.

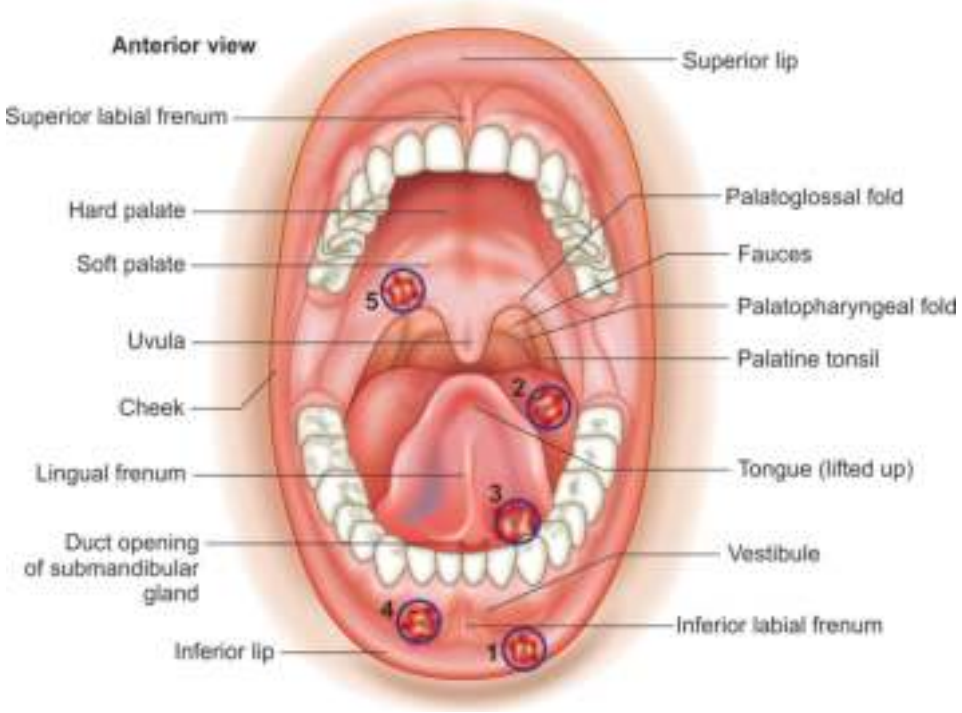


Fig. 19.1: Locations and frequency of oral carcinoma (1–5 most common to least common).

In 50% of cases, human papillomavirus 16 (HPV-16) is the etiological agent in oropharyngeal squamous cell carcinoma.

Molecular Pathogenesis

All cell lines derived from squamous cell carcinoma show multiple loss of chromosomes. Short arms of acrocentric chromosomes (13, 14, 15, 21 and 22) are deleted in 70% of cases. Cytogenetic changes in oral squamous cell carcinoma are given in Table 19.3.

Molecular pathogenesis of squamous cell carcinoma is a multistep process involving the sequential activation of oncogenes and inactivation of tumor suppressor genes in the clonal population of cells. At the molecular level, amplification and overexpression have been observed in epidermal growth factor receptor (EGFR), and apoptosis-inhibiting BCL-2 gene. The high frequency of these aberrations suggests that these genomic alterations play an important role in the pathogenesis of oral squamous cell carcinoma.

- **Transition from normal to hyperplasia:** First change is loss of chromosomal 9p21 and 3p during this process. Hypermethylation and loss of heterozygosity at this locus leads to inactivation of p16, an inhibitor of cyclin-dependent kinase.
- **Mild/moderate dysplasia to severe dysplasia:** Subsequent loss of heterozygosity at 17p with mutation of p53 tumor suppressor gene progresses to severe dysplasia in oral cavity.
- **Severe dysplasia to SCC:** Finally, amplification and overexpression of the cyclin D1 gene located on 11q13 and genomic deletions of 4q, 6p, 8p, 13q and 14q cause progression of severe dysplasia to squamous cell carcinoma in oral cavity.

Table 19.3 Cytogenetic changes in oral squamous cell carcinoma in descending order

Chromosomal Abnormality	Frequency
Chromosome 18q	100%
Chromosome 10p	80%
Chromosome 8p	70%
Chromosome 3p	60%
Chromosome 9p	–
Chromosome 17p	–
Chromosome 4p	–
Chromosome 6p	–
Chromosome 13q	–
Chromosome 14q	–

Surgical Pathology: Squamous Cell Carcinoma in Oral Cavity

Gross Morphology

- Gross morphology of tumor depends on stage of the presentation of oral carcinoma. Initial lesion is either opaque white or irregular warty areas.
- Later, the tumor undergoes ulceration. Ulcer has firm rolled edges and indurated bed.

Light Microscopy

- **Squamous cell carcinoma:** It ranges from well-differentiated keratinizing to poorly differentiated, sometimes shows sarcomatoid to basaloid morphology. Tumor may be slow to rapidly growing invading cervical lymph nodes. Low-grade well-differentiated squamous cell carcinoma is associated with smokeless tobacco (Figs 19.2 and 19.3).
- **Verrucous carcinoma:** It occurs in buccal mucosa and gums of lower jaw. Patient presents with bulky, soft, cauliflower-like growth that becomes infected giving foul smell. Under light microscopy, tumor shows well-differentiated carcinoma. Rete ridges are markedly thickened and growing down into underlying tissues in a characteristically bullous pattern. At a later stage, tumor invades stroma.

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in oral squamous cell carcinoma on histopathological sections.

Markers	Differentiation of Tumor	Expression
Cytokeratin 1,10	Well-differentiated keratinized SCC	Positive
Cytokeratin 4,13	Well-differentiated non-keratinized SCC	Positive
Cytokeratin 8,18	Poorly-differentiated SCC	Positive

Mode of Spread

- **Direct invasion:** Tumor has tendency to invade either deeply or widely within the subepithelial tissue. Invasion of mandible creates obstacle to complete surgical excision.



Fig. 19.2: Squamous cell carcinoma of lower lip (arrow).

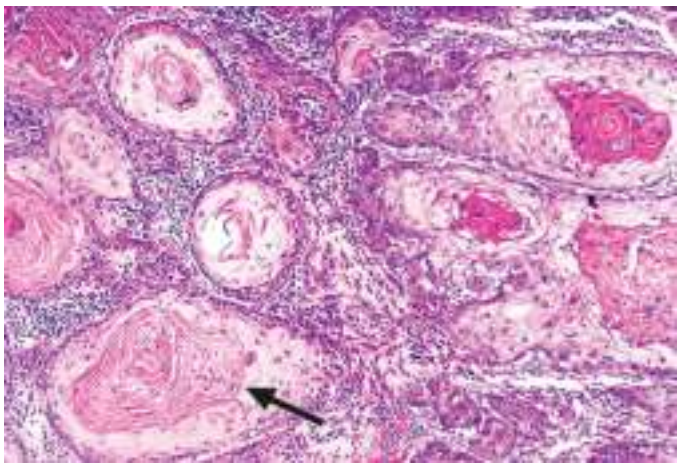


Fig. 19.3: Well differentiated squamous cell carcinoma (arrow) (400X).

- **Lymphatic route:** Lymph node metastases are more often present at the time of clinical presentation in patients with oral carcinoma. Lymph nodes are seen in the submandibular region. At later stage, tumor may metastasize to mediastinal lymph nodes.
- **Hematogenous route:** Oral carcinoma may metastasize to distant organs such as lungs, liver and bones via hematogenous route.

Prognosis

- Prognosis of oral carcinoma depends on location, depth of invasion, stage, histologic grading, desmoplastic reaction, tissue eosinophilia, lymph node metastases and DNA ploidy.
- Presence of florid desmoplastic reaction in squamous cell carcinoma of lip is associated with increased incidence of metastases and associated with aggressive behavior.
- Tumor infiltrated by eosinophils has favorable prognosis.
- Lymph node metastases are used in staging system. Extracapsular spread is an indicator of poor prognosis.
- The nondiploid tumors tend to be clinically more advanced than diploid tumors.
- Overexpression of p16 has favorable prognosis.
- Overexpression of p21 gene and TROP2, loss of H antigen and amplification of 3q26.3 is associated with poor prognosis. Survival rate and staging of oral carcinoma are given in Tables 19.4 and 19.5.

KAPOSI SARCOMA

Kaposi sarcoma occurs due to herpesvirus 8 in the oral cavity. It is most frequently associated with AIDS and located on the palate. It is locally aggressive endothelial tumor.

Surgical Pathology: Kaposi Sarcoma	
Light Microscopy	
Tumor is composed of bizarre-shaped blood vessels with stands of spindle cells and extravasated red blood cells.	
Immunohistochemistry Technique	
Lining cells of vascular structures are positive for CD31, CD34, podoplanin and nuclear expression of HHV8.	
Markers	**Expression**
▪ CD31 ▪ CD34 ▪ Podoplanin ▪ HHV8	▪ Positive ▪ Positive ▪ Positive ▪ Positive

Table 19.4 Overall 5-year survival rate of oral carcinoma

Location	Survival Rate
Lower lip	90%
Anterior aspect of tongue	60%
Posterior part of tongue, floor, tonsil, gum, hard palate	40%
Soft palate	20–30%

Table 19.5 Survival rate and staging of oral carcinoma

Stage	Tumor	Lymph Node Metastases	Distant Metastases	5-Year Survival
Stage 0	*Tis* (Tumor *in situ*)	N0	M0	100%
Stage I	T1 (≤2 cm)	N0	M0	90%
Stage II	T2 (>2 to 4 cm)	N0	M0	77%
Stage III	▪ T1, T2 ▪ T3 (>4 cm)	▪ N1 (≤3 cm) ▪ N0, N1	▪ M0 ▪ M0	61%
Stage IVA	▪ T1, T2, T3 ▪ T4a (advanced invading tissue)	▪ N2 (>3–6 cm single) ▪ N0, N1, N2	▪ M0 ▪ M0	32%
Stage IVB	▪ T4b ▪ Any T	▪ Any N ▪ N3 (>6 cm)	▪ M0 ▪ M0	25%
Stage IVC	Any T	Any N	M1	3.5%

Prognosis

Evolution of Kaposi sarcoma is modified by treatment that includes surgery, radiotherapy and chemotherapy.

EPITHELIAL PRECURSOR ORAL LESIONS

Leukoplakia

Leukoplakia is a clinical term describing irregular white mucosal patches of oral mucosa. On clinical examination, lesion may be homogenous, nodular, erythroplakia or proliferative verrucous leukoplakia. WHO criteria used in diagnosis of dysplasia of oral mucosa are shown in Table 19.6.

- Leukoplakia results from hyperkeratosis, usually secondary to chronic irritation. It is caused by oral tobacco products and excessive alcohol intake.
- Leukoplakia is usually a benign disorder. However, it may represent dysplasia (mild, moderate or severe or carcinoma *in situ*) progressing to squamous cell carcinoma. Oral intraepithelial neoplasia is categorized into grade I (mild dysplasia), grade II (moderate dysplasia) and grade III (severe dysplasia or carcinoma *in situ*). Biopsy is required to rule out squamous dysplasia or squamous cell carcinoma (Fig. 19.4).

Granuloma Pyogenicum

Granuloma pyogenicum is a reactive vascular lesion most commonly seen on tongue, lips, or buccal mucosa especially in pregnant women. Minor trauma to tissues in these regions permits invasion of microorganisms (Fig. 19.5).

Table 19.6 WHO criteria used in diagnosis of dysplasia of oral mucosa

Histologic Architecture
▪ Irregular epithelial stratification ▪ Loss of polarity of basal cells ▪ Drop-shaped rete ridges ▪ Increased number of mitoses ▪ Abnormally superficial mitoses ▪ Premature keratinization in single cells (dyskeratosis) ▪ Keratin pearls within rete ridges
Cytologic Features
▪ Anisocytosis (variation in size) ▪ Cellular and nuclear pleomorphism ▪ Anisonucleosis (abnormal variation in nuclear size) with prominent nucleoli ▪ Increased nucleocytoplasmic ratio ▪ Atypical mitotic figures

Patient presents with small <1.0 cm elevated, red or purple, soft mass, with a smooth, lobulated, ulcerated surface in the oral cavity most frequent on the gingiva.

Surgical Pathology: Granuloma Pyogenicum

Light Microscopy

- Histologic examination shows nodule composed of highly vascular granulation tissue that shows varying degrees of acute and chronic inflammation.
- Granuloma pyogenicum later transforms to less vascular mass resembling fibroma.



Fig. 19.4: Leukoplakia of the oral cavity (arrow).

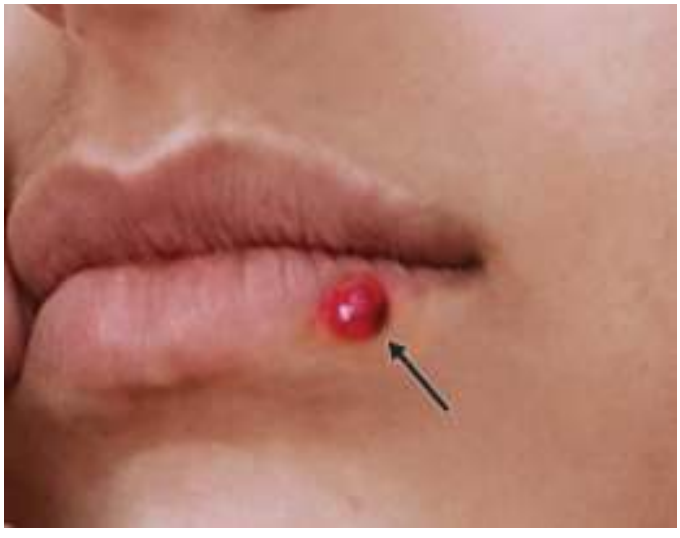


Fig. 19.5: Granuloma pyogenicum on the lip (arrow).

DISORDERS OF SALIVARY GLANDS

ANATOMY OF SALIVARY GLANDS

The salivary gland system comprises three pairs of major glands—parotid, submandibular and sublingual glands. Minor salivary glands are dispersed in the submucosa of oral cavity, lips, gingiva, floor of the mouth, cheek, hard and soft tongue, tonsillar areas and oropharynx (Figs 19.6 and 19.7).

- The functional unit of salivary glands is the secretory acinus and related ducts, and myoepithelial cells. Acini may be serous, mucous or mixed.
- The parotid glands are almost purely serous and the parenchyma is divided into lobules by fibrous septa. Parotid glands are divided into superficial and deep portions by the facial nerve. Each parotid gland drains via Stensen's duct and enters into the mouth.
- The submandibular glands are located in the groove between the lower jaw and tongue. These drain via Wharton's duct into the mouth.
- The sublingual glands are situated in the sublingual tissue of the mandible. Each gland drains via Bartholin's duct into the mouth. In some cases, sublingual gland's main excretory duct joins the main submandibular duct.

INFLAMMATORY DISORDERS

Sialadenitis may be caused by viral or bacterial infection, immune-mediated mechanisms, or occlusion of the salivary ducts by stones (sialolithiasis).

ACUTE SIALADENITIS

Acute sialadenitis is most often caused by bacterial or viral infection. Oral cavity is the source of bacterial

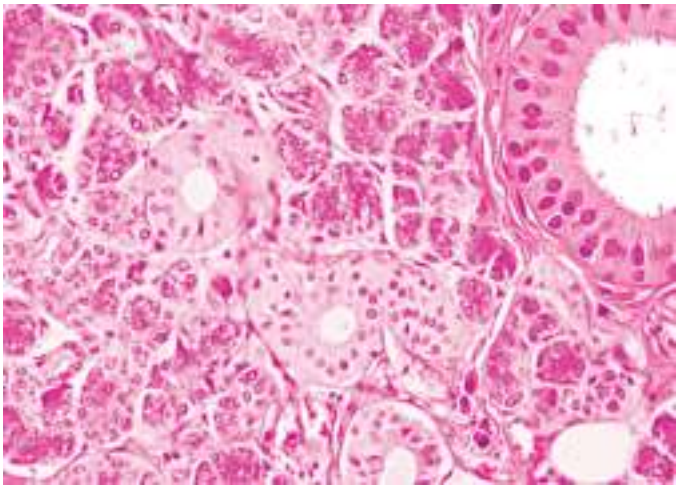


Fig. 19.7: Histology of salivary gland. Salivary gland is made up of secretory acini and ducts (400X).

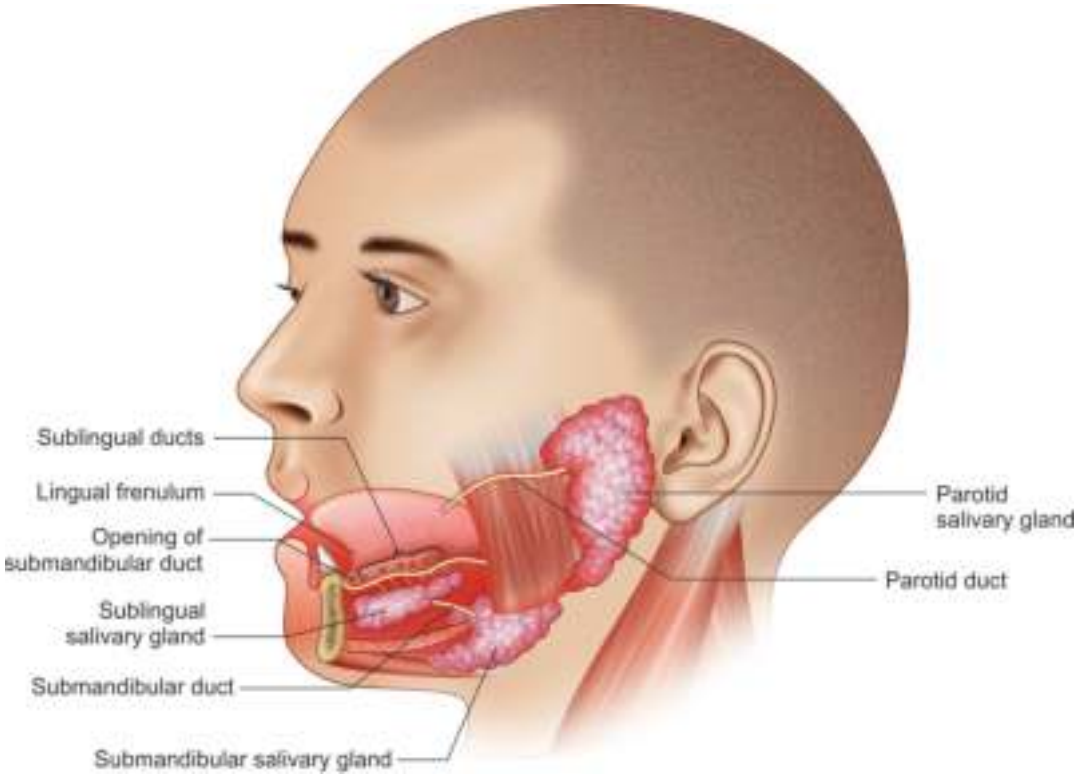


Fig. 19.6: Major salivary glands. These are salivary, submandibular and sublingual glands.

infection. Bacterial sialadenitis occurs when saliva flows through the affected duct is decreased due to salivary calculi. Acute sialadenitis is seen mainly in association with chronic xerostomia. Causative organisms include *Staphylococcus aureus* followed by *Streptococcus viridans*. Salivary gland shows purulent exudate and bacterial colonies. Patient presents with painful enlargement of one or both salivary glands. Examination of the duct orifices may show the presence of pus discharging into the oral cavity. Acute sialadenitis leads to abscess formation in salivary gland.

VIRAL SIALADENITIS

Mumps is the most common cause of deafness in 80% of cases among the postnatal viral infections, which causes bilateral parotitis with increased serum amylase most commonly in children <14 years of age. The incubation period is 16–18 days. Salivary gland shows infiltration by chronic inflammatory cells (Fig. 19.8).

Complications

Complications of viral sialadenitis include orchitis (unilateral; without sterility risk), oophoritis, aseptic meningitis (most common extrasalivary complication) and pancreatitis (increased serum amylase level).

AUTOIMMUNE SIALADENITIS

Autoimmune sialadenitis is relatively common. Sjögren's syndrome is a chronic inflammatory disease of the salivary and lacrimal glands. Sjögren's syndrome is characterized by keratoconjunctivitis sicca (dry eyes), and xerostomia (dry mouth), due to lymphocytic infiltration and parenchymal destruction of the parotid and lacrimal glands. It may be associated with a systemic collagen vascular disease. Malignant lymphoma is a frequent complication of autoimmune sialadenitis.

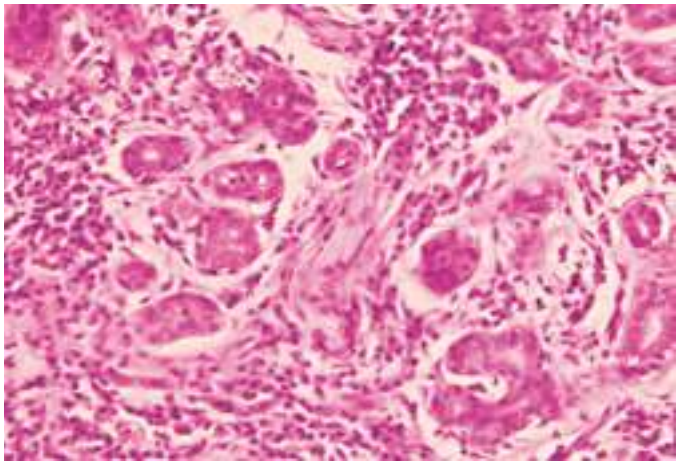


Fig. 19.8: Chronic sialadenitis. It shows infiltration by chronic inflammatory cells (400X).

Surgical Pathology: Autoimmune Sialadenitis

Light Microscopy

- Parotid and lacrimal glands show infiltration by lymphocytes.
- Late on, in the course of the disease, the affected glands become atrophic associated with fibrosis and fatty infiltration of the parenchyma.

Etiopathogenesis

Autoimmune sialadenitis is most likely to autoimmune origin associated with rheumatoid arthritis and Sjögren's syndrome. Autoantibodies demonstrated in Sjögren's syndrome are anti-SS (Ro) and anti-SS (La). Rheumatoid factor and antisalivary gland precipitinin are demonstrated in rheumatoid arthritis.

CHRONIC OBSTRUCTIVE SIALADENITIS

Chronic obstructive sialadenitis most often occurs in submandibular gland, producing a characteristic histologic morphology.

Surgical Pathology: Chronic Obstructive Sialadenitis

Light Microscopy

- Dilated ducts contain eosinophilic glycoproteins.
- Squamous metaplasia is demonstrated in ducts.
- There is expansion of interlobular connective tissue resulting in fibrosis.
- The acini show marked atrophy.
- A mild to moderate chronic inflammatory infiltrate is seen.

CHRONIC RECURRENT PAROTITIS

Chronic recurrent parotitis occurs between the ages of 5–15 years. Girls are more commonly affected than boys. Patient presents with swelling, tenderness over parotid glands, fever and malaise lasting for 7–10 days. Its etiopathogenesis is still not well known.

Surgical Pathology: Chronic Recurrent Parotitis

Light Microscopy

Affected parotid gland shows acinar atrophy and fibrosis.

CYSTIC DISORDERS

Mucocele

Mucocele (cyst-like pool of mucus) lined by granulation tissue, develops near a minor salivary gland. It results from mucus leakage caused by rupture of obstructed or traumatized ducts. Ranula is a large mucocele of salivary gland, characteristically localized to the floor of the mouth.

TUMORS OF SALIVARY GLANDS

Salivary gland tumors have diverse histopathology. These are relatively uncommon constituting 2–6% of all head and neck neoplasms. Their histologic and behavioral diversity as well as their proximity to important head and neck structures that pose considerable management challenges.

- Fine needle aspiration cytology is the popular method for diagnostic evaluation of salivary gland masses due to their superficial nature and easy accessibility for the procedure.
- The majority of tumors of the parotid gland are benign; in contrast, about 50% of the tumors of the submandibular gland are malignant. Pleomorphic adenoma is the most common benign salivary gland tumor. Mucoepidermoid carcinoma is the most common malignant tumor of salivary gland. Recurrence after surgical removal is common, because remnants of tumor may be left behind.
- The majority of salivary gland tumors occur in the parotid gland in 80% of cases overall. Approximately 80% of salivary gland tumors are benign and 20% of tumors are malignant.
- Approximately 15% of tumors occur in submandibular glands. Tumors may be benign in 50% and malignant in 50% of cases. About 5% of salivary gland tumors occur in submandibular glands. Around 40% of tumors are benign and 60% of tumors are malignant.
- Irradiation is only etiologic factor of salivary gland tumors. Adults or children receiving therapeutic irradiation in head and neck are at higher risk for development of salivary gland tumors. An increase in the incidence of salivary gland tumors has also been reported amongst survivors of the nuclear bomb attacks on Hiroshima and Nagasaki in Japanese population.
- A number of viruses have been implicated in the pathogenesis of salivary gland tumors. There is a strong association between Epstein-Barr virus (EBV) and lymphoepithelial carcinomas, but this appears to be largely restricted to Asian patients (Fig. 19.9).

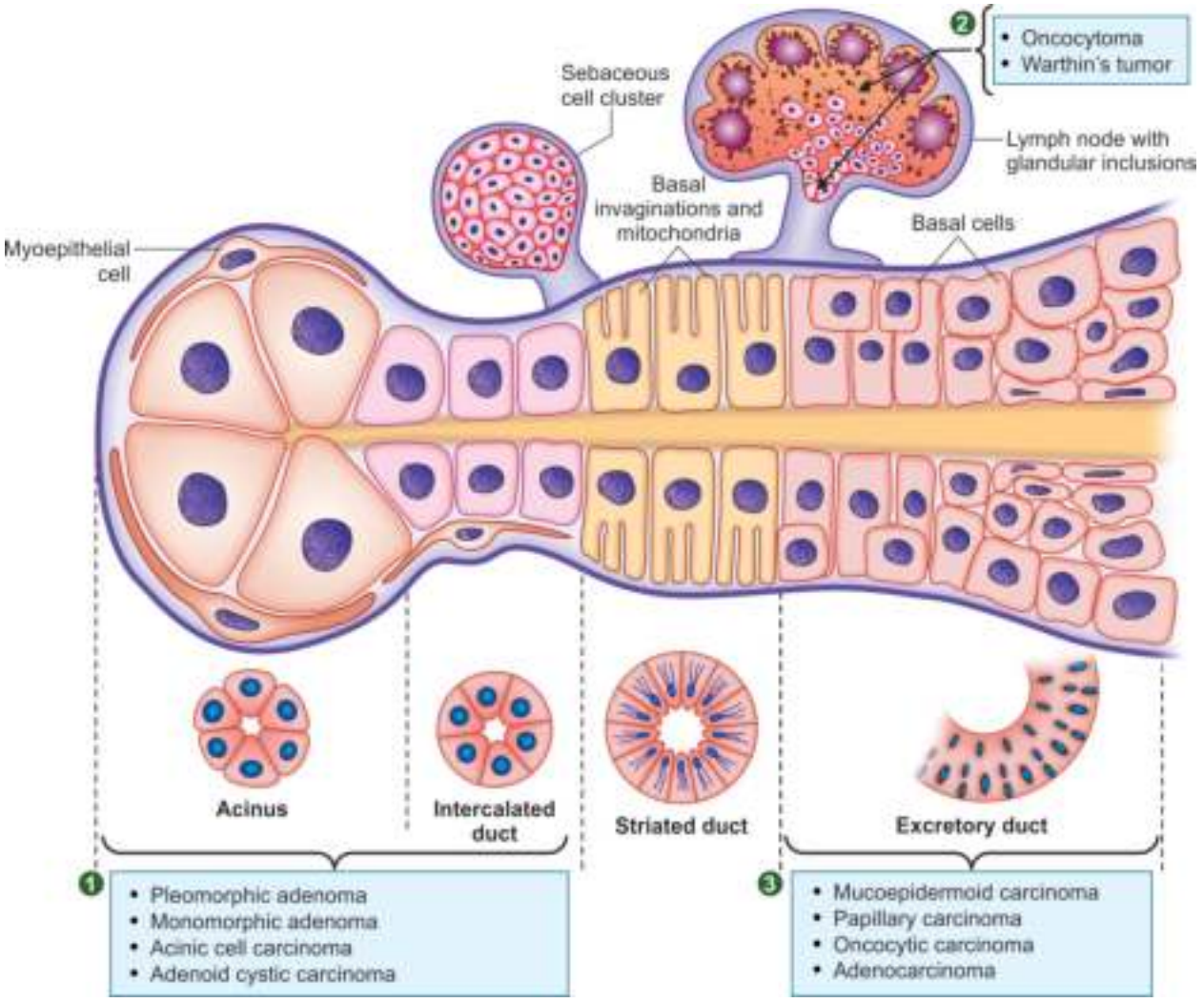


Fig. 19.9: Tumors of salivary glands.

The 2024 WHO classification of salivary gland tumors is given in Table 19.7. Salient features of malignant tumors of salivary glands are given in Table 19.8.

PLEOMORPHIC ADENOMA

Pleomorphic adenoma is commonest benign salivary gland tumor. It is slow-growing painless mass.

Table 19.7 The 2024 WHO classification of salivary gland tumors

Malignant Tumors

- Mucoepidermoid carcinoma
- Adenoid cystic carcinoma
- Acinic cell carcinoma
- Polymorphous adenocarcinoma
- Clear cell carcinoma
- Basal cell adenocarcinoma
- Intraductal carcinoma
- Adenocarcinoma, not otherwise specified
- Salivary duct carcinoma
- Myoepithelial carcinoma
- Epithelial-myoepithelial carcinoma
- Carcinoma ex pleomorphic adenoma
- Secretory carcinoma
- Sebaceous adenocarcinoma
- Carcinosarcoma
- Poorly differentiated carcinoma
  - Undifferentiated carcinoma
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma
- Lymphoepithelial carcinoma
- Squamous cell carcinoma
- Oncocytic carcinoma
- Uncertain malignant potential sialoblastoma

Benign Tumors

- Pleomorphic adenoma
- Myoepithelioma
- Basal cell adenoma
- Warthin tumor
- Oncocytoma
- Lymphadenoma
- Cystadenoma
- Sialadenoma papilliferum
- Ductal papillomas
- Sebaceous adenoma
- Canalicular adenoma and other ductal adenomas
- Sclerosing polycystic adenoma
- Keratocytoma
- Intercalated duct adenoma
- Striated duct adenoma

Non-neoplastic Epithelial Lesions

- Sclerosing polycystic hyperplasia
- Nodular oncocytic hyperplasia
- Lymphoepithelial sialadenitis
- Intercalated duct hyperplasia

Benign Soft Tissue Lesions

- Hemangioma
- Lipoma/sialolipoma
- Nodular fasciitis

Hematolymphoid Tumors

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma).

Contd...

Table 19.8 Salient features of important malignant tumors of salivary glands

Tumor	Sites	Age Group	Molecular Genetics	Features	Light Microscopy	Immunomarkers
Muco-epidermoid carcinoma	Parotid gland and minor salivary glands	Young to elderly	Translocation 11q21, 19p13	Slow growing mass, spreads by direct invasion, lymphatic and hematogenous route	Low- to high-grade	MUC4 positivity better prognosis
Adenoid cystic carcinoma	Submandibular, minor salivary and parotid glands	5th decade	Losses of 12p, 6p23q, 13q21	Tumor invades perineural space	Cribriform or solid pattern	CEA, keratin, S-100 protein, muscle specific actin and Ki-67
Acinic cell carcinoma	Parotid glands	5th decade	–	Clinical course variable, spreads via lymphatic and hematogenous route	Solid or papillary cystic or follicular pattern	The acinic cells show PAS positive diastase resistant granules. These cells are negative for mucin stains
Polymorphous low-grade carcinoma	Minor salivary glands	4th decade	–	Mass in palate	Tumor cells forming glandular structures	Keratin, vimentin, S-100 protein, CEA, GFAP, muscle specific actin, EMA, galectin 3 and BCL-2

Parotid gland is the most common site followed by submandibular gland and minor salivary glands. It was called mixed salivary gland tumor because the stroma is showing areas mimicking cartilaginous differentiation.

Pleomorphic adenoma has a biphasic appearance, which represents an admixture of epithelial and chondromyxoid stromal elements. Mesenchymal chondroid areas occur due to metaplastic changes in pleomorphic adenoma. It rarely becomes malignant.

Age and Sex Distribution

Pleomorphic adenoma affects between 40 and 60 years of age. It is more common in women than men (4:1).

Location

Pleomorphic adenoma most frequently occurs in major salivary gland usually in superficial region of parotid gland above the facial nerve in 90% and deep region of parotid gland below facial nerve in 10% of cases. It also occurs in submandibular gland in 45% of cases and minor salivary glands, e.g. palate, upper lip and mucosa in 45% of cases.

Molecular Genetic Alterations

Chromosomes 3p21, 8q12 and 12q13-15 rearrangements and the PLAG-1 and HMGI-C genes are demonstrated in pleomorphic adenomas.

Clinical Features

Patient presents with slow growing, painless and firm nontender mass in parotid gland. Lateral palate is the most common site in minor salivary gland. In all locations, small tumors are nontender and mobile on palpation but may become fixed with large growth. Facial nerve paralysis in association with pleomorphic adenomas almost never occurs, even with extremely large tumors.

Surgical Pathology: Pleomorphic Adenoma

Gross Morphology

- Pleomorphic adenoma in parotid gland is encapsulated smooth or lobulated tumor, clearly demarcated from the surrounding normal salivary gland.
- The tumor often shows small projections protruding through capsule. If the tumor is enucleated, these projections are left behind resulting in recurrence of tumor after surgical excision.
- Cut surface is solid, rubbery and containing areas of gelatinous myxoid stroma.
- Pleomorphic adenoma arising in minor salivary glands is never encapsulated (Figs 19.10 and 19.11).

Light Microscopy

There is considerable variation in the histologic features within single tumor. Therefore, multiple samples are necessary to study the complete morphology of the tumor. Tumor may be composed of epithelial and stromal component in varying proportions.

- **Epithelial component of pleomorphic adenoma:** Epithelial component is believed to originate from intercalated ducts. Tumor is composed of gland-like epithelial component surrounded by a myxomatous stroma often containing foci of bone or cartilage. Epithelial cells are arranged in nests, solid sheets, some forming duct-like structures.
- **Stromal component of pleomorphic adenoma:** The epithelial cells are embedded in chondromyxoid stroma. The stroma shows accumulation of basophilic material with the staining reaction of connective tissue mucins. The myxoid stroma may be bulky invaded by stromal cells.
- **Other findings in pleomorphic adenoma:** It may show foci of mucinous and squamoid differentiation mimicking mucoepidermoid carcinoma. It may also show adenoid cystic-like pattern.



Fig. 19.10: Pleomorphic adenoma of parotid gland. The multilobulated appearance of this tumor is shown. It must be removed together with some surrounding parotid gland, or else some of the irregular projections around its margins will be left behind and the tumor will recur.

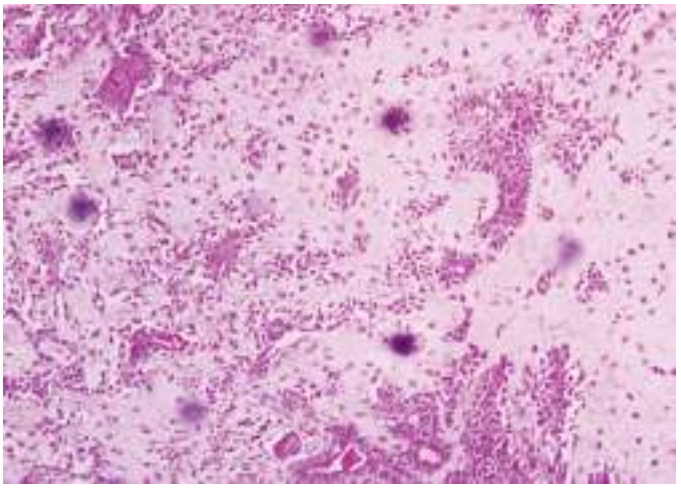


Fig. 19.11: Pleomorphic adenoma of salivary gland. Tumor is composed of mixtures of epithelial component forming duct-like structures, myoepithelial/stromal cells within myxoid stroma, and foci of chondroid/osseous metaplasia (400X).

Malignant Potential

Minimally invasive carcinoma is defined as an extension of <1.5 mm beyond capsule of pleomorphic adenoma and associated with an excellent prognosis. Malignancy in pleomorphic adenoma encompasses these entities: carcinoma ex pleomorphic adenoma and carcinosarcoma. Staining for HER2/neu can be used to detect early development of carcinoma in pleomorphic adenoma. Carcinosarcoma has been associated with poor prognosis.

Metastasizing Pleomorphic Adenoma

Metastasizing pleomorphic adenoma is defined as a histologically benign pleomorphic adenoma that manifests local or distant metastases in multiple organs such as bone, lung, lymph nodes and rarely kidneys.

Surgical Intervention

Often, pleomorphic adenoma is difficult to excise completely because of its proximity to the facial nerve. It is likely to recur after resection due to tumor spillage in the wound. Recurrence of pleomorphic adenoma represents local growth in the form of multiple nodules within fibrous and adipose tissue and does not reflect malignancy.

WARTHIN'S TUMOR

Warthin's tumor accounts for 8% of all tumors in the parotid glands. It is a multicentric tumor, also known as **papillary cystadenoma lymphomatosum** or **adenolymphoma**. The term adenolymphoma should not be confused with any form of lymphoma. Warthin's tumor is monomorphic adenoma with heavy lymphocyte infiltrate in the stroma. Recurrence after excision of tumor is rare. Rarely, squamous cell carcinoma or lymphoma may arise in Warthin's tumor.

Origin

Warthin's tumor most often occurs in parotid glands. It arises from hydrotropic salivary duct epithelium within immediately subjacent lymph node or it may be due to cystic changes of the epithelium in the inflammatory infiltrate.

Age and Sex Predilection

Warthin's tumor is the only tumor of the salivary glands, which is more common in men (especially tobacco smokers) than in women. It generally occurs after the age of 30 years, with most arising after age 50 years. Bilateral tumors occur in 10% of cases.

Molecular Genetic Alterations

Translocations of chromosomes 11q21 and 19p13 are demonstrated in Warthin's tumor.

Clinical Features

Patient presents with slow-growing painless firm or rubbery or nodular mass. A few patients may present with rapidly growing tumor associated with pain or pressure symptoms.

Imaging Technique

Radionuclide scanning (Tc 99m) is helpful in diagnosing patients with Warthin's tumor and oncocytoma.

Surgical Pathology: Warthin's Tumor

Gross Morphology

- Tumor forms soft well-circumscribed encapsulated brown mass usually located superficially within the parotid gland.
- Cut surface shows irregular multiple cysts of variable diameter containing variably viscous fluid.
- The cyst fluid has a characteristic motor oil quality.
- The lymphoid component makes up the solid areas of the tumor (Figs 19.12 and 19.13).

Light Microscopy

Tumor is composed of mixture of epithelial and lymphoid elements.

- **Epithelial element:** Clefts and cysts are lined by pink staining double layer epithelium. Inner layer consists of tall columnar cells with granular cytoplasm (oncocytes) towards the luminal aspect lining the cystic spaces. Outer layer is composed of cuboidal cells along the basement membrane. Electron microscopy reveals presence of large number of mitochondria accounting for the granular cytoplasm, suggesting common feature for oncocytes in all epithelial tissues.
- **Stromal lymphoid tissue:** The epithelial cells are forming papillary projections into cystic glandular spaces embedded in dense stromal lymphoid tissue. The stroma contains florid lymphoid infiltrate in which some normal lymphoid follicles with germinal centers are present. Malignancy may develop either in the epithelial or lymphoid component.
- **Other findings:** Occasional goblet cells may be present within the epithelium mimicking oncocystic mucoepidermoid carcinoma. Squamous metaplasia with atypia may mimic squamous cell carcinoma if biopsy taken after fine needle aspiration.

Differential Diagnosis

It is worth mentioning that oncocytes are also demonstrated in oncocytic tumors. Lymphoid aggregates are demonstrated in lymphoepithelial cyst, chronic sialadenitis and Sjögren's syndrome. Differences between pleomorphic adenoma and Warthin's tumor of salivary glands are given in Table 19.9.

Therapeutic Correlation

Warthin's tumor is treated by surgical resection with preservation of facial nerve.



Fig. 19.12: Warthin's tumor (adenolymphoma) of the parotid gland. Cut surface of this benign tumor is multicystic. The cysts are filled with mucus. It is the second most frequent tumor of the parotid gland, after pleomorphic adenoma.

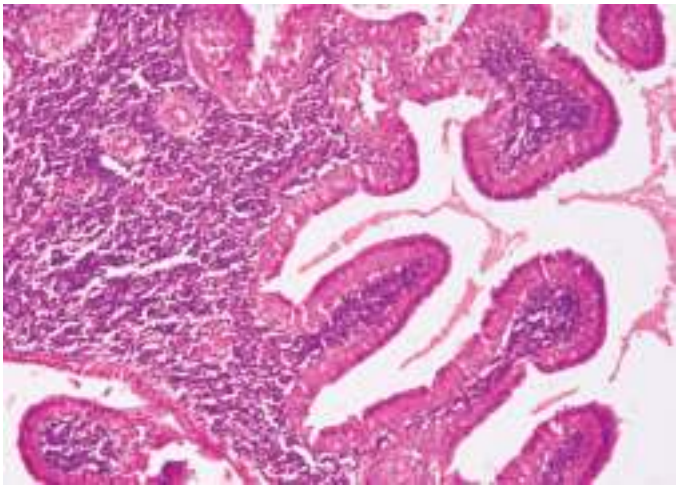


Fig. 19.13: Warthin's tumor. Tumor is composed of mixture of epithelial and lymphoid elements. Clefts and cysts are lined by pink staining double layer epithelium. These epithelial cells are forming papillary projections into cystic glandular spaces embedded in dense lymphoid tissue (400X).

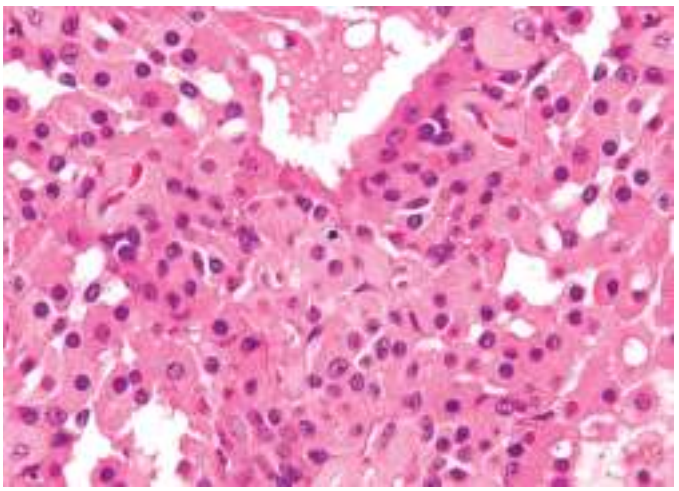


Fig. 19.14: Oncocytoma. Tumor is composed of cords of uniform polyhedral cells with distinct cell membrane, granular eosinophilic cytoplasm, central round vesicular nuclei, and thin fibrous stroma (400X).

ONCOCYTOMA

Oncocytoma is rare benign salivary gland tumor that affects elderly men and women. Recurrence rate is low after surgical excision (Fig. 19.14).

Site of Origin

Oncocytoma is mainly located in parotid gland in 80% of cases, submandibular gland in 10% of cases and rest in minor salivary glands (palate, buccal mucosa or tongue).

Clinical Features

Patient presents with slow-growing painless, firm, mobile, and nontender mass in superficial lobe of parotid gland.

Table 19.9 Differences between pleomorphic adenoma and Warthin's tumor of salivary glands

Parameters	Pleomorphic Adenoma	Warthin's Tumor
Origin	Intercalated ducts	Striated ducts
Glands involved	Parotid gland and minor salivary glands, e.g. palate, upper lip and mucosa (10%)	Parotid gland
Frequency	70–80%	10%
Sex predilection	Females preponderance	Males > females
Number	Solitary (90%)	Solitary (90%) and multiple (10%)
Laterality	Unilateral (most often)	Unilateral (most often)
Clinical features	Nodular firm inconsistency	Smooth soft and cystic lesion
Histology	Epithelial cells are embedded in chondromyxoid stroma	Double layer pink staining epithelium and lymphoid aggregates
Recurrence	Common after surgical excision	Uncommon after surgical excision

Surgical Pathology: Oncocytoma of Salivary Glands
Gross Morphology
Tumor is homogenous with a smooth surface. It may be divided into lobules by fibrous septae.
Light Microscopy
▪ Tumor is composed of uniform epithelial cells arranged in sheets or cords. ▪ Tumor cells contain abundant mitochondria constituting 60% of the cell volume, which impart a brightly eosinophilic look.
Electron Microscopy
Tumor cells contain hyperplasia of mitochondria constituting 60% of cell volume.

MYOEPITHELIOMA

Myoepithelioma is a rare benign tumor arising in parotid gland and minor salivary glands (hard or soft palate). It is occasionally seen in the submandibular glands. Local recurrence is more common than pleomorphic adenoma after surgical excision. It equally affects men and women over 50 years of age. Patient presents with slow-growing asymptomatic mass.

Surgical Pathology: Myoepithelioma	
Gross Morphology	
Tumor is well circumscribed similar to a pleomorphic adenoma but without the myxoid stroma.	
Light Microscopy	
Histologic variants of myoepithelioma include spindle cell type, hyaline type, plasmacytoid type or clear cell type. ▪ **Spindle cell variant of myoepithelioma:** It occurs in parotid gland. It is composed of spindle cells arranged in sheets and cords. ▪ **Plasmacytoid pattern of myoepithelioma:** It is less common but the most frequently encountered pattern in palate tumors. ▪ **Combined pattern of myoepithelioma:** It demonstrates a combination of the spindle and plasmacytoid cells. This variant is uncommon.	
Immunohistochemistry Technique	
Panel of immunomarkers is used to analyze expression in myoepithelioma on histopathologic sections.	
Markers	**Expression**
▪ S-100 protein ▪ Pancytokeratin ▪ Smooth muscle actin ▪ p63	▪ Positive ▪ Positive ▪ Positive ▪ Positive

MONOMORPHIC ADENOMA

Monomorphic adenoma is rare salivary gland tumor affecting 60–70 years of age group. Histologic variants of monomorphic adenoma include basal cell adenoma (most common in parotid gland), canalicular adenoma (minor salivary glands multifocal in origin), sebaceous adenoma, glycogen-rich adenoma and clear cell adenoma. Tumor is easily excised. Recurrence is rare after surgical excision.

Clinical Features

Patient presents with slow growing painless firm to fluctuant 1–2 cm nodule.

Surgical Pathology: Monomorphic Adenoma	
Gross Morphology	
Tumor is well circumscribed solid to cystic without capsule. Cut surface is tan to pink.	
Light Microscopy	
Monomorphic adenoma shows various histologic patterns. ▪ Monomorphic adenoma is composed of cords of cells forming duct-like structures in a background of fibrous stroma. ▪ Canalicular adenoma is composed of two rows of cells opposed to each other giving beaded appearance.	
Immunohistochemistry Technique	
Panel of immunomarkers is used to analyze expression in canalicular adenoma on histopathological sections.	
Markers	**Expression**
▪ S-100 protein ▪ Cytokeratin	▪ Positive ▪ Positive

MUCOEPIDERMOID CARCINOMA

Mucoepidermoid carcinoma is most common malignant tumor of the salivary glands. It most often occurs in the parotid glands. It may arise in minor salivary glands. Mucoepidermoid carcinoma is characterized by mucous, intermediate, epidermoid cells, columnar, clear cells and oncocytic features. All mucoepidermoid carcinomas are potentially malignant with metastatic potential regardless of their microscopic appearance.

Age Group

Mucoepidermoid carcinoma occurs more frequently in women than in men that affects of tumor young to elderly persons.

Molecular Genetic Alterations

Translocations of chromosomes 11q21 and 19p13 are demonstrated in mucoepidermoid carcinoma resulting in MECT-MAMAL2 fusion gene. The median survival of MECT-MAMAL2 fusion gene positive patients is >10 years. On the other hand, median survival of fusion gene negative patients is 1.6 years. Elevated HER2/neu gene expression and gene amplification have been demonstrated in mucoepidermoid carcinoma.

Clinical Features

Patient presents with slow-growing asymptomatic mass in salivary glands, very similar to that of benign tumor.

Surgical Pathology: Mucoepidermoid Carcinoma

Gross Morphology

Tumor is well circumscribed partially encapsulated with infiltrative edges. Cut surface is firm, smooth with cystic areas.

Light Microscopy

Mucoepidermoid carcinoma may be of low, intermediate or high grade. Histologic features and grading mucoepidermoid carcinoma are based on cystic appearance, necrosis, neural invasion, mitotic figures and anaplasia (Tables 19.10 and 19.11).

- **Low-grade mucoepidermoid carcinoma**: Tumor is composed of mixture of squamoid (epidermoid), mucus-secreting cells (eccentric nuclei). Squamoid cells are less than mucus cells. The proportion of different cell types and their architectural configuration (including cyst formation) varies. Tumor cells are arranged in sheets, nests and duct-like patterns. Keratin pearl formation may be seen in occasional case (Fig. 19.15).
- **Intermediate-grade mucoepidermoid carcinoma:** Tumor is composed of squamoid and mucus cells in equal proportion. Tumor cells show pleomorphism and are arranged in sheets, nests and duct-like patterns. Many mitotic figures may be demonstrated (Fig. 19.16).
- **High-grade mucoepidermoid carcinoma:** Tumor shows solid growth pattern with predominant epidermoid cells and increased cellularity with cellular atypia and frequent mitotic figures. It may be mistaken for squamous cell carcinoma (Fig. 19.17).

Differential Diagnosis

Differential diagnosis of mucoepidermoid carcinoma includes pleomorphic adenoma, acinic cell carcinoma and necrotizing sialometaplasia.

Histochemistry

Positive histochemical staining for mucin is seen in mucoepidermoid carcinoma rather than squamous cell carcinoma. Tumor with MUC4 positivity has better prognosis than MUC1 positivity.

Immunohistochemistry Technique

Low-grade mucoepidermoid carcinoma shows positivity for CK7 and CK14. Epidermoid cells and intermediate cells express p63 and p40.

Mode of Spread

Mucoepidermoid carcinoma may spread by direct invasion, lymphatic route and hematogenous route, i.e. lung, liver, bone and brain (Table 19.12).

Prognosis

Low-grade mucoepidermoid carcinoma has an excellent prognosis with 5-year survival in 90%, but high-grade tumors are associated with 5-year survival in 20–40% of cases.

Table 19.10 Histopathologic features and scores of mucoepidermoid carcinoma of salivary gland

Light Microscopic Findings	Scoring System
Cystic component >20%	2
Neural invasion	2
Necrosis	3
Mitotic figures 4 or greater than/high power field	3
Anaplasia	4

Table 19.11 Tumor grading and scoring of mucoepidermoid carcinoma

Tumor Grade	Scoring System
Low-grade neoplasm	0–4
Intermediate-grade neoplasm	5–6
High-grade neoplasm	7 or more

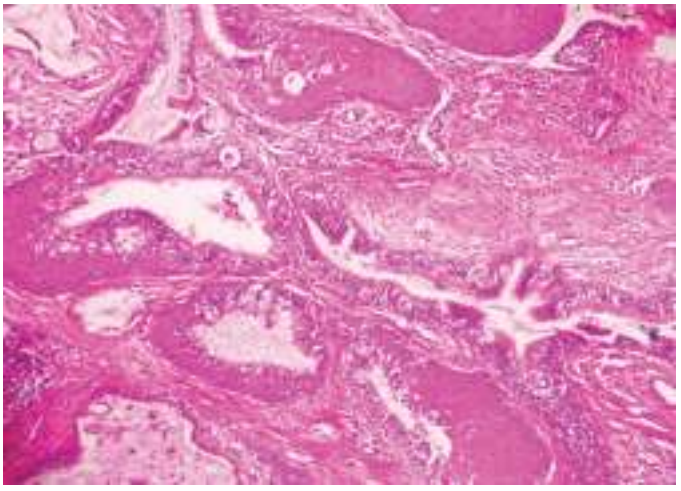


Fig. 19.15: Mucoepidermoid carcinoma (low-grade tumor). It is chiefly composed of mucus cells. Epidermal cells are less than mucus cells (400X).

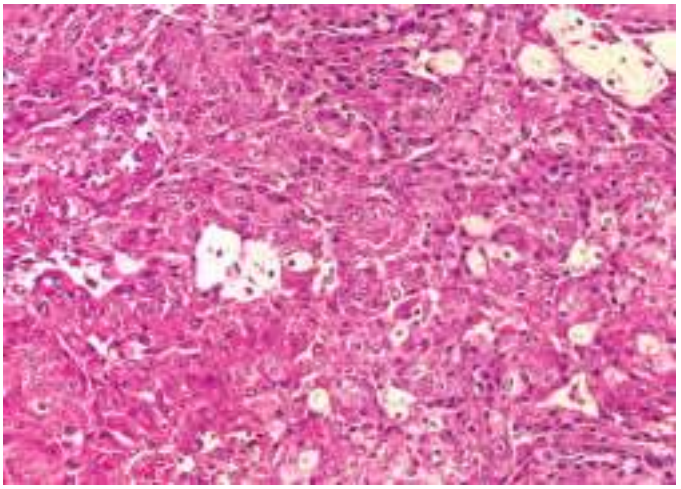


Fig. 19.16: Mucoepidermoid carcinoma (intermediate-grade tumor). It is composed of mucus and squamoid cells in equal proportion. Tumor cells are pleomorphic and showing mitotic figures (400X).

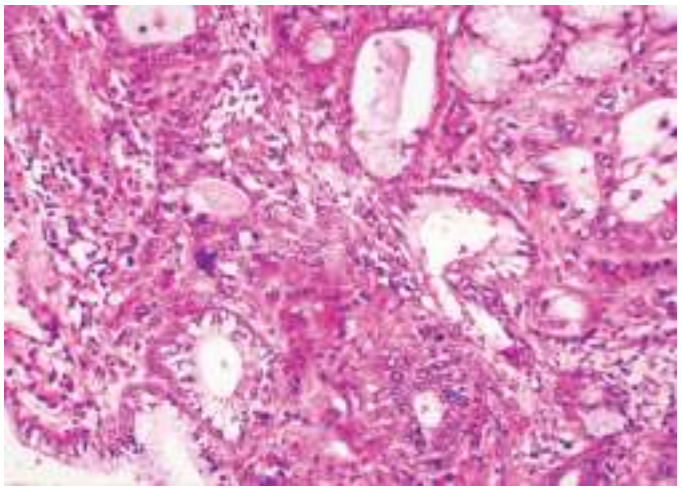


Fig. 19.17: Mucoepidermoid carcinoma (high-grade tumor). It is chiefly composed of squamoid cells and smaller population of mucus cells (400X).

ADENOID CYSTIC CARCINOMA

Adenoid cystic carcinoma is second most common malignant tumor of salivary gland. It most often invades perineural spaces (Figs 19.18 to 19.20).

- Tumor grows in cribriform and/or solid pattern. Solid pattern is associated with poor prognosis.



Fig. 19.18: Adenoid cystic carcinoma. It is well circumscribed solid tumor light tan with infiltrative margins.

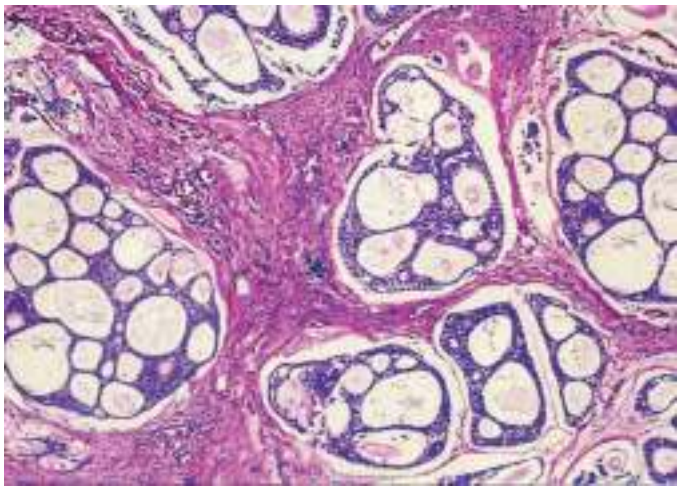


Fig. 19.19: Adenoid cystic carcinoma. It is composed of inner ductal cells and outer myoepithelial cells (400X).

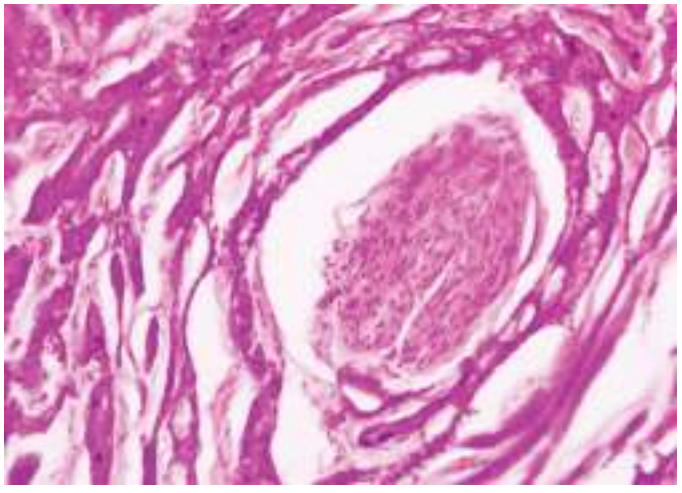


Fig. 19.20: Adenoid cystic carcinoma. It shows perineural invasion (400X).

Biphasic tumor shows ductal and myoepithelial differentiation.

- Tumor has a relentless clinical course with fatal outcome. Growth pattern is most commonly infiltrative and hence recurrence is common due to perineural invasion after surgical excision.

Table 19.12 Mode of spread and locations of mucoepidermoid carcinoma of salivary gland

Location of Tumor	Mode of Spread	Metastases
Parotid gland (most common site)	Lymphatic route	Pre-auricular and submandibular lymph nodes
Submandibular gland	Lymphatic route	Submandibular and upper jugular lymph nodes
Palate region	Direct invasion	Upper respiratory tract and base of skull
Lip region	Lymphatic route	Submental lymph nodes
Oral cavity	Lymphatic route	Submandibular, post-auricular and upper accessory nodes in neck level II then to levels III, IV and V

There may be widespread metastases to lung, liver, bone, and brain via hematogenous route.

Location

Adenoid cystic carcinoma occurs in submandibular gland (15% of cases), parotid gland (2% of cases), sublingual and minor salivary glands (15–20% of cases). It is a slow-growing tumor with extensive perineural invasion causing pain.

Age and Sex Predilection

Tumor affects equally men and women with peak in the 5th decade. It tends to recur after surgery.

Molecular Genetic Alterations

Frequent losses at 12q (33%), 6q23-q, 13q21-q22 and 19q have been reported in 40% of cases. About 60–90% of tumors carry MYB-NF1B and MYBL1-NF1B fusion.

Clinical Features

Patient with adenoid cystic carcinoma in major salivary gland especially in submandibular gland presents with asymptomatic slow-growing mass but highly malignant neoplasm followed by pain due to perineural invasion.

- Hypoglossal nerve involvement by tumor leads to impairment of tongue movements.
- Lingual nerve invasion by tumor causes tingling sensation of tongue. Facial nerve paralysis may also occur.
- Minor salivary gland adenoid cystic carcinoma is characterized by a submucosal mass with or without pain and ulceration.

Surgical Pathology: Adenoid Cystic Carcinoma

Gross Morphology

Tumor is well circumscribed but without capsule. It is invariably infiltrative tumor invading surrounding normal tissue. Despite its name, it is solid tumor that rarely displays obvious cystic spaces on cut surface.

Light Microscopy

Tumor is a basaloid tumor consisting of epithelial and myoepithelial cells in variable morphologic configurations. It shows two patterns: cribriform (most common), tubular or solid.

- **Cribriform pattern of tumor:** In adenoid cystic carcinoma, if the cystic spaces are large and numerous, it is termed cribriform pattern. It is often referred to as swiss cheese pattern. The tumor cells are small monomorphic with scant cytoplasm arranged in nests around cylindrical spaces that may contain mucinous or hyalinized material.
- **Solid pattern of tumor:** Adenoid cystic carcinoma is composed of sheets of tumor cells with no intervening spaces. The tumor is graded as 1, 2 and 3. Grade 1 tumor is composed of tubular and cribriform pattern. Grade 2 tumor contains 30–70% solid areas. Grade 2 tumor comprising >70% solid areas has poor prognosis.

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in adenoid cystic carcinoma on histopathological sections.

Markers	Expression
Ductal cells	
▪ CK-7	▪ Positive
▪ CAM 5.2	▪ Positive
Myoepithelial markers	
▪ Smooth muscle actin (SMA)	▪ Positive
▪ S-100 protein	▪ Positive
▪ Calponin	▪ Positive
▪ p40	▪ Positive
▪ p63	▪ Positive
▪ GFAP	▪ Positive
▪ Cytokeratins	▪ Positive

Differential Diagnosis

Differential diagnosis of adenoid cystic carcinoma includes basaloid squamous carcinoma, polymorphous low-grade adenocarcinoma, epithelial-myoepithelial carcinoma and membranous variant of basal cell adenoma.

Mode of Spread

- **Direct invasion:** Adenoid cystic carcinoma invades surrounding connective tissue involving perineural spaces. Tumor may permeate bones and penetrate through nose or antrum.
- **Hematogenous route:** Adenoid cystic carcinoma may metastasize to distant organs especially lungs.

Prognostic Factors

Prognostic factors that influence survival include histologic patterns, location of tumor, clinical stage, bone involvement and status of surgical margins.

- Tubular and cribriform patterns of adenoid cystic carcinoma have less aggressive course.
- Tumor with solid pattern of cells, tumor suppressor p53 gene mutation and foci of dedifferentiation has been associated with poor prognosis.

Therapeutic Correlation

Patient is treated by complete surgical excision of tumor and postoperative radiation therapy. Since tumor shows perineural invasion, hence facial nerve is also sacrificed along with tumor.

ACINIC CELL CARCINOMA

Acinic cell carcinoma is rare malignant tumor accounting for 2–3% of tumors in parotid glands, in which at least neoplastic cells demonstrate serous acinar cell differentiation with PAS-diastase positive zymogen

secretory granules. Salivary ductal cells are also present as a component of this neoplasm. Approximately 80–90% of these tumors occur within parotid glands.

Age Group

Acinic cell carcinoma is low-grade malignancy of salivary glands especially in children and young age group. Peak incidence is seen in the 5th decade especially women.

Clinical Features

Patient presents with slow-growing asymptomatic tumor in parotid glands. Acinic cell carcinoma may transform to high-grade adenocarcinoma or poorly differentiated carcinoma that presents with rapid growth, pain and facial nerve palsy. Clinical course of acinic cell carcinoma is characterized by metastases in 8–10% and local recurrence after surgical excision in 12–20% of patients. Treatment is surgical excision.

Surgical Pathology: Acinic Cell Carcinoma

Gross Morphology

- Tumor is well circumscribed without a capsule measuring 1–3 cm in largest dimension.
- Cut surface appears lobular with gray-white, friable, solid and cystic areas.

Light Microscopy

- Tumor is composed of acinic cells arranged in solid or papillary cystic or follicular pattern. The acinic cells show close resemblance to serous acini of salivary glands.
- Tumor cells show differentiation towards acinar cells and ductal cells; but lack myoepithelial differentiation. But there is loss of normal lobular architecture and lacking normal ducts in the tumor nodule.
- The surrounding stroma often shows presence of lymphoid infiltrate.
- Acinic cell carcinoma is graded based on nuclear and architectual features (Fig. 19.21).

Histochemistry

The acinic cells show PAS positive diastase resistant granules. These cells are negative for mucin stains.

Mode of Spread

Acinic cell carcinoma initially metastasizes to cervical lymph nodes and subsequently to more distant organs especially in the lungs.

Prognostic Factors

Histologic grading has been unsuccessful to predict prognosis. Poor prognostic factors include necrosis, extraglandular extension, increased pleomorphism, high mitotic rate, and prominent infiltration of nerves and blood vessels.

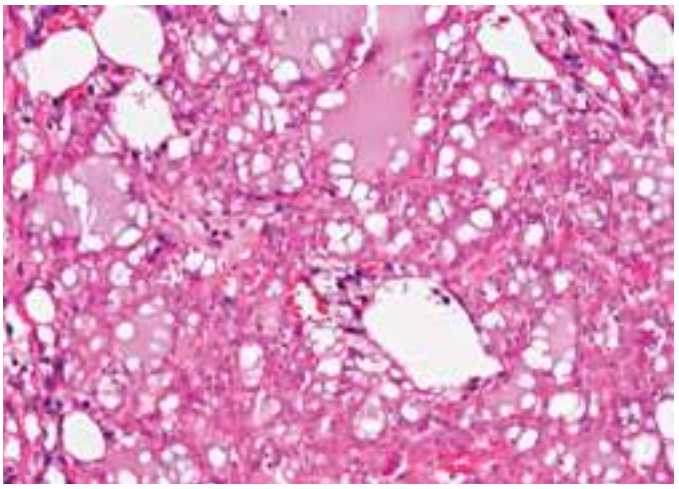


Fig. 19.21: Acinic cell carcinoma. It shows vacuolated cells similar in size to intercalated duct-type cells but have clear cytoplasmic vacuoles, which sometimes distend the cellular membranes (400X).

Prognosis

Circumscribed well-differentiated acinic cell carcinoma with abundant lymphoid aggregates has excellent prognosis.

POLYMORPHOUS LOW-GRADE ADENOCARCINOMA

Polymorphous low-grade adenocarcinoma is second common tumor of minor salivary glands. Most common sites include palate (60%), buccal mucosa, retromolar region, upper lip, and the base of the tongue. It affects over 40 years of age with equal frequency in men and women. It has infiltrative growth pattern with low metastatic potential (Fig. 19.22).

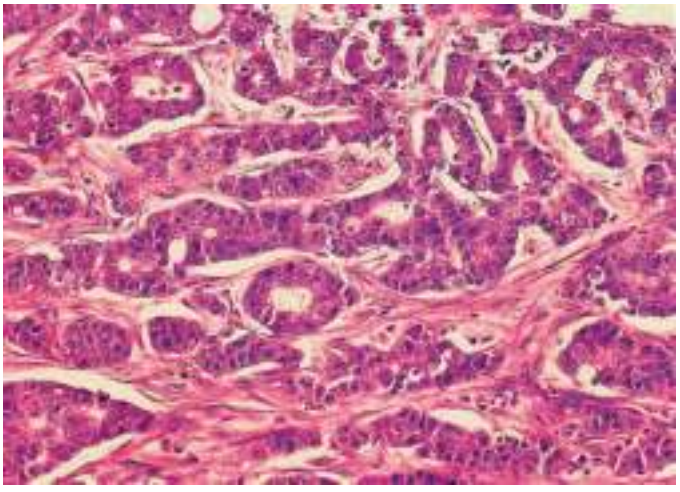


Fig. 19.22: Adenocarcinoma. It shows presence of glandular structures and absence of squamoid cells (400X).

Clinical Features

Painless mass in the palate is the most common clinical sign of polymorphous low-grade adenocarcinoma. Bleeding or ulceration of the overlying mucosa occurs occasionally.

Surgical Pathology: Polymorphous Low-grade Adenocarcinoma

Gross Morphology

- Tumor is solid firm circumscribed with irregular border invading into surrounding tissue.
- Cut surface is lobulated with yellow tan.

Light Microscopy

Tumor is composed of tumor cells forming glandular structures (*see* Fig. 19.22).

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in polymorphous low-grade adenocarcinoma on histopathologic sections.

Markers	Expression
CK-7	Positive (100%)
Vimentin	Positive (100%)
S-100 protein	Positive (97%)
CEA	Positive (54%)
GPAF	Positive (15%)
SMA	Positive (13%)
EMA	Positive (12%)
Galectin 3	Positive (many cases)
BCL-2	Positive (many cases)

Metastases

Tumor may metastasize to regional lymph nodes and distant organs (lung and bone) via hematogenous route.

CARCINOSARCOMA

Carcinosarcoma is a rare aggressive malignant salivary gland tumor composed of epithelial and mesenchymal components. It may occur in pleomorphic adenoma and malignant tumors (undifferentiated and adenocarcinoma) except acinic cell carcinoma.

- Most commonly, carcinosarcoma exists in the form of an undifferentiated carcinoma (30%) or adenocarcinoma (25%).
- Approximately, 25% of patients will have lymph node metastasis on clinical presentation. Currently recommended treatment includes radical surgery, neck dissection for palpable nodes and postoperative radiotherapy.

SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma (SCC) is rare malignant tumor of salivary gland seen over 60 years of age. Men are more affected than women. High-grade mucoepidermoid carcinoma, metastatic SCC to the gland or intraglandular nodes and direct extension of a SCC must first be excluded to establish diagnosis.

- Gross and histologic appearance is similar to SCC seen in other sites. It varies from well-differentiated with keratinization to poorly differentiated without keratinization. It is an aggressive tumor, which may metastasize to regional lymph nodes.
- Treatment of SCC of salivary gland consists of surgical resection, neck dissection and postoperative radiation.

DISORDERS OF JAW

ANATOMY OF JAW

Jaw harbors odontogenic apparatus of deciduous and permanent dentitions.

- The teeth gums are composed of enamel, dermal papillae and tooth follicle.
- The enamel is composed of ectodermally derived epithelial cells, the ameloblasts.
- The dental papillae are derived from ectomesenchyme, forming dentine.
- The tooth follicle is also derived from ectomesenchyme that surrounds developing tooth and provides support to tooth and perichondrium.
- Odontogenic tissue may be source of cysts and tumors. Normal structure of tooth is shown in Fig. 19.23.

DENTAL AND PERIODONTAL DISORDERS OF JAW

DENTAL CARIES

Dental caries is the most prevalent chronic disease of the calcified tissues of the teeth. It begins with the disintegration of the enamel prisms, accumulation of debris and superadded *Streptococcus mutans* infections. These changes produce a small pit or fissure in the enamel (Fig. 19.24A to C).

Cavity Formation in Dentin

Reaching of infection at dentinoenamel junction leads to spread of infection laterally penetrating into the dentin along the dentinal tubules. It leads to formation of cavity in the dentin producing a flask-shaped lesion

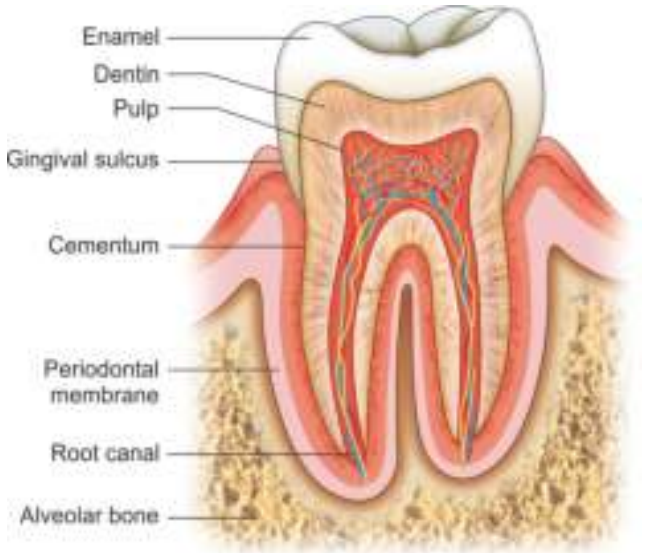


Fig. 19.23: Normal structure of tooth.

with a narrow orifice. Microorganisms may invade vascular pulp of the tooth.

Clinical Features

Patient may develop pulpitis that results in periapical granulation tissue and periodontitis of the marginal gingiva.

PERIODONTAL DISEASE

Periodontal disease refers to acute and chronic periodontal inflammatory disorders of the soft tissues surrounding the teeth. It leads to loss of supporting bone. Adults with poor oral hygiene develop chronic periodontal disease. There may be a strong family history of periodontal disease. Chronic periodontitis causes loss of more teeth in comparison to caries.

CYSTIC LESIONS OF JAW

Odontogenic and nonodontogenic cysts of jaw are given in Tables 19.13 and 19.14.

Table 19.13 Odontogenic cysts of jaw

Developmental Odontogenic Cysts
Dentigerous cysts (follicular cyst)
Keratocystic odontogenic tumor (odontogenic keratocyst)
Lateral periodontal cyst
Gingival cyst of adults
Calcifying cystic odontogenic tumor (calcifying odontogenic cyst, Gorlin cyst)
Glandular odontogenic cyst
Inflammatory Odontogenic Cysts
Periapical (radicular) cyst
Residual periapical (radicular) cyst

Table 19.14 Nonodontogenic cysts of jaw

Epithelial-lined Nonodontogenic Cysts (Fissural Cyst)
Nasopalatine duct cysts (fissural cysts)
Globulomaxillary cyst
Median palatal cysts
Nonepithelial-lined Nonodontogenic Cysts
Simple bone cysts
Aneurysmal bone cysts

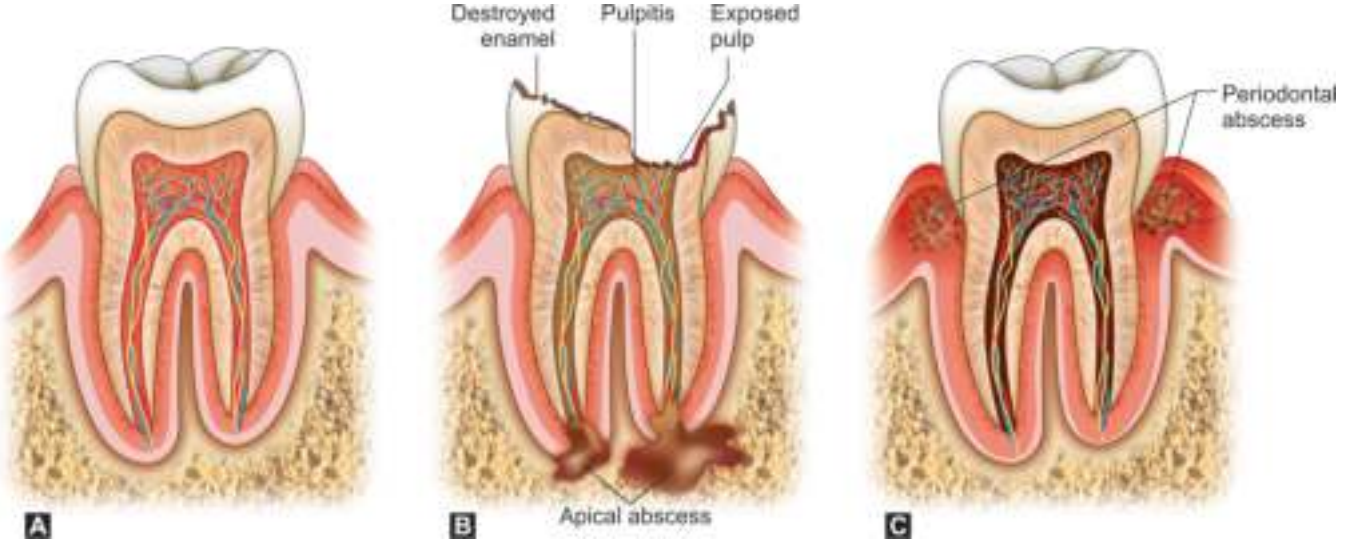


Fig. 19.24: Pathogenesis of dental caries. (A) Normal structure of tooth, (B) apical abscess, (C) periodontal abscess.

DENTIGEROUS CYST (FOLLICULAR CYST)

Dentigerous cyst is a unilocular cyst, which forms in association with the crown of an impacted molar or canine tooth. Enucleation and excision of the associated tooth are the treatment of choice.

- Patient presents with asymptomatic, well defined expansile radiolucent lesion.
- On histologic examination, dentigerous cyst is lined by cuboidal to flattened epithelial cells. There is presence of focal keratinization, mucous cells, inflammatory cells and dystrophic calcification.

KERATOCYSTIC ODONTOGENIC TUMOR (ODONTOGENIC KERATOCYST)

Odontogenic keratocyst is symptomatic unilocular cyst in the mandible or maxilla affecting 2nd to 4th decades of life that contains keratinous debris. On histologic examination, cyst shows palisading basal cells covered by a few layers of squamous cells under parakeratotic surface. Lack of rete ridges is diagnostic feature. Rarely carcinoma may arise from the cyst.

LATERAL PERIODONTAL CYST

Lateral periodontal cyst occurs in lateral surface of roots of mandibular premolar teeth during 5th to 7th decades. Periodontal cyst is well demarcated, radiolucent on radiograph. On light microscopy, cyst shows a thin layer of nonkeratinized epithelium with focal thickening. There is no inflammation surrounding the cyst.

GINGIVAL CYST

Gingival cyst occurs in the buccal gingiva of the mandible near the premolar and molar teeth during 5th to 6th decades. Gingival cyst may be single measuring <1 cm in diameter or multiple. On histologic examination, cyst is lined by a thin layer of epithelium. There is no inflammation in surrounding soft tissue.

CALCIFYING ODONTOGENIC CYST

Calcifying odontogenic cyst occurs as a radiolucent asymptomatic lesion in maxilla or mandible during 2nd and 3rd decades of life. On light microscopy, cyst shows a proliferating layer of columnar palisaded basal cells similar to ameloblasts. Superficial layer has large cells with eosinophilic cytoplasm lacking nuclei termed as ghost cells. Calcification may be seen. Recurrence is uncommon after enucleation of cyst.

GLANDULAR ODONTOGENIC CYST

Glandular odontogenic cyst is referred to sialo-odontogenic cyst. It occurs as a radiolucent lesion in anterior region of mandible or maxilla. Patient most often presents with painful swelling.

On histologic examination, cyst contains nonkeratinized epithelium, mucus secreting cells and focal solid areas. There may be presence of ghost cells and hyaline bodies. Surgical excision is the treatment of choice.

RADICULAR (PERIAPICAL CYST)

Radicular cyst (periapical cyst) is associated with nonviable carious teeth. It occurs in the apical third of tooth root during 3rd to 7th decades of life.

- Patient presents with painful swelling. On histologic examination, cyst shows inflammation surrounding the nonkeratinizing epithelium.
- There is presence of cholesterol clefts, foamy macrophages, dystrophic calcification, and intraepithelial hyaline bodies (Rushton bodies). Residual cyst is a variant of radicular cyst occurring at the site of extracted tooth.

NASOPALATINE DUCT CYST

Nasopalatine duct cyst is the most common lesion affecting 4th to 6th decades of life. It arises from moderate-sized nerves and small muscular arteries within incisive canal.

- Patient presents with swelling of the anterior palate with pain. It is well circumscribed radiolucent lesion demonstrated on radiograph.
- On histologic examination, cyst may be lined by stratified squamous, pseudostratified columnar cells within the incisive canal. Surgical enucleation is the treatment of choice.

GLOBULOMAXILLARY CYST

Globulomaxillary cyst originates from epithelium entrapped during fusion of the globular portion of the medial nasal process within maxillary process especially between the lateral incisors.

- Cyst is well circumscribed radiolucent demonstrated on radiograph.
- On histologic examination, cyst is lined by stratified squamous epithelium. Occasionally, it is lined by pseudostratified columnar ciliated epithelium. Enucleation is the treatment of choice.

MEDIAN PALATAL CYST

Median palatal cyst is believed to originate from epithelium entrapped along the embryonic line of fusion of the lateral palatal shelves of the maxilla affecting young adults.

- Patient presents with firm or fluctuant swelling in the midline of the hard palate posterior to the incisive

papilla. It is well-circumscribed radiolucent lesion in the midline of the hard palate demonstrated on radiograph.

- On histologic examination, cyst is lined by stratified squamous epithelium. Surgical enucleation is the treatment of choice.

SIMPLE BONE CYST

Simple bone cyst is known with multiple names such as unicameral bone cyst or solitary bone cyst, progressive bone cyst, traumatic bone cyst or hemorrhagic bone cyst, which is well circumscribed osteolytic lesion in the posterior mandible in young <20 years of age. Maxilla may be affected in some cases.

On histologic examination, cyst is lined by fibrovascular tissue with hemosiderin laden macrophages. There is presence of reactive bone and osteoclasts.

ANEURYSMAL BONE CYST

Aneurysmal bone cyst is rapidly enlarging blood-filled cystic lesion affecting first three decades of life. It occurs in mandible in 60% or maxilla in 40% of cases.

- The cystic lesion may be isolated or associated with chondroblastoma or osteoblastoma.
- On histologic examination, aneurysmal bone cyst shows fibrotic stroma with multinucleated giant cells, macrophages and hemosiderin granules. There may be presence of ossification (Fig. 19.25).

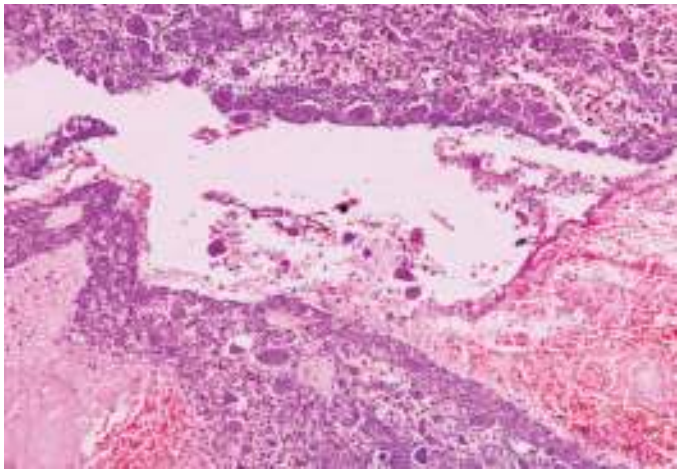


Fig. 19.25: Aneurysmal bone cyst shows fibrotic stroma with multinucleated giant cells, macrophages and hemosiderin granules (100X).

ODONTOGENIC TUMORS

Odontogenic tumors are derived from epithelial, ectomesenchymal and/or mesenchymal elements of the tooth forming apparatuses. It is worth mentioning that peripheral (extraosseous) equivalents of most central odontogenic tumors located in the mandibular or maxillary gingiva arise either from surface mucosa or dental lamina nests in the submucosa or sinonasal tract. WHO classification of tumors of odontogenic and maxillofacial bone tumors is given in Table 19.15.

Table 19.15 WHO classification of tumors of odontogenic and maxillofacial bone tumors

Odontogenic Carcinomas
- Ameloblastic carcinoma
- Primary intraosseous carcinoma, not otherwise specified
- Sclerosing odontogenic carcinoma
- Clear cell odontogenic carcinoma
- Ghost cell odontogenic carcinoma

Odontogenic Carcinosarcoma

Odontogenic Sarcoma

Benign Odontogenic Tumors
- Benign epithelial odontogenic tumors
  - Ameloblastoma
  - Ameloblastoma, unicystic type
  - Ameloblastoma, extraosseous/peripheral type
  - Metastasizing ameloblastoma
  - Squamous odontogenic tumor
  - Calcifying epithelial odontogenic tumor
  - Adenomatoid odontogenic tumor
- Benign mixed epithelial and mesenchymal odontogenic tumors
  - Ameloblastic fibroma
  - Primordial odontogenic tumor
  - Odontoma
  - Odontoma, compound type
  - Odontoma, complex type
  - Dentinogenic ghost cell tumor
- Benign mesenchymal odontogenic tumors
  - Odontogenic fibroma
  - Odontogenic myxoma/myxofibroma
  - Cementoblastoma
  - Cemento-ossifying fibroma

Odontogenic Cysts of Inflammatory Origin
- Radicular cyst
- Inflammatory collateral cysts

Odontogenic and Non-odontogenic Developmental Cysts
- Dentigerous cyst
- Odontogenic keratocyst
- Lateral periodontal cyst and botryoid odontogenic cyst
- Gingival cyst
- Glandular odontogenic cyst
- Calcifying odontogenic cyst
- Orthokeratinized odontogenic cyst
- Nasopalatine duct cyst

Contd...

Contd...

Table 19.15 WHO classification of tumors of odontogenic and maxillofacial bone tumors (*Contd...*)

Malignant Maxillofacial Bone and Cartilage Tumors
▪ Chondrosarcoma
▪ Chondrosarcoma grade 1
▪ Chondrosarcoma grade 2/3
▪ Mesenchymal chondrosarcoma
▪ Osteosarcoma, not otherwise specified
▪ Low grade central osteosarcoma
▪ Chondroblastic osteosarcoma
▪ Parosteal osteosarcoma
▪ Periosteal osteosarcoma
Benign Maxillofacial Bone and Cartilage Tumors
▪ Chondroma
▪ Osteoma
▪ Melanotic neuroectodermal tumor of infancy
▪ Chondroblastoma
▪ Chondromyxoid fibroma
▪ Osteoid osteoma
▪ Osteoblastoma
▪ Desmoplastic fibroma
Fibro-osseous and Osteochondromatous Lesions
▪ Ossifying fibroma
▪ Familial gigantiform cementoma
▪ Fibrous dysplasia
▪ Cemento-osseous dysplasia
▪ Osteochondroma
Giant Cell Lesions and Bone Cysts
▪ Central giant cell granuloma
▪ Peripheral giant cell granuloma
▪ Cherubism
▪ Aneurysmal bone cyst
▪ Simple bone cyst
Hematolymphoid Tumors
Solitary plasmacytoma of bone

Contd...

ADENOMATOID ODONTOGENIC TUMOR

Adenomatoid odontogenic tumor most often occurs in anterior maxilla. It affects especially females during second decade of life. It is slow-growing tumor with progressive growth.

Surgical Pathology: Adenomatoid Odontogenic Tumor
Gross Morphology
Tumor is predominantly cystic simulating an odontogenic cyst.
Light Microscopy
▪ Tumor is composed of odontogenic epithelium embedded in a mature connective tissue. ▪ Tumor shows numerous duct-like structures lined by cuboidal or columnar cells. ▪ Hyaline and calcified deposits may be scattered throughout epithelial lining duct-like structures.

CALCIFYING EPITHELIAL ODONTOGENIC TUMOR (PINDBORG TUMOR)

Calcifying epithelial odontogenic tumor most often occurs in mandibular premolar–molar area in association with an embedded tooth. Tumor may occur in gingiva. It occurs during 4th and 5th decades with no sex predilection. Tumor may be invasive. It may recur after surgical excision. It is less aggressive tumor than ameloblastoma.

Surgical Pathology: Calcifying Epithelial Odontogenic Tumor
Gross Morphology
Tumor is solid. Cystic variety has been reported.
Light Microscopy
▪ Tumor is composed of closely packed polyhedral epithelial cells and scanty stroma. ▪ Tumor cells show mild pleomorphism. ▪ There is presence of eosinophilic homogenous amyloid-like material that becomes calcified.

SQUAMOUS ODONTOGENIC TUMOR

Squamous odontogenic tumor is a benign locally infiltrative tumor that occurs in anterior maxilla and posterior mandible. Tumor in the maxillary region tends to be more aggressive. On radiograph, tumor appears as a well-circumscribed semicircular radiolucency surrounded by sclerotic border.

Surgical Pathology: Squamous Odontogenic Tumor
Light Microscopy
Tumor is composed of nests and islands of well differentiated squamous epithelium lacking atypia located within a collagenous stroma of low to moderate cellularity.

AMELOBLASTIC FIBROMA

Ameloblastic fibroma most often affects younger age group. It has benign clinical course.

Surgical Pathology: Ameloblastic Fibroma
Gross Morphology
Tumor is solid. But cystic variant has been reported.

Light Microscopy

- Tumor is composed of neoplastic fibrous stroma enclosing thin strip of ameloblastic epithelium. The presence of mesenchymal component is diagnostic feature of this tumor.
- Epithelial component has two layers of cells.
- There may be presence of hard tooth structures such as dentin.
- Occasionally stellate reticulum is present.

ODONTOMA

Odontoma features production of calcified parts of teeth. It occurs in the alveolar ridge of the mandible or maxilla. There are three variants: complex, compound and ameloblastic odontomas.

Complex Odontoma

Complex odontoma most often occurs in molar areas of the mandible especially of females. It is a benign poorly differentiated tumor forming enamel, dentin or cementum in uncoordinated fashion. It is incidentally detected on routine radiographic examination.

Compound Odontoma

Compound odontoma most often occurs in the anterior region of maxilla. It is a benign well-differentiated tumor composed of masses of small denticles.

Ameloblastic Odontoma

Ameloblastic odontoma is aggressive tumor. It is currently regarded as immature complex odontoma. Tumor is solid and cystic. Tumor is composed of prominent epithelial component resembling ameloblastoma. It recurs after surgical excision.

CEMENTOMA

Cementoma is relatively common non-neoplastic disorder. It most often occurs in mandibular incisor region especially affecting females. It is restricted to small region surrounding apices of teeth. Lesion is asymptomatic and does not require treatment.

Surgical Pathology: Cementoma

Light Microscopy

- Cementoma is composed of curvilinear trabeculae resembling ginger root or irregularly shaped cementum-like masses.
- It should be differentiated from cemento-ossifying fibroma, which shows thin isolated trabeculae with prominent osteoblastic rimming.

ODONTOGENIC MYXOMA, MYXOFIBROMA AND FIBROMA

Odontogenic myxoma is derived from dental papilla (tooth germ origin), hence qualifies for odontogenic origin. It is related to malformed or missing teeth. It occurs during 2nd and 3rd decades in mandible and maxilla. It is cystic lesion demonstrated on radiograph. Recurrence is common after surgical removal. Odontogenic fibroma most often occurs in maxilla anterior to the molar teeth especially affecting females.

Surgical Pathology: Odontogenic Myxoma

Gross Morphology

Tumor is cystic in consistency.

Light Microscopy

- Tumor is composed of loose stellate cells with long branching odontogenic epithelium.
- Odontogenic fibroma differs from myxoma by the presence of fibrous tissue and odontogenic epithelial rests.
- Myxofibroma is composed of myxomatous and fibrous components in equal proportion.

Immunohistochemistry Technique

Tumor cells of odontogenic myxoma are immunoreactive for vimentin but negative for S-100 protein.

AMELOBLASTOMA (ADAMANTINOMA)

Ameloblastoma is the most common of the epithelial odontogenic tumors. It constitutes 1% of tumors and cysts of jaw during 3rd to 5th decades. Tumor arises from epithelial lining of dentigerous cyst, remnants of the dental lamina and enamel organ or basal layer of oral mucosa. Ameloblastoma occurs in the mandibular region in >08% and in molar ramus region in 70% of cases.

Surgical Pathology: Ameloblastoma

Gross Morphology

Traditionally, ameloblastoma has been divided into solid and multicystic, but this distinction is arbitrary.

Light Microscopy

Histologic variants of ameloblastoma are follicular, plexiform, acanthomatous, papilliferous-keratotic, granular cell, desmoplastic, vascular and dentinoameloblastoma. Two or more histologic patterns may coexist within the same tumor. Out of these follicular and plexiform histologic variants are more common.

- **Follicular variant of ameloblastoma:** Tumor is composed of outermost tall columnar cells with polarization of the nuclei away from basement membrane resembling inner dental epithelium of the developing tooth follicle (ameloblastic layer). The inner (central) portion of the epithelial island is composed of loose network of cells resembling stellate reticulum. Squamous metaplasia within the stellate reticulum gives rise to the acanthomatous variant.
- **Plexiform variant of ameloblastoma:** Tumor is composed of irregular nests and interdigitating cords of epithelial cells with a minimum of stroma (Figs 19.26 and 19.27).

Immunohistochemistry Technique	
Panel of immunomarkers is used to analyze expression in ameloblastoma on histologic sections. Granular cell ameloblastoma is positive for cytokeratin and CD68 but negative for S-100 protein. Tumor cells exhibit strong reactivity for cytokeratins.	
Markers	**Expression**
▪ CK5, CK14, CK8, CK18, CK19	▪ Positive
▪ Calretinin	▪ Positive (stellate reticulum areas)
▪ S-100 protein	▪ Negative
Electron Microscopy	
On electron microscopy study, tumor cells exhibit clear cut evidence of epithelial differentiation in the form of tonofilaments and complex desmosomes.	

Mode of Spread

Ameloblastoma has invasive property and tendency to recur after surgical excision. It is due to invasion and recurrence properties, this is placed under borderline (low-grade malignant) tumor category by oral pathologists and WHO classification of tumors of odontogenic and maxillofacial bone tumors. Distant metastases have been documented and in instances.



Fig. 19.26: Ameloblastoma of maxilla. Tumor is multilocular and located in maxilla arising from the enamel organ.

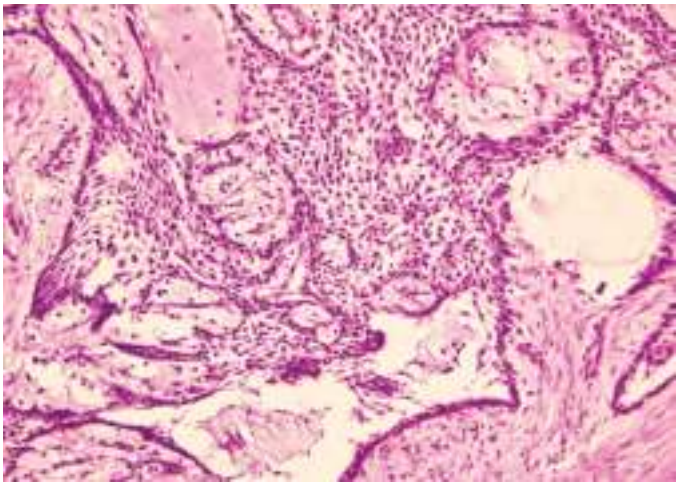


Fig. 19.27: Ameloblastoma. Tumor is composed of outermost tall columnar cells with polarization of the nuclei from basement membrane. The inner (central) portion of the epithelial island is composed of loose network of cells resembling stellate reticulum (100X).

Differential Diagnosis

Ameloblastoma has to be differentiated from focal reactive hyperplasia in a dentigerous cyst, unicystic ameloblastoma. Minimal diagnostic criteria for ameloblastoma are presence of a palisaded basal layer with stellate reticulum-like epithelium above immunoreactivity for calretinin supports the diagnosis.

Management

Enucleation of the ameloblastoma is most often curative. Local recurrence may occur after surgical excision if ameloblastic islands remain embedded in the cyst wall.

AMELOBLASTIC CARCINOMA

Ameloblastic carcinoma has microscopic architectural features of ameloblastoma with additional malignant component. Tumor shows lack of differentiation, increased cellularity, marked atypia, numerous mitoses, vascular and neural invasion.

ODONTOGENIC CARCINOMA

Odontogenic carcinoma is restricted to primary intraosseous carcinoma lacking ameloblastic-like pattern. It is considered to be of odontogenic origin either *de novo* or arising in a odontogenic cyst.

Clear Cell Odontogenic Carcinoma

Clear cell odontogenic carcinoma most often occurs in jaw. It simulates metastatic clear cell carcinoma of salivary gland. Tumor is composed of clear cells arranged in nests surrounded by mature collagenous stroma. Tumor cells lack glycogen and mucin.

Sclerosing Odontogenic Carcinoma

Sclerosing odontogenic carcinoma is composed of polygonal monomorphic epithelial cells arranged in small nests and thin cords embedded in abundant sclerosing hyaline stroma. Tumor cells exhibit skeletal muscle and neural infiltration.

AMELOBLASTIC FIBROSARCOMA

Ameloblastic fibrosarcoma morphology is similar to ameloblastic fibroma except it shows increased cellularity, cytologic atypia and aggressive clinical course. It has diminished or absent epithelial component. Some tumors containing combined features of ameloblastic carcinoma and fibrosarcoma are termed **ameloblastic carcinosarcoma**.

Table 19.16 Nonodontogenic tumors

Benign Fibro-osseous Lesions
▪ Fibrous dysplasia ▪ Juvenile ossifying fibroma: Trabecular and psammomatoid variants ▪ Cementosseous fibroma: Periapical or florid variants
Giant Cell Lesions
▪ Central (intraosseous) giant cell granuloma ▪ Brown tumor of hyperparathyroidism ▪ Cherubism ▪ Aneurysmal bone cyst

NONODONTOGENIC TUMORS

Nonodontogenic tumors are classified in Table 19.16.

FIBROUS DYSPLASIA

Fibrous dysplasia may be polyostotic or monostotic associated with somatic mutation of GNAS1 gene. Polyostotic form is associated with Albright syndrome. Mean age at the time of diagnosis is 25–35 years.

Clinical Features

Patient presents with painless unilateral swelling of maxilla or mandible.

Imaging Technique

On radiograph, lesion varies from cystic or translucent to sclerotic or radiopaque with ill-defined margins.

Surgical Pathology: Fibrous Dysplasia
Light Microscopy
▪ Fibrous dysplasia is composed of C-shaped or Chinese figure-like trabeculae of woven or immature bone within proliferating fibroblastic stroma. ▪ These trabeculae lack rimming by osteoblasts.
Immunohistochemistry Technique
Fibrous dysplasia shows stronger immunoreactivity for osteocalcin.

FIBROMA GROUP LESIONS

Fibroma group lesions are derived from periodontal ligament. These have predilection for molar–premolar region of mandible. These lesions are more amenable to surgical enucleation or curettage. These lesions are differentiated from fibrous dysplasia.

Ossifying Fibroma

Ossifying fibroma is composed of lamellar bone with osteoblastic rimming.

Cemento-ossifying Fibroma

Cemento-ossifying fibroma is composed of rounded psammoma-like masses resembling cement.

Juvenile (Aggressive) Ossifying Fibroma

Juvenile ossifying fibroma is actively growing affecting young persons and composed of cellular fibrous stroma.

CENTRAL GIANT CELL GRANULOMA

Central giant cell granuloma is most common cystic lesion affecting children and younger age group. It occurs in mandible or maxilla. Tumor occurs as a result of trauma resulting in organization of slow minute recurrent hemorrhages. These lesions are treated by surgical removal or curettage.

Peripheral giant cell granuloma is characterized by development of cystic mass on the gingiva or the alveolar process as a result of unusual proliferative reaction to local injury. It is seen as a mass covered by mucous membrane, which can be ulcerated.

Surgical Pathology: Central Giant Cell Granuloma
Light Microscopy
▪ This cellular lesion is composed of numerous multinucleated giant cells embedded in a fibrous stroma that also contains ovoid or spindle-shaped mesenchymal cells. ▪ Osteoclast-like giant cells have patchy distribution usually associated with areas of hemorrhage.

CHERUBISM

Cherubism is hereditary autosomal dominant disorder characterized by intraosseous fibrous swellings of the jaw. The gene responsible for cherubism has been mapped on chromosome 4p16.3. On histologic examination, it is indistinguishable from central giant cell granuloma. Young patient may present with bilateral involvement of mandible and maxilla.

BROWN TUMOR OF HYPERPARATHYROIDISM

Osteoclastic bone resorption results to osteitis fibrosa cystica (also known as brown tumor) in jaw and long bones. The lesion is expansile and multilocular with solid and cystic areas. Cystic areas have a brown color due to presence of abundant hemosiderin.

Recent Advances: Salivary Gland Tumor Specimen Handling in Surgical Pathology

- Although fine needle aspiration cytology (FNAC) is a diagnostic tool for preoperative evaluation of salivary gland tumors. Histopathology remains the gold standard for final diagnosis of tumors of major salivary glands (parotid, submandibular and sublingual glands), and minor salivary glands (mucous-secreting glands in the lining membrane of the aerodigestive tract). Pleomorphic adenoma and Warthin tumor arise in major salivary glands especially in parotid gland. Malignant tumors usually occur in the minor salivary glands especially in palate region. Proper diagnosis of malignant tumors with accurate staging is very important in the management of these cases.
- Ultrasound-guided fine needle aspiration cytology (FNAC) is recommended for salivary gland tumors to determine, where the tumor is benign or malignant. Computed tomography (CT) and magnetic resonance imaging (MRI) can confirm salivary gland malignant tumor infiltration in the adjacent skeletal muscle and to see if the tumor has spread to regional lymph nodes in the neck. CT scan has utility in proven malignancy to exclude distant metastases in lung, bone, brain, and liver associated with poor prognosis.
- Diagnosis of salivary gland tumors can be challenging due to varied and overlapping histologic features and immunohistochemical staining patterns of various histologic tumor types including technical limitations such as sampling errors, crush artifact and limited size tumor.
- Pathologist should take into considerations of clinical history, radiologic findings information, macroscopic tumor site, unifocal/multifocal tumor, tumor laterality, tumor size, tumor extent (extra-parenchymal extension, extraglandular soft tissue, facial nerve, ear canal, mandible, skull base, carotid artery encasement), histologic patterns (i.e. tubular and cribriform architecture of polymorphous adenocarcinoma, squamous differentiation in mucoepidermoid carcinoma, solid architecture of adenoid cystic carcinoma, mucin secreting tumor, Warthin tumor with oncocytic epithelial cells lining papillary and cystic structures in a lymphoid stroma, high-grade cytology, epithelial and lymphoid elements), and ancillary studies.
  - Recognition of specific histologic patterns can help narrow the differential diagnosis. Several important clinical features provide an important initial framework for the assessment of biopsy and resected surgical specimens.
  - Pathologist should follow reporting protocol including histologic grade, lymphovascular invasion, perineural invasion, tumor margin status, regional lymph nodes status with tumor, and number of lymph nodes examined, and distant sites in lung, bone, brain, and liver, if applicable.
- Molecular genetic alterations in salivary gland tumors can be identified by routine karyotype analysis or through fluorescence *in situ* hybridization (FISH) technique, which are helping to identify potential targets that may improve treatment for some of these salivary gland tumors.
  - Pleomorphic adenoma and carcinoma ex pleomorphic adenoma demonstrate PLAG1 rearrangements (8q12) or HMGA2 rearrangements (12q14.15). Adenoid cystic carcinoma is a highly malignant tumor, which arises in minor salivary glands and demonstrates chromosomal translocation t(6;9) → MYB-NFIB. Mucoepidermoid carcinoma is a highly malignant tumor, which involves mostly the parotid gland followed by the minor salivary glands and submandibular gland and demonstrates chromosomal translocation t(11;19 → CRTC1-MAML2).
  - Secretory carcinoma of salivary gland shows chromosomal translocation t(12;15) → ETC-NTRK3 fusion. Polymorphous adenocarcinoma demonstrates PRKD1 E710D hotspot activating mutations. Cribriform adenocarcinoma of the minor salivary gland shows PRKD1 rearrangements. Clear cell carcinoma demonstrates chromosomal translocation t(12;22) → EWSR1-ATF1.
- Surgical excision of benign tumors in parotid gland completely cures the patients. Malignant tumors in the submandibular gland are treated by adjuvant chemotherapy following surgery except in cases of small, low-grade tumors that have been completely excised. In the event of intraoperative tumor spillage, most cases need long-term follow-up for clinical observation. During parotidectomy, it is most important to preserve facial nerve function. Neck dissection is recommended in all cases of malignant tumors in parotid gland except low-grade small tumors.

CHAPTER

20

Gastrointestinal Tract and Peritoneum

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

- Gastrointestinal tract: overview
  - Embryology
  - Histology
    - GIT divisions and referred pain in related disorders
- Gastrointestinal tract disorders
  - Amyloidosis of GIT

DISORDERS OF ESOPHAGUS

- Esophagus: overview
  - Anatomy
  - Physiology
  - Developmental anomalies
- Inflammatory lesions
  - Esophagitis
  - Gastroesophageal reflux disease
  - Barrett esophagus
- Gastric Non-neoplastic disorders
  - Esophageal diverticulum
  - Esophageal achalasia
  - Hiatus hernia
  - Scleroderma including CREST syndrome
  - Esophageal stricture
  - Postcricoid webs (rings)
  - Esophageal varices
  - Schatzki ring
  - Vater's syndrome
  - Boerhaave's syndrome
  - Mallory-Weiss syndrome
- Esophagus: tumors
  - Benign polypoid lesions
  - Squamous cell carcinoma
  - Adenocarcinoma
  - Neuroendocrine tumor
  - Mesenchymal tumors
  - Other tumors

DISORDERS OF STOMACH

- Stomach: overview (anatomy and physiology)
- *Helicobacter pylori*-induced gastrointestinal tract disorders
- Inflammatory disorders
- Peptic ulcer disease
- Gastric polyps
- Neoplastic disorders of stomach
  - Gastric carcinoma
  - Gastric MALT lymphoma
  - Gastrointestinal stromal tumors
  - Gastric neuroendocrine tumors
- Non-neoplastic disorders

DISORDERS OF SMALL INTESTINE

- Anatomy and histology
- Congenital anomalies
- Malabsorption syndrome
- Diarrhea
- Infectious disorders
- Obstructive disorders
- Vascular disorders
- Polyps and tumors

DISORDERS OF LARGE INTESTINE

- Anatomy and physiology
- Neuromuscular disorders
- Diverticular disorders
- Obstructive disorders
- Inflammatory bowel disease
- Colitis: other causes
- Vascular disorders of colon
- Neoplastic disorders of large intestine
- Multiple polyposis syndromes
- Malignant tumors of colon
  - Hereditary nonpolyposis colon cancer
  - Colorectal carcinoma

DISORDERS OF APPENDIX

- Anatomy and histology
- Inflammatory disorders
- Neoplastic disorders of appendix

DISORDERS OF ANUS AND PERIANAL REGION

- Anatomy and histology
- Vascular disorders of anal canal
- Non-neoplastic disorders of anal canal
- Neoplastic disorders of anal canal

DISORDERS OF PERITONEUM

- Anatomy and histology
- Non-neoplastic disorders of peritoneum
- Neoplastic disorders of peritoneum (primary and secondary neoplasms)

GASTROINTESTINAL TRACT DISORDERS: DIAGNOSTIC APPROACH

GASTROINTESTINAL TRACT: OVERVIEW

EMBRYOLOGY

Most gastrointestinal tract organs are derived from endoderm, the innermost layer of developing embryo. During embryologic development, most visceral organs develop between 3 and 8 weeks. Blood flows through ductus venosus in the fetus via umbilical vein into inferior vena cava. Umbilical vein gives rise to the adult structure 'falciform ligament'.

- Diaphragm is derived from septum transversum, dorsal mesentery of the esophagus, pleuroperitoneal folds and body wall.

- Ventral pancreatic bud gives rise to main pancreatic duct, uncinate process, and head of the pancreas. Dorsal pancreatic bud ultimately gives rise to accessory pancreatic duct, pancreatic duct, the body, isthmus and tail of the pancreas.
- Primitive gut is divided into foregut (pharynx to duodenum), midgut (duodenum to transverse colon) and hindgut (distal transverse colon to rectum).
- Foregut is supplied by celiac trunk, midgut by superior mesenteric artery and hindgut by inferior mesenteric artery.

Developmental Anomalies of GIT

Developmental anomalies of GIT are cleft lip, cleft palate, atresia and stenosis of GIT, and tracheoesophageal fistula mentioned as under:

- **Cleft lip:** It occurs due to failure of fusion of the maxillary and medial nasal processes during embryonic development resulting in a gap in the upper lip while palate remains intact.
- **Cleft palate:** It occurs due to failure of fusion of the lateral palatine processes, the nasal septum, and/or the median palatine processes. The resulting gap leaves the nasal cavity in communication with the oral cavity.
- **Atresia and stenosis of GIT:** The normal GIT is a tube that develops from the cords of embryonic cells. The cells forming the central part of these cords of embryonic cells undergo apoptosis. If these centrally located cords of embryonic cells do not undergo apoptosis, it results in the absence of GIT lumen. The affected part of GIT becomes atretic. Duodenal atresia is the most common disorder usually associated with Down syndrome. Duodenal atresia is characterized by bile stained vomiting at birth. Abdominal radiograph demonstrates '**double bubble**' appearance of the duodenum.
- **Tracheoesophageal fistula (TEF):** It is the most common congenital anomaly. Most frequent type of tracheoesophageal fistula is distal tracheoesophageal fistula with proximal esophageal atresia.

HISTOLOGY

Entire GIT has a relatively uniform structure. It consists of four layers, i.e. innermost mucosa, submucosa, muscular coat and outermost serosa (adventitia). Adventitia in esophagus is linked with other thoracic organs.

GIT Divisions and Referred Pain in Related Disorders

Depending on the innervation of GIT, pain is referred in different regions.

- **Foregut:** It extends from esophagus to second part of duodenum, where common bile duct enters in this region. It comprises esophagus, stomach and duodenum. Visceral pain arising from foregut is referred to epigastrium.
- **Midgut:** It starts from the duodenum just distal to entry of common bile duct and extends to middle and distal thirds transverse colon known as **Cannon's point**. Midgut includes terminal ileum, tip of the appendix, and adjacent colon. Visceral pain arising from midgut radiates to periumbilical region. Pain in acute appendicitis is felt around periumbilical region. Pain in acute appendicitis is localized to right iliac fossa due to inflammation of the peritoneum.
- **Hindgut:** It starts from Cannon's point to distal structures of GIT. It includes small portion of transverse colon, descending colon and rectum.Visceral pain arising from hindgut radiates to suprapubic region.

GASTROINTESTINAL TRACT DISORDERS

Main disorders of the gastrointestinal tract include developmental disorders (e.g. atresia, stenosis, diverticulosis and fistula), inflammatory (e.g. esophagitis, inflammatory bowel disease), functional disorders, circulatory disorders and tumors. Obstructive disorders of gastrointestinal tract are given in Table 20.1.

Table 20.1 Obstructive disorders of gastrointestinal tract

Disorder	Features
Duodenal atresia	Duodenal atresia, most commonly associated with Down's syndrome, is characterized by vomiting of bile stained material at birth and an abdominal radiograph with the **double bubble sign**
Hirschsprung's disease (congenital megacolon)	▪ Disorder is characterized by congenital absence of ganglion cells due to gene mutation (endothelin 3) usually in the submucosal and muscular coat of large bowel ▪ Aganglionic segment of colon is permanently contracted causing intestinal obstruction. This obstruction leads to dilatation of proximal normal (ganglionic) segment ▪ Rectum is always involved, and this is the preferred site for biopsy. Sigmoid colon and proximal are involved, but entire colonic involvement is very rare

Contd...

Table 20.1 Obstructive disorders of gastrointestinal tract (*Contd...*)

Disorder	Features
Acquired megacolon	Disorder may be associated with Down's syndrome and Chagas' disease
Adhesions	The adhesions are commonly acquired from previous abdominal surgery
Direct inguinal hernia	Direct inguinal hernia occurs through the posterior wall of the inguinal canal in the center of Hesselbach's triangle
Indirect inguinal hernia	Indirect inguinal hernia is most common cause of intestinal obstruction, in which small bowel extends into the scrotal sac, lateral to the triangle of Hesselbach
Umbilical hernia	Umbilical hernia occurs in children and adults. Ascites and pregnancy precipitate hernias in adults
Incisional hernia	Incisional hernia occurs in previously surgical incision sites
Intussusception	▪ Intussusception is an invagination of one segment of proximal bowel (usually the terminal ileum) into the distal bowel (usually the cecum), causing intestinal obstruction and ischemia of inner loop; ileocecal (most common), jejunojejunal, ileoileal and colocolic ▪ Intussusception primarily affects children due to active peristalsis and lymphoid hyperplasia. In adults, pedunculated polyps are common cause of intussusception
Volvulus	▪ Volvulus is twisting of a mobile loop of the intestine (usually the sigmoid colon) around its own mesenteric root with subsequent obstruction and infarction. It is also found in the small intestine. It occurs in small intestine and sigmoid colon ▪ Complications of volvulus include strangulation, gangrene and obstruction
Malrotation of intestine	During fetal life, defective rotation of intestine occurs. As a result, large bowel does not descend into right iliac fossa. These patients are prone to develop volvulus and internal hernia
Paralytic ileus	▪ Paralytic ileus is a lack of intestinal peristalsis associated with stagnation of intestinal contents ▪ The most common causes include abdominal surgery persisting for 2–3 days, peritonitis, shock and vascular collapse
Gallstone ileus	Gallstone ileus occurs when a chronically inflamed gallbladder forms a fistula with the small intestine at ileocecal valve
Meconium ileus	Meconium ileus is a potential complication of a newborn with cystic fibrosis

Most important symptoms and signs pertaining to the GIT include dysphagia, vomiting, hematemesis, hematochezia, melena, colics, diarrhea and constipation. Causes of dysphagia are given in Table 20.2. Causes of gastrointestinal tract bleeding are given in Table 20.3.

AMYLOIDOSIS OF GIT

Amyloidosis occurs in tongue, stomach, and rectal mucosa. Biopsy must be taken from rectum (80% sensitivity), tongue and stomach. Fine needle aspiration of abdominal fat is ideal to establish diagnosis in 90%

Table 20.2 Causes of dysphagia

Mechanical Obstruction ▪ Esophageal webs ▪ Esophageal rings ▪ Esophageal stricture ▪ Esophageal scar ▪ Esophageal achalasia
Inflammatory Disorders
Eosinophilic Esophagitis
Neurological Disorders ▪ Injury to vagus nerve ▪ Diabetes mellitus involving autonomic nervous system ▪ Chagas disease (*Trypanosoma cruzi*) causing enlarged esophagus ▪ Dorsal motor neuron lesions
Infiltrative Disorders ▪ Amyloidosis ▪ Malignant tumors ▪ Sarcoidosis

Table 20.3 Causes of gastrointestinal tract bleeding

Upper Gastrointestinal Tract Bleeding
Esophageal varices
Esophageal neoplasms
Mallory-Weiss lacerations
Gastritis
Peptic ulcer disease
Lower Gastrointestinal Tract Bleeding
Rapid upper gastrointestinal bleeding
Diverticulosis
Infectious colitis
Angiodysplasia
Inflammatory bowel disease
Neoplasms
Anal fissure
Hemorrhoids

of cases. Sections obtained from biopsy should be stained by Congo red stain. Amyloid may be demonstrated by electron microscopy and immunofluorescence microscopy. Mass spectrometry distinguishes AL from AA.

EXTRANODAL LYMPHOMAS OF GASTROINTESTINAL TRACT

Gastrointestinal tract is the most common primary site of extranodal lymphomas, which account for 20–40% of all lymphomas. Gastric region is most often involved followed by the ileocecal region and the colon. Multiple lymphomas of the gastrointestinal tract occur in small proportion of cases. Predisposing factors of gastrointestinal tract lymphomas include *Helicobacter pylori* infection, celiac disease, ulcerative colitis and Crohn's disease. The development of different histologic types of lymphoma appears to be related to chronic antigenic stimulation, inadequate immune regulation, or a combination of these factors. Lymphomas of the gastrointestinal tract are given in Table 20.4. Clinical features and most common extranodal lymphomas of gastrointestinal tract are given in Table 20.5.

Table 20.4 Lymphomas of the gastrointestinal tract

Gastrointestinal Tract Region	Histologic Variant of Lymphoma
Gastric region	▪ B cell lymphoma ▪ Diffuse large B cell lymphoma (DLBCL) ▪ Extranodal marginal zone lymphoma (MZL) also known as MALT lymphoma ▪ Burkitt lymphoma ▪ Mantle cell lymphoma (MCL) ▪ Follicular lymphoma ▪ Peripheral T cell lymphoma (PTCL)
Small intestine	▪ B cell lymphoma ▪ Diffuse large B cell lymphoma (DLBCL) ▪ Extranodal marginal zone lymphoma (MZL) also known as MALT lymphoma ▪ Burkitt lymphoma ▪ Mantle cell lymphoma ▪ Follicular cell lymphoma ▪ T cell/NK cell lymphoma ▪ Peripheral T cell lymphoma, not otherwise specified ▪ NK/T cell lymphoma, nasal type
Colon region	▪ B cell lymphoma ▪ Diffuse large B cell lymphoma (DLBCL) ▪ Extranodal marginal zone lymphoma (MZL) also known as MALT lymphoma ▪ Burkitt lymphoma ▪ Mantle cell lymphoma ▪ Follicular cell lymphoma ▪ T cell/NK cell lymphoma ▪ Enteropathy-associated T cell lymphoma ▪ Peripheral T cell lymphoma, not otherwise specified
Anal region	▪ B cell lymphoma ▪ Diffuse large B cell lymphoma (DLBCL)

Table 20.5 Clinical features and most common extranodal lymphomas of gastrointestinal tract

Symptoms are distinctive for various types of lymphomas. The most common clinical presentation associated with GIT lymphoma includes abdominal pain, palpable mass, diarrhea, nausea, vomiting, fever, perforation and bleeding. Bulky lymphomas in the ileocecal region may cause intussusception

Gastric Region

- Gastric region is most common site of lymphoma in 55–75% of cases
- Most common histologic types of lymphomas in the gastric region include diffuse large B cell lymphoma (DLBCL) and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, also known as MALT lymphoma

Small Intestine

- Small intestine is next most common site of GIT lymphoma in 15–35% of cases
- Diffuse large B cell lymphoma (DLBCL) and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, also known as MALT lymphoma are most common lymphomas in small intestine especially in the ileocecal region
- Majority of B cell lymphomas arise in the small intestine in elderly persons. Mesenteric lymph node involvement is common
- Extra-abdominal spread is unusual. Patient presents with melena or obstruction

Large Intestine

- Large intestine is the primary site of GIT lymphomas in 3–20% of cases
- Most common lymphomas in large intestine include diffuse large B cell lymphoma (DLBCL) and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, also known as MALT lymphoma

Anal Region

Anal region is less common site of lymphoma. Most common histologic variant is diffuse large B cell lymphoma (DLBCL)

DISORDERS OF ESOPHAGUS

ESOPHAGUS: OVERVIEW

ANATOMY

Esophagus is a long hollow muscular tube. It consists of four layers. With the exception of infradiaphragmatic portion, esophagus is lined by nonkeratinized stratified squamous epithelium. The basal layer of lining epithelium constitutes 15% of total thickness of epithelium. The lower two-thirds of the esophagus has thick muscularis mucosa. Gastroesophageal reflux leads to increased thickness of lining epithelial layer.

PHYSIOLOGY

Esophagus participates in propulsion of food from the mouth to the stomach. It also prevents reflux of gastric contents from the stomach back into esophagus.

DEVELOPMENTAL ANOMALIES

Common congenital anomalies of the esophagus include tracheoesophageal fistula, esophageal atresia and esophageal cysts.

Tracheoesophageal Fistula

Tracheoesophageal fistula is a congenital disorder characterized by direct communication between trachea and esophagus. Food passing through fistula enters the trachea, resulting in choking, coughing and cyanosis on attempts at food intake soon after birth.

- Newborn may develop pneumonia as a result of milk reflux into the lungs resulting in distension of the stomach by air. This disorder requires immediate surgical correction. It occurs in three distinct variants.
- Upper esophagus ends in a blind pouch (esophageal atresia). The lower portion of esophagus communicates with the trachea near the tracheal bifurcation. It is the most common variant of tracheoesophageal fistula (90%). Maternal polyhydramnios (increased amniotic fluid) is frequently associated abnormality.
- In second most common variant of tracheoesophageal fistula, the upper portion of esophagus communicates with the trachea. The lower esophageal segment is not connected to the upper esophagus.
- In a third variant, there is a fistulous connection between the trachea and a completely patent esophagus.

Clinical Pearls: Symptoms Related to Esophageal Disorders

- **Dysphagia:** It refers to difficulty in swallowing of solid food (sign of obstruction) or solids as well as liquids (motor dysfunction). Obstruction of esophagus occurs due to strictures, webs, rings, tumor or functional disorders (achalasia or paralysis).
- **Odynophagia:** It refers to pain on swallowing. Most common causes include gastroesophageal reflux disease (GERD) and infectious esophagitis (e.g. virus and fungi).
- **Heartburn:** It refers to burning sensation in retrosternal region as a result of regurgitation of acid in mouth most often associated with gastroesophageal reflux disease.

Pathology Pearls: Gross Examination and Tissue Handling

- Various esophageal diseases are studied by tissue sections obtained by standard endoscopic biopsy, endoscopic mucosal resection (EMR) and esophagectomy (Table 20.6).
- Esophageal biopsy is considered more usful to establish gastroesophageal disease (GERD) in children than adults.

INFLAMMATORY LESIONS

ESOPHAGITIS

The esophagus, covered by squamous epithelium, is relatively resistant to infection. Esophagitis occurs due to gastroesophageal reflux disease, chemicals, cytotoxic drugs, uremia and irradiation.

- In immunocompromised host in AIDS, various opportunistic infectious agents cause esophagitis

Table 20.6 Gross examination and tissue handling of esophagus

Procedure	Characteristics
Standard endoscopic biopsy	Several small tissue fragments measuring 1–5 mm consist of mucosa and muscularis mucosae
Endoscopic biopsy with jumbo forceps	Several tissue fragments measuring 4–8 mm consist of mucosa and submucosa
Endoscopic mucosal resection (EMR)	En bloc resection of superficial malignant and premalignant lesions measuring 1–2 mm
Esophagectomy	Tissue specimens consist of esophagus, proximal stomach and attached soft tissue with lymph nodes

such as herpes simplex type 1, and cytomegalovirus. *Candida albicans* causes white mucosal painful patches in esophagus debilitated persons.

- Gastrointestinal tract disorders that can cause extensive eosinophilic infiltration in the esophageal mucosa include eosinophilic esophagitis, reflux esophagitis, parasitic infection, eosinophilic gastritis and allergic reaction to medication or food. Eosinophilic esophagitis involves middle-third of esophagus in children. Differences between reflux and eosinophilic esophagitis are given in Table 20.7.
- Diagnostic criteria of eosinophilic esophagitis include dysphagia, endoscopic evidence of concentric mucosal rings, presence of ≥15 eosinophils per high power field on histologic examination of esophageal biopsy.

GASTROESOPHAGEAL REFLUX DISEASE

Under physiologic state, lower esophageal sphincter (LES) prevents the entry of the gastric contents in the esophagus. Gastroesophageal reflux disease is popularly known as heartburn.

Gastroesophageal reflux disease refers to chronic backflow of digestive juices (hydrochloric acid and bile) into the esophagus due to transient relaxation of lower esophageal sphincter resulting in damage to epithelial lining of esophagus. Gastroesophageal reflux disease (GERD) is shown in Fig. 20.1A and B.

Predisposing Factors

Predisposing factors for GERD include hiatus hernia (most common), reflux esophagitis, incompetent lower esophageal sphincter, increased intra-abdominal pressure (pregnancy), scleroderma, decreased lower esophageal sphincter tone as a result of alcohol, smoking and caffeine.

Pathogenesis

Gastroesophageal reflux disease causes breach in mucosal barrier, which may allow invasion of infectious agents (e.g. *Candida albicans*, herpes simplex virus or cytomegalovirus and bacteria) resulting in acute inflammation of the lower-third of esophagus as well as metaplastic change in lining epithelium.

Table 20.7 Differences between reflux and eosinophilic esophagitis

Parameters	Reflux Esophagitis	Eosinophilic Esophagitis
Age group	Adults	Children
Sex predilection	Male and female equally affected	Males > females
Symptom	Heartburn	Dysphagia
Endoscopic findings	Variable	Stricture, ring
Histopathological examination	<5 eosinophils per high power field	>20 eosinophils per high power field, eosinophilic microabscesses
Treatment	Gastroesophageal reflux disease	Topical steroids
Complications	Barrett esophagus, stricture	Stricture

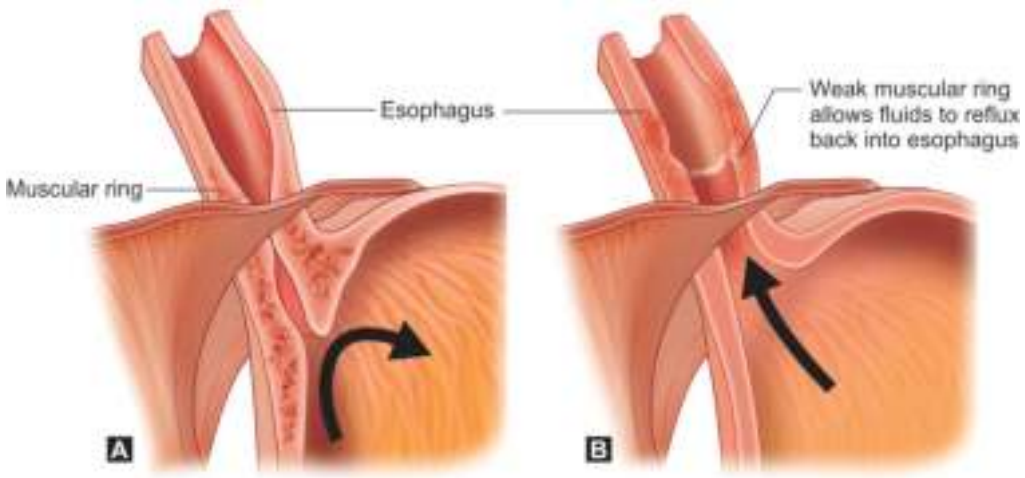


Fig. 20.1: Gastroesophageal reflux disease. (A) Normal esophagus, (B) esophagus is involved as a result of gastroesophageal reflux.

Clinical Features

Patient presents with acute epigastric pain at night (when reflux is worse), nocturnal cough, nocturnal asthma and hematemesis. Symptoms are relieved by antacids.

Complications

Chronic irritation of the esophageal epithelium due to gastroesophageal reflux disease (GERD) may cause several complications: Barrett esophagus, esophageal ulcers, stricture formation and respiratory injury are described as under.

- **Barrett esophagus:** It refers to replacement of the esophageal squamous epithelium by glandular columnar epithelium resembling gastric or colonic epithelium.
- **Esophageal ulcers:** These lesions may bleed or undergo superadded infection.
- **Stricture formation:** Chronic irritation to esophageal lining results in fibrosis forming esophageal stricture.
- **Respiratory injury:** Aspiration of gastric contents in the airways cause injury to larynx and lungs.

BARRETT ESOPHAGUS

Barrett esophagus is a pathologic change characterized by replacement of the esophageal squamous epithelium by glandular columnar epithelium resembling gastric or colonic epithelium. There is increased risk for adenocarcinoma of esophagus in 5–10% of cases.

Pathogenesis

Chronic gastroesophageal reflux disease induces esophagitis and glandular metaplasia in distal third of esophagus due to injury induced by gastric contents (acid, bile). Healing occurs by ingrowth of immature pluripotent stem cells resulting in replacement of esophageal squamous epithelium by columnar epithelium (similar to gastric or intestine) in distal third of esophagus. It is known as Barrett esophagus.

Surgical Pathology: Barrett Esophagus

Light Microscopy

- Definitive diagnosis of Barrett esophagus is established by demonstration of goblet cells with acidic mucin.
- Histologic examination reveals replacement of the esophageal squamous epithelium by glandular columnar epithelium (similar to gastric or intestinal columnar epithelium) in a patchy or circumferential manner, known as Barrett esophagus.
- Underlying connective tissue usually shows signs of chronic inflammation. Barrett esophagus shows glandular metaplasia (Fig. 20.2).

Histochemistry

Histochemical stain with Alcian blue/periodic acid–Schiff (AB/PAS/pH 2.5) is used to highlight goblet cells in a case of Barrett esophagus.

Immunohistochemistry Technique

Panel of immunohistochemical markers applied at site of Barrett's esophageal intestinal metaplasia at the gastroduodenal junction includes CK7, CK20, DAS1, MUC1 and MUC2.

Markers	Expression
▪ CK7	▪ Positive (surface and deep region)
▪ CK20	▪ Positive (surface region only)
▪ DAS1	▪ Positive
▪ MUC1	▪ Positive
▪ MUC2	▪ Positive

Clinical Features

Patient presents with painful swallowing (odynophagia), ulceration, hematemesis, and esophageal stricture.

Diagnostic Approach and Management

Diagnostic criteria for Barrett esophagus are endoscopic evidence of replacement of gray squamous mucosa by pink glandular mucosa, i.e. 'salmon red patches'.

- In the presence of low-grade dysplasia, endoscopy should be repeated in 6–12 months. Low-grade dysplasia is treated to resolve gastroesophageal reflux disease (GERD) and inflammation. Then rebiopsy should be performed in 3–6 months.
- High-grade dysplasia in Barrett esophgus is treated by endoscopic resection of the lesion. Keeping in view that lesion may undergo malignant transformation.

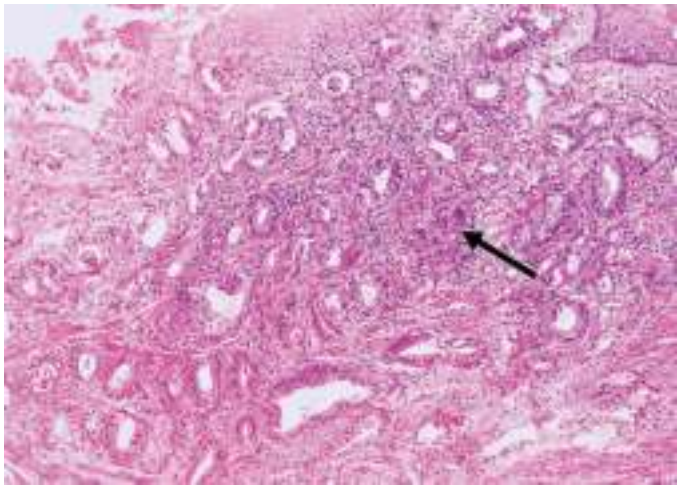


Fig. 20.2: Barrett esophagus. It shows dysplasia of gastric type of mucosa as a result of metaplasia. Goblet cells in columnar mucosa are seen (arrow).

High-grade dysplasia in Barrett esophagus is treated by photodynamic therapy, endoscopic mucosectomy and laser ablation.

Complications

Barrett esophagus is a definite risk factor for development of adenocarcinoma in 5–10% of cases.

- Severe dysplasia must be followed up clinically in every case, and partial resection of the esophagus is recommended in some cases.
- Other complications of Barrett esophagus besides malignancy are reflux esophagitis, aspiration pneumonia, esophageal stricture, esophageal ulcer, perforation and bleeding.

NON-NEOPLASTIC DISORDERS

ESOPHAGEAL DIVERTICULUM

Esophageal diverticulum refers to herniation (outpouching) of one or more layers of the esophageal wall. True diverticulum consists of mucosal, muscular, and serosal layers.

False (pulsion) diverticulum is created by increased intraesophageal pressure that results in herniation of mucosa, submucosa and muscularis propria through weak wall. Esophageal diverticula occur in three characteristic locations.

- **Zenker's diverticulum:** It is most common esophageal diverticulum; present above the upper esophageal sphincter due to defect in the cricopharyngeus muscle. It affects older than 60 years.
- **Traction diverticulum:** It is present near the midpoint of the esophagus due to motor abnormalities.
- **Epiphrenic diverticulum:** It is located above the lower esophageal sphincter due to motor abnormalities associated with achalasia (persistent constriction of the lower esophageal sphincter).

ESOPHAGEAL ACHALASIA

During swallowing, vasoactive peptides synthesized by ganglion cells in myenteric plexus result in relaxation of lower esophageal sphincter. Achalasia refers to loss of esophageal peristalsis and relaxation of lower esophageal sphincter resulting in dysphagia.

Pathogenesis

- **Congenital causes of esophageal achalasia:** Absence of ganglion cells in myenteric plexus in lower esophageal sphincter causes loss of esophageal peristalsis and relaxation of lower esophageal sphincter, it is known as '**achalasia**'. Proximal esophagus above LES (lower esophageal sphincter) is dilated.
- **Acquired causes of esophageal achalasia:** *Trypanosoma cruzi* infection (Chagas' disease) is the most important cause of achalasia in South America. Food is retained within the esophagus, and esophagus undergoes hypertrophy and dilatation.

Clinical Features

Patient presents with dysphagia for solids and liquids. Achalasia may lead to esophageal squamous cell carcinoma in about 5% of cases.

Surgical Pathology: Esophageal Achalasia
Light Microscopy
Histologic examination reveals absence of ganglion cells in the myenteric plexus of the lower esophageal sphincter, analogous to Hirschsprung's disease.

HIATUS HERNIA

Hiatus hernia is a protrusion of the stomach through an enlarged esophageal hiatus in the diaphragm. Patient presents with heartburn and regurgitation. Large herniation carries a risk of gastric volvulus or intrathoracic gastric dilation. There are two types of hiatal hernia—sliding and paraesophageal.

- **Sliding hiatal hernia:** It refers to protrusion of the proximal stomach upward above the diaphragm through a widened diaphragmatic hiatus (most common in 90% of cases). Patient presents with nocturnal epigastric distress, hematemesis, and dysphagia (stricture formation). Patient may develop Barrett's esophagus (glandular metaplasia) resulting in risk for adenocarcinoma in 5–10% of cases.
- **Paraesophageal hernia:** The gastroesophageal junction is in the normal location, but only small portion of the stomach rolls up (less common in 10% of cases) and may be strangulated by the diaphragm (Fig. 20.3).

SCLERODERMA

Scleroderma is an autoimmune disease associated with systemic increased synthesis of collagen in blood vessels, skin and internal organs. Anti-Scl-70 antibodies to nuclear topoisomerase are virtually specific for this autoimmune disease.

Lower esophageal sphincter is relaxed and dilated. There is absence of peristalsis in the proximal esophagus that causes dysphagia.

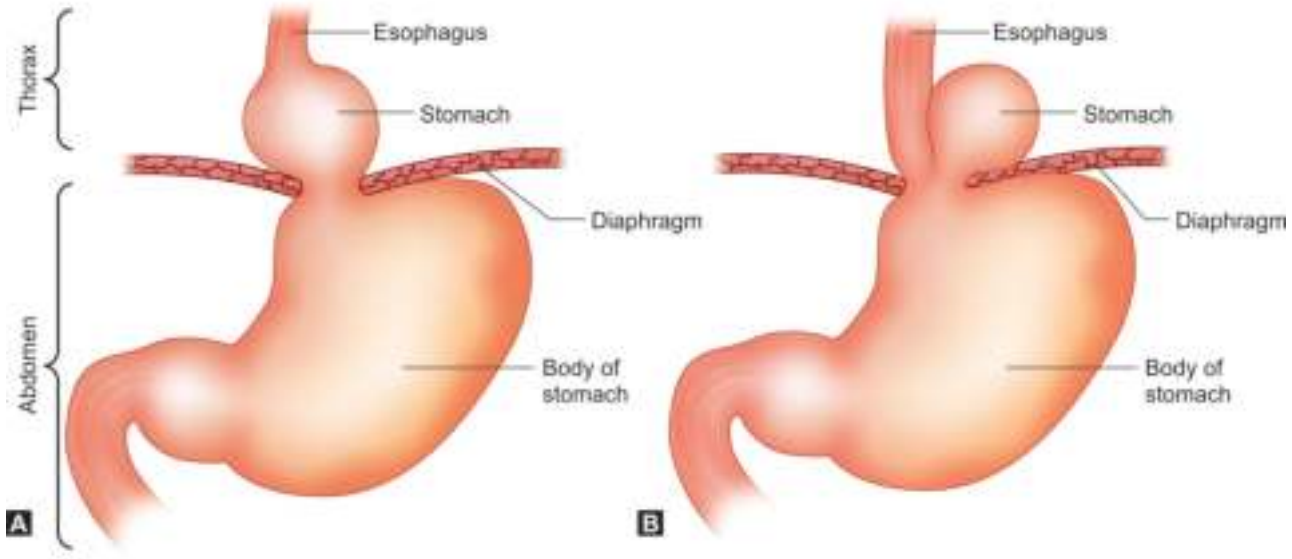


Fig. 20.3: Hiatal hernia. (A) Sliding hiatal hernia, (B) paraesophageal hiatal hernia.

Clinical Pearls: CREST Syndrome

- CREST syndrome, a variant of progressive systemic sclerosis, is also associated with esophageal motility problems.
- Patient presents with dysphagia, intermittent episodes of ischemia of the fingers, marked by pallor, paresthesia and pain known as Raynaud phenomenon.

ESOPHAGEAL STRICTURE

Esophageal stricture most often results from prolonged esophageal gastric acid reflux. It may also be caused by accidental ingestion of corrosive alkali or acid.

Strong acid produces esophageal coagulative necrosis, and strong alkali results in liquefactive necrosis, which heals with stricture formation in esophagus, and marked by progressive dysphagia.

POSTCRICOID WEBS (RINGS)

Postcricoid webs are thin mucosal membranes that projecting into the lumen of the esophagus most often seen in '**Plummer-Vinson syndrome**' also known as '**Paterson-Kelly syndrome**'.

Clinical Features

Patient presents with iron deficiency anemia, postcricoid webs in the esophagus resulting in dysphagia, glossitis, leukoplakia, koilonychia (spoon nails) and achlorhydria. There is increased risk for development of carcinoma of oropharynx and upper esophagus in Plummer-Vinson syndrome.

ESOPHAGEAL VARICES

Left gastric vein drains blood from distal esophagus and proximal stomach into the portal vein. Portal hypertension causes esophageal varices due to dilatation of left gastric vein.

- Esophageal veins are dilated veins immediately beneath the mucosa in the lower-third of esophagus in cirrhosis. These veins are linked to the portal system through gastroesophageal anastomoses. Left gastric vein is also dilated in these patients.
- Esophageal varices >5 mm in diameter are prone to rupture and life-threatening hemorrhage. Endoscopy is the primary diagnostic tool for confirming esophageal varices and treated by sclerotherapy.
- Esophageal varices cause hematemesis in liver cirrhosis, lacerations in Mallory-Weiss syndrome, esophagitis due to chemical agents or infection; and esophageal perforation in Boerhaave's syndrome.

SCHATZKI RING

Due to Schatzki ring, patient develops subepithelial semicircular fibrous strand in the wall of the esophagus resulting in narrowing of lumen at gastroesophageal junction. Patient presents with dysphagia. The lesion may be demonstrated by endoscopy. It is treated by balloon dilatation catheter technique.

VATER'S SYNDROME

Vater's syndrome is characterized by vertebral abnormalities, esophageal atresia, tracheoesophageal fistula, renal disease and absent radius bone.

BOERHAAVE'S SYNDROME

Boerhaave's syndrome is characterized by rupture of distal esophagus due to vomiting or retching regurgitating (forceful vomiting).

MALLORY-WEISS SYNDROME

Mallory-Weiss syndrome is characterized by mucosal tear of distal esophagus and upper stomach due to violent retching leading to bleeding. It is typically encountered in chronic alcoholics.

ESOPHAGUS: TUMORS

Leiomyoma, squamous cell carcinoma and adenocarcinoma are common tumors of esophagus. Histologic examination of esophageal malignancy reveals squamous cell carcinoma in 60% and adenocarcinoma in 40% of cases. Squamous cell carcinoma occurs worldwide. Primary melanoma of esophagus is extremely rare, but melanoma from other site may metastasize to esophagus. Revised 2019 WHO classification of esophageal tumors is given in Table 20.8. Benign and malignant tumors of esophagus are discussed below.

Table 20.8 Revised 2019 WHO classification of esophageal tumors

Benign Epithelial Tumors and Precursor Lesions
▪ Squamous cell papilloma, NOS
▪ Squamous papillomatosis
▪ Esophageal squamous dysplasia (intraepithelial neoplasia), low-grade
▪ Esophageal squamous dysplasia (intraepithelial neoplasia), moderate grade
▪ Esophageal glandular dysplasia (intraepithelial neoplasia), low-grade
▪ Esophageal glandular dysplasia (intraepithelial neoplasia), high-grade
Malignant Epithelial Tumors
▪ Squamous cell carcinoma, NOS
• Verrucous squamous cell carcinoma
• Squamous cell carcinoma, spindle cell variant
• Basaloid squamous cell carcinoma
▪ Adenocarcinoma, NOS
▪ Adenoid cystic carcinoma
▪ Mucoepidermoid carcinoma
▪ Adenosquamous carcinoma
▪ Carcinoma, undifferentiated, NOS
▪ Neuroendocrine tumors
• Neuroendocrine tumor grade 1
• Neuroendocrine tumor grade 2
• Neuroendocrine tumor grade 3
▪ Neuroendocrine carcinoma, NOS
• Large cell neuroendocrine carcinoma
• Small cell neuroendocrine carcinoma
▪ Mixed neuroendocrine and non-neuroendocrine neoplasm (MiNEN)
• Combined small cell—adenocarcinoma
• Combined small cell—squamous cell carcinoma

Adapted from 2019 WHO classification of tumors of digestive system.
Term NOS, not otherwise specified.
Other less common tumors derived from soft tissue are leiomyoma, lipoma, gastrointestinal stromal tumors (benign, ulcerated, potentially malignant and malignant), leiomyosarcoma and rhabdomyosarcoma. Metastatic esophageal tumors are breast carcinoma and lung carcinoma.

BENIGN POLYPOID LESIONS

Fibrovascular Polyps

Fibrovascular polyps are pedunculated intraluminal growth filling esophageal lumen. On histologic examination, polyps are composed of fibrovascular core covered by benign squamous mucosa.

Inflammatory Polyps

Inflammatory polyps are often related to gastroesophageal reflux disease (GERD) and present near the squamocolumnar junction of esophagus. On endoscopy, inflammatory polyp resembles adenocarcinoma. On histopathological examination, polyp is composed of inflamed squamous and/or foveolar mucosa.

Squamous Papilloma

Squamous papilloma is most often related to GERD. On histopathologic examination, tumor is composed of fibrovascular core covered by benign reactive squamous epithelium with varying degrees of inflammation.

SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma is most common malignant tumor of the esophagus. It frequently affects 50–60 years old age group and is more common in men than women. It most often occurs in esophageal lower portion (30%) followed by middle portion (50%) and upper portion (20%). Distribution of esophageal carcinoma is shown in Fig. 20.4.

Predisposing Factors

Risk factors, clinical/endoscopic features, histologic features, immunohistochemistry, molecular genetic alterations and prognosis of esophageal squamous cell carcinoma are given in Table 20.9.

- Cigarette smoking and alcohol are well-known risk factors for esophageal squamous cell carcinoma.

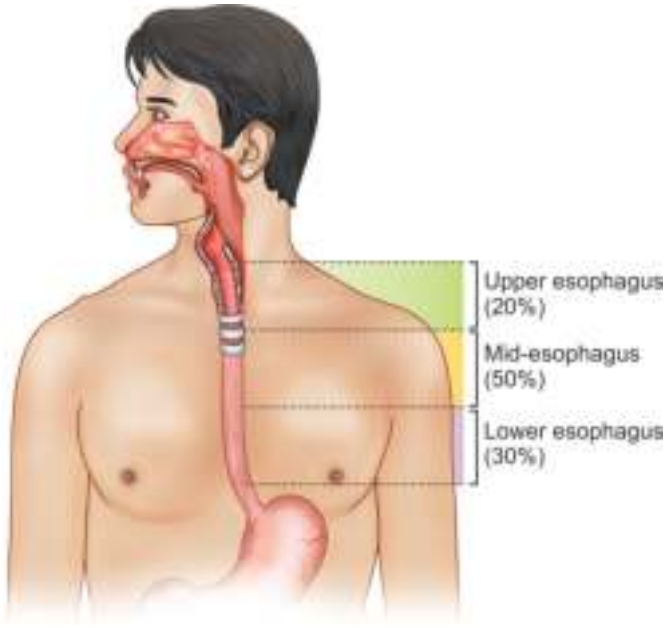


Fig. 20.4: Distribution of esophageal carcinoma.

- Other predisposing factors include Plummer-Vinson syndrome, achalasia, tylosis and Barrett esophagus. Human papillomavirus plays an etiologic role in esophageal carcinogenesis.
- Polycyclic hydrocarbons are aromatic hydrocarbons derived from animal fat by process of broiling of meats (e.g. smoked meat and fish). Ingestion of smoked meat containing polycyclic hydrocarbons undergoes hydroxylation in liver forming epoxides, results in forming covalent adducts, which bind to cellular DNA, RNA and proteins and alter the genome. Exposure to polycyclic hydrocarbons may produce cancers of oral cavity, esophagus (upper- and middle-third) and pancreas (Table 20.10).
- In immunocompromised state, patients may develop esophageal carcinoma, colorectal carcinoma, Burkitt lymphoma, diffuse large B cell lymphoma and Kaposi sarcoma in gastrointestinal tract.

Table 20.9 Risk factors, clinical/endoscopic features, histologic features, immunohistochemistry, molecular genetic alterations and prognosis of esophageal squamous cell carcinoma

Parameters	Features
Risk factors	
Socioeconomic status	Low socioeconomic status
Tobacco chewing/smoking	Most important risk factor, tobacco specific nitrosamines and polycyclic aromatic hydrocarbons main carcinogenic substances
Alcohol consumption	▪ Excessive alcohol consumption increases risk, acetaldehyde metabolite causes DNA abnormalities and structural and functional alterations of proteins ▪ Persons with ALDH2 and ALDH1 gene mutations lead to excessive accumulation of acetaldehyde
Drinking hot beverages	Consumption of hot beverages increase risk in Southern Brazil, Islamic Republic Iran
Dietary habits	Pickled vegetables associated with esophageal carcinoma
Increased body mass index	Associated with increased risk
Genetic factors	Tylosis autosomal dominant disorder due to RHBDF2 gene mutation on 17q25.3 increases risk in 90% of cases
Associated medical disorders	Plummer-Vinson syndrome related to iron deficiency, achalasia, radiotherapy to the chest area and caustic ingestion injury
Clinical and endoscopic features	
Age group	60–70 years
Esophagus involved	Middle third > distal third > proximal third
Morphology of growth	Fungating growth (most common), ulcerative growth, or infiltrative growth
Prognosis and survival	Aggressive tumor with poor prognosis and five-year survival
Histologic features	
Differentiation	▪ *Well-differentiated SCC:* Tumor cells are arranged in sheets, ample keratinization, formation of squamous pearls, intercellular bridges, few basaloid cells ▪ *Moderately differentiated SCC (most common):* Variable keratinization and significant proportion of basaloid cells are present ▪ *Poorly differentiated SCC:* Tumor cells are arranged in small or large nests, predominantly basaloid cells, or marked pleomorphism

Contd...

Table 20.9 Risk factors, clinical/endoscopic features, histologic features, immunohistochemistry, molecular genetic alterations and prognosis of esophageal squamous cell carcinoma (*Contd...*)

Parameters	Features
Histologic subtypes	Basaloid squamous cell carcinoma, spindle cell SCC (carcinosarcoma), verrucous squamous cell carcinoma
Immunohistochemistry technique	
TP63	Positive
CK5/CK6	Positive
CK7	Positive
CK14	Positive
CENP-H (centromere protein H)	Positive (associated with poor prognosis)
Molecular genetic alterations	
Genes that regulate cell cycle	TP53 gene mutation (60–90%), cell regulatory gene mutations such as CDKN2A(p16), RB1, CHEK1, CHEK2, NFEL2, CCND1, CDK4, CDK6, MDM2
Genes that control cell differentiation	NOTCH1 and NOTCH3 gene mutations (30%)
EGFR (HER1) signaling pathway	EGFR overexpression (60–75%), mutations and/or amplification in *ras* and AKT family oncogenes (75%)
Epigenetic factors	▪ DNA methylation, chromatin modification, RNA editing and loss of genomic printing ▪ Promotor hypermethylation-related silencing of CDKN2A results in overexpression of p53
Inherited factors	Tylosis (missense gene RHBDF2) results in overactivation of EGFR signaling pathway
Copy-number alterations and structural rearrangements	Gains of chromosomes 3, 10, 12 and 20 result from mutations and amplifications of genes
Other genetic abnormalities	Gene mutations of SNPs and TP53 due to acetaldehyde
Prognostic factors	
Tumor stage	Tumor, node, metastasis—metastatic classification
Tumor differentiation	Prognosis better in well differentiated squamous cell carcinoma, and poor in poorly differentiated carcinoma

Table 20.10 Chemical carcinogens associated with esophageal carcinoma

Chemical Carcinogen	Mechanisms of Entry in Body	Associated Cancers
Polycyclic hydrocarbons	Tobacco smoke, ingestion of smoked meat and fish	▪ Esophagus carcinoma (middle and distal) ▪ Oral carcinoma ▪ Pancreas carcinoma ▪ Lung carcinoma ▪ Renal pelvis transitional cell carcinoma ▪ Urinary bladder transitional cell carcinoma

Molecular Pathogenesis: Esophageal Squamous Cell Carcinoma

- Squamous cell carcinoma of esophagus develops by stepwise progression with genetic alterations driving progression from histologically normal squamous epithelium to intraepithelial neoplasia (dysplasia) to high-grade neoplasia (dysplasia) to invasive squamous cell carcinoma.
- TP53 tumor suppressor gene mutation is a key early driver mutation. Genetic abnormalities demonstrated at the intra-epithelial neoplasia stage include aneuploidy, copy-number genetic alterations, changes related to the amplification of genes such as EGFR and silencing of the CDKN2A (p16) by hypermethylation. Normally, cyclin-dependent kinases act as cell cycle regulatory proteins, that control the expression of various genes.
- Other gene mutations related to cell cycle regulators include NFE2L2, CHEK1, CHEK2, and amplifications of cell-cycle regulatory genes (CCND1, CDK4, CDK6 and MDM2). NOTCH1 and NOTCH3 gene mutations have demonstrated in 25% of cases.
- Oncogene activation and tumor suppressor gene mutation associated with esophageal carcinoma are given in Tables 20.11 and 20.12.

Table 20.11 Oncogenes, their mode of activation and associated with esophageal carcinoma

Category	Proto-oncogene	Mode of Activation	Associated Malignancies
Cyclins and cyclin-dependent kinases (cell cycle regulators)			
Cyclins	CCND1 (cyclin D1)	▪ Amplification ▪ Amplification ▪ Translocation	▪ Esophageal carcinoma ▪ Multiple myeloma ▪ Breast carcinoma ▪ Mantle cell lymphoma

Table 20.12 Tumor suppressor gene mutation associated with esophageal carcinoma

Gene	Protein	Function	Familial Tumors	Sporadic Cancers
Cell cycle progression inhibitors				
CDKN2A	$p16^{INK4a}$ and p14 ARF	▪ p16 inhibits CDK ▪ p14 indirectly activates p53 tumor suppressor	Familial melanoma	▪ Esophageal carcinoma ▪ Pancreas carcinoma ▪ Breast carcinoma

Locations

- Squamous cell carcinoma may occur in any portion of esophagus. But it most commonly develops in the middle region (most common site) and lower-thirds in areas of normal anatomic constrictions. Distally located tumor often invades the stomach. It is an aggressive cancer associated with rapid progression and short survival.
- Growth protrudes into the esophageal lumen and spreads by local extension into adjacent structures, i.e. trachea, bronchi, or aorta, or diffuse infiltration into the esophageal wall.

Clinical Features

Patient presents with dysphagia, weight loss, and anorexia. Occasionally, pain or hematemesis may occur.

Surgical Pathology: Esophageal Squamous Cell Carcinoma

Gross Morphology

- The tumor is usually circumferential and often ulcerated (most common) with sharply demarcated margins. It may be a fungating growth obstructing esophageal lumen.
- Cut surface of tumor is gray white, which invades muscular wall, extends out and involves trachea (Fig. 20.5).



Fig. 20.5: Squamous cell carcinoma of esophagus: It shows fungating growth invading wall (arrow).

Light Microscopy

Squamous cell carcinoma of esophagus has both vertical and horizontal growth of neoplastic squamous epithelium beyond the basement membrane. Grading of the tumor is based on the degree of cytological atypia, mitotic activity and presence of keratinization (Fig. 20.6).

- **Well-differentiated (grade 1) SCC:** Tumor is composed of large cells with abundant eosinophilic cytoplasm, ample keratinization, formation of squamous pearls, intercellular bridges, few basaloid cells; arranged in sheets. Cytologic atypia is minimal with low mitotic activity. The invasive margin of the tumor is pushing.
- **Moderately differentiated (grade 2 most common) SCC:** Tumor demonstrates cytological atypia, mitoses, surface parakeratosis and infrequent keratinization, significant proportion of basaloid cells.
- **Poorly differentiated (grade 3) SCC:** Tumor is composed of predominantly basaloid cells with marked pleomorphism arranged in small or large nests.

Immunohistochemistry Technique

Expression of cytokeratin (CK5/CK6, CK7, CK14) in squamous cell carcinoma is related to the degree of differentiation of tumor. Tumor shows positivity for TP63. Centromere protein H (CENP-H) is one of the fundamental components of the human active kinetophore. High expression of CENP-H is related to poor prognosis in squamous cell carcinoma.

Markers	Expression
▪ TP63	▪ Positive
▪ CK5/CK6	▪ Positive
▪ CK7	▪ Positive
▪ CK14	▪ Positive
▪ CENP-H	▪ Positive (associated with poor prognosis)

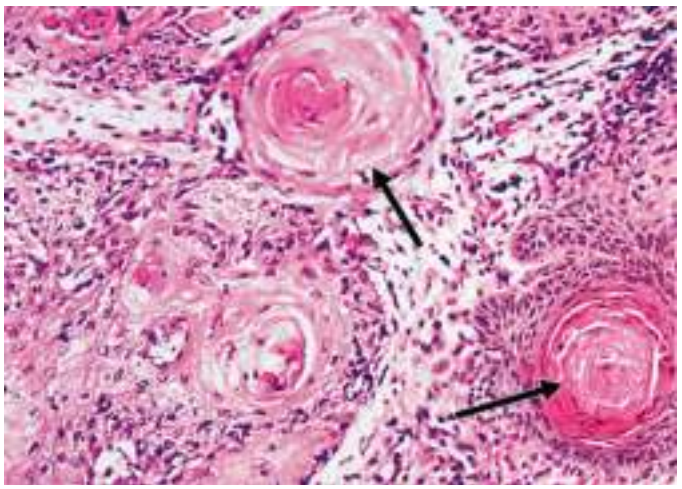


Fig. 20.6: Squamous cell carcinoma of esophagus: Tumor is composed of large cells with abundant eosinophilic cytoplasm, keratinization, keratin pearls (arrows) and intercellular bridges arranged in sheets (400X).

Mode of Spread

- **Local spread:** Esophageal squamous cell carcinoma invades in longitudinal and transverse directions. Longitudinal spread may involve gastric region. There may be formation of tracheoesophageal fistula.
- **Lymphatic spread:** Esophagus has abundant lymphatic drainage.
  - Tumor first spreads beneath the mucosa, then locally by lymphatic channels that drains into periesophageal area below the diaphragm and upward into the cervical lymph nodes.
  - The tumor may also metastasize to submucosa of stomach, probably through the submucosal lymphatic channels. Lymph node metastases occur early in the course of the disease. This is the main reason for treatment failure.
- **Hematogenous spread:** Tumor metastasizes via hematogenous route to the liver, lungs and adrenal glands.

ADENOCARCINOMA

Adenocarcinoma most often occurs in the lower-third of the esophagus especially in the setting of Barrett esophagus. Gastroesophageal reflux disease plays key role in the pathogenesis of Barrett disease.

Barrett esophagus is a pathologic change characterized by replacement of the esophageal squamous epithelium by glandular columnar epithelium resembling gastric or colonic epithelium. There is increased risk for adenocarcinoma of esophagus in 5–10% of cases. Risk factors for esophageal adenocarcinoma are given in Table 20.13.

Surgical Pathology: Esophageal Adenocarcinoma

Gross Morphology

Tumor is a small polypoidal, elevated, flat, occult or even depressed lesion. It is located close to gastroesophageal junction.

Light Microscopy

- Tumor is composed of infiltrative tubular or papillary structures. Signet ring cells with mucin production may be demonstrated. Presence of signet cell must be differentiated from signet ring cell gastric carcinoma composed of >50% signet cells.
- Esophageal adenocarcinoma may be well-differentiated with >95% of tumor composed of glands, moderately differentiated in 50–90% of cases and poorly differentiated in <50% of cases. Well-differentiated esophageal adenocarcinoma poses diagnostic challenge (Fig. 20.7).

Immunohistochemistry Technique

More than 90% of esophageal adenocarcinoma express CK7. Approximately 40% of these tumors also express CD20.

Markers	Expression
▪ CK7	▪ Positive (90%)
▪ CD20	▪ Positive (40%)

NEUROENDOCRINE TUMOR

Neuroendocrine tumor is an epithelial neoplasm with prominent neuroendocrine differentiation. Tumor can occur in middle or distal esophagus in elderly persons. Pathologic spectrum of neuroendocrine tumor can be low-grade, high-grade or large cell variant.

Table 20.13 Risk factors for esophageal adenocarcinoma

Risk Factors	Comments
Gastroesophageal reflux disease (GERD)	Most important risk factor for esophageal adenocarcinoma, Barrett disease
Abdominal obesity	Abdominal obesity related to adipokines increase GERD, Barrett disease
Male sex	Higher androgen levels increase risk
Tobacco smoking	Tobacco smoking increases Barrett esophagus and esophageal adenocarcinoma
Helicobacter pylori infection	*Helicobacter pylori* associated with atrophic gastritis and esophageal adenocarcinoma
Anti-reflux therapies	Long-term use of proton-pump inhibitors increases risk
Medications	Large doses of nonsteroidal anti-inflammatory drugs increase risk

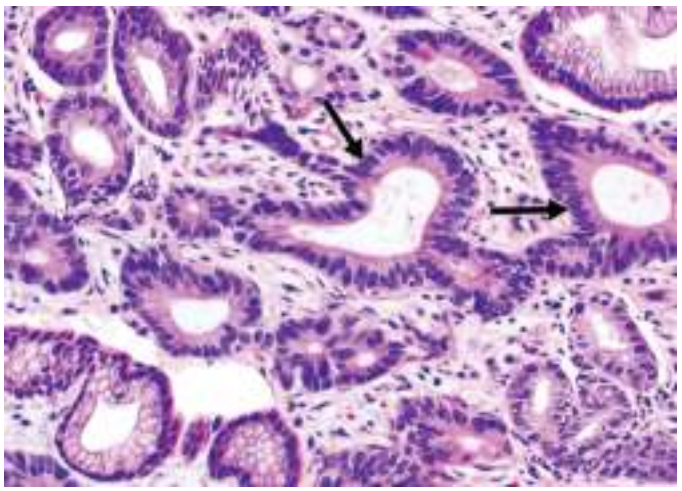


Fig. 20.7: Adenocarcinoma of esophagus (arrows) (400X).

Clinical Features

Neuroendocrine tumor of esophagus is usually an incidental finding on radiological studies or upper gastrointestinal tract endoscopy.

- High-grade neuroendocrine tumor of esophagus causes dysphagia, weight loss and chest pain.
- Tumor is diagnosed on biopsy or surgical resection.

Surgical Pathology: Neuroendocrine Tumor

Gross Morphology

Neuroendocrine tumor of esophagus is a small polypoidal or fungating or deeply invasive.

Light Microscopy

- **Well differentiated (low-grade) tumor:** Tumor is composed of uniform, small, bland tumor cells arranged in solid trabecular, gyriform or glandular patterns. It may have paneth cell differentiation. Patient has favorable prognosis.
- **Poorly differentiated (high-grade) tumor:** Tumor can be large cell or small cell tumors.
  - Large cell tumor is composed of nests of pleomorphic, large cells with prominent nucleoli and a moderate amount of cytoplasm, prominent crush artifact and Azzopardi phenomenon, necrosis, high mitotic activity and lymphovascular invasion. Tumor cells are arranged in solid or cribriform patterns. Patient has poor prognosis.

Histologic Grading of Tumor

- **Grade 1 tumor:** It shows <2 mitoses/10 HPF or <2% Ki-67 index.
- **Grade 2 tumor:** It shows 2–20 mitoses/10 HPF or 3–20% Ki-67 index.
- **Grade 3 tumor:** It shows >20 mitoses/10 HPF or >20% Ki-67 index.

Immunohistochemistry Technique

Neuroendocrine tumors show positivity for synaptophysin, chromogranin A, neuron-specific enolase (NSE) and CD56 (neural cell adhesion molecule—NCAM).

Markers	Expression
▪ Synaptophysin	▪ Positive
▪ Chromogranin A	▪ Positive
▪ NSE (neuron-specific enolase)	▪ Positive
▪ CD56 (NCAM)	▪ Positive

MESENCHYMAL TUMORS

Leiomyoma

Leiomyoma is the most common mesenchymal tumor of esophagus. It is demonstrated as an intramural, well circumscribed growth in the middle or distal esophagus. Cut surface is gray white. Histologic examination reveals benign smooth muscle bundles arranged in whirling pattern.

Gastrointestinal Stromal Tumor

Gastrointestinal stromal tumor (GIST) occurs as intraluminal mass in distal portion of esophagus. The tumor is CD117+.

Granular Cell Tumor

Granular cell tumor is demonstrated as incidental submucosal lesion. On histologic examination, tumor is composed of bland cells with granular cytosplasm arranged in sheets and nests. Cytoplasm shows positivity with S-100 and PAS stain.

Vascular Tumors

Capillary or cavernous hemangiomas appear as polypoidal intramural masses. Lymphangiomas are small sessile to pedunculated growth in the middle- to lower-third of esophagus.

OTHER TUMORS

Melanoma

Primary melanoma most often occurs as pigmented, polypoidal mass in the middle or distal portion of esophagus.

Lymphoma

Primary lymphoma rarely occurs in esophagus. On histologic examination, diffuse large B cell lymphoma, mucosa-associated lymphoid tissue lymphoma and T cell lymphoma have been described.

Metastases

Esophagus is uncommonly involved due to metastases. Breast carcinoma, lung carcinoma and melanoma may metastasize to the esophagus.

DISORDERS OF STOMACH

STOMACH: OVERVIEW (ANATOMY AND PHYSIOLOGY)

Stomach is divided into five regions: cardia, fundus, body (corpus), antrum and pylorus. The pylorus ends in a strong muscular sphincter at the gastroduodenal junction. The pylorus is a muscular zone of stomach controlling passage of food into duodenum. Cardiac region is poorly defined extending up to 5 cm distal to gastroesophageal junction. Stomach also has lesser and greater curvatures.

HISTOLOGY

Stomach has five layers: mucosa, submucosa, smooth muscle, subserosa and serosa. Mucosa of the stomach is composed of tubular glands. The cardiac region consists of mucus secreting glands. Fundus and body regions of stomach consist of glands that synthesize acid pepsin juices. Pylorus region consists of endocrine cells, which synthesize gastrin hormone.

Pathology Pearls: Gastric Glands in Body of Stomach

Gastric gland consisting of surface mucosal cells form gastric pits of foveola. It consists of three portions: isthmus, neck and base. Parietal cells are located in isthmus. Neck of stomach contains parietal cells, mucosal cells and stem cells.

- **Chief cells (zymogenic cells):** Chief cells synthesize pepsinogen. Hydrochloric acid synthesized by parietal cells converts pepsinogen into pepsin. Pepsin breaks certain peptide bonds in proteins. Chief cells secrete gastric lipase, which splits short chain triglycerides into fatty acids and monoglycerides.
- **Parietal cells (oxyntic cells):** Parietal cells secrete intrinsic factor, which is needed for absorption of vitamin B_{12}, required for erythropoiesis. Deficiency of vitamin B_{12} causes macrocytic anemia. Parietal cells also secrete hydrochloric acid, which kills microorganisms in food, denatures proteins and converts pepsinogen into pepsin.
- **Mucus surface and neck cells:** Mucus cells secrete mucus, which forms a protective barrier that prevents digestion of stomach wall.
- **Enteroendocrine G cells:** Enteroendocrine G cells stimulate chief cells to secrete pepsinogen and parietal cells to secrete HCl. It provides acidic pH (pH 1.8). It converts proenzyme pepsinogen into active pepsin. Pepsin converts proteins into peptides. Rennin is a proteolytic enzyme found in stomach of infants which helps in the digestion of milk proteins.
- **Brunner's glands:** Brunner's glands present in the duodenum synthesize alkaline fluid to buffer gastric acid and mucus for protection and lubrication.

VESSELS AND NERVES

Blood Supply

Most of the blood supply to stomach is derived from the celiac artery. Left and right gastric arteries are present along the lesser curvature. Left and right gastroepiploic arteries are present along great curvature. Proximal stomach also receives blood supply from inferior phrenic and short gastric arteries.

Venous Drainage

Venous drainage parallels the arterial supply of stomach. Left and right gastric vein drains into the portal vein. Right gastroepiploic veins drain into superior mesenteric vein, while left gastroepiploic vein drains into the splenic vein.

Lymphatic Drainage

Lymphatic drainage from superior gastric, suprapyloric, pancreaticoduodenal and inferior gastric/subpyloric regions of stomach drain into celiac group of lymph nodes and thoracic duct.

Innervation

Stomach is supplied by vagus nerve (parasympathetic system) and sympathetic system *via* the celiac plexus.

HELICOBACTER PYLORI-INDUCED GASTROINTESTINAL TRACT DISORDERS

Helicobacter pylori is transmitted by fecal–oral route. The bacterium resides within the mucosal layer lining pits beneath the bicarbonate-rich mucous barrier, where the pH approaches neutrality. *Helicobacter pylori* does not survive in metaplastic glands. Mucus and bicarbonate secretion are defense mechanisms against acid and pepsin (Fig. 20.8A and B). Role of *Helicobacter pylori* in gastrointestinal tract disorders is given in Table 20.14.

HELICOBACTER PYLORI-INDUCED MUCOSAL INJURY

Helicobacter pylori induces mucosal injury by following mechanisms. Role of *Helicobacter pylori* in gastrointestinal disorders is shown in Fig. 20.9A to D.

Attachment of Helicobacter Pylori to Mucosal Cells

The pathogen attaches to mucosa with the help of cell adhesion molecules.

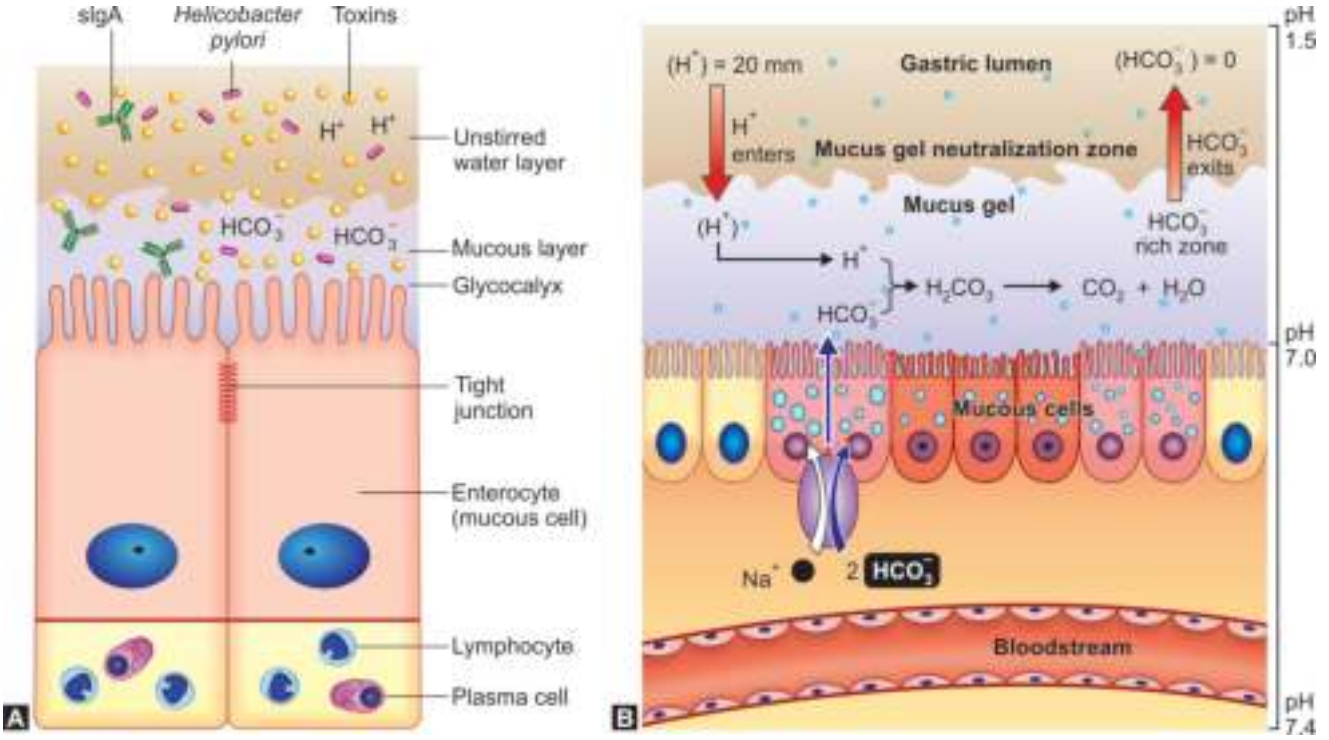


Fig. 20.8A and B: Gastroduodenal mucus and bicarbonate secretion into the adherent layer of mucus gel creates a pH gradient with a neural pH at the epithelial surfaces in stomach and duodenum. the mucus gel (rich in mucus and bicarbonate) acts as a barrier and provides the first line of mucosal protection against luminal acid and pepsin.

Table 20.14 Role of *Helicobacter pylori* in gastrointestinal disorders

Acute Erosive Gastritis
Chronic Gastritis
▪ Chronic gastritis type A (autoimmune gastritis autoantibodies against parietal cells and intrinsic factor in 10%) ▪ Chronic gastritis type B (non-autoimmune gastritis due to *Helicobacter pylori* in 90%) ▪ Chronic gastritis type AB
Granulomatous Gastritis
Chronic atrophic gastritis with extensive inflammation and glandular atrophy (glands widely spaced)
Lymphocytic Gastritis (Associated with Celiac Disease)
▪ Giant fold gastritis ▪ Some forms of autoimmune gastritis

Contd...

Hyperplasia and Metaplasia	
▪ G cell hyperplasia	▪ Intestinal metaplasia
Large Gastric Folds	
Ménétrier disease (hypertrophic gastropathy, i.e. idiopathic enlargement of mucosal folds)	
Peptic Ulcer Disease (10%)	
▪ Gastric ulcer	▪ Duodenal ulcer
Gastric Polyps	
Hyperplastic polyps	
Malignant Tumors (1–2%)	
▪ Gastric adenocarcinoma ▪ Gastric MALT (MALT, mucosa-associated lymphoid tissue) lymphoma	

Gastritis occurs in all cases.

Role of VacA Exotoxin

VacA exotoxin released by *Helicobacter pylori* enhances the ability of bacteria to colonize in the gastric epithelium and contributes to the pathogenesis of peptic ulcer disease and gastric carcinoma. VacA exotoxin disrupts gastric epithelial function due to vacuoles formation in the epithelium, '**cytochrome c**' released induced apoptosis of epithelium and blockade T cell response.

Role of DNA Segment (CagA) Effector Molecules

CagA effector molecules of *Helicobacter pylori* alter signaling pathway and tight junctions. These also induce IL-8 synthesis.

Role of Immunologic T and B Cells

The T and B cells synthesize thrombotic platelet activating factor (PAF), which acts on bacterial gene

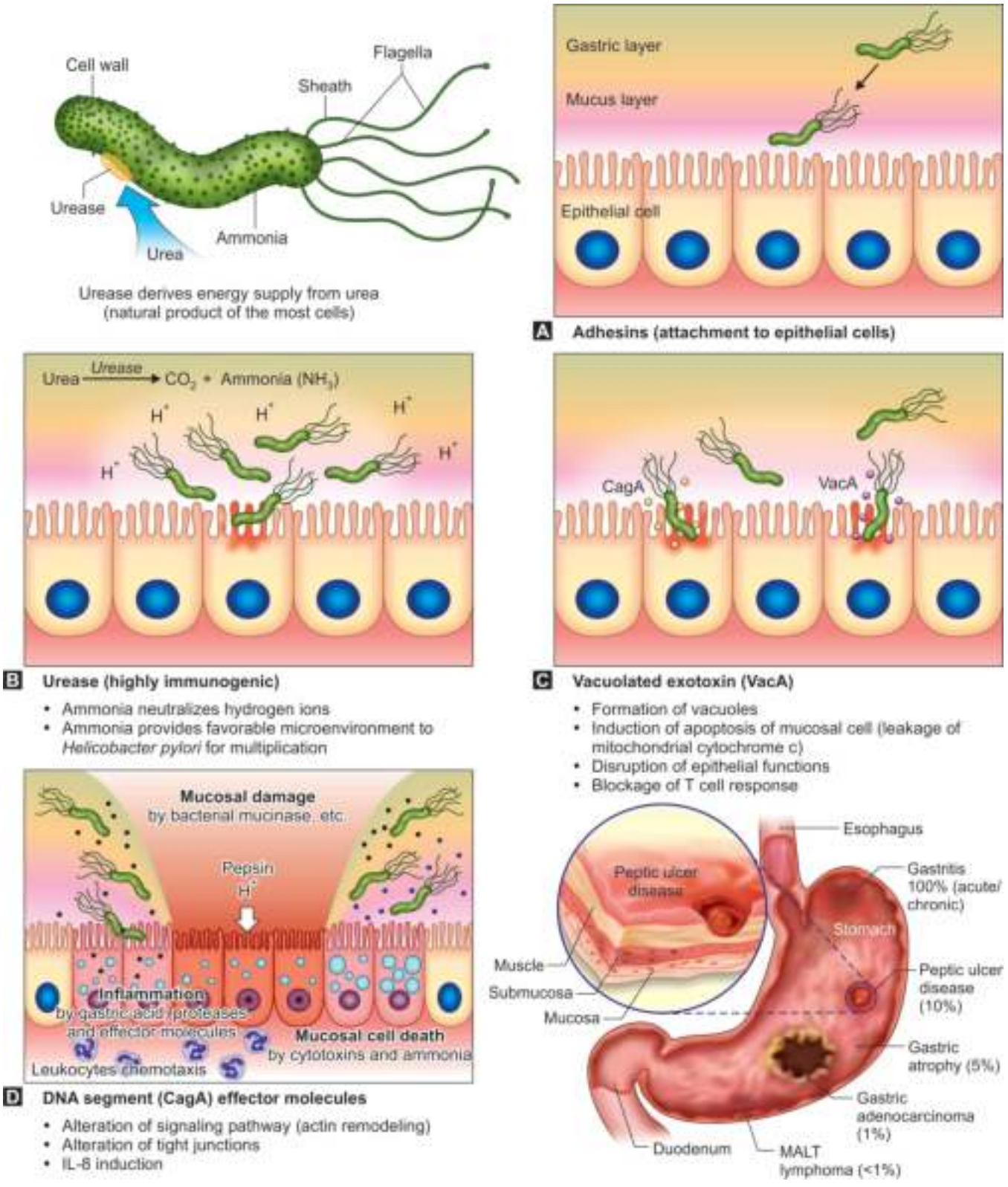


Fig. 20.9A to D: Role of *Helicobacter pylori* in gastrointestinal tract disorders.

products of *Helicobacter pylori* resulting in synthesis of proteases, phospholipases, and urease responsible for mucosal injury.

Role of Urease

Urease of *Helicobacter pylori* derives energy from urea. Urease converts urea into ammonia and CO_2.

Ammonia neutralizes hydrogen ions present in mucus layer of gastric mucosa and provides favorable microenvironment for growth of *Helicobacter pylori*.

- Ammonia stimulates gastrin, which enhances hydrochloric acid production resulting in mucosal injury due to impaired duodenal bicarbonate levels.
- Myeloperoxidase converts ammonia into monochloramine, which causes mucosal injury.

Role of Protease Enzyme

Protease breaks down mucus glycoproteins and destroys mucosal barrier of gastrointestinal tract.

Role of Phospholipase

Phospholipase damages surface epithelial cells of gastrointestinal tract. It also enhances release of leukotrienes, which attract polymorphonuclear cells resulting in release of myeloperoxidase.

Pathology Pearls: Diagnostic Tests for *Helicobacter pylori*

Tests for diagnosis of *Helicobacter pylori* infection fall into two categories: (i) Noninvasive and (ii) invasive.

- **Urea breath test:** Radiolabeled urea is administered to the patient. Urease produced by *Helicobacter pylori* combines with CO_2, which can be detected on breath.
- **Serology:** Antibody to *Helicobacter pylori* can be demonstrated in serum of patient.
- **Stool examination:** *Helicobacter pylori* can be demonstrated in stool of patient.
- **Culture:** *Helicobacter pylori* can be cultured from the biopsy specimen.
- **Rapid urease test:** It is also known as Campylobacter like organism (CLO) test. The basis of the test is the ability to synthesize urease enzyme, which catalyzes the conversion of urea to ammonia.
- **Histology:** *Helicobacter pylori* can be demonstrated by Giemsa, Warthin-Starry and Steiner stain in biopsy specimen.
- **Molecular study:** *Helicobacter pylori* (bacterial antigen) can be demonstrated by immunohistochemistry technique and polymerase chain reaction technique in biopsy specimen.

HELICOBACTER PYLORI-INDUCED GIT MALIGNANCIES

World Health Organization has designated *Helicobacter pylori*—a potential human carcinogen. It is strongly associated with gastric adenocarcinoma (lower portion) and gastric B cell lymphoma of the mucosa-associated lymphoid tissue (MALT).

- *Helicobacter pylori* causes gastric adenocarcinoma (lower portion) through its role in the development of chronic gastritis, gastric atrophy and intestinal metaplasia, hypochlorhydria, intragastric bacterial growth converting dietary amines into nitrosamines (potent carcinogenic agent), sustained epithelial proliferation, genomic mutation, dysplasia, early gastric carcinoma and advanced gastric carcinoma.
- Consumption of smoked fish and meat is rich in nitrosamines. Nitrosamines are also derived from nitrates and nitrites being used as preservative for salted pickled vegetables.
- In the advanced gastric carcinoma; urea breath test or antibody test for *Helicobacter pylori* must be performed.

INFLAMMATORY DISORDERS

Gastritis is inflammation of the mucosa of the stomach. It may be acute or chronic gastritis.

ACUTE EROSIVE GASTRITIS

Acute erosive gastritis occurs due to nonsteroidal anti-inflammatory drugs, cigarette smoking, heavy alcohol intake, biliary regurgitation, severe burns (Curling's ulcer), AIDS (cytomegalovirus) and brain injury. Nonsteroidal anti-inflammatory drugs cause hemorrhagic gastritis by disrupting bicarbonate-rich mucous barrier.

Surgical Pathology: Acute Erosive Gastritis

Light Microscopy

Gastric mucosa shows multiple erosions not extending beyond the muscularis mucosa and acute inflammation, necrosis, and hemorrhage.

CHRONIC GASTRITIS

Chronic gastritis refers to atrophy of gastric glands secondary to chronic inflammation. *Helicobacter pylori*-induce chronic gastritis, eosinophilic gastritis, lymphocytic gastritis and granulomatous gastritis.

- On gross examination, gastric mucosa is inflamed (Fig. 20.10). On histologic examination, chronic gastritis reveals atrophy of the gastric glands as well as lamina propria, intestinal metaplasia and lymphocytic infiltrate in the atrophic mucosa (Fig. 20.11).
- As *Helicobacter pylori* does not survive in the metaplastic intestinal glands, hence not demonstrated in biopsies. Histologic types of chronic gastritis include type A, type B and type AB.

Atrophic Gastritis

Atrophic gastritis is reserved for chronic gastritis with intestinal metaplasia. The crypts of the gastric

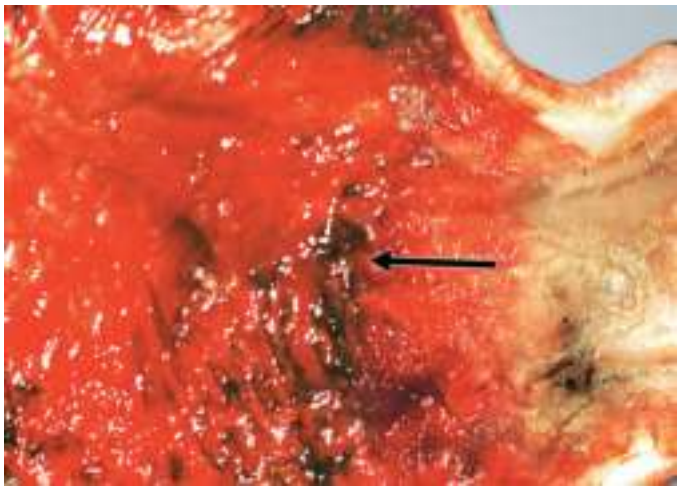


Fig. 20.10: Chronic gastritis. It shows inflamed gastric mucosa (arrow).

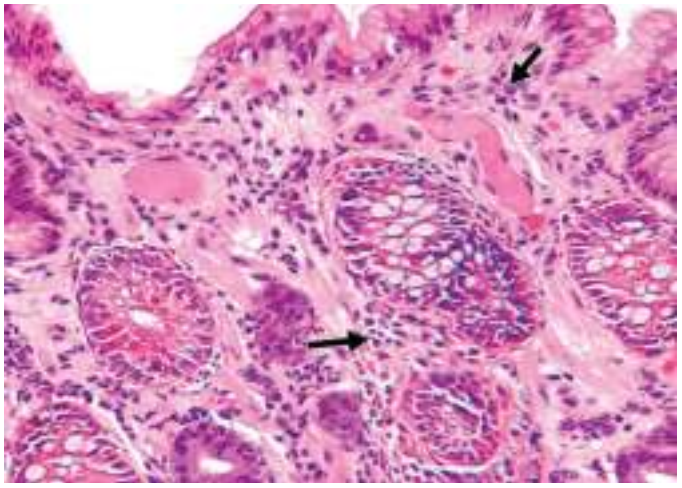


Fig. 20.11: Chronic gastritis light microscopy shows chronic inflammatory infiltrate in gastric region with intestinal metaplasia (arrows) (400X).

corpus are replaced by intestinal glands with goblet cells. There is elongation of glandular necks with extended epithelial regeneration and dysplasia. Risk for gastric carcinoma is increased in patients with chronic atrophic gastritis.

Chronic Atrophic Gastritis

Chronic Gastritis Type A/Autoimmune Gastritis

Chronic gastritis type A is an autoimmune-mediated chronic gastritis involving gastric fundus and body. Autoimmune diseases associated with autoimmune gastritis are given in Table 20.15.

Autoantibodies are formed against parietal cells and intrinsic factor. These autoantibodies are detected in serum and gastric secretions. Patient develops megaloblastic anemia due to vitamin B_{12} deficiency and achlorhydria. Bone marrow shows megaloblastic erythropoiesis. Peripheral blood smear shows macrocytes, leukopenia and thrombocytopenia. Polymorphonuclear cells show hypersegmented nuclei. Patient may develop gastric adenocarcinoma, in 10% of cases, carcinoid tumor and chronic thyroiditis.

Table 20.15 Autoimmune diseases associated with autoimmune gastritis

Hashimoto's thyroiditis
Thyrotoxicosis
Celiac disease
Insulin-dependent diabetes mellitus
Juvenile autoimmune thyroid disease
Dermatitis herpetiformis
Vitiligo
Idiopathic hypoadrenalism
Idiopathic hypoparathyroidism

Surgical Pathology: Chronic Atrophic Gastritis Type A

Light Microscopy

Histologic examination of gastric biopsy shows following features:

- There is diffuse mucosal damage and atrophy of the oxyntic acid producing mucosa within the gastric fundus and body.
- Antral mucosa lacks inflammation.
- Deep gastric glands are infiltrated by lymphocytes, macrophages and plasma cells.
- Intestinal metaplasia and antral G cell hyperplasia are seen.

Chronic Gastritis Type B/Non-autoimmune Gastritis

Helicobacter pylori resides in the gastric antrum and pylorus. It is associated with chronic gastritis type B.

These patients are also associated with gastroduodenal ulcers, gastric adenocarcinoma and MALT (mucosa-associated lymphoid tissue) lymphoma. Differences between chronic gastritis type A and type B are given in Table 20.16.

Chronic Gastritis Type AB

Chronic gastritis type AB is associated with combination of *Helicobacter pylori* and dietary factors, which predisposes to gastric adenocarcinoma.

Granulomatous Gastritis

Granulomatous gastritis occurs in patient suffering from Crohn's disease, sarcoidosis and infectious agent, e.g. Mycobacterium tubercle bacilli, fungi, cytomegalovirus and *Helicobacter pylori*.

Table 20.16 Differences between chronic atrophic gastritis type A and type B

Parameters	Chronic Atrophic Gastritis Type A (Autoimmune)	Chronic Atrophic Gastritis Type B (Non-autoimmune)
Patient population	Older age group (White women)	Adults (worldwide)
Pathogenesis	▪ Autoimmune mechanism ▪ Autoantibodies against parietal cells and intrinsic factor	▪ Nonimmune mechanism ▪ *Helicobacter pylori*
Clinical manifestations	▪ Pernicious anemia (megaloblastic erythropoiesis) ▪ Decreased gastric acid secretion	▪ Abdominal pain and dyspepsia ▪ Upper gastrointestinal bleeding
Light microscopy	▪ Chronic gastritis ▪ Progressive destruction of glands in fundus region ▪ Intestinal metaplasia (pyloric region) and entero-chromaffin cells hyperplasia	▪ Chronic gastritis ▪ Destruction of glands absent ▪ Intestinal metaplasia (pyloric region, if body involved)
Serum gastrin level	Elevated	Normal or low
Complications	▪ Gastric adenocarcinoma (10%) ▪ Carcinoid tumors ▪ Chronic thyroiditis	▪ Gastric adenocarcinoma (lower gastric region) ▪ Gastric MALT lymphoma (mucosa-associated lymphoid tissue lymphoma) ▪ Gastroduodenal ulcers (peptic ulcer disease)
Clinical course	Chronic aggressive course	Chronic less aggressive course

Chronic atrophic gastritis type AB caused by Helicobacter pylori and dietary factors predisposes to gastric adenocarcinoma.
Eosinophilic gastritis is related to allergens and characterized by transmural infiltration by eosinophils and muscular hypertrophy.

PEPTIC ULCER DISEASE

Peptic ulcer disease occurs in duodenum, stomach, and lower end of the esophagus including Meckel's diverticulum. *Helicobacter pylori* is most often associated with duodenal ulcers in 90% and gastric ulcers in 65% of cases. Eradication of *Helicobacter pylori* contributes to the healing of peptic ulcers.

Peptic ulcer disease is most often solitary, but may be multiple in 20% of cases. Vagotomy is performed only in the treatment of complicated persistent duodenal ulcers resistant to other treatment modalities. Pathogenesis and clinical course of peptic ulcer disease is shown in Fig. 20.12.

LOCATIONS

Duodenal peptic ulcer most often occurs in first portion of the duodenum just close to the pylorus, lesser curvature of stomach and gastric antrum. It is associated with hypersecretion of gastric acid and pepsin and *Helicobacter pylori* infection. Most common cause of duodenal ulcer is *Helicobacter pylori* infection.

- *Helicobacter pylori* increases gastric acid secretion and impairs mucosal defenses. Gastric peptic ulcer most often occurs at lesser curvature just at the junction of the body and pyloric antrum. Differences between duodenal and gastric peptic ulcers are given in Table 20.17.

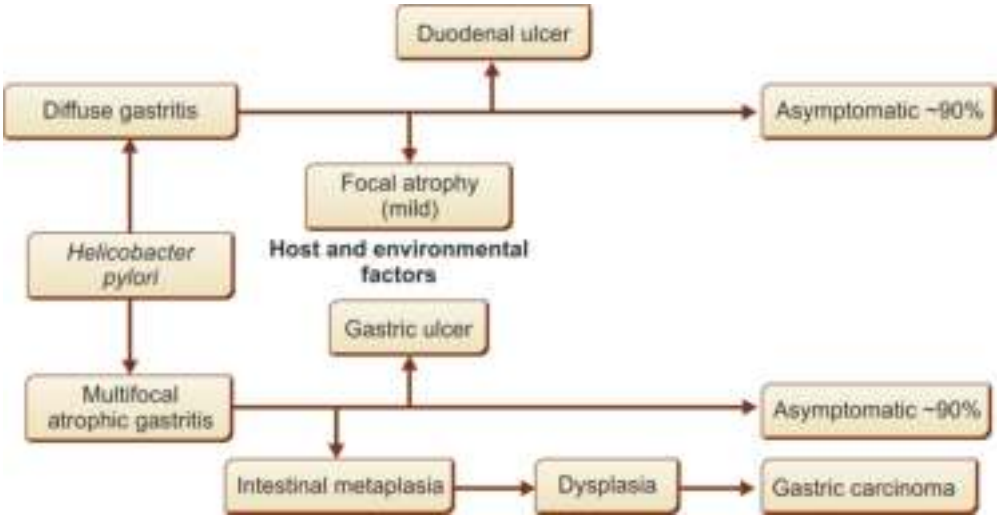


Fig. 20.12: Pathogenesis and clinical course of peptic ulcer disease.

Table 20.17 Differences between duodenal and gastric peptic ulcers

Parameters	Duodenal Ulcer	Gastric Ulcer
Age group	25–50 years of age	More than 50 years
Sex predilection	Men > women	Men and women equally affected
Risk factors	MEN 1, cirrhosis, chronic obstructive pulmonary disease, chronic renal failure, hyperparathyroidism	Smoking delays healing of ulcer
Frequency	More often (75% cases)	Less often (25%)
Increased incidence	Blood group O	Blood group A
Etiology	*Helicobacter pylori* is responsible for defective mucosal barrier in 80–90% of cases	Due to disruption of mucosal barrier due to mucosal ischemia (reduced PGE), bile reflux, delayed gastric emptying
Basal acid output (BAO) and maximal acid output (MAO)	Acid levels high	Normal to low. May be increased due to enhanced gastrin secretion
Number of peptic ulcer	Solitary	Solitary
Location	Anterior portion of first part of duodenum in 90% cases and posterior portion	Lesser curvature of antrum (same location for cancer)
Perforation	Ulcer present in posterior portion of duodenum may perforate into pancreas results in pancreatitis	Perforation is less common
Clinical manifestations	Burning epigastric pain 1–3 hours after eating. Epigastric pain relieved by eating	Epigastric pain exacerbated by eating
Malignant transformation	Duodenal ulcers never undergo malignant transformation	Malignant transformation is more common
Complications	▪ Bleeding from gastroduodenal artery, perforation (air under diaphragm, pain radiates to left or right shoulder) ▪ Gastric outlet obstruction and pancreatitis	▪ Bleeding from left gastric artery ▪ Perforation of gastric ulcer
Endoscopy accuracy	90–95%	90–95%
Upper GIT barium study	Diagnostic in 70–80% of cases	Diagnostic in 70–80% of cases
Histopathological examination	Not essential as not undergoing malignant transformation	Essential to rule out malignant transformation in 1–4% of cases
Treatment	▪ Avoid NSAIDs, alcohol, smoking ▪ Eradication of *Helicobacter pylori* ▪ Highly selective vagotomy in resistant cases	▪ Avoid NSAIDs, alcohol, smoking ▪ Eradication of *Helicobacter pylori* ▪ Hemigastrectomy without vagotomy in resistant cases

- Other sites of peptic ulcer disease are gastroesophageal junction in the settings of Barrett esophagus/GERD, Meckel diverticulum containing ectopic gastric mucosa, and mucosa adjacent to the anastomoses line in patient underwent gastrointestinal tract surgery.

ETIOPATHOGENESIS

Since the gastric ulcer patients do not contain unusually high quantities of hydrochloric acid or pepsin, it is postulated that glands are damaged by back diffusion of hydrogen ions (most important). *Helicobacter pylori*, spicy food, alcohol and drug aspirin disrupt mucosal barrier resulting in mucosal injury due to back diffusion of hydrogen ions. *Campylobacter pylori*, a common cause of gastritis has been associated with peptic ulcer disease and gastric carcinoma.

Helicobacter Pylori

- *Helicobacter pylori* increases gastric acid secretion and apparently impair both gastric and duodenal mucosal defenses resulting in duodenal ulcer (90%) and gastric ulcer (65%).
- *Helicobacter pylori* synthesizes urease, phospholipase and protease which cause breakdown of glycoproteins resulting in defective mucosal barrier.
- Increased permeability of the gastric mucosa to hydrogen ion leads to back diffusion of hydrogen ions resulting in injury to the gastric mucosa (mucosal ischemia due to reduced PGE).
- Eradication of *Helicobacter pylori* infection is curative of peptic ulcer disease in most patients.

Bile Reflux and Delayed Gastric Emptying

These conditions may also cause peptic ulcer disease.

Dietary Factors and Acid Secretion

Alcohol, spicy food, and substances that stimulate acid secretion may play a pathogenetic role in peptic ulcer disease. Duodenal ulcer is always associated with hypersecretion of gastric acid and pepsin.

- Due to increased parietal cell mass, basal acid output and maximal acid output are increased in duodenal ulcer.
- On the other hand, basal acid output (BAO) and maximal acid output (MAO) are normal to low in gastric ulcer.

Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs such as aspirin inhibit prostaglandin synthesis resulting in damage to mucosal barrier resulting in peptic ulcer disease.

Tobacco Smoking

The incidence of peptic ulcer disease is two-fold greater in tobacco smokers.

Zollinger-Ellison Syndrome

Recurrent intractable peptic ulcer disease or peptic ulcer in aberrant sites, i.e. the jejunum is characteristic of the Zollinger-Ellison syndrome with excess gastrin production from a gastrinoma (islet cell tumor of the pancreas).

Genetic Factors

Increased frequency of occurrence of peptic ulcer disease in persons with blood group O suggests that genetic factors may play a pathogenetic role in peptic ulcer disease.

Hyperparathyroidism

Peptic ulcer disease is sometimes associated with primary hyperparathyroidism.

Multiple Endocrine Neoplasia 1 (MEN 1)

Peptic ulcer disease may occur in MEN 1, an autosomal dominant disorder characterized by adenomas or hyperplasia of pituitary, thyroid, parathyroid, adrenal and pancreatic islet cells.

Hypercalcemia increases serum gastrin, thereby stimulating gastric acid secretion. Defensive and aggressive factors in relation to peptic ulcer disease are shown in Fig. 20.13.

CLINICAL FEATURES

Patient with gastric ulcer presents with immediate epigastric pain just after food intake. While pain occurs within 1–3 hours after food intake in duodenal ulcer.

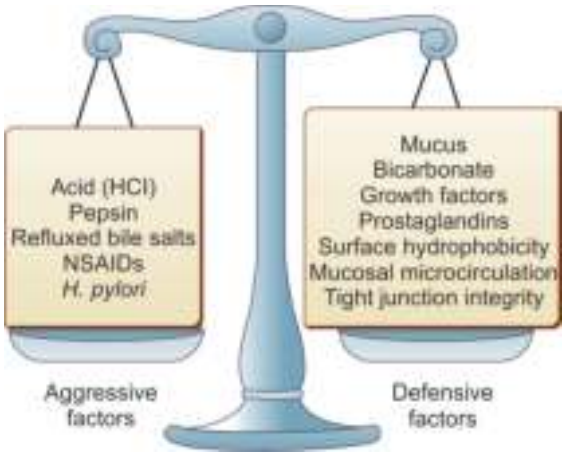


Fig. 20.13: Defensive and aggressive factors in relation to peptic ulcer disease.

Surgical Pathology: Peptic Ulcer Disease

Gross Morphology

Morphologic features of peptic ulcer disease are described in Fig. 20.14.

- **Size and shape:** Most peptic ulcers are small round or oval defect of the mucosa measuring 1–2 cm in diameter, and rarely exceeding 4 cm in size.
- **Base of the ulcer:** Base of the ulcer is smooth and clean due to action of hydrochloric acid and pepsin, but it may be covered with fibrin.
- **Margin of ulcer:** Gastric ulcer shows radiating folds of gastric mucosa starting at the ulcer with punched out margins appearing as radiating fold in a spoke-like fashion, in contrast to gastric carcinoma, which often has an irregular necrotic base and firm and raised margins.
  - The distinction between gastric peptic ulcer and ulcerated gastric carcinoma must be established by biopsy.
  - In contrast to malignant gastric ulcer, peptic ulcer disease usually heals with conservative management.

Light Microscopy

On histologic examination, peptic ulcer shows following four zones from superficial layer to deep base. The mucosal defect may extend all the way to the muscle layer (Figs 20.15 and 20.16).

- **Floor/base:** It is superficial zone of peptic ulcer and composed of necrotic fibrinous amorphous debris.
- **Zone of acute inflammatory exudates:** This zone is infiltrated by numerous neutrophils. Inflammation is present beneath floor/base of ulcer, and merges with granulation tissue.
- **Granulation tissue:** Granulation tissue is composed of blood vessels, fibroblasts, macrophages, lymphocytes and plasma cells. Granulation tissue gradually transforms into a zone of fibrous scarring.
- **Zone of fibrous scarring:** Granulation tissue rests on a solid fibrous or collagenous scar. If the ulcer extends into the muscle layer, it may erode blood vessels and cause bleeding.

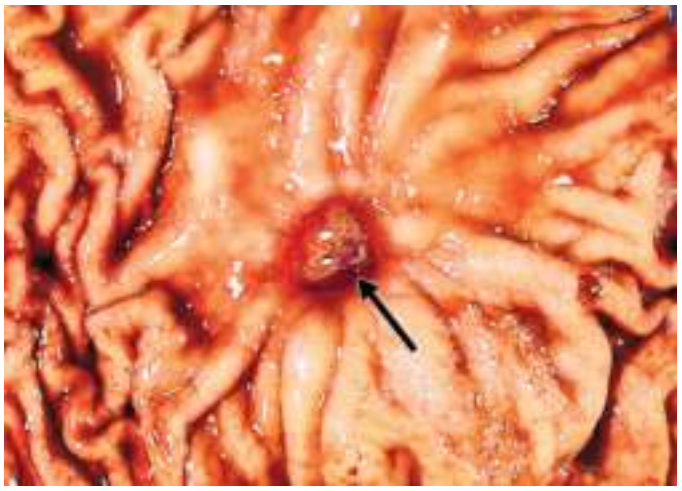


Fig. 20.14: Peptic ulcer disease. A partial gastrectomy was performed because of hematemesis. There was a bleeding artery in the base of the ulcer (arrow).

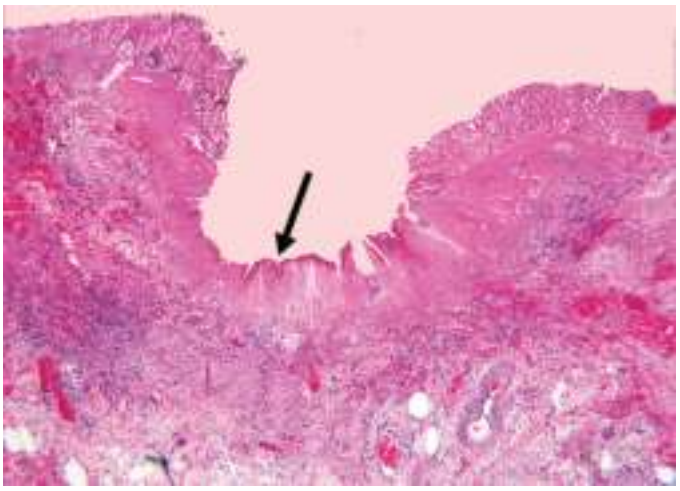


Fig. 20.15: Chronic peptic ulcer. It shows base composed of necrotic fibrinous amorphous debris, overlying inflammatory infiltrate, granulation tissue and fibrous tissue (arrow) (100X).

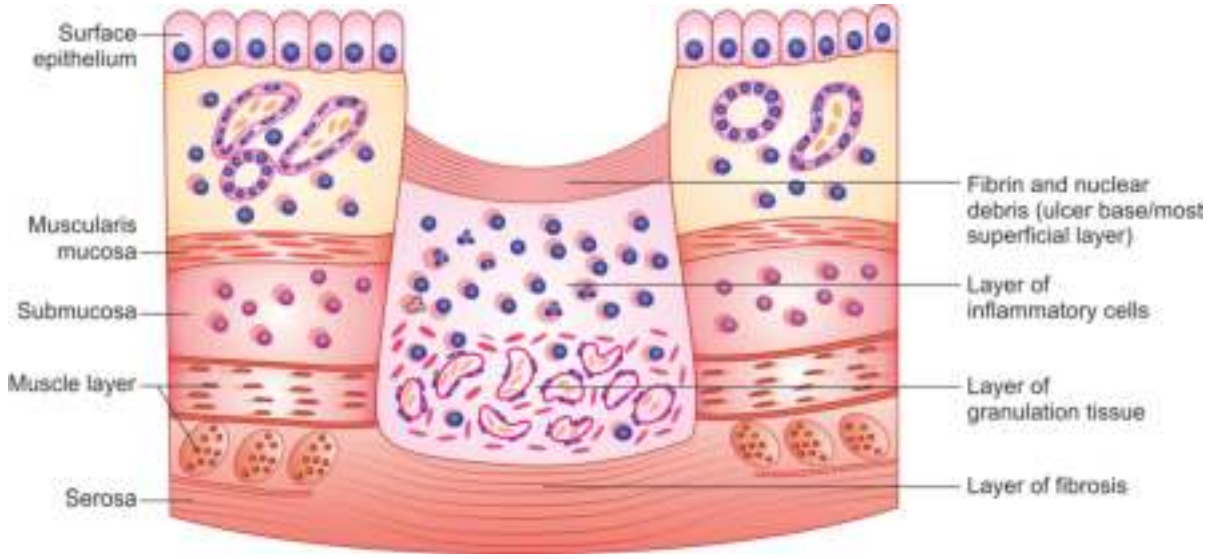


Fig. 20.16: Peptic ulcer disease shows four zones from superficial layer to deep base: superficial floor/base, zone of inflammatory exudate, zone of granulation and zone of fibrous scarring.

Clinical Pearls: Complications of Peptic Ulcer Disease

Complications of peptic ulcer disease include hemorrhage, perforation, stenosis, pancreatitis and malignant transformation. Reasons for surgery in patient with peptic ulcer disease are hemorrhage, perforation, obstruction and exclusion of malignant transformation of peptic ulcer disease.

- **Hemorrhage:** Hemorrhage is the most common complication of peptic ulcer disease. Patient presents with hematemesis with melena (black stools containing blood). Ulcer erodes the wall of arteries supplying to the region. Gastroduodenal artery bleeds in duodenal ulcer, whereas bleeding occurs from left gastric artery in gastric ulcer. Severe bleeding is life-threatening in 10–20% of patients. Melena is commonly seen in patients who suffer from chronic peptic ulcer disease.
- **Perforation:** Duodenal ulcer most often perforates than gastric ulcer disease in 10% of cases. Patient develops peritonitis and paralytic ileus. Air is demonstrated under the diaphragm (pneumoperitoneum) by imaging techniques. Perforation with severe hemorrhage is responsible for hypotensive shock with fatal outcome patients. Fibrosis around healed gastric ulcer causes narrowing in center of the stomach giving hourglass deformity. Patient with healed ulcer in duodenum develops pyloric stenosis.
- **Pancreatitis:** Perforation of posterior wall of duodenal ulcer may cause peritonitis or involvement of liver. Pancreatitis is accompanied by dull pain and elevated serum amylase and lipase.
- **Malignant transformation:** Most gastric ulcers are benign, but small percentage of cases may undergo malignant transformation. Duodenal ulcers never undergo malignant transformation. Peptic ulcer disease shows distribution of benign ulcers in stomach and duodenum and malignant gastric ulcers (Fig. 20.17 and B). Differences between benign and malignant ulcers are given in Table 20.18.

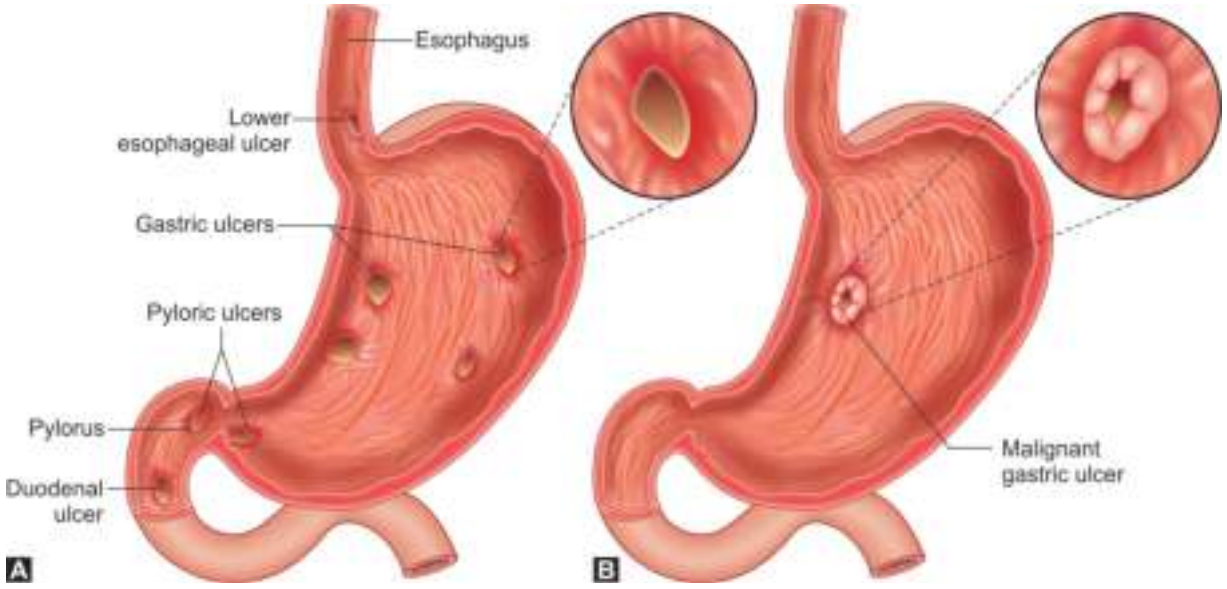


Fig. 20.17: (A) Peptic ulcer disease shows distribution of ulcers in stomach and duodenum, (B) malignant gastric ulcer.

Table 20.18 Differences between benign and malignant gastric ulcers

Parameters	Benign Gastric Ulcer	Malignant Gastric Ulcer
Age group	Younger age group	Older age group
History	Longer history	Shorter history
Pain abdomen	Common	Less common
Gender	Male predominance	Male and female equally affected or male predominance
Site of ulcer	Antrum, lesser curvature of stomach	Along greater curvature of stomach
Base of ulcer	Clear and hemorrhagic	Necrotic debris
Gastric mucosal folds	Radiating mucosal folds	Interrupted mucosal folds
Margins of ulcer	Sharply punched-out	Irregular heaped up
Barium meal study	Sharp punched-out lesion	Irregular lesion
Light microscopy	Composed of necrotic floor base, numerous neutrophils, granulation tissue and fibrosis	Showing features of malignancy, that may disseminate to distant organs

GASTRIC POLYPS

The term gastric polyp usually refers to any lesion which projects above the mucosal plane into the lumen of the stomach. Gastric polyps can be divided into neoplastic and non-neoplastic categories.

- **Gastric hyperplastic polyps:** These are composed of elongated, dilated, branching foveola and inflammed lamina propria. These may occur in Osler-Weber-Rendu syndrome. Size of the polyp is >2 cm seen in >50 years of age.
- **Fundal gland polyps:** These are composed of cystically dilated spaces partially lined by parietal cells. These may occur in familial adenomatous polyposis (FAP) coli syndrome or sporadic cases.
- **Inflammatory fibroid polyps:** Inflammatory fibroid polyp most commonly occurs in the gastric antrum. On histologic examination, it is composed of a submucosal collection of bland spindle cells in a background of dilated vascular channels and inflammatory cells especially eosinophils. Spindle cells are CD34+.
- **Gastric adenomatous polyps:** These can develop anywhere in the stomach. These growths can often be precursor of gastric carcinoma.
- **Gastric hamartomatous polyps:** These are demonstrated in patients with Peutz-Jeghers syndrome, Cowden's disease and juvenile polyposis.

NEOPLASTIC DISORDERS OF STOMACH

Most common benign gastric neoplasms include hyperplastic polyps, fundic gland polyps and adenomas. Malignant tumors are gastric adenocarcinoma (90–95%), gastrointestinal tract lymphoma (3%), gastrointestinal tract stomal tumors, e.g. leiomyoma (1%), carcinoid

tumors (1%) and diffuse gastric carcinoma [known as '**linitis plastica**' (**leather bottle stomach**) results in a thickened rigid stomach caused by the extensive infiltration of neoplastic cells in the gastric wall].

- According to Bormann classification of advanced gastric adenocarcinoma includes: type 1 (polypoid fungating growth), type 2 (ulcerative with elevated distinct borders), type 3 (ulcerative with elevated indistinct borders); and type 4 (diffuse with indistinct borders).
- Risk factors for developing gastric adenocarcinoma include chronic gastritis; dietary nitrates/smoked food/pickled vegetables, *Helicobacter pylori* infection, history of adenomatous polyps and decreased intake of fresh fruits/vegetables. Patient presents with weight loss, abdominal pain, dysphagia, anorexia, nausea and vomiting.
- Primary gastric adenocarcinoma involves mucosa, whereas secondary tumors (e.g. breast carcinoma, malignant melanoma and leukemic infiltration) involve submucosa and muscularis. Revised 2019 WHO classification of tumors of the stomach is given in Table 20.19. Risk factors for development of gastric carcinoma is given in Table 20.20.
- **Krukenberg tumor** is metastasis from breast lobular carcinoma and diffuse gastric carcinoma known as 'linitis plastica' to the ovaries. Histologically, tumor shows signet-ring cell features.
- **Virchow node** is firm palpable left supraclavicular lymph nodes that represents metastatic carcinoma, typically from a primary carcinoma in the thorax and abdomen.

Pathology Pearls: Gastric Dysplasia Types

Gastric dysplasia is of three types described as under.

- **Adenomatous gastric dysplasia:** It is intestinal/type 1 dysplasia. It may be tubular or villus resembling colonic adenoma.
- **Hyperplastic gastric dysplasia:** It is gastric foveolar/type 2 dysplasia. It most often occurs in a case of chronic atrophic gastritis with immature intestinal metaplasia.
- **Tubule neck gastric dysplasia:** It is also known as globoid dysplasia. Its histopathologic features are similar to the invasive diffuse type of gastric adenocarcinoma. In tubule neck dysplasia, there is presence of aggregates of enlarged clear cells with vacuolated cytoplasia and dysplastic nuclei.

GASTRIC CARCINOMA

Gastric carcinoma is the most common tumor, which constitutes 90–95% of all gastric cancers. It is often

Table 20.19 Revised 2019 WHO classification of tumors of the stomach

Benign Epithelial Tumors and Precursor Lesions
■ Glandular intraepithelial neoplasia, low-grade
■ Glandular intraepithelial neoplasia, high-grade • Serrated dysplasia, low-grade • Serrated dysplasia, high-grade • Intestinal-type dysplasia • Foveolar type (gastric-type) dysplasia • Gastric pit/crypt dysplasia
■ Intestinal-type adenoma, low-grade
■ Intestinal-type adenoma, high-grade • Sporadic intestinal-type gastric adenoma • Syndromic intestinal-type gastric adenoma
■ Adenomatous polyp, low-grade dysplasia
■ Adenomatous polyp, high-grade dysplasia
Malignant Epithelial Tumors
■ Adenocarcinoma, NOS • Tubular adenocarcinoma • Parietal adenocarcinoma • Adenocarcinoma with mixed subtypes • Papillary adenocarcinoma, NOS • Micropapillary carcinoma, NOS • Mucoepidermoid carcinoma • Mucinous adenocarcinoma • Signet ring cell carcinoma • Poorly cohesive carcinoma • Medullary carcinoma with lymphoid stroma • Hepatoid adenocarcinoma • Paneth cell carcinoma
■ Squamous cell carcinoma, NOS
■ Carcinoma, undifferentiated, NOS • Large cell carcinoma with rhabdoid phenotype • Pleomorphic carcinoma • Sarcomatoid carcinoma • Carcinoma with osteoclast-like giant cells
■ Gastroblastoma
■ Neuroendocrine tumors, NOS • Neuroendocrine tumor, grade 1 • Neuroendocrine tumor, grade 2 • Neuroendocrine tumor, grade 3 • Gastrinoma, NOS • Somatostatinoma, NOS • Enterochromaffin cell carcinoid • Enterochromaffin like (ECL) cell malignant
■ Neuroendocrine carcinoma, NOS • Large cell neuroendocrine carcinoma • Small cell neuroendocrine carcinoma
■ Mixed neuroendocrine–non-neuroendocrine neoplasm (MiNEN)

Adapted from 2019 WHO classification of tumors of digestive system. Other less common tumors derived from soft tissue are leiomyoma, gastrointestinal stromal tumors, glomus tumor, granular cell tumor, Kaposi sarcoma, synovial sarcoma. Primary lymphoma may occur. Stomach may be involved due to metastases from breast carcinoma, melanoma and leukemia.

Contd...

Table 20.20 Risk factors for development of gastric carcinoma

Risk Factors	Comments
Host factors/predisposing conditions	
Gender	More common in men than women
Age group	>50 years especially in blood group A
Geographical region	Japan, Finland, Iceland, Russia, China and Southern and Eastern USA
Tobacco chewing or smoking	Higher risk for carcinoma at esophageal gastric region increased
Ménétrier disease (hypertrophic gastropathy)	Higher risk for gastric carcinoma due to unknown reason
Common variable immunodeficiency (CVID)	Higher risk for gastric carcinoma and MALT lymphoma
Gastric adenoma	Size >2 cm increased risk for gastric carcinoma
Previous partial gastrectomy for peptic ulcer	Higher risk for gastric carcinoma in gastric cancer after many years
Environmental factors	
Helicobacter pylori	▪ Single most important factor in the development of gastric cancer ▪ *Helicobacter pylori* gastritis affects 40–75% of persons are at high risk ▪ Cag positive *Helicobacter pylori* has ability tumor suppressor genes such as TP53 and RUNX3 ▪ Increases the risk for gastric carcinoma by five to six folds ▪ Intestinal type adenocarcinoma of stomach ▪ Gastric MALT lymphoma
Chronic gastritis	▪ Hypochlorhydria favors colonization of *Helicobacter pylori* ▪ Intestinal metaplasia in chronic gastritis is a precursor lesion
Pernicious anemia	Hypochlorhydria due to decreased synthesis of hydrochloric acid by parietal cells favors colonization of *Helicobacter pylori*
Dietary factors/nutritional factors	▪ Diet rich in nitrosamines in preserved food (nitrates are converted to nitrites) ▪ Consumption of smoked fish rich in nitrosamines
Occupational exposure to coal, asbestos, rubber products	Higher risk for gastric carcinoma
Epstein-Barr virus	Higher risk for gastric carcinoma (intestinal type and linitis plastica)
Radiation	▪ Dose-dependent increase in gastric carcinoma occurs in Japanese atomic bomb survivors suggesting ionization radiation may play role in the gastric carcinoma ▪ Radiotherapy of the abdomen for lymphoma and testicular tumors in young patient increase the risk for development of gastric carcinoma
Inherited cancer syndrome	
Familial gastric carcinoma (E-cadherin gene mutation)	Higher risk for gastric carcinoma (linitis plastica variant)
β-Catenin gene mutation	Higher risk for gastric carcinoma (linitis plastica variant)
Familial adenomatous polyposis (FAP) due to APC gene mutation	Higher risk for gastric carcinoma at younger age
Li-Fraumeni syndrome (p53 gene mutation)	Higher risk for gastric carcinoma at younger age
Peutz-Jeghers syndrome (STK1 gene mutation)	Higher risk for gastric carcinoma
p16 gene mutation	Higher risk for gastric carcinoma
Hereditary nonpolyposis colorectal cancer syndrome (Lynch syndrome): MLH1, MSH2, MSH6, PMS1, PMS2, TGF	Higher risk for gastric carcinoma
Family history of gastric carcinoma in first degree relatives	Higher risk for gastric carcinoma
BRCA1 and BRCA2	Higher risk for gastric carcinoma

discovered at advanced stage. Practically all gastric adenocarcinomas arise from generative or basal cells of foveolae. Intestinal metaplasia is precursor lesion of gastric adenocarcinoma. Locations and frequency of gastric carcinoma are given in Table 20.21. Distribution of gastric carcinoma is shown in Fig. 20.18A and B.

Table 20.21 Location and frequency of gastric carcinoma

Location	Frequency
Pyloantrum region (anterior and posterior walls and lesser curvature of stomach)	50–60%
Cardiac region of stomach	25%
Body and fundus region	25%
Greater curvature of stomach	12%

Predisposing Factors

- **Gender:** Gastric carcinoma is more common in men than women.
- **Age group:** There is a sharp increase in frequency of gastric carcinoma in people over the age of 50 years especially in men. Most people with gastric carcinoma are diagnosed in their late 60s and 80s.
- **Geographical incidence:** Incidence of gastric carcinoma is higher in Japan, Finland, and Iceland, Chile, Russia, China and Southern and Eastern Europe, but low incidence in USA. Its incidence is higher in South India than rest of the country.
- ***Helicobacter pylori:*** Incidence of gastric carcinoma in the lower (distal) part of the stomach parallels the presence of chronic gastritis secondary to *Helicobacter pylori*. Persistent infection by *Helicobacter pylori* is associated with chronic atrophic gastritis, sustained epithelial proliferation, genomic mutation, dysplasia, early gastric carcinoma and advanced gastric adenocarcinoma, MALT (mucosa-associated lymphoid tissue) lymphoma and carcinoid tumors. *Helicobacter pylori* induces mucosal injury and converts dietary amines into nitrosamines (carcinogenic agent).
- **Occupational exposure:** Workers in the coal, asbestos, and rubber industries seem to have a higher risk for gastric carcinoma.
- **Dietary factors:** Diet rich in nitrosamines increases the risk for gastric carcinoma. Consumption of smoked fish and meat is rich in nitrosamines. Nitrosamines are also derived from nitrates and nitrites being used as preservative for salted pickled vegetables. Dietary nitrates and nitrites are converted to nitrosamines by *Helicobacter pylori*. On the other hand, eating lots of fresh fruits and vegetables appear to lower the risk for gastric carcinoma. Green leafy vegetables and citrus fruits act as antioxidants and thus are protective against gastric carcinoma. A high intake of animal fat has also been considered a protective factor against gastric carcinoma.
- **Tobacco chewing or smoking:** Tobacco chewing or smoking increases gastric carcinoma risk in the upper portion of the stomach near the esophagus. The rate of gastric carcinoma is about double in tobacoo smokers.
- **Blood group A:** For unknown reasons, people with blood group A have a higher risk of developing gastric carcinoma in 4% of cases.
- **Previous gastric surgery:** Partial gastrectomy performed in peptic ulcer disease increases risk for gastric carcinoma after many years of surgery. It is due to decreased secretion of gastric acid, which allows more nitrite-producing bacteria to be present. Reflux of bile from the small intestine into the stomach after surgery may also increase the risk for gastric carcinoma.

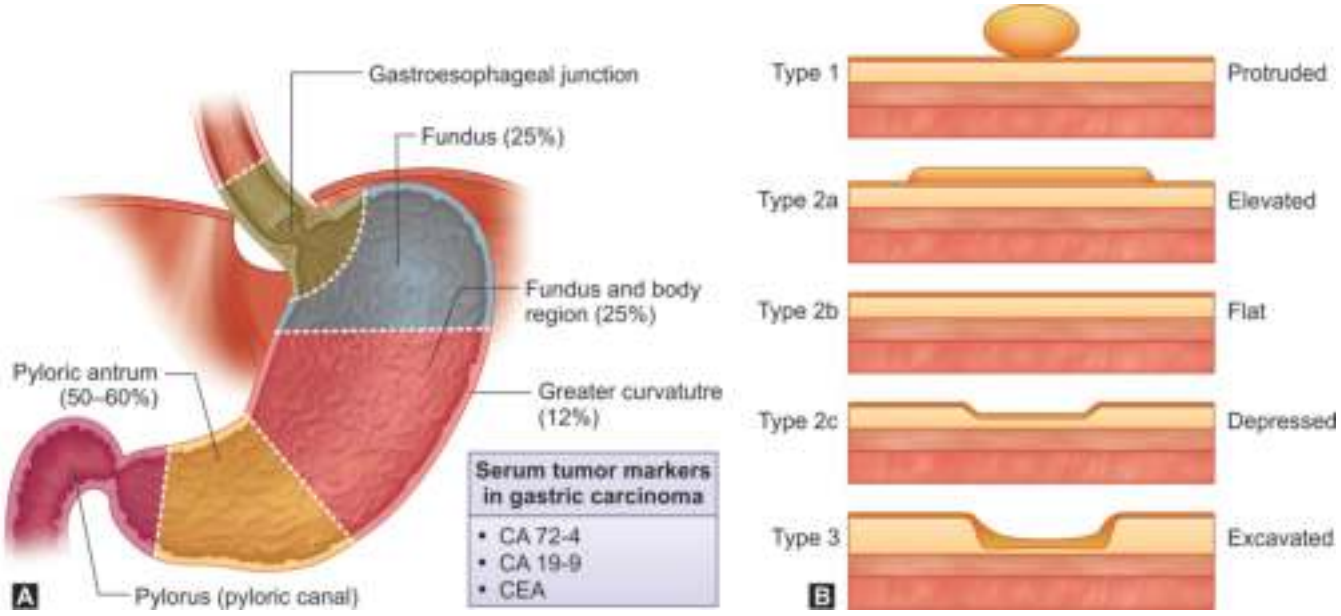


Fig. 20.18: (A) Distribution of gastric carcinoma including serum tumor markers. (B) Endoscopic classification of early gastric cancer (ECG) is defined as involvement of mucosa and submucosa, irrespective of lymph node status. Advanced gastric carcinoma involves muscular coat and beyond.

- **Pernicious anemia:** Intrinsic factor synthesized by parietal cells is required for absorption of vitamin B_{12}. Autoantibodies formed against intrinsic factor cause megaloblastic anemia. Risk for gastric carcinoma increases in patients suffering from pernicious anemia.
- **Ménétrier disease (hypertrophic gastropathy):** This disorder is characterized by excessive growth of gastric lining resulting in large mucosal folds. There is low level of gastric acid in this disorder. Due to unknown reason, there is increased risk for gastric carcinoma.
- **Inherited cancer syndromes:** Some inherited conditions may raise a person's risk for gastric carcinoma. Gene mutations such as E-cadherin, β-catenin, APC tumor suppressor gene, fibroblast growth factor, DNA repair genes and FGF3 participate in the pathogenesis of gastric carcinoma. Oncogenes, tumor suppressor gene mutations and chemical carcinogens associated with gastric carcinoma are given in Tables 20.22 to 20.24.
  - **Germline mutation of E-cadherin (CDH1):** Under physiologic state, E-cadherin (CDH1) participates in adhesion of intercellular junctions of gastric mucosal cells. A minority of gastric carcinoma cases is related to germline defects of E-cadherin (CDH1). Mutation of E-cadherin causes impairment of intercellular junctions of gastric mucosal cells. It seems a key step in development of gastric signet ring adenocarcinoma with diffuse infiltration resulting in a linitis plastica (leather bottle) gross appearance.
  - **Mutation of β-catenin:** Under physiologic state, β-catenin, a protein binds to E-cadherin. Mutation of β-catenin is associated with gastric signet ring

Table 20.22 Oncogenes, their mode of activation associated with gastric carcinoma

Category	Proto-oncogene	Mode of Activation	Associated Malignancies
Growth factors			
Fibroblast growth factors (FGFs)	FGF3	Amplification	▪ Osteosarcoma ▪ *Gastric carcinoma* ▪ Bladder cancer ▪ Breast cancer ▪ Melanoma
Growth factor receptors			
Receptor for KIT ligand	KIT	Point mutation	▪ *Gastrointestinal tumors* ▪ Seminoma ▪ Leukemia

Table 20.23 Tumor suppressor gene mutations associated with gastric carcinoma

Gene	Protein	Function	Familial Tumors	Sporadic Cancers
Mitogenic signaling pathway inhibitors				
APC	Adenomatous polyposis coli (locus 5p21), mutation by deletion or nonsense	APC protein inhibits Wnt signaling activator pathway, thus prevents nuclear transcription. It degrades β-catenin	Familial polyposis coli with malignant transformation	▪ *Gastric carcinoma* ▪ Colon carcinoma ▪ Pancreas carcinoma ▪ Thyroid carcinoma ▪ Melanoma
Inhibitors of invasiveness and metastases				
CDH1	E-cadherin	Participates in cell adhesion and inhibits cell motility	*Familial gastric carcinoma*	▪ *Gastric carcinoma* ▪ Breast lobular carcinoma

Table 20.24 Chemical carcinogens associated with gastric carcinoma

Chemical Carcinogen	Examples	Associated Cancers
Nitrosamines	Nitrates and nitrites widely used as fertilizers and as food additives converted to nitrosamines by gut bacteria in hypochlorhydria	*Gastric carcinoma*
Occupational asbestosis	Asbestos in building material	▪ *Gastric carcinoma* ▪ Lung carcinoma ▪ Mesothelioma

carcinoma (**linitis plastica variant**). There is increased risk of development of lobular carcinoma breast.

- **Hereditary nonpolyposis colorectal cancer (HNPCC):** This inherited genetic disorder is also known as Lynch syndrome which increases risk for gastric carcinoma (linitis plastica). In most cases, HNPCC is caused by a defect in either MLH1 or MSH2 gene, but other genes can cause HNPCC, including MLH3, MSH6, TGFBR2, PMS1, and PMS2.
- **Familial adenomatous polyposis (FAP):** It is caused by mutations in the APC gene. People with this disorder are at higher risk for colorectal carcinoma and gastric carcinoma.
- **BRCA1 and BRCA2 mutations:** Women who carry mutations of the inherited breast cancer genes BRCA1 or BRCA2 may also have a higher rate of gastric carcinoma.
- **Li-Fraumeni syndrome:** Li-Fraumeni syndrome is caused by a mutation in the TP53 gene. There is increased risk for gastric carcinoma at young age in patient suffering from this syndrome.
- **Peutz-Jeghers syndrome:** It is caused by mutations in the gene STK1. This disorder is characterized by hamartomatous polyps in the stomach, intestines, nose, airways and lungs and dark freckle-like spots on the lips and inner cheeks. The patient with Peutz-Jeghers syndrome is at increased risk for development of cancers of the breast, colon, pancreas, stomach, and several other organs.
- **A family history of gastric carcinoma:** People with first-degree relatives (parents, siblings, or children) who have had gastric carcinoma are more likely to develop this disease.
- **Common variable immunodeficiency (CVID):** People with CVID have an increased risk for gastric carcinoma. The immune system in these patients fails to synthesize antibodies against pathogens. There is increased risk for atrophic gastritis, pernicious anemia, gastric carcinoma and gastric MALT lymphoma.

Gastric Carcinoma: Classification

Gastric carcinoma is classified on the basis of depth of invasion (e.g. early and advanced gastric adenocarcinoma), macroscopic growth pattern (e.g. flat mucosal lesions, exophytic, ulcerative and diffuse infiltrating types) and histologic types (e.g. intestinal adenocarcinoma and diffuse infiltrative type, e.g. linitis plastica).

Classification of gastric carcinoma based on macroscopic growth patterns, endoscopic, histologic features and involvement of gastric wall is given in Table 20.25.

Table 20.25 Classification of gastric carcinoma based on macroscopic growth patterns, endoscopic, histologic features and involvement of gastric wall

Characteristics	Comments
Classification of gastric adenocarcinoma based on macroscopic growth patterns	
Flat/depressed lesion	Lesion without obvious tumor confined to mucosa (lesion slightly elevated, flat or depressed)
Exophytic/polypoidal/fungating lesion	Solid tumor projecting into the gastric lumen
Excavated/ulcerative lesion	Shallow or deep erosive crater in the wall of stomach mimicking gastric ulcer
Borrmann classification based on macroscopic appearance of advanced gastric adenocarcinoma	
Type 1 advanced gastric adenocarcinoma	Polypoid fungating growth
Type 2 advanced gastric adenocarcinoma	Ulcerative with elevated distinct borders
Type 3 advanced gastric adenocarcinoma	Ulcerative with elevated indistinct borders
Type 4 advanced gastric adenocarcinoma	Diffuse with indistinct borders
Endoscopic classification of early gastric carcinoma	
Type 1 (exophytic/polypoidal/fungating mass)	Solid tumor growth protruding into the gastric lumen as a polypoidal or nodular mass
Type 2a (slightly elevated lesion)	Superficial growth slightly elevated above mucosal surface
Type 2b (flat)	Growth pattern flat
Type 2c (growth depressed)	Growth pattern depressed below mucosal surface
Type 3 (excavated lesion)	Shallow or deep erosive crater in the wall of stomach may closely resemble chronic peptic ulcer disease

Contd...

Table 20.25 Classification of gastric carcinoma based on macroscopic growth patterns, endoscopic, histologic features and involvement of gastric wall (*Contd...*)

Characteristics	Comments
Lauren classification of gastric adenocarcinoma depending on histologic type	
Intestinal type of gastric adenocarcinoma	▪ Polypoid fungating or ulcerated mass (Borrmann type 1 and type 2) ▪ Cohesive tumor cells forming recognizable glands regardless of their degree of cytologic differentiation
Diffuse infiltrative gastric adenocarcinoma (linitis plastica)	▪ Linitis plastica type mass (diffuse involvement of gastric wall) (Borrmann type 3 and type 4) ▪ Discohesive tumor cells (signet ring cells) resembling histiocytes with little differentiation penetrate through the gastric wall, most abundant in superficial portions of the gastric mucosa. Submucosal fibrosis with or without mucosal ulceration
Classification of gastric adenocarcinoma based on involvement of gastric wall	
Early gastric adenocarcinoma	▪ Mucosa and submucosa without penetration of muscular coat regardless of lymph node status ▪ Risk factors for recurrence of early gastric carcinoma include poor differentiation, vascular invasion and occult metastasis ▪ Excellent prognosis ▪ Five-year survival (93–95%)
Advanced gastric adenocarcinoma	▪ Mucosa, submucosa and penetration into muscular coat and beyond ▪ Early gastric carcinoma takes eight years to become advanced invasive disease ▪ Poor prognosis ▪ Five-year survival (5–15%)

According to Depth of Invasion of Tumor

According to depth of invasion, gastric adenocarcinoma has been divided into early and advanced gastric adenocarcinoma. Approximately, 5-year survival rate after surgical resection in early gastric adenocarcinoma has been found to be 93–99%, in comparison with 5–15% in advanced gastric adenocarcinoma.

- **Early gastric adenocarcinoma:** Early gastric adenocarcinoma is confined to the mucosa or submucosa without penetration of the muscularis coat regardless of the lymph node status of the patient. It has an excellent prognosis. It takes 8 years to become more invasive. It is diagnosed by barium study or endoscopy and biopsy. Risk factors for recurrence in early gastric adenocarcinoma include vascular invasion, poor differentiation and presence of occult metastases.
- **Advanced gastric adenocarcinoma:** When gastric adenocarcinoma infiltrates the muscular coat or beyond, it is termed advanced gastric adenocarcinoma with poor prognosis.

According to Growth Patterns of Tumor

Based on gross appearance, gastric carcinoma has been classified into following types:

- **Flat gastric mucosal lesions:** Early intramucosal carcinomas may present in the form of mucosal patches or loss of rugae. Flat mucosal lesions are not visible on endoscopy. High-risk people are screened yearly with endoscopy and cytologic examination of gastric brushings for early detection of gastric carcinoma especially in Japan.
- **Exophytic gastric carcinoma:** Intestinal type of gastric adenocarcinoma is polypoidal or fungating growth (cauliflower) protruding into the lumen of the stomach.
- **Ulcerated gastric carcinoma:** It is most often >2 cm in diameter along the lesser curvature, pylorus and antrum in majority of cases. Ulcerated gastric carcinoma also known as malignant ulcer has rolled elevated edges (crate-like) and not clearly demarcated from the normal mucosa. Benign peptic ulcers have sharp regular margins. Biopsy is essential to differentiate benign gastric ulcer from malignant ulcer.
- **Diffuse infiltrative gastric carcinoma:** It is also known as linitis plastica. It is diffuse infiltrative type of gastric carcinoma converting the gastric wall into rigid thick structure known as leather bottle appearance.

Histologic Variants of Gastric Carcinoma

According to Lauren's classification, two major categories of gastric carcinoma exists, which have been designated intestinal type of gastric adenocarcinoma (53%), diffuse infiltrative type/linitis plastica (33%) and the remainders are heterogeneous in composition.

Table 20.26 Comparison of intestinal type of gastric adenocarcinoma and diffuse type of gastric carcinoma based on Lauren classification

Features	Intestinal Type of Gastric Adenocarcinoma	Diffuse Type of Gastric Carcinoma
Epidemiology	Epidemic seen in high risk population	Endemic seen in high risk population
Age group	>55 years	Younger persons
Sex predilection	Males > females	Males and females equally affected
Frequency	53%	33%
Precursor lesion (chronic gastritis)	Present	Absent
Role of *Helicobacter pylori*	Present	Absent
Role of dietary nitrosamines and consumption of smoked fish	Present	Absent
Location of tumor	▪ Gastric lesser curvature, pylorus and antrum (50–60%) ▪ Cardiac region (25%) ▪ Fundus and body of stomach (uncommon sites)	Diffuse involvement of gastric wall by tumor cells (signet ring cells) especially in superficial mucosal region
Gastric peristalsis	Present	Absent
Molecular genetic alterations	▪ APC gene mutation ▪ p53 gene mutation ▪ p16 gene mutation	E-cadherin gene mutation
Gross morphology	Polypoid fungating or ulcerated mass (Borrmann type 1 and type 2)	Linitis plastica type mass (diffuse involvement of gastric wall) (Borrmann type 3 and type 4)
Endoscopy	Polypoid fungating mass (Borrmann type 1 and type 2)	Depressed or excavated (Paris system type 2c, type 3)
Light microscopy	Cohesive tumor cells forming recognizable glands regardless of their degree of cytologic differentiation	▪ Dyscohesive tumor cells (signet ring cells) resembling histiocytes with little differentiation penetrate through the gastric wall, most abundant in superficial portions of the gastric mucosa ▪ Submucosal fibrosis with or without mucosal ulceration
Immunohistochemistry	▪ HER2/neu positive (well differentiated tumor and hepatoid variant) ▪ Cytokeratins positive ▪ EMA (epithelial membrane antigen) positive	Hep Par 1 positive
Metastasis	▪ Regional lymph nodes ▪ Discrete hepatic metastases	Diffuse, transperitoneal metastases involving ovaries (Krukenberg tumor)
Prognosis	Better survival	Poor survival

According to Borrmann classification of advanced gastric carcinoma include: Type 1 (polypoid fungating growth), type 2 (ulcerative with elevated distinct borders), type 3 (ulcerative with elevated indistinct borders), and type 4 (diffuse with indistinct borders).

- Extracellular mucin release in either types of gastric carcinoma can result in formation of large mucin lakes that dissect tissue planes. Intestinal gastric adenocarcinoma and diffuse infiltrative (linitis plastica) gastric carcinoma are described as under.
- Comparison of intestinal type of gastric adenocarcinoma and diffuse type of gastric carcinoma based on Lauren classification is given in Table 20.26.

Intestinal Type of Gastric Adenocarcinoma

Intestinal type of gastric adenocarcinoma arises from precursor lesions in stomach. It is associated with autoimmune-mediated chronic gastritis (type A), *Helicobacter pylori* associated chronic gastritis (type B), dietary nitrosamines and ingestion of smoked food.

Surgical Pathology: Intestinal Type of Gastric Adenocarcinoma

Gross Morphology

Tumor is solid polypoidal (fungating) mass projecting into the lumen of the stomach. It may undergo ulceration (Figs 20.19 and 20.20).

Light Microscopy

- On histologic examination, gastric adenocarcinoma may exhibit papillary, tubular, mucinous, poorly cohesive or mixed patterns.
- Tumor is composed of tumor cells with hyperchromatic nuclei and arranged in well-defined glandular and papillary structures.
- These glandular structures are lined by tall columnar cells containing mucin resembling colonic adenocarcinoma (Fig. 20.21).

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in intestinal type of gastric adenocarcinoma on tissue sections are cytokeratins (CKs), epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA) and CDX2.

Markers	Expression
▪ Cytokeratins (CKs)	▪ Positive
▪ EMA	▪ Positive
▪ CEA	▪ Positive
▪ CDX2	▪ Positive (specific marker)

Diffuse Infiltrative Type of Gastric Carcinoma

Diffuse infiltrative type of gastric carcinoma (linitis plastica) does not develop from precursor lesions in stomach. It is not associated with *Helicobacter pylori*. It constitutes 10% of all gastric carcinomas. No true tumor mass is seen macroscopically.

In a family with CDH1 gene mutation in case of hereditary diffuse gastric carcinoma, prophylactic total gastrectomy is recommended after attaining age of 20 years. BRCA2 gene mutation is also demonstrated in diffuse infiltrative type of gastric carcinoma (linitis plastica). Linitis plastica spreads to the muscular coat of the stomach wall and makes it thicker and more rigid.

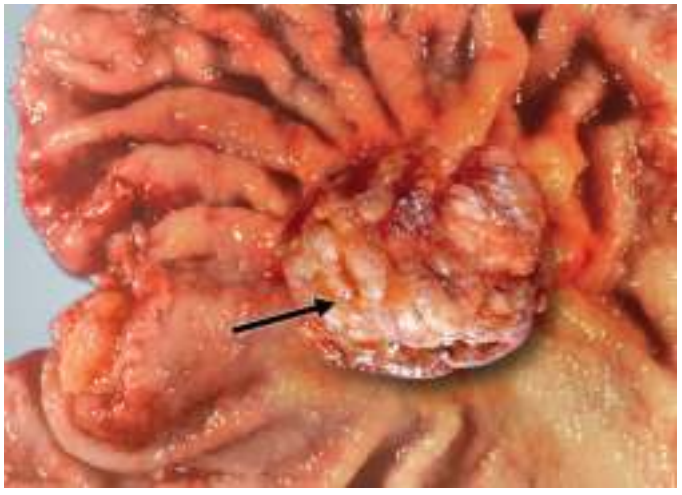


Fig. 20.19: Polypoid adenocarcinoma of the stomach. The patient was treated by partial gastrectomy (arrow).



Fig. 20.20: Gastric carcinoma: It shows ulceroproliferative growth (arrows). (*Courtesy*: Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

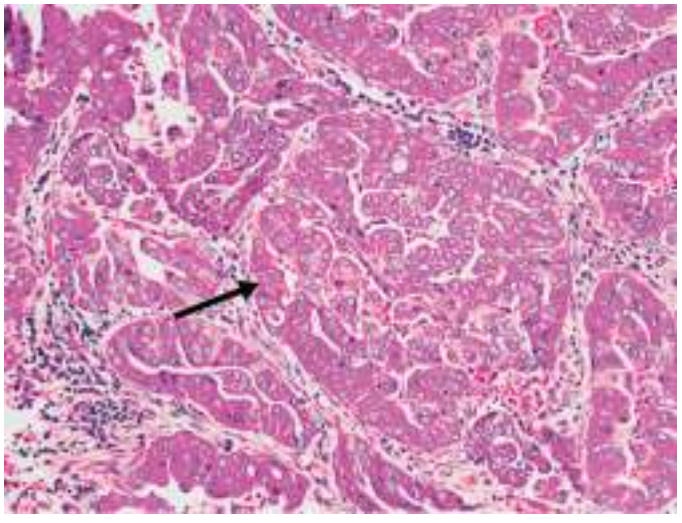


Fig. 20.21: Gastric adenocarcinoma (arrow). Tumor is composed of neoplastic cells with hyperchromatic nuclei cells are arranged in well-differentiated glandular and papillary structures lined by tall columnar cells (400X).

Surgical Pathology: Diffuse Infiltrative Type of Gastric Carcinoma (Linitis Plastica)

Gross Morphology

- The stomach becomes stiff rigid walls due to infiltration of tumor cells in all gastric layers and extensive fibrosis (desmoplastic reaction) giving radiologic leather bottle appearance of stomach. It has been referred to as a linitis plastica.
- Peristalsis is absent in linitis plastica variant of gastric carcinoma (Fig. 20.22).

Light Microscopy

- Tumor is composed of small cells, containing abundant mucin pushing the nuclei to the periphery called **signet ring cells**. Glandular differentiation is not seen.

- Tumor cells permeate mucosa as well as gastric wall individually or in small clusters. These are often confused with macrophages. Submucosal fibrosis is present with or without mucosa ulceration (Fig. 20.23).

Pathology Pearls: Mode of Spread with Gastric Carcinoma

Gastric carcinoma especially diffuse infiltrative type (linitis plastica variant) spreads by local/direct extension, lymphatic, hematogenous and transcoelomic routes.

- **Local/direct route:** Gastric carcinoma invades gastric wall (full thickness), duodenum, pancreas, liver and retroperitoneum.
- **Lymphatic route:** Gastric carcinoma invades submucosal and subserosal lymphatics and draining lymph nodes depending on location of gastric carcinoma.
  - Supraclavicular metastasis is known as 'Virchow sign'. Virchow lymph node is supraclavicular lymph node, which filters the lymph from the thoracic duct prior to its entry into the systemic veins.
  - Tumor located in proximal stomach and gastroesophageal junction metastasizes to mediastinal and pericardial lymph nodes.
  - Tumor located in body of stomach metastasizes to lymph nodes present in lesser and greater curvature.
  - Tumor located in distal part of stomach metastasizes to lymph nodes present in periduodenal, peripancreatic and porta hepatic lymph nodes.
- **Hematogenous route:** Intestinal type of gastric adenocarcinoma via hematogenous route metastasizes to liver, lung and bone.
- **Transcoelomic route:** Diffuse infiltrative type of gastric carcinoma (linitis plastica variant) metastasizing to the ovaries is known as **Krukenberg tumor**. The tumor cells contain abundant mucin, displacing nucleus to one side giving signet ring-like cells.

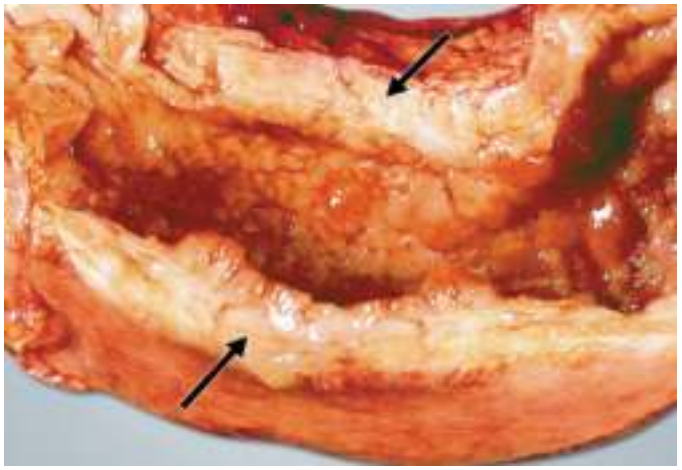


Fig. 20.22: Diffuse infiltrative type of gastric carcinoma (linitis plastica). In linitis plasica, the tumor cells infiltrate beneath the mucosa and produce marked fibrosis and thickening of the stomach wall (arrows).

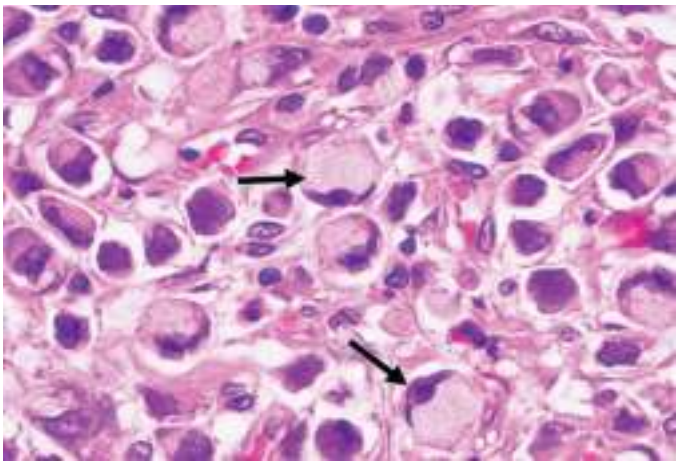


Fig. 20.23: Linitis plastica (diffuse infiltrative type) gastric carcinoma showing tumor cells containing abundant mucin pushing the nuclei to the periphery called signet ring cells (arrows) (400X).

Clinical Pearls: Mary Joseph Nodule in Periumbilical Region

- Mary Joseph nodule is formed due to metastases from gastric carcinoma (50%), colon carcinoma, pancreatic carcinoma in the subcutaneous region of periumbilical region. It is named after nurse Mary Joseph, who noticed nodule in periumbilical region.
- Other primary sites include ovarian and endometrial carcinoma. Proposed mechanism of spread is via direct transperitoneal, lymphatics, hematogenous, falciparum ligament, median umbilical ligament and remnant of vitelline duct routes.

Clinical Features

Weight loss and epigastric pain are the most common symptoms of gastric carcinoma. Other symptoms include vomiting, hematemesis or melena, anorexia, abdominal mass, dysphagia, diarrhea and iron deficiency anemia. Patient develops symptoms depending on involvement of organs due to metastases.

Clinical signs include cachexia, anemia, epigastric mass, hepatomegaly, palpable supraclavicular lymph node. Virchow node is supraclavicular lymph node, which filters the lymph from the thoracic duct prior to its entry into the systemic veins.

Pathology Pearls: Laboratory Diagnosis of Gastric Carcinoma

Gastric carcinomas are diagnosed by following diagnostic tools described as under.

- **Serum tumor markers:** CA 72-4 and carcinoembryonic antigen (CEA) are used to detect gastric adenocarcinoma. Serum CA 72-4 is also increased in colon carcinoma. Serum CEA is also increased nonseminomatous germ cell testicular tumor, colon carcinoma, gastric carcinoma, pancreatic carcinoma, lung carcinoma and breast carcinoma.
- **Serum pepsinogen level:** It is a specific marker for intestinal metaplasia precursor of gastric carcinoma.

- **Gastric function tests:** Gastric hydrochloric acid is decreased in gastric adenocarcinoma resulting in achlorhydria or hypochlorhydria.
- **Exfoliative cytology:** Examination of tumor cells obtained by gastric lavage is a diagnostic tool. Gastric lavage with chymotrypsin softens mucus over gastric carcinoma.
- **Endoscopy:** It is performed to know the extent of growth and obtain biopsies from representative areas.
- **Imaging techniques:** Barium meal study is useful in diagnosis of diffuse infiltrative type of gastric carcinoma (linitis plastica). It shows persistent, irregular, filling defect. USG, CT scan and MRI are done to rule out secondary deposits in celiac lymph nodes and distant organs, i.e. liver and ovaries. Double contrast barium swallow has 90% accuracy, but it is difficult to differentiate between malignant and benign peptic ulcers. CT scan is useful for evaluation of distant disease.
- **Histologic examination:** Biopsies are processed and sections are examined. Histochemical stain mucicarmine demonstrates mucin.
- **Immunohistochemistry and FISH techniques:** Gastric carcinomas that are HER2/neu positive can be treated with drugs that target the HER2/neu protein, such as trastuzumab (Herceptin®). The biopsy sample may be tested in two different ways:
  - Immunohistochemistry (IHC) technique: In this test, special antibodies that stick to the HER2/neu protein are applied to the sample, which cause cells to change color if many copies are present. This color change can be seen under a microscope. The test results are reported as 0, 1+, 2+, or 3+.
  - Fluorescent *in situ* hybridization (FISH) technique: This test uses fluorescent pieces of DNA that specifically stick to copies of the HER2/neu gene in cells, which can then be counted under a special microscope.

GASTRIC MALT LYMPHOMA

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is the most common form of extranodal lymphoma of B cell origin (mucosa-associated lymphoid tissue). It is low-grade lymphoma associated with *Helicobacter pylori* infection.

Demonstration of light chain restriction is helpful in differentiating MALT lymphoma from reactive lymphoid lesions.

Predisposing Factors

Predisposing factors for gastric MALT lymphoma include AIDS, celiac disease and Mediterranean α-heavy chain disease. During immunocompromised state, patients may develop diffuse large B cell lymphoma, Burkitt lymphoma, esophageal carcinoma, colorectal carcinoma and Kaposi sarcoma.

Pathogens Linked Gastrointestinal Cancers

- World Health Organization has designated *Helicobacter pylori*—a potential human carcinogen. *Helicobacter pylori* is strongly associated with gastric adenocarcinoma in lower half of gastric region and low-grade B cell lymphoma of the MALT. Some of MALT lymphomas regress after eradication of *Helicobacter pylori* infection with antibiotics.
- *Borrelia burgdorferi*, a spirochete and *Campylobacter jejuni* have been associated with gastrointestinal tract lymphoma.

Molecular Genetic Alterations

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma shows three types of chromosomal translocation: (a) t(11;18) (p21;q21) is most common type of translocation. It predicts resistance to remission after eradication of *Helicobacter pylori* with antibiotic therapy, and (b) t(1;14) (p22;q22), and (c) t(14;18) (q32;p21).

NF-κB transcription factor promotes B cell growth and their survival. Monoclonality is demonstrated by restricted expression of κ or λ immunoglobulin light chain, which differentiates from reactive lymph node lesions.

Clinical Features

Patient presents with nonspecific symptoms such as weight loss, dyspepsia, and abdominal pain.

Surgical Pathology: Gastric MALT Lymphoma

Gross Morphology

Gastric MALT lymphoma replaces normal mucosa (Fig. 20.24).

Light Microscopy

- Gastric mucosa and submucosa are infiltrated by small cleaved lymphocytes forming follicles, lymphoepithelial lesion and Dutcher bodies.
- Lymphoepithelial lesion consists of >3 lymphocytes, that displacing adjacent epithelial cells and destroying the gastric gland.
- Gastric mucosa may show erosion, intestinal metaplasia, atrophy and *Helicobacter pylori* (Fig. 20.25).

Immunophenotyping

Panel of immunomarkers used to analyze expression in gastrointestinal tract MALT lymphoma includes CD20, CD79a, CD21, CD35,CD43, IgM, IgA, IgG and BCL-2. Comparison of gastric mucosa-associated lymphoid tissue (MALT) lymphoma and chronic lymphocytic gastritis is given in Table 20.27.

Markers	Expression
CD20	Positive
CD79a	Positive
CD35	Positive
CD43	Positive
IgM>IgA>IgG	Positive (25%)
BCL-2	Positive

▪ IgD	▪ Negative
▪ CD5	▪ Negative
▪ CD10	▪ Negative
▪ BCL-16	▪ Negative
▪ Cyclin D1	▪ Negative

There is no definite immunohistochemical marker for gastric MALT lymphoma. Panel of immunohistochemical markers is apple to assess architecture of infiltration lymphoid cells, immunophenotype and chain restriction.

Prognosis

Gastric MALT lymphoma has a considerably better prognosis than gastric carcinoma (5-year survival rate is 45%).

GASTROINTESTINAL STROMAL TUMORS

Gastrointestinal stromal tumors (GISTs) are defined as cellular, spindle cells, epithelioid or occasionally pleomorphic mesenchymal tumors, which express c-KIT (CD117) protein and demonstrated by immunohistochemistry a few exceptions occur due to fixation defects, sampling errors, following STI-571B therapy and some tumors lacking c-KIT (CD117) expression.

- Gastrointestinal stromal tumors originate from stromal stem cells that are precursors of smooth muscle cells (GI fibroblasts or pacemaker cells of Cajal controlling peristalsis). These tumors may be associated with neurofibromatosis and Carney's triad (GIST, paraganglioma and pulmonary chondroma).
- A gain-of-function mutation in KIT or *PDGFRA* gene may exist. Gastrointestinal stromal tumors with a KIT mutation respond to Gleevec (imatinib) therapy.

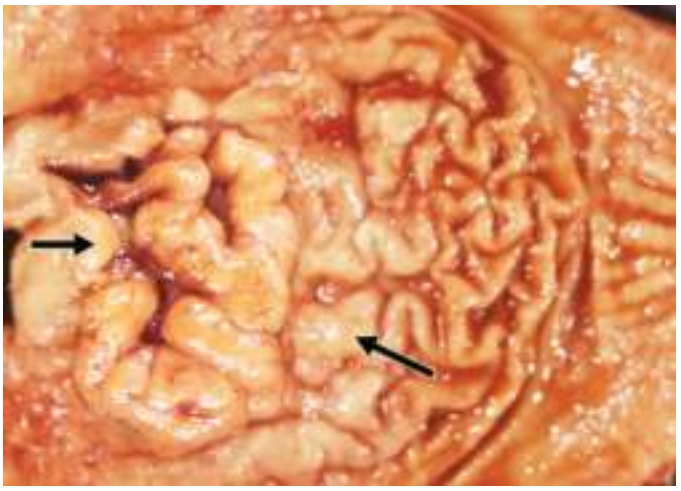


Fig. 20.24: Gastric lymphoma. Tumor is replacing most of normal gastric mucosa (arrows).

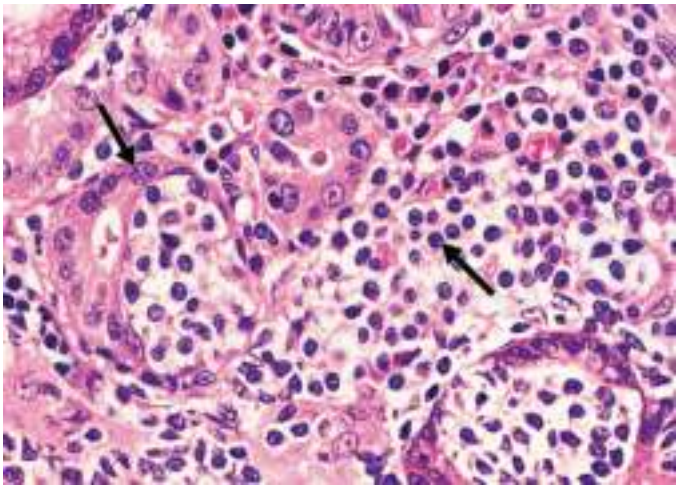


Fig. 20.25: Gastric lymphoma. Tumor shows small lymphocytes forming follicles (arrows) (400X).

Table 20.27 Comparison of mucosa-associated lymphoid tissue (MALT) lymphoma and chronic lymphocytic gastritis

Feature	Gastric MALT Lymphoma	Chronic Lymphocytic Gastritis
Light microscopy		
Lymphocyte number	Significantly increased	Significantly increased
Lymphocyte distribution	About three or four lymphocytes arranged in clusters (forming lymphoid follicles)	Single lymphocyte arranged in linear fashion in the gastric epithelium
Interfollicular lymphocytes	Malignant B cells with irregular nuclear contour	Mature T cells
Cytologic atypia of lymphocytes	Present	Absent
Perilymphocytic halo	Common	Uncommon
Significant gastric epithelial destruction	Present	Absent
Diffuse lamina propria infiltration and destruction by atypical lymphocytes	Present	Absent
Plasma cells	Present in variable number beneath surface lining epithelium shows light chain restriction	Present diffusely in lamina propria and lacking light chain restriction

Contd...

Table 20.27 Comparison of mucosa-associated lymphoid tissue (MALT) lymphoma and chronic lymphocytic gastritis (*Contd...*)

Feature	Gastric MALT Lymphoma	Chronic Lymphocytic Gastritis
Immunohistochemistry		
Immunomarkers	▪ CD19 (pan B cell marker) ▪ CD20 (pan B cell marker) ▪ CD22 ▪ CD79a ▪ κ Light chain ▪ λ Light chain ▪ BCL-2	▪ CD2 ▪ CD3 (pan T cell marker) ▪ CD4 (T helper cell marker) ▪ CD5 ▪ CD7 ▪ CD8 (cytotoxic T cell marker)

Tumor size and mitotic rate features determine the risk of aggressive behavior.

Locations

Gastrointestinal stromal tumors (GISTs) occur in entire gastrointestinal tract such as stomach (50%), small intestine (25%), large intestine (10%), and extra-gastrointestinal sites. Gastric stromal tumors are usually submucosal and covered by intact mucosa.

Molecular Genetic Alterations

Approximately 75–80% of GISTs have KIT gene mutations. Mutations in platelet-derived growth factor receptor-α (*PDGFRA*) have been demonstrated in 10% of cases.

Surgical Pathology: Gastrointestinal Stromal Tumors

Gross Morphology

GISTs range from a few mm to >40 cm with mean size of 6 cm. Tumor is well circumscribed. Cut surface is smooth, lobulated with whorled-silk appearance. Prominent fibrohyaline areas undergoing calcification may be demonstrated.

Light Microscopy

Gastrointestinal stromal tumors are classified into three categories based on histopathological examination: (a) spindle cell, (b) epithelioid, and (c) mixed cell variants.

- Tumor may be composed of fascicles and bundles of spindle cells or epithelioid cells with vacuolated cytoplasm or mixture of spindle and epithelioid cells with focal pleomorphism.
- Benign gastrointestinal stromal tumors are small and measuring 2–5 cm and may show a few mitoses.
- Malignant stromal tumors measure >6 cm and show areas of hemorrhage, necrosis, high mitoses and features of anaplasia. Proposed guidelines for assessing malignant potential of gastric GISTs are given in Table 20.28.

Immunohistochemistry Technique

Panel of markers is used to analyze expression in gastrointestinal stromal tumors on histopathologic sections.

Markers	Expression
▪ c-KIT oncogene (CD117) encoding tyrosine kinase*	▪ Positive (95% of cases)
▪ DOG1 (protein of unknown function)	▪ Positive (most specific marker)
▪ CD171 (cell adhesive molecule)	▪ Positive
▪ Protein kinase C theta	▪ Positive
▪ Vimentin	▪ Positive
▪ Smooth muscle actin encoded by ACTA2 gene located on 10q–24q	▪ Positive
▪ CD34	▪ Positive
▪ Desmin	▪ Positive (5% of cases)

**CD117 positivity is seen in GIST cells, melanocytes, mast cells and germ cells.*

Phenotype Categories

A gastrointestinal stromal tumor shows histologic sign of smooth muscle differentiation is called leiomyoma or leiomyosarcoma.

Table 20.28 Proposed guidelines for assessing malignant potential of gastric GISTs

Tumor Size	Mitoses	Predictable Behavior
≤2 cm	≤5 mitoses/50 HPFs	Benign, metastasis rate or tumor-related mortality: 0%
>2–10 cm	5 mitoses/50 HPFs	Very low malignant potential, metastasis rate or tumor-related mortality: <3%
>10 cm	≤5 mitoses/50 HPFs, or ≤5 cm >5 mitoses/50 HPFs	Low to moderate malignant potential, metastasis rate or tumor-related mortality: 12–15%
>5 cm	>5 mitoses/50 HPFs	High malignant potential, metastasis rate or tumor-related mortality: 49–86%

GIST shows neural differentiation in autonomic nervous system is known as GANT. GISTs may show dual smooth muscle and neural differentiation.

Behavior

Mitotic count, size, and location help in predicting the behavior of gastrointestinal stromal tumors. GISTs in small intestine most often behave like leiomyosarcoma as opposite to the stomach. GISTs are considered to be of low malignant potential and removed surgically.

Management

Surgical resection of gastrointestinal stromal tumor and treatment with imatinib cure the patients. Therapeutic agent imatinib inhibits the tyrosine kinase activity of c-KIT and *PDGFRA*.

GASTRIC NEUROENDOCRINE TUMORS

Gastric neuroendocrine tumors are present in stomach (body and fundus) and proximal duodenum. These tumors synthesize histamine, somatostatin and serotonin.

- There are three types of gastric neuroendocrine tumor. Type 1 neuroendocrine tumor is associated with autoimmune chronic atrophic gastritis. Type 2 neuroendocrine tumor is associated with multiple endocrine neoplasia type 1 (MEN 1) and Zollinger-Ellison syndrome. Type 3 neuroendocrine tumor occurs in sporadic cases.
- Duodenal neuroendocrine tumors are associated with Zollinger-Ellison syndrome or neurofibromatosis type 1 or sporadic. Carcinoid (neuroendocrine) tumors are discussed in details under small intestinal tumors.

GASTRIC NON-NEOPLASTIC DISORDERS

CONGENITAL PYLORIC STENOSIS

Congenital pyloric stenosis is a male-dominant disorder and more common in identical twins. It is characterized by hypertrophy of the circular smooth muscle in the pyloric region of the stomach resulting in obstruction of the outlet of stomach. The only consistent microscopic abnormality is hypertrophy of the circular muscle coat.

Clinical Features

Patient presents with projectile vomiting of non-bile stained fluid 2–4 weeks after birth. On clinical examination, palpable mass is observed through the abdominal wall in the epigastric region. Surgical resection within first 6 months of life gives excellent results.

MÉNÉTRIER DISEASE

Ménétrier disease (giant hypertrophic gastritis) is characterized by extreme enlargement of gastric rugae (greater curvature in the fundus and body of the stomach) and sometimes by severe loss of plasma proteins from the altered mucosa. The disease occurs due to cytomegalovirus in children and adults. Overexpression of TGF-α is seen in adulthood form. Affected patients have an increased risk of gastric carcinoma.

PANCREATIC HETEROTROPIC RESTS

Pancreatic heterotropic rests most commonly occur in the wall of the stomach.

HYPERPLASTIC (REGENERATIVE) POLYPS

Hyperplastic (regenerative) polyps are non-neoplastic polyps that may be associated with chronic blood loss.

TRICHOBEZOAR

Trichobezoar is usually seen in long-haired girls or young women who eat their own hair as a nervous habit. Trichobezoar may grow by accretion to form a complete cast of the stomach. Strands of hair may extend into the bowel as far as the transverse colon known as Rapunzel syndrome (Fig. 20.26).



Fig. 20.26: Trichobezoar shows hair forming cast in stomach. (*Courtesy*: Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

DISORDERS OF SMALL INTESTINE

ANATOMY AND HISTOLOGY

Small intestine is 6–7 meters long. It comprises duodenum, jejunum and ileum. It begins at the distal gastric pylorus and ends at ileocecal region. It consists of mucosa, submucosa, muscular coat and serous coat.

- Small intestine is lined by villus mucosa. The individual villus consists of finger-like projections, which is lined by tall columnar absorptive cells, goblet cells, crypt cells, neuroendocrine cells and Paneth cells. The lamina propria contains plasma cells. Peyer's patches composed of aggregates of lymphocytes are distributed throughout the mucosa of small intestine.
- Duodenum contains Brunner's glands in the submucosa. The entire mucosa rests on muscularis mucosae. The smooth muscle layer delineates the lamina propria from the submucosa.

CONGENITAL ANOMALIES

MECKEL'S DIVERTICULUM

Meckel's diverticulum is a remnant of the omphalomesenteric duct. It causes outpouching of four layers of small intestine (true diverticulum) in the antimesenteric aspect of ileum, within 60–100 cm from ileocecal valve. Intermittent painless rectal bleeding is the classic presentation due to acid-related bleeding of aberrant mucosa (remnant of embryonic yolk sac) in children under age of two years. The Tc-99m pertechnetate scan also known as Meckel's scan is diagnostic. Meckel's diverticulum follows the rule of 2s:

- 2% of population affected
- 2% feet from the ileocecal valve
- 2 inches in length
- 2:1 male to female ratio
- 2 types of tissue (gastric and pancreatic tissue)
- Occurs in the first 2 years of age.

Surgical Pathology: Meckel's Diverticulum
Gross Morphology
Meckel's diverticulum shows outpouching of small intestine (Fig. 20.27).
Light Microscopy
Meckel's diverticulum may contain heterotropic rests such as pancreatic tissue, gastric, duodenal, or colonic mucosa (Fig. 20.28).

Clinical Features

Most patients are generally asymptomatic. Patient may develop bleeding in Meckel's diverticulum owing to the presence of heterotropic rests of gastric or pancreatic mucosa.

Complications

Approximately 2% of patients may develop ectopic peptic ulcers, ileoumbilical fistula, intussusception, volvulus, intestinal obstruction, perforation, diverticulitis and mucocele.

HETEROTROPIC GASTRIC MUCOSA

Presence of heterotropic gastric full thickness fundic type oxyntic mucosa produces small nodule or sessile



Fig. 20.27: Meckel's diverticulum. It shows outpouching of small intestine (arrow).

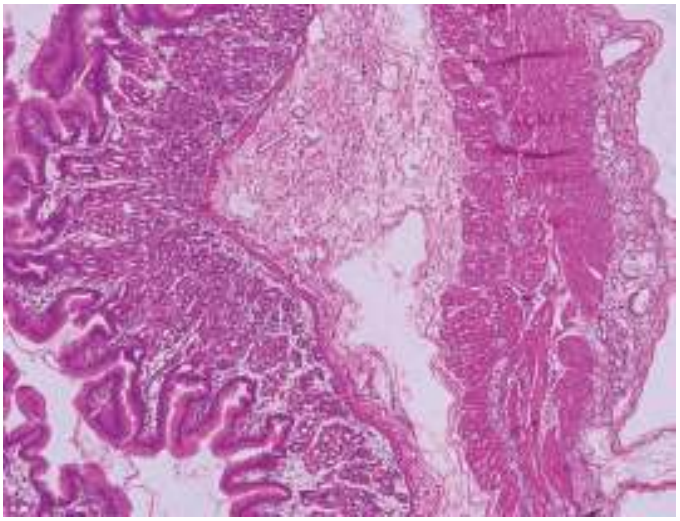


Fig. 20.28: Meckel's diverticulum. It shows heterotropic rests of gastric mucosa (100X).

polyp in the duodenal bulb. This entity differs from foveolar surface metaplasia of duodenal mucosa seen in secondary to inflammation of duodenum by *Helicobacter pylori*.

HETEROTROPIC PANCREAS

Patient with heterotropic pancreas presents with mass in duodenum. On histologic examination of duodenal mass, the lesion is composed of pancreatic ducts and acini with or without islets of Langerhans.

MALROTATION OF SMALL INTESTINE

During fetal life, defective rotation of small intestine occurs. As a result, large bowel does not descend into right iliac fossa. These patients are prone to develop volvulus and internal hernia.

DUPLICATION OF SMALL INTESTINE

Duplication of the small intestine may be tubular or cystic. Approximately 75% are not contagious with the lumen of the associated segment of small intestine. Duplications comprise all the layers of the segment.

MALABSORPTION SYNDROME

Malabsorption syndrome is defined as increased fecal excretion of fat with concurrent deficiencies of vitamins, minerals, carbohydrates, and proteins. Bile salts and fatty acids are required to micellarize monoglycerides and fatty acids. Small intestinal villi are required to reabsorb micelles. Deficiency of pancreatic lipase leads to malassimilation of the fats in the small bowel. Mechanisms of malabsorption are given in Table 20.29.

Etiopathogenesis

Malabsorption syndrome is caused by pancreatic insufficiency, chronic pancreatitis, bile salts/bile acid deficiency due to cirrhosis, cholestasis and small bowel disease (e.g. celiac disease).

Table 20.29 Mechanisms of malabsorption syndrome

Mechanisms	Causes
Impaired intraluminal digestion	▪ Pancreatic insufficiency ▪ Defective bile secretion
Primary mucosal cell abnormality	▪ Lactose intolerance ▪ Bacterial overgrowth
Reduced surface area	▪ Celiac disease ▪ Tropical sprue ▪ Inflammatory bowel disease
Obstruction of lymphatics	▪ Lymphoma ▪ Tuberculosis
Infections	▪ Enterocolitis ▪ Parasitic infection

- Other causes of malabsorption syndrome include Whipple disease, Crohn's disease and diseases obstructing the intestinal lymphatics.
- Small bowel disorders linked to malabsorption syndrome include celiac disease, refractory sprue, collagenous sprue, autoimmune enteropathy, common variable immunodeficiency, microvillus inclusion disease, lymphangiectasia, disaccharidases deficiency and abetalipoproteinemia.
- Causes of malabsorption syndrome are given in Table 20.30. Causes of clinical manifestations of malabsorption syndrome are given in Table 20.31.

Clinical Features

Patient presents with steatorrhea, weight loss, diarrhea, and malnutrition. Steatorrhea denotes passage of excessive sticky stools that float as a result of mal-digestion of fats by small bowel as a result of pancreatic insufficiency.

Complications

Patient develops complications as a result of deficiencies of vitamins (e.g. fat-soluble and water-soluble), pitting edema (e.g. hypoproteinemia), and recurrent infections, and dimorphic anemia (e.g. iron and folate deficiency).

Table 20.30 Causes of malabsorption syndrome

Primary Causes	Secondary Causes
Celiac disease	Mucosal damage (Crohn's disease, tubercular intestine, NHL intestine, radiation)
Whipple disease	Chronic pancreatitis causing pancreatic insufficiency
Refractory sprue	Liver disorders (cirrhosis, cholestasis causing bile salts/bile acid deficiency)
Collagenous sprue	Surgical resection of small intestine
Autoimmune enteropathy	Parasitic infestations (hookworm, roundworm, Giardia)
Common variable immunodeficiency	Drugs administration (methotrexate, neomycin)
Microvillus inclusion disease and disaccharidases deficiency	Impaired transport (lymphangiectasia and abetalipoproteinemia)

Table 20.31 Clinical manifestations of malabsorption syndrome

Dietary Constituents	Site of Absorption	Essential Requirement	Clinical Manifestations
Carbohydrates			
Starch	Small intestine	▪ Amylase ▪ Maltase ▪ Isomaltase-α-dextrin	▪ Diarrhea ▪ Flatulence ▪ Abdominal discomfort
Sucrose	Small intestine	Sucrase	▪ Dehydration ▪ Abdominal discomfort
Lactose	Small intestine	Lactase	▪ Dehydration ▪ Abdominal discomfort
Fats, proteins, water and electrolytes			
Fat	Upper jejunum	▪ Pancreatic lipase ▪ Bile salts ▪ Functioning lymphatic channels	▪ Weight loss ▪ Steatorrhea ▪ Fat-soluble vitamins deficiency
Proteins	Small intestine	Pancreatic enzymes (e.g. trypsin, chymotrypsin, elastin)	Edema, loss of muscle mass, weakness
Water and electrolytes	Mainly small intestine	Osmotic gradient	Diarrhea, dehydration, muscle cramps
Vitamins			
Vitamin B_{12}	Ileum	Intrinsic factor	Megaloblastic anemia, glossitis, peripheral neuropathy
Folic acid	Upper jejunum	Absorption may be impaired by anticonvulsant drugs	Glossitis, cheilosis
Vitamin A	Upper jejunum	Bile salts	Night blindness, exophthalmia, corneal irritation
Vitamin D	Upper jejunum	Bile salts	Bone pain, fractures, tetany
Vitamin K	Upper jejunum	Bile salts	Easy bruising and bleeding
Vitamin E	Upper jejunum	Bile salts	Uncertain
Minerals			
Calcium	Duodenum	Vitamin D and parathormone	Bone pain, fractures, tetany
Iron	Duodenum and jejunum	Normal pH (hydrochloric acid)	Iron deficiency anemia, glossitis

Laboratory Diagnosis

General screening tests for malabsorption syndrome disorders include 24 hours fecal fat and serum β-carotene. The gold standard test to document fat malabsorption is the quantitative 72 hours fecal stool test. Decreased absorption of D-xylose indicates small bowel disease. It is useful in identifying assimilation of proteins in the small bowel (decreased uptake of the pentose sugar).

CELIAC DISEASE

Celiac disease is an autoimmune disease caused by sensitivity to gluten in cereal products. Autoantibodies to gluten result in flattening or destruction of intestinal villi. It is also known as celiac sprue, nontropical sprue or gluten-induced enteropathy. Genetic and immune-mediated mechanisms may be involved in the pathogenesis of celiac disease. Patient presents with chronic diarrhea, abdominal distension and malabsorption syndrome.

Pathology Pearls: Diagnostic Criteria of Celiac Disease

Diagnostic criteria of celiac disease are described as under:

- **Clinical features:** Patient presents with clinical manifestations of malabsorption syndrome and/or iron deficiency anemia.
- **Serum autoantibodies assay:** There is presence of serum IgA antibodies to gliadin (AGA-IgA), tissue transglutaminase (tTg-IgA), endomysium (EMA-IgA-1) and IgG to gliadin (AGA-IgG).
- **Histologic examination:** Intestinal biopsy shows villus atrophy and elongation of crypts due to hyperplasia. Lamina propria shows infiltration by macrophages, lymphocytes and plasma cells.
- **Response to treatment with gluten-free diet:** Patient is advised to take gluten-free diet.

Genetic Predisposition

Incidence of celiac disease increases in association with human leukocyte antigens (HLAs), HLA-B8 and HLA-DW3, type 1 diabetes mellitus and dermatitis herpetiformis, an autoimmune IgA-mediated vesicular disease of skin associated with celiac disease. Genetic factors in celiac disease are given in Table 20.32.

Diseases associated with celiac disease include intestinal adenocarcinoma, enteropathy associated T cell lymphoma, dermatitis herpetiformis, lymphocytic colitis and lymphocytic gastritis.

Etiopathogenesis

Gluten is a name for group of proteins present in grains such as wheat, oat, rye, barley and spelt.

- **Physiologic state:** Normally, digestive enzymes split gluten into small chains of amino acids resulting in absorption of gluten by intestinal villi.
- **Pathologic state:** In celiac disease, digestive enzymes split gluten into long chain amino acid known as gliadin. Disease producing gliadin is absorbed into intestinal villi. Autoantibodies to gliadin result in triggering inflammatory response. Intestinal biopsy (e.g. proximal jejunum and distal ileum exhibiting villus atrophy) is essential for diagnosis. Pathogenesis of celiac disease is shown in Fig. 20.29.

Surgical Pathology: Celiac Disease

Light Microscopy

- Normal intestinal villi are slender and much longer than crypts.
- In celiac disease, intestinal biopsy shows villus atrophy (complete loss of villi), elongation of crypts due to hyperplasia.
- Overall thickness of the mucosa may not be reduced significantly as a result of crypt hyperplasia. Enterocyte damage may be evidenced by flattening and/or cytoplasmic vacuolization.
- Lamina propria shows infiltration by macrophages, lymphocytes and plasma cells. These changes can revert to normal in response to a gluten-free diet.
- Disorders that may mimic gluten-sensitive enteropathy are given in Table 20.33. Light microscopy of celiac disease is shown in Fig. 20.30A and B.

Clinical Features

Children suffering with celiac disease present with diarrhea, i.e. pale, bulky, frothy, foul-smelling stools, failure to thrive, growth retardation, weight loss, weakness and abdominal distension with nonspecific symptoms or anemia.

Table 20.32 Genetic factors in celiac disease

Categories	Examples
Human leukocyte antigens (HLAs)	▪ HLA DQ2 (class II) ▪ HLA DQ8 (class II) ▪ HLA-DW3
Polymorphism of immune regulatory genes determining mucosal epithelial cell polarity	▪ IL-2 ▪ IL-21 ▪ CCR3 ▪ SH2B3
Associated disorders	▪ Diabetes mellitus type 1 ▪ Sjögren's syndrome ▪ Ataxia-telangiectasia ▪ Autism ▪ IgA nephropathy ▪ Down's syndrome ▪ Turner's syndrome ▪ Dermatitis herpetiformis

Complications

Celiac disease is complicated by splenic atrophy and less commonly, large T cell lymphoma of the small bowel and small intestinal obstruction.

Laboratory Diagnosis

Diagnostic modalities of celiac disease include 24 hours fecal fat excretion, D-xylose test, anti-tissue transglutaminase circulating autoantibodies and intestinal biopsy. Demonstration of autoantibodies in serum may also be used for screening prior to definitive diagnosis by intestinal biopsy.

Treatment

A gluten-free diet cures celiac disease. Gluten is not present in corn, quinoa, potatoes, nuts, rice or soy. Flour made from these foods, vegetables and meat deficient in gluten are advised in celiac disease.

REFRACTORY SPRUE

Refractory sprue refers to unresponsiveness to a gluten-free diet. Relapse of symptoms occurs despite gluten restriction. It is not distinguishable from classic celiac disease. Some gastroenterologists consider refractory sprue as a type of T cell lymphoma. There may be presence of numerous neutrophils on histopathological examination.

COLLAGENOUS SPRUE

Collagenous sprue is characterized by flattening of mucosal villi and deposition of collagen fibers in subepithelial region of small intestine.

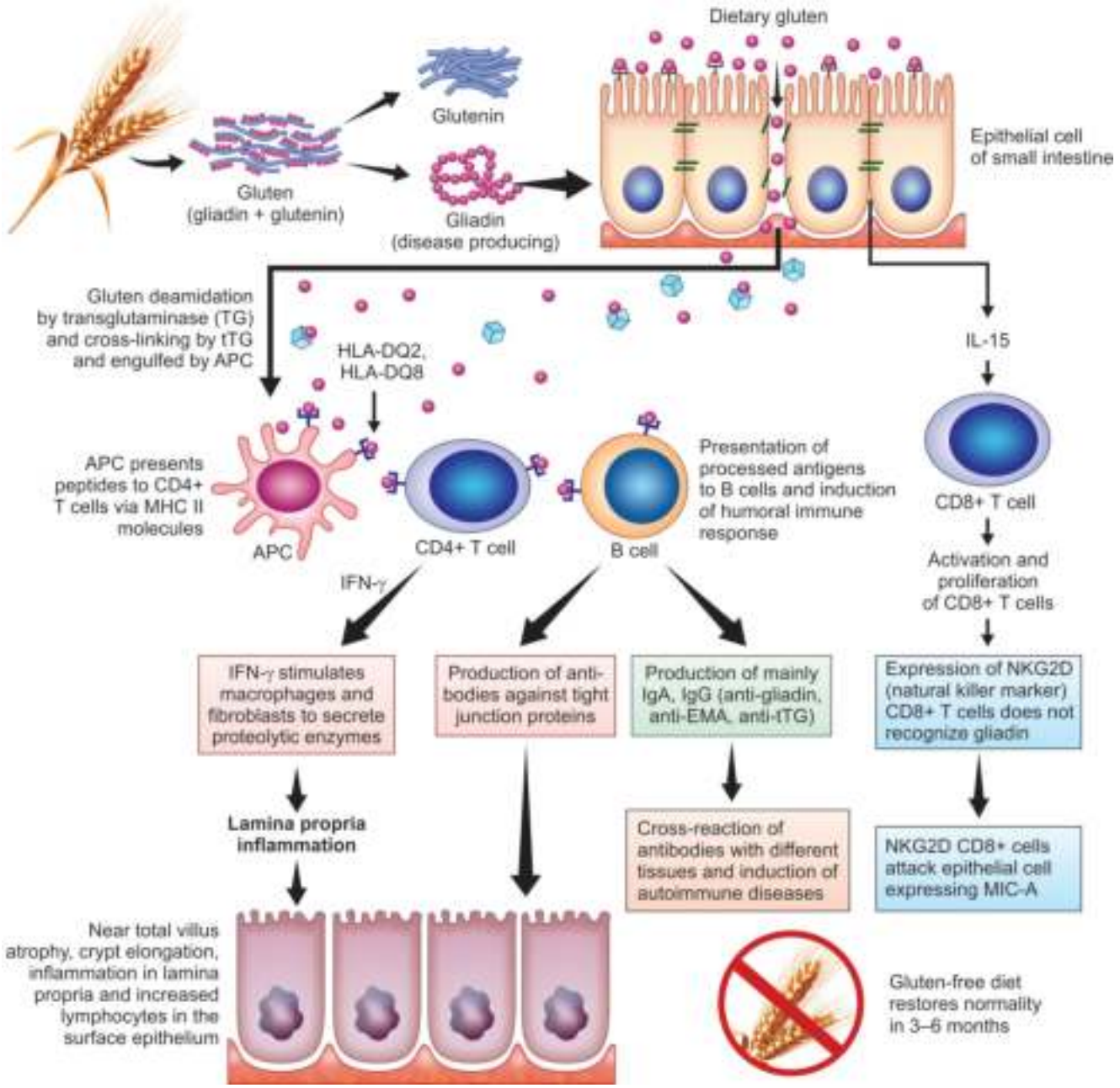


Fig. 20.29: Pathogenesis of celiac disease. Sensitivity to gliadin (disease producing) causes villus atrophy due to epithelial cell destruction, crypt hyperplasia due to expansion of epithelial cells in the proliferative compartment at the bases of the crypts inflammation of lamina propria and increased number of natural killer cells and CD8+ cytotoxic T cells in the epithelium.

Table 20.33 Disorders that may mimic gluten-sensitive enteropathy

Celiac disease/gluten-sensitive enteropathy	HIV enteropathy
Tropical sprue	Food allergies
Helicobacter pylori infection	Giardiasis
Bacterial overgrowth	Crohn's disease
Autoimmune enteropathy	Zollinger-Ellison disease
Nonsteroidal anti-inflammatory drug administration	Systemic autoimmune diseases
Dermatitis herpetiformis	Viral enteritis

Contd...

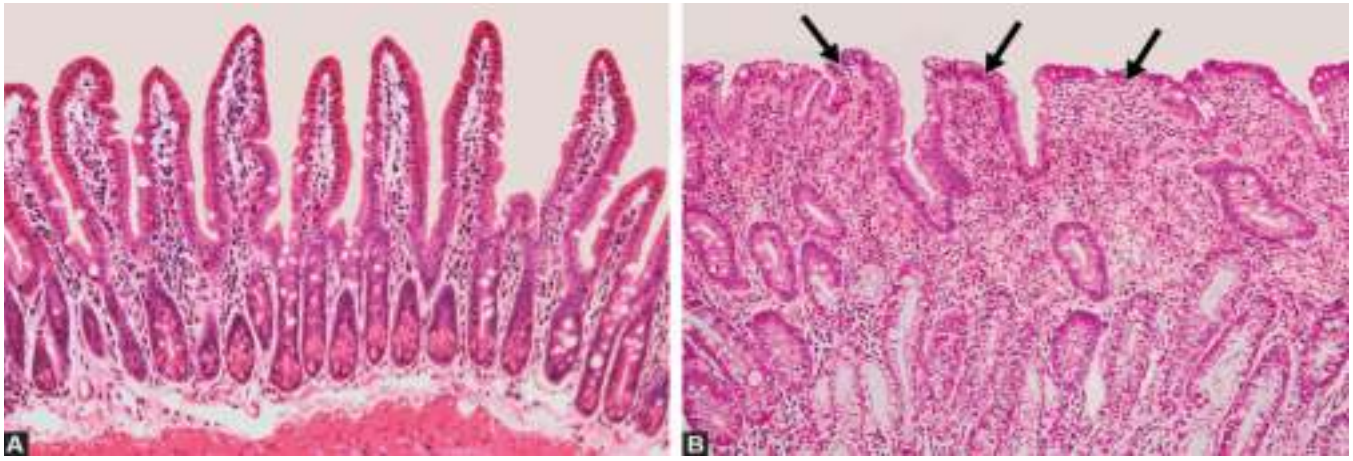


Fig. 20.30: Celiac disease. (A) Shows normal villi of small intestine, (B) shows villus atrophy and chronic inflammatory cells in lamina propria in celiac disease (arrows) (100X).

AUTOIMMUNE ENTEROPATHY

Autoimmune enteropathy shares clinical and histopathologic features with celiac disease. It involves both small intestine and large intestine. Clinical response to corticosteroids may help to establish diagnosis.

Surgical Pathology: Autoimmune Enteropathy

Light Microscopy

- Intestinal biopsy study shows increased intraepithelial CD8+ cytotoxic T cells and plasma cells.
- There is presence of villus atrophy exhibiting blunted or flattened appearance.
- Crypt hyperplasia is demonstrated.
- Apoptotic bodies are apparent.

EOSINOPHILIC GASTROENTERITIS

Eosinophilic gastroenteritis involves mucosa of stomach and intestine. On histologic examination, the mucosa shows infiltration by eosinophils. There is presence of cryptitis and crypt abscess formation. Parasitic infestations, food allergy to cow's milk protein, drug reaction, connective tissue disorders and tumors must be excluded.

COMMON VARIABLE IMMUNODEFICIENCY

Common variable immunodeficiency is characterized by loss of plasma cells in lamina propria. Histologic examination shows variable degree of blunting of villi, lymphocytic infiltration in epithelium and lymphoid aggregates. Giardia should be searched in the biopsies.

MICROVILLUS INCLUSION DISEASE

Microvillus inclusion disease is an autosomal recessive disorder causing diarrhea in infancy. Basic defect is loss of normal brush border on the luminal surface of the enterocytes as a result of incorporation of brush border into the cytoplasm appearing as apical microvillus inclusions. Microvillus inclusion disease is diagnosed by histologic, immunohistochemical, periodic acid–Schiff (PAS) stain, electron microscopy and immunohistochemistry (carcinoembryonic antigen, CD10 or villin). Histologic examination shows diffuse villus atrophy lacking inflammatory response.

INTESTINAL LYMPHANGIECTASIA

Intestinal lymphangiectasia is characterized by abnormal dilated lymphatic channels of small intestine, which may be congenital or secondary. It is an additional cause of malabsorption syndrome. It leads to marked protein loss with resultant hypoproteinemia and generalized edema.

DISACCHARIDASE DEFICIENCY

Lactose is one of the most common disaccharides in dairy products. The intestinal brush border contains disaccharidases that are important for cleavage of lactose to free glucose and galactose for absorption.

Acquired Disaccharidase Deficiency

Acquired disaccharidase deficiency is a widespread disorder of carbohydrate absorption. It leads to milk intolerance. The symptoms of this disease typically begin in adolescence, when patients complain of flatulence and diarrhea after the ingestion of dairy products. Histologic changes in the intestine are not significant.

Congenital Lactase Deficiency

Congenital lactase deficiency may be lethal if not recognized. Infants are unable to break down lactose in breast milk.

Table 20.34 Infectious causes of watery diarrhea

Pathogen	Clinical Presentation	Comments
Salmonella typhi	▪ Vomiting ▪ Fever ▪ Pain abdomen	Associated with ingestion of undercooked beef or chickens
Vibrio cholerae	▪ Vomiting ▪ Dehydration within 12–48 hours ▪ 'Rice water' stool	Cholera toxin in water
Clostridium difficile	Pseudomembranous colitis	Associated with infection by *Clostridium difficile* in patient receiving broad-spectrum antibiotics suppressing normal bacterial flora of intestine
Escherichia coli	▪ Osmotic diarrhea (3–6 days) ▪ Fever	▪ Infant diarrhea ▪ Traveler's diarrhea ▪ Tropical sprue
Rotavirus	▪ Severe dehydration ▪ Nausea ▪ Vomiting	Infant diarrhea during winter
Norwalk virus	▪ Mild diarrhea ▪ Nausea ▪ Vomiting	▪ Fecal–oral transmission ▪ Usually seen in cruise ships
Adenovirus	Moderate vomiting	Second most common cause of infant diarrhea
Giardia lamblia	▪ Steatorrhea (small intestine) ▪ Foul smelling stool	History of camping/hiking
Cryptosporidium	Severe dehydration due to large volume fluid loss	Seen in immunocompromised persons especially HIV infection

ABETALIPOPROTEINEMIA

Abetalipoproteinemia is an inherited disorder, which impairs the normal absorption of fats and certain vitamins from the diet. Patient develops symptoms due to deficiency of vitamins in the body. Peripheral blood examination shows acanthocytes.

DIARRHEA

Diarrhea is passage of >250 gm of stool per day (normal 100 g/day). Diarrhea may be acute (<3 weeks duration) and chronic (>4 weeks duration). Infectious causes of watery diarrhea are given in Table 20.34. Causes and mechanisms of diarrhea are given in Table 20.35. Signs and symptoms associated with chronic diarrhea are given in Table 20.36.

Table 20.35 Causes and mechanisms of diarrhea

Mechanism	Causes
Secretory diarrhea	▪ Rotavirus ▪ *Vibrio cholerae* ▪ *Giardia lamblia*
Osmotic diarrhea	▪ Disaccharidase deficiency ▪ Lactulose therapy
Exudative diarrhea	▪ Shigella (diagnosed by stool culture) ▪ *Salmonella typhi* ▪ Inflammatory bowel disease
Malabsorption	▪ Celiac disease ▪ Tropical sprue ▪ Inflammatory bowel disease

Table 20.36 Signs and symptoms associated with chronic diarrhea

Signs and Symptoms	Chronic Diarrhea	
Arthritis	▪ Ulcerative colitis ▪ Crohn's disease	▪ Whipple's disease
Liver disease	▪ Ulcerative colitis ▪ Crohn's disease	▪ Colon carcinoma with metastases
Fever	▪ Ulcerative colitis ▪ Crohn's disease ▪ Amoebic colitis	▪ Tubercular intestinal disease ▪ Whipple disease
Neuropathy	▪ Amyloidosis	▪ Diabetic diarrhea
Severe weight loss	▪ Malabsorption syndrome	▪ Inflammatory bowel disease
Lymphadenopathy	▪ GIT lymphoma ▪ AIDS ▪ Ulcerative colitis	▪ Crohn's disease ▪ Whipple's disease
Erythema nodosum	▪ Ulcerative colitis	▪ Crohn's disease
Dermatitis herpetiformis	Celiac disease	
Flushing	Carcinoid tumors	
Pyoderma gangrenosum	Ulcerative colitis	

PATHOGENS ASSOCIATED WITH DIARRHEA

- **Rotavirus:** It is the most common childhood cause of watery diarrhea without blood in children <2 years of age. It is transmitted by the fecal–oral route. Rotavirus is demonstrated in duodenal biopsy specimens in 50% of cases.
- **Norwalk virus:** It is most common cause of diarrhea in childhood and adults.
- **Food poisoning:** Patient presents with gastroenteritis from ingestion of contaminated food. Microbial pathogens include *Staphylococcus aureus*, *Clostridium botulinum* (in canned foods; toxins block cholinergic nerves in infants), *Bacillus cereus* (contaminated fried rice), *Clostridium perfringens* and Salmonella.
- ***Clostridium difficile*:** Patient develops pseudomembranous colitis caused by *Clostridium difficile* due to antibiotic-induced (most commonly ampicillin and clindamycin). It is best diagnosed by toxin assay of stool. It is treated with metronidazole.
- ***Shigella sonnei:*** It is transmitted by the fecal–oral route. Patient presents with bloody diarrhea as a result of pseudomembranous inflammation in the ileum and colon.
- ***Salmonella typhimurium:*** It is transmitted by fecal–oral route from animal reservoirs (poultry turtles). Patient develops enterocolitis.
- ***Salmonella typhi:*** It is transmitted by fecal–oral route. Pathogen invades Peyer's patches and produces septicemia during the first week. Blood is the best culture medium for pathogen isolation. Second week of the infection is marked by diarrhea. In addition, patient has classic triad of bradycardia, absolute neutropenia, and hepatosplenomegaly. It may produce chronic carrier state. The organism persists in gallbladder for one year after infection.
- ***Campylobacter jejuni:*** It is contracted by eating contaminated poultry or drinking contaminated milk. It is the most common invasive bacterial enterocolitis in the United States. Patient presents with bloody diarrhea with crypt abscesses in intestine, and the ulcers resembling ulcerative colitis.
- ***Vibrio cholerae:*** It produces a powerful enterotoxin that stimulates adenyl cyclase in the small bowel, leading to severe secretory diarrhea (rice water stool). It is best treated with a glucose/electrolyte solution.
- ***Escherichia coli (E. coli):*** *E. coli* pathogen produces toxin-induced and invasive diarrheas. Heat labile strain stimulates cyclic AMP producing secretory diarrhea. Enteroinvasive strain is associated with an invasive enterocolitis. Enterohemorrhagic strain associated with the O157:H7 serotype contaminating raw ground beef, may produce hemolytic uremic syndrome and hemorrhagic or pseudomembranous colitis. Enteropathogenetic strain produces mild diarrhea in infants and children.

Clinical Pearls: Types of Diarrhea

Osmotic Diarrhea

Nonabsorbable osmotic substances (e.g. mannitol, sorbitol, sugar-free gum or magnesium sulfate from Epsom salt) stimulate osmotic influx of water and electrolytes into the intestinal lumen producing osmotic diarrhea. Intake of milk products by children due to genetic deficiency of lactase enzyme causes withdrawing of water into the lumen, resulting in a hypotonic diarrhea without mucosal inflammation.

Secretory Diarrhea

- In secretory diarrhea, the intestinal cells secrete more water than they can absorb. *Vibrio cholerae* without causing intestinal mucosal inflammation secretes toxin that activates the adenyl cyclic AMP-dependent water pump in the cell membrane.
- The intestinal cells secrete chloride accompanied by water into the lumen producing secretory diarrhea. *E. coli* toxin that is heat stable toxin stimulates water influx through mechanisms that are not cyclic AMP dependent.
- Other causes of secretory diarrhea include Rotavirus, Norwalk virus. Neuroendocrine tumors (e.g. carcinoids and pancreatic islet cell tumors) synthesize vasoactive amines (e.g. serotonin and other polypeptides) responsible for secretory diarrhea.

Exudative (Invasive) Diarrhea

- Exudative diarrhea is caused by pathogens (e.g. *E. coli, Clostridium difficile* and Shigella).
- The organisms cause inflammation of intestinal mucosa with production of inflammatory exudates.
- Fecal smears show leukocytes. Other causes include ulcerative colitis, Crohn's disease, lymphomas and carcinomas.

INFECTIOUS DISORDERS

TROPICAL SPRUE AND BACTERIAL OVERGROWTH

Tropical sprue and bacterial overgrowth simulate celiac disease. It may involve the entire small intestine with more severe disease in distal segment. Clinical history and travel history are important in establishing diagnosis.

WHIPPLE DISEASE (TROPICAL SPRUE)

Tropical sprue is also known as Whipple disease, which occurs due to *Tropheryma whipplei*, in that often responds well to tetracycline. It may involve the entire small intestine with more severe disease in distal segment. It may resemble celiac disease, but megaloblastic anemia dominates the clinical picture. Intestinal biopsy shows only partial villus atrophy.

Etiopathogenesis

Whipple disease is caused by *Tropheryma whipplei* bacteria, which usually affects 30–60 years of age group, involving multiple organs such as small intestine (jejunum and ileum), joints (arthralgia) and cardiac and neurologic manifestations. The organism blocks lymphatic channels of small intestine and interferes with uptake of fat (malabsorption). Draining mesenteric lymph nodes, liver, lung, heart, brain, bone and synovial membrane are also infiltrated by macrophages carrying organisms.

Clinical Features

Patient presents with fever, chronic diarrhea, joint pain, gray-brown skin pigmentation, and generalized lymphadenopathy; antibiotics are curative.

Surgical Pathology: Whipple Disease (Tropical Sprue)

Gross Morphology

- Small intestinal wall is thickened and edematous with dull-colored serosa.
- Mesentery is thickened and indurated with mesenteric lymphadenopathy.

Light Microscopy

- Histologic examination shows blunting and widening of the villi of jejunum and ileum.
- Lamina propria is infiltrated by large number of foamy macrophages widening of the villi. Histologic changes in Whipple disease are shown in Fig. 20.31A and B.

Histochemistry

- The *Tropheryma whipplei* organisms are present in macrophages of intestinal mucosa, which are visualized by diastase resistant PAS stain and Gomori's methenamine silver as rod-shaped in liver, lung, heart, brain, bone and synovial membrane are also infiltrated with organisms carrying macrophages.
- These organisms may demonstrated by polymerase chain reaction and electron microscopy.

Treatment

These patients are treated by antibiotics therapy.

GIARDIASIS

Giardiasis is caused by *Giardia lamblia*. It does not induce significant villus architectural changes. The parasite is demonstrated on the luminal surface of normal appearing mucosa on histopathologic examination. The parasite is mistaken as cytoplasmic debris. Giemsa stain and trichrome stain highlight the parasite. Histologic findings demonstrating *Giardia lamblia* are shown in Fig. 20.32.

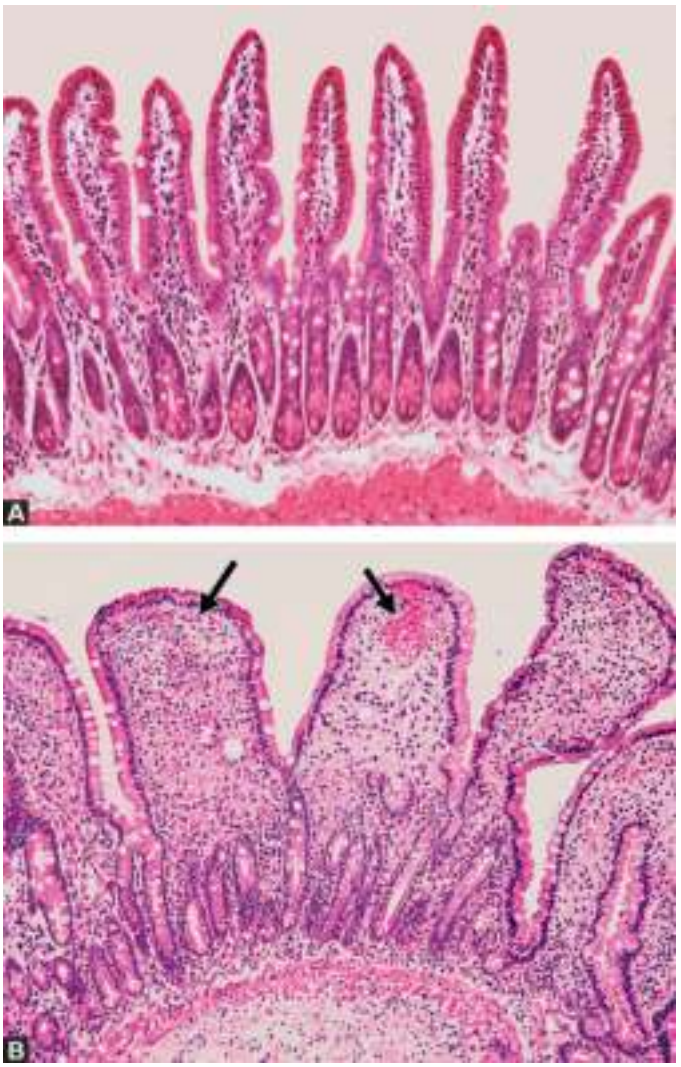


Fig. 20.31: Histologic changes in Whipple disease. (A) It shows normal villi of small intestine, (B) it shows blunting and widening of the villi of jejunum and lamina propria is infiltrated by large number of foamy macrophages (arrows) (100X).

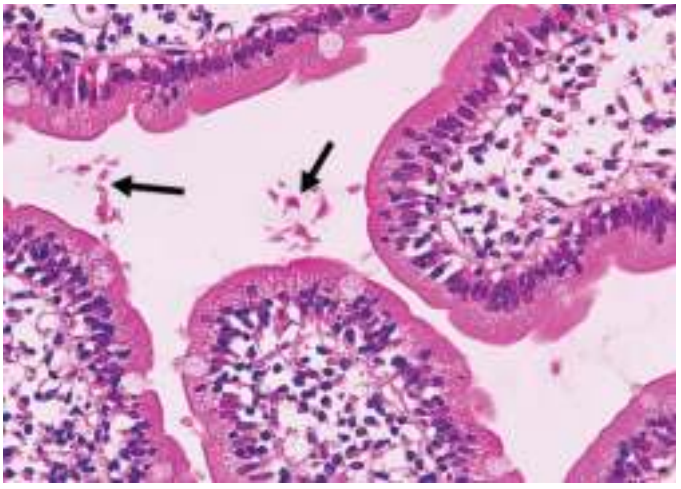


Fig. 20.32: Giardiasis. *Giardia lamblia* parasites are seen on the mucosal side of intestine (arrows).



Fig. 20.33: *Ascaris strongyloides. Strongyloides stercoralis* (roundworm nematode) causing gangrene of small intestine (arrows). (*Courtesy:* Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

ASCARIS STRONGYLOIDIASIS

Strongyloidiasis is a human disease caused by nematode roundworm called *Strongyloides stercoralis*. It most often infects small intestine and rarely colon. The parasite is diagnosed by demonstration of larvae, eggs and adult worms embedded in the crypts. Eosinophils, sometimes with Charcot-Leyden crystals may be present. Gastric strongyloidiasis may occur in association with human T-lymphotropic virus causing adult T cell lymphoma. Multiple roundworms of *Strongyloides stercoralis* are shown in Fig. 20.33.

TUBERCULAR INTESTINAL ULCER

Tuberculosis is worldwide health problem caused by tubercle bacilli (homis strain). It is transmitted by inhalation of droplets (most common), ingestion of infected milk and skin inoculation (handling infected specimens).

Mycobacterium tubercle bacilli reach the intestine by either ingestion of infected sputum or ingestion of unpasteurized milk. Tuberculosis affects terminal ileum and most often ileocecal region.

Primary Intestinal Tuberculosis

Mycobacterium tubercle bacilli (bovis strain) reach the intestine due to ingestion of unpasteurized milk. Draining lymph nodes of small intestine are enlarged and matted with caseous necrosis known as 'tabes mesenterica'. It most often heals by fibrosis and dystrophic calcification.

Secondary Intestinal Tuberculosis

- Mycobacterium tubercle bacilli reach the intestine by swallowing of infected sputum in a case of pulmonary tuberculosis. Intestinal lesions are more prominent than draining lymph nodes.
- The tubercular lesions begin in the Peyer's patches (lymphoid follicles) and spread through lymphatic channels resulting in tubercular ulcer in terminal ileum especially in ileocecal region. It produces circumferential ulcers perpendicular to long axis of small intestine. Fibrosis in ileocecal region is responsible for stricture formation. Tubercular stricture formation of intestine is shown in Fig. 20.34.
- Hyperplastic ileocecal tuberculosis is a variant of intestinal tuberculosis characterized by thickening of terminal ileum, cecum and ascending colon with mucosal ulceration. On clinical examination, hyperplastic ileocecal tuberculosis is palpable and mistaken for cecal carcinoma.

Surgical Pathology: Intestinal Tuberculosis

Light Microscopy

- Histologic examination of intestine reveals epithelioid cell granulomas, caseous necrosis and Langhans' giant cells.
- Crohn's disease also produces stricture formation, in which segmental involvement of intestine occurs with skip areas thickened wall resembles pipe-like and shows irregular linear ulcers in the mucosa.

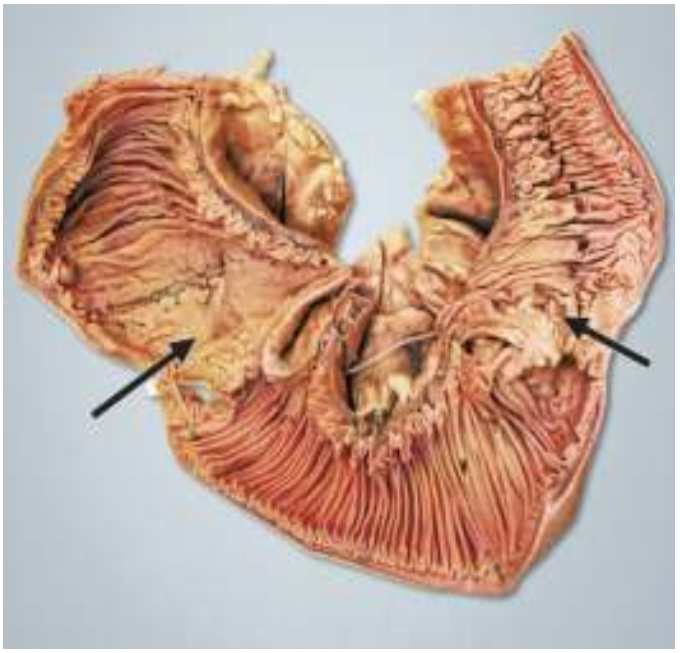


Fig. 20.34: Tubercular stricture of intestine (arrows). (*Courtesy:* Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

INTESTINAL ULCERS IN TYPHOID FEVER

Typhoid fever is a systemic disease caused by *Salmonella typhi* bacilli. Patient presents with continuous fever with relative bradycardia, abdominal symptoms and psychosis. There is involvement of mononuclear phagocytic system with nodule formation in the Peyer's patches of lower ileum.

Mode of Transmission

Salmonella typhi has three antigenic structures: O antigens, H antigens and Vi antigens. The organism is transmitted by fecal–oral route.

Clinical Features

Patient may present with step-ladder rise of fever during first week and continued during second and third weeks, headache, nausea, vomiting, abdominal tenderness, rose spots (2–4 mm on trunk) and hepatosplenomegaly.

Clinical manifestations and laboratory findings in typhoid fever are given in Table 20.37. Differences between tubercular and typhoid ulcers are given in Table 20.38.

Pathogenesis

Ingested *Salmonella typhi* pathogen invades the mucosa, engulfed by macrophages and transported to regional lymph nodes. Multiplication of pathogen occurs in the lymphoid tissue resulting in bacteremia. *Salmonella typhi* reinfects lymphoid tissue and liberates endotoxin leading to delayed hypersensitivity reaction. Erythrophagocytosis is demonstrated in histopathologic examination of tissue sections.

Extraintestinal Lesions

Proliferation of reticuloendothelial cells occurs in spleen (sinus histiocytes), liver (Kupffer's cells), lymph nodes and bone marrow. Patient develops splenomegaly, hepatomegaly and lymphadenopathy.

Intestinal Pathology

Terminal ileum and cecum are involved due to *Salmonella typhi* infection. There are four stages lasting for 4 weeks.

- **Hyperplasia of Peyer's patches during first week:** In the first week of infection, hyperplasia of Peyer's

Table 20.37 Clinical manifestations and laboratory findings in typhoid fever

Clinical Features	First Stage (1st Week)	Second Stage (2nd Week)	Third Stage (3rd Week)	Fourth Stage (4th Week)
Rose spots	Present on 5th day	Persists till 10th day	Absent	Absent
Splenomegaly	Slightly palpable on 6th day	Moderate enlarged	Moderate enlarged	Moderate enlarged
Psychosis and confusion	Absent	Present on 10th day	Present	Absent
Leukopenia	Present on 5th day	Present	Present	Absent
Blood culture	+++	+++	+/–	–
Stool culture	–	+	+++	–
Widal reaction	–	++	+++	–

Table 20.38 Differences between tubercular and typhoid ulcers

Characteristics	Tubercular Ulcer	Typhoid Ulcer
Causative organism	Mycobacterium tubercle bacilli	*Salmonella typhi*
Site of gut involvement	Ileocecal region	Terminal ileum (most common), may occur in jejunum or colon
Lymphoid involvement	Peyer's patches	Peyer's patches and also lymphoid follicles
Arrangement of ulcers	Transverse axis of intestine	Longitudinal axis of intestine
Size of ulcer	Large	Small
Margin	Irregular ulcer may encircle gut	Regular well circumscribed
Base of ulcer	Caseous material (creamy white)	Black due to sloughing of mucosa
Perforation of ulcer	Absent	Common
Bleeding from ulcer	Absent	Common
Stricture formation	Present as a result of fibrosis	Absent
Light microscopy	Caseous necrosis, epithelioid granulomas, Langhans' giant cells and demonstration of acid-fast bacilli	Erythrophagocytosis and presence of organisms in macrophages

patches (lymphoid tissue) of terminal ileum occurs in the mucosa and extending to submucosa in the terminal ileum. Mucosa is projecting on the surface. Blood culture is positive for *Salmonella typhi* but stool culture is negative.

- **Necrosis of Peyer's patches during second week:** Necrosis of Peyer's patches occurs from center to periphery exhibiting yellow or greenish-brown discoloration. Blood culture and stool culture are positive.
- **Mucosal ulceration of intestine during third week:** In the third week of infection, mucosal surface of ileum shows round to oval deep ulcers along the long with axis of intestine on gross examination. Stool culture and Widal reaction are positive. These ulcers may perforate leading to hemorrhage.
- **Healing of intestinal ulcer during fourth week:** Healing of intestinal ulcers occurs in the 4th week of infection. Widal reaction is positive. Typhoid ulcers in small intestine are shown in Figs 20.35 and 20.36.

OBSTRUCTIVE DISORDERS

Partial or complete obstruction of lumen of small intestine is a serious clinical disorder. Small bowel obstruction can be simple, strangulated (interrupting blood supply) and closed loop type (isolating from rest of small intestine).

Small bowel obstruction occurs due to adhesions from previous surgery and congenital bowel deformities, which can be due to intraluminal (e.g. tumor, foreign bodies), intramural and extra-intestinal etiology.

DUODENAL ATRESIA

Duodenal atresia occurs either due to failure of the gut to canalize or failure of segment to develop during fetal growth. The normal gastrointestinal system is a tube that develops from cords of embryonic cells. The embryonic cells forming the central part of these cords undergo apoptosis resulting in formation of lumen. If the centrally located cells do not undergo apoptosis, the lumen never forms and the affected segment of the GIT system will be atretic (i.e. unpassable).

Duodenal atresia is most commonly associated with Down syndrome and characterized by vomiting of bile stained material at birth. Abdominal radiograph demonstrates double bubble sign.

MECONIUM ILEUS

Meconium ileus is a bowel obstruction that occurs when the meconium in child's intestine is thicker and sticker than normal meconium, creating a blockade in the ileum. Meconium ileus in babies with cystic fibrosis may be complicated by volvulus and intestinal atresia.

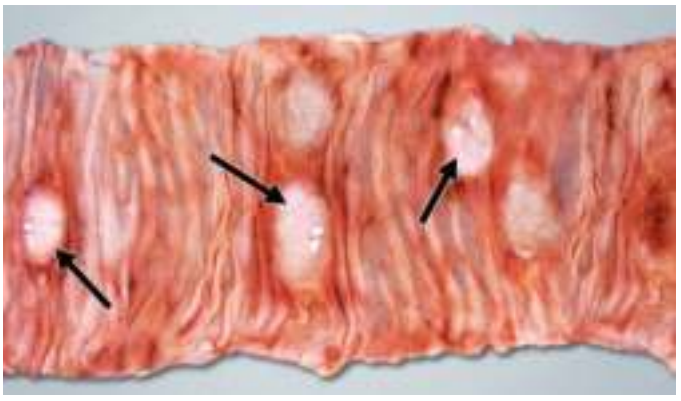


Fig. 20.35: Typhoid ulcers in small intestine. The Peyer's patches are prominent. Round to oval ulcers with hemorrhage are present along the long axis of intestine (arrows).

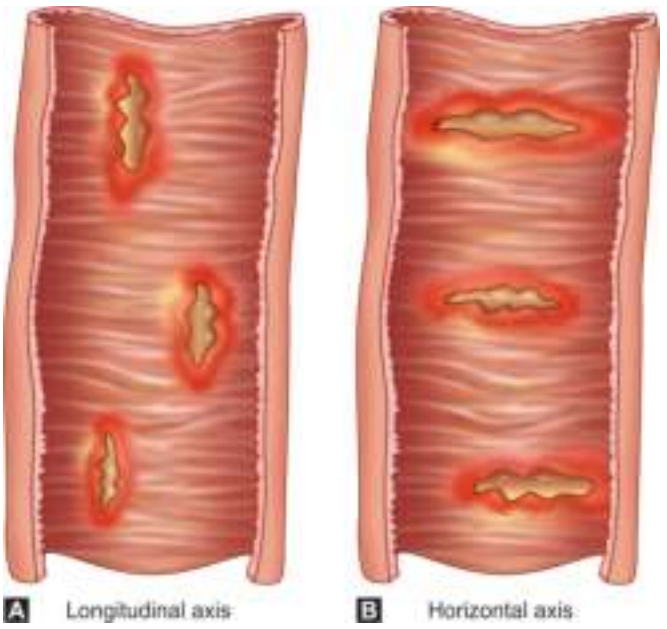


Fig. 20.36: Ulcers in small intestine. (A) Typhoid ulcers, (B) tubercular ulcers.

INTESTINAL INTUSSUSCEPTION

Intestinal intussusception is an invagination of one segment of proximal bowel (usually the terminal ileum) into the distal bowel (usually the cecum), causing intestinal obstruction.

- Intestinal intussusception is a common cause of intestinal obstruction in children due to active peristalsis between 3 months and 6 years of age.
- In adults, intestinal intussusception occurs due to pedunculated polyps or malignant growth. Small pedunculated tumors carried by peristalsis may pull forward the loop to which such a tumor is attached.
- Based on the location, intestinal intussusception may be ileocecal (most common), jejunojejunal, ileoileal and colocolic. The inner loop of the gut may become necrotic unless the invagination is everted surgically.

Pathology

Intestinal intussusception progresses to tissue necrosis of segment of intestine as a result of decreased blood flow, swelling and inflammation. It can lead to intestinal perforation and infection. Septic shock and dehydration can occur very rapidly. Gross morphology of intussusception is shown in Fig. 20.37.

Clinical Features

Child presents with signs of obstruction (paroxysmal abdominal colicky pain) and rectal bleeding (currant jelly stools) from bowel infarction. On clinical examination, abdominal distension and decreased bowel sounds are observed. Features of dehydration include rapid pulse, pallor and marked sweating.

Diagnostic Evaluation

Abdominal radiograph may show absence of gas in right upper quadrant. Ultrasonography may be done to locate area of telescoped bowel.

PARALYTIC ILEUS

Paralytic ileus refers to lack of intestinal peristalsis associated with stagnation of intestinal contents. The most common causes of paralytic ileus include abdominal surgery persisting for 2–3 days, peritonitis, shock and vascular collapse.

GALLBLADDER STONE ILEUS

In some cases of gallbladder stone disease, gallstone erodes through the gallbladder into the adjacent small bowel forming cholecystoenteric fistula. Gallbladder stone enters ileum through the fistulous tract and blocks the intestine known as gallstone ileus. It can be very serious disorder which requires immediate surgery. It primarily occurs in patients over age 65, and can sometimes be fatal.

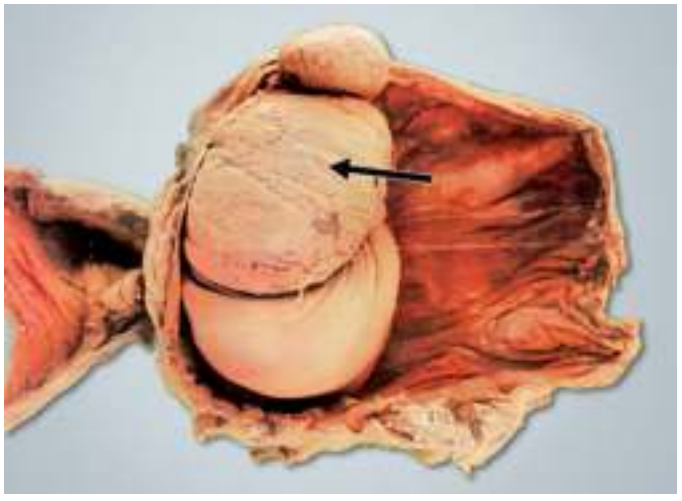


Fig. 20.37: Intussusception. It shows invagination of one segment of proximal bowel (usually the terminal ileum) into the distal bowel (arrow). (*Courtesy:* Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

INGUINAL HERNIA

In inguinal hernia, the hernial contents are intestine, omentum, or other abdominal contents that pass through the inguinal canal opening into the hernial sac.

- Direct inguinal hernia occurs through the posterior wall of the inguinal canal in the center of Hesselbach's triangle.
- Indirect inguinal hernia is most common cause of intestinal obstruction, in which small bowel extends into the scrotal sac, lateral to the triangle of Hesselbach.

ADHESIONS

Adhesions are most common cause of gastrointestinal obstruction. These are most commonly acquired from previous abdominal surgery.

UMBILICAL HERNIA

Umbilical hernias occur in children and adults. Ascites and pregnancy precipitate hernias in adults. Umbilical hernia can cause segment of bowel out of abdomen and produce symptoms.

VENTRAL INCISIONAL HERNIA

Ventral incisional hernia occurs in previous surgical incision sites. In a strangulated ventral hernia, bowel gets tightly caught within an opening in abdominal wall.

VASCULAR DISORDERS

SMALL BOWEL INFARCTION

Occlusion of superior mesenteric artery is most common cause of small bowel infarction. Small intestine is supplied by superior mesenteric artery. Colon receives blood from inferior mesenteric artery. Atheromatous plaques are common in superior mesenteric artery due to its oblique origin from the abdominal aorta.

Etiopathogenesis

Decreased blood supply to small intestine occurs due to thrombus over an atheromatous plaque of supplying blood vessel, emboli from left heart, hypovolemic shock, vasospasm (digitalis), venous thrombus (polycythemia) and mechanical obstruction (volvulus). Systemic arterial thrombi may embolize to mesenteric arteries resulting in small bowel infarction.

Clinical Features

Patient presents with diffuse abdominal pain, nausea, vomiting and bloody diarrhea.

Complications

In transmural gut infarction; there is danger of gut perforation with mucosal ulcerations and formation of pseudomembrane.

POLYPS AND TUMORS

Polyps and tumors of the small intestine make up a small percentage of gastrointestinal tract neoplasms. The most common malignant tumors are neuroendocrine (carcinoid) tumors, lymphoma, adenocarcinoma, and GISTs.

Non-neoplastic lesions are more common in small intestine. Revised 2019 WHO classification of tumors of small intestine and ampulla of Vater is given in Table 20.39.

ADENOCARCINOMA

Adenocarcinoma of small intestine is much less common than adenocarcinoma of colon. Approximately 40–50% of cases have tumor in the duodenum in the region of ampulla of Vater. It may also occur in jejunum or ileum. Disorders associated with an increased risk for small intestinal carcinoma are given in Table 20.40.

Surgical Pathology: Adenocarcinoma of Small Intestine

Gross Morphology

- Duodenal adenocarcinoma is relatively circumscribed with polypoidal appearance and central ulceration.
- Adenocarcinoma in jejunum or ileum shows circumferential involvement with extensive ulceration and annular appearance.

Light Microscopy

- Tumor is well differentiated adenocarcinoma producing mucin.
- Scattered neuroendocrine cells may be present.

NEUROENDOCRINE (CARCINOID) TUMORS

Carcinoid tumors are neuroendocrine tumors of low-grade malignancy. These are most commonly located in the submucosa of the intestinal tract, e.g. appendix (40% cases), terminal ileum (25% cases), colon, rectum (10–20% cases), duodenum (2–5% cases), esophagus and stomach.

- Carcinoid tumors are multicentric in stomach and ileum. Peak incidence is in the sixth decade. The tumors are well differentiated and composed of cells similar to those of normal counterparts.

Table 20.39 Revised 2019 WHO classification of tumors of small intestine and ampulla of Vater

Benign Epithelial Tumors and Precursor Lesions
▪ Adenomatous polyp, low-grade dysplasia
▪ Adenomatous polyp, high-grade dysplasia • Intestinal-type adenoma, low-grade • Intestinal-type adenoma, high-grade • Serrated dysplasia, low-grade • Serrated dysplasia, high-grade • Noninvasive pancreatobiliary papillary neoplasm with low-grade dysplasia • Noninvasive pancreatobiliary papillary neoplasm with high-grade dysplasia • Intra-ampullary papillary-tubular neoplasm
Malignant Epithelial Tumors
▪ Adenocarcinoma, NOS
▪ Mucinous adenocarcinoma
▪ Signet ring cell carcinoma
▪ Medullary carcinoma, NOS
▪ Adenocarcinoma, intestinal type
▪ Pancreatobiliary-type carcinoma
▪ Tubular adenocarcinoma
▪ Neuroendocrine tumor, NOS • Neuroendocrine tumor, grade 1 • Neuroendocrine tumor, grade 2 • Neuroendocrine tumor, grade 3 • Gastrinoma, NOS • Enterochromaffin-like cell carcinoid • Extra-adrenal paraganglioma, NOS
▪ Neuroendocrine carcinoma, NOS • Large cell neuroendocrine carcinoma • Small cell neuroendocrine carcinoma
▪ Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN)

Adapted from 2019 WHO classification of tumor of digestive system.
Term NOS (not otherwise specified).
Other less common tumors derived from soft tissue are leiomyoma, gastrointestinal stromal tumors, lipoma, angiosarcoma, Kaposi sarcoma. Hematolymphoid neoplasms are Burkitt lymphoma, follicular lymphoma, diffuse large B cell lymphoma and T cell lymphoma.

Table 20.40 Disorders associated with an increased risk for small intestinal carcinoma

Sporadic adenomatous polyps
Familial adenomatous polyposis (FAP)
Hereditary nonpolyposis colon cancer syndrome (Lynch syndrome)
Juvenile polyposis syndrome
Crohn's disease
Celiac disease
Gardener's syndrome
Peutz-Jeghers syndrome
α-Chain disease

Table 20.41 Features of neuroendocrine (carcinoid) tumors in gastrointestinal tract

Parameters	Foregut Carcinoids (Proximal to Ligament of Treitz)	Midgut Carcinoids	Hindgut Carcinoids
Structures	▪ Stomach (body and fundus) ▪ Proximal duodenum but rare in esophagus	▪ Terminal ileum ▪ Jejunum	▪ Tip of appendix ▪ Colorectal region
Size	1–2 cm or more	<3.5 cm	Appendix (up to 1 cm) and colon up to 5 cm
Frequency	2–5%	60–80%	10–20%
Substances synthesized	Histamine, somatostatin and serotonin	Serotonin, substance P and polypeptide YY	Serotonin, substance P
Clinical course	Asymptomatic course	Aggressive course due to greater depth of local invasion, necrosis and mitosis with poor outcome	Rectal carcinoids producing polypeptide hormones, colonic carcinoid may metastasize
Tumor cells	Argyrophilic cells	Argentaphilic cells	Argyrophilic cells
Carcinoid syndrome	Rare	Most common	Rare

- Goblet cell carcinoid tumor consisting of mucin secreting and goblet-like cells is known as adenocarcinoid tumor with aggressive behavior than classic carcinoid tumor. Features of neuroendocrine (carcinoid) tumors in gastrointestinal tract are given in Table 20.41.

Neuroendocrine Cells

Neuroendocrine cells of the gut utilize amino acids, or derivatives of amino acids, as chemical messengers mediating paracrine and neuroendocrine effects. They are known as APUD cells (amine precursor uptake and decarboxylation).

- **Enterochromaffin cells:** Enterochromaffin cells are named after their staining characteristics after chromate fixation. These are found in groups at the bases of the intestinal crypts small intestine. These cells synthesize 5-HT (5-hydroxytryptamine) and kallikrein.
- **Nonchromaffin enteroendocrine cells:** These cells are dispersed as single cells in the crypt and villus epithelium, and bowel wall.

Neuroendocrine Tumors: Categories

Neuroendocrine tumors are categorized into three grades (G1, G2 and G3) according to mitotic count and Ki-67 proliferative index. The term neuroendocrine carcinoma is reserved for poorly differentiated neuroendocrine tumor with marked nuclear atypia and high mitotic rate (>20 high power field). Classification and grading of neuroendocrine tumors (carcinoids) of the GI tract are given in Table 20.42.

Surgical Pathology: Neuroendocrine Tumors of Intestine

Gross Morphology

Following formalin fixation, tumor acquires yellow color. Infiltration of the submucosa by tumor is the rule.

Light Microscopy

- Neuroendocrine tumors of small intestine are composed of neuroendocrine cells.
- Tumor cells are round with uniform nuclei, scanty cytoplasm and indistinct cell borders. Tumor cells are arranged in solid nests, cords separated by thin fibrous septa.
- Tumor cells may form rosettes around blood vessels, which secrete hormones into the systemic circulation (Fig. 20.38).

Histochemical Staining

Silver staining demonstrates black granules in the cytoplasm of tumor cells. Argentaffin cells containing granules have the property of reducing silver salts without the help of reducing agent, while argyrophil cells reduce silver salts to metallic silver (black color) only after addition of a reducing agent.

Electron Microscopy

Tumor cells show cytoplasmic granules, which are stained red with eosin, yellowish brown with chrome salts, black with iron-hematoxylin and metallic black with silver salts.

Immunohistochemistry Technique

Panel of immunohistochemical markers used in gastrointestinal tract carcinoid (neuroendocrine) tumors includes synaptophysin, chromogranin A, neuron-specific enolase (NSE), CD56 (neuronal cell adhesion molecule—NCAM), cytokeratins (AE1/AE3 and CAM 5.2 epithelial marker) and CDX2 (polyclonal marker).

Markers	Expression
▪ Synaptophysin	▪ Positive
▪ Chromogranin A	▪ Positive
▪ NSE (neuron-specific enolase)	▪ Positive
▪ CD56 (NCAM)	▪ Positive
▪ AE1/AE3 and CAM 5.2	▪ Positive
▪ CDX2	▪ Positive (50%)

Table 20.42 Classification and grading of neuroendocrine tumors (carcinoids) of the GI tract

Classification	Grade	Cytological Features	Mitotic Count
Neuroendocrine tumor	1 2	▪ Cytological bland ▪ Cytological bland	▪ <2 per HPF and/or 2% Ki-67 index ▪ 2–20 per HPF and/or 3–20% Ki-67 index ▪ >20 per 10 HPF and/or >20% Ki-67 index
Neuroendocrine carcinoma (NEC)	3	Tumor displaying at least 30% component of adenocarcinoma	
Hyperplastic or preneoplastic lesions	–	–	–

Grading requires mitotic count in at least 50 HPFs and Ki-67 percentage in at least 500–2000 cells within the area of strongest nuclear labeling.

Metastases

Carcinoid tumor of appendix and rectum is seldom malignant. Most carcinoid tumors invade submucosa and muscularis and sometimes penetrate bowel wall. Metastases may be seen in 5% of cases having tumor <1 cm size. However, large carcinoid tumor >2 cm size in ileum and appendix has tendency to spread to regional lymph nodes and distant organs such as liver, lungs and bones in 66% of cases.

Carcinoid Syndrome

Neuroendocrine tumors metastasizing to the liver can lead to carcinoid syndrome as a result of synthesis of 5-HT (5-hydroxytryptamine), serotonin, and bradykinin. 5-HT increases gut motility resulting in diarrhea, excessive bowel sounds (borborygmi) and abdominal pain.

- Kallikrein produces flushing of skin, bronchospasm and diarrhea. 5-HT is inactivated in the liver by monoamine oxidase to form 5-hydroxyindoleacetic acid (5-HIAA), and this is excreted in the urine.
- Urinary 5-hydroxyindoleacetic acid (5-HIAA) may be elevated in these patients.
- Patient presents with cutaneous flushing, watery diarrhea, abdominal cramps, bronchospasm and valvular lesions of the right side of the heart. Clinical manifestations of neuroendocrine (carcinoid) tumors of small intestine are given in Table 20.43.

Carcinoid Heart Disease

The products synthesized by carcinoid tumor such as 5-HT, serotonin, and bradykinin enter into hepatic veins and affect the right side of the heart. The patient develops tricuspid valve incompetence and pulmonary valve stenosis as a result of formation of fibrous plaques. Smooth muscle cells within endocardium may undergo proliferation as a result of stimulation by kallikrein.

GASTROINTESTINAL STROMAL TUMORS

Gastrointestinal stomal tumors (GISTs) are most common mesenchymal neoplasms that originate primarily in the wall of gastrointestinal tract.

- Clinical behavior ranges from benign/low-grade to malignant tumors. Malignant gastrointestinal stromal tumors can disseminate to liver and intra-abdominal structures associated with fatal outcome.
- Instead of dividing GISTs into benign/low-grade to malignant; risk of progression to malignant phenotype is based on anatomic location, size and mitotic activity of gastrointestinal stromal tumors.

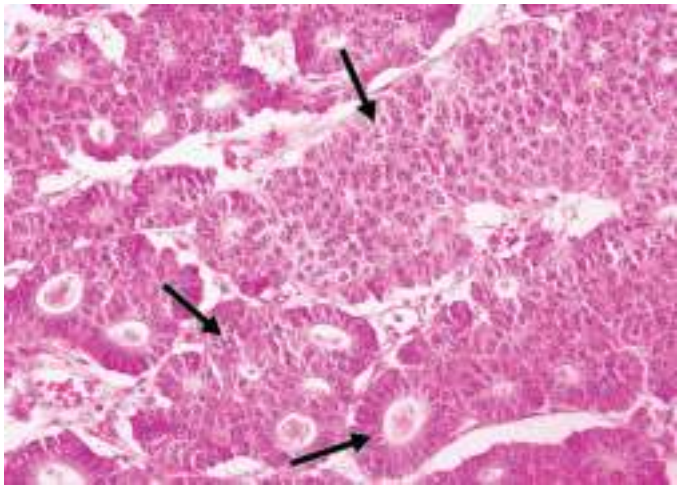


Fig. 20.38: Neuroendocrine tumor of ileum is composed of uniform cells with stippled chromatin (arrows) (400X).

Table 20.43 Clinical manifestations of neuroendocrine (carcinoid) tumors of small intestine

Organs Involved	Clinical Manifestations
Liver	Metastases
Vascular phenomenon	Flushing, cyanosis and telangiectasia
Respiratory system	Bronchoconstriction
Heart	Pulmonary and tricuspid valvular disease
Blood and tissue cells	5-Hydroxytryptamine (5-HT) and serotonin
Urine analysis	↑ 5-Hydroxyindoleacetic acid (5-HIAA) excretion
Skin	Hyperpigmentation
Legs	Edema

Table 20.44 Classification of gastrointestinal stromal tumors

Benign Tumors
Benign gastrointestinal stromal tumor
Intermediate (Locally Aggressive) Tumors
Gastrointestinal stromal tumor of uncertain malignant potential
Malignant Tumors
Malignant gastrointestinal stromal tumor

Classification of gastrointestinal stromal tumors is given in Table 20.44.

Origin, Location and Age Distribution

Gastrointestinal stromal tumors (GISTs) originate from stromal stem cells, that are precursors of smooth muscle cells (i.e. gastrointestinal fibroblasts or pacemaker cells of Cajal controlling peristalsis).

- Most common sites of gastrointestinal stromal tumor are stomach (60%), small intestine (30%), colon, appendix (10%), rectum and gallbladder.
- Rare sites of extra-gastrointestinal stromal tumors are omentum, mesentery, retroperitoneum and pelvis. It usually affects adult males with median age of 58 years.
- GISTs may be associated with neurofibromatosis and Carney triad (GIST, paraganglioma and pulmonary chondroma). It can affect children and young adults in the setting of Carney syndrome.

Molecular Genetic Alterations

Approximately 90% of GISTs demonstrate c-KIT (CD117) mutation. Mutation in *PDGFRA* is demonstrated in 10% of cases. A gain of function in c-KIT (CD117) or *PDGFRA* gene may coexist. GISTs with c-KIT (CD117) mutation respond to Gleevec (imatinib) therapy.

Clinical Features

Patient presents with anemia due to ulceration and bleeding of GIST, fatigue, pain abdomen and distension abdomen. GIST is detected as an incidental finding in surgical specimens such as gastrectomy for gastric carcinoma. Carney's syndrome is characterized by Carney's triad (gastric GIST, extra-adrenal paraganglioma and pulmonary chondroma) in children.

Radiologic Findings

CT and MRI scans are performed to diagnose gastrointestinal stromal tumor. Endoscopy and ultrasonography are useful diagnostic techniques to detect gastrointestinal stromal tumors.

Surgical Pathology: Gastrointestinal Stromal Tumors (GISTs) of Small Intestine

Gross Morphology

- Gastrointestinal stromal tumors are either unilobular or multilobular with smooth circumscribed borders. These may vary from small mural nodule to large pedunculated mass in serosa.
- Duodenal GISTs measure 5 cm in diameter; and 7 cm in diameter of GIST in jejunum and ileum. Ulceration is commonly demonstrated in large tumor.
- Cut surface of tumor is fleshy and cystic with areas of hemorrhage and necrosis (Fig. 20.39).

Light Microscopy

- Gastrointestinal stromal tumors are composed of either epithelioid or spindle-shaped cells. Majority of GISTs show combined cytomorphologic features of epithelioid and spindle-shaped cells. Cardinal diagnostic features of GISTs include lack of pleomorphism and low mitoses.
- Epithelioid GIST is composed of epithelioid tumor cells with round-elongated nuclei, fine chromatin, inconspicuous nucleoli and abundant fibrillary cytoplasm; and arranged in either in sheets or nests.
- Spindle cell GIST is composed of spindle-shaped neoplastic cells with round-elongated nuclei, fine chromatin, inconspicuous nucleoli and abundant fibrillary cytoplasm; and arranged in fascicular pattern. Spindle cell GISTs may demonstrate smooth muscle component, and neural differentiation shows nuclear palisading resembling schwannoma (Fig. 20.40).

Electron Microscopy

Analysis of GISTs shows lack of differentiation, i.e. incomplete differentiation of or neuroaxonal differentiation.

Histochemistry

A small set of GISTs has acellular collagen bundles known as 'skeinoid fibers', that are demonstrated by PAS stain.

Differential Diagnosis

Gastrointestinal stromal tumor should be differentiated from leiomyoma, leiomyosarcoma, schwannoma, desmoid fibromatosis, malignant melanoma and carcinoma.

Immunohistochemistry Technique

Gastrointestinal stromal tumors show positivity for c-KIT (CD117) encoding tyrosine kinase, DOG1 (most specific marker), CD34 (75%), and smooth muscle actin (40%), c-KIT (CD117) staining is most often diffuse and strong membranous, cytoplasmic or paranuclear globular staining pattern, protein kinase c-theta, smooth muscle actin encoded by ACTA2 gene, vimentin and desmin. About 5% of GISTs are negative for KIT (CD117). Majority of these tumors are of epithelioid GISTs show *PDGFRA* gene mutations.

Markers	Expression
c-KIT (CD117)	Positive (90%)
DOG1	Positive
PDGFRA	Positive
CD34	Positive (75%)
Smooth muscle actin	Positive (40%)
Protein kinase c-theta	Positive
Vimentin	Positive
Desmin	Positive (5%)
S-100 protein	Negative

Molecular Genetic Alterations

Loss of material from the short arm of chromosome 1 and monosomy for chromosome 14 and chromosome 22 are most common cytogenetic profile in GISTs. Loss of material from the short arm of chromosomes 9 and 11 is less common and associated with aggressive clinical course.

Prognosis, Clinical Behavior and Treatment

Mitotic count, size, and location help in predicting the behavior of GISTs. GISTs in small intestine most often behave like leiomyosarcoma as opposite to the stomach. GISTs are considered to be of low-malignant potential and are removed surgically. Proposed guidelines for predicting malignant behavior of GISTs are given in Table 20.45.

- Majority of GISTs exhibit benign clinical course. Recurrence and metastases are demonstrated in 50% of cases.
- GISTs have tendency for intra-abdominal spread and metastasize to liver. Lymph node metastasis is extremely rare. Lungs are the most common site of metastasis outside the abdomen.

DIFFUSE LARGE B CELL LYMPHOMA

Diffuse large B cell lymphoma (DLBCL) can arise from the abundant lymphoid tissue of the small intestine, which accounts for 50% of all lymphomas. Patient may present with malabsorption syndrome when there is diffuse involvement.

Surgical Pathology: Diffuse Large B Cell Lymphoma
Gross Morphology
Large polypoidal or ulcerated circumferential tumor mass of DLBCL is seen in small intestine (Fig. 20.41).
Light Microscopy
Tumor is composed of sheets of large lymphoid cells with vesicular nuclear chromatin, prominent nucleoli, irregular contours and scanty cytoplasm (Fig. 20.42).

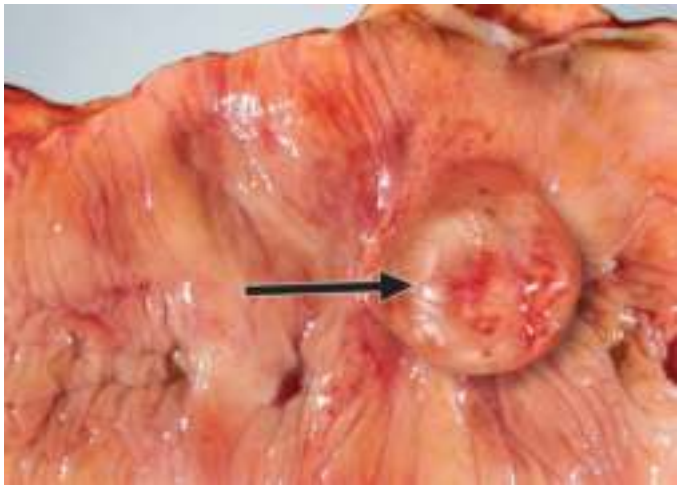


Fig. 20.39: Gastrointestinal stromal tumor. It shows well circumscribed tumor projecting towards lumen of intestine (arrow). Cut surface of tumor may show hemorrhage and necrosis.

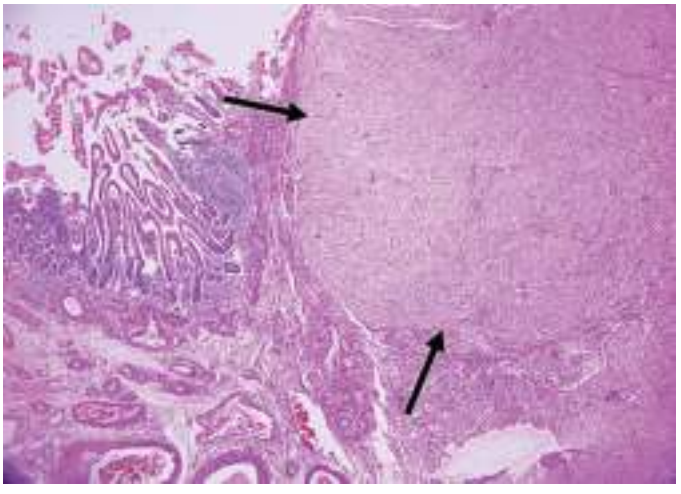


Fig. 20.40: Gastrointestinal stromal tumors. Tumor is composed of epithelioid and spindle cells arranged in sheets. Tumor lacks pleomorphism and mitosis (arrows) (100X).

Table 20.45 Proposed guidelines for predicting malignant behavior of gastrointestinal stromal tumors

Tumor Size	Light Microscopy	Clinical Behavior	Metastases and Mortality
≤2 cm	≤5 mitoses/50 HPF	Benign course	Prognosis excellent
≤2 cm and ≥5 cm	≤5 mitoses/50 HPF	Low malignant potential	Metastases and fatal outcome (4%)
≥5 cm and ≤10 cm	≤5 mitoses/50 HPF	Moderately malignant	Metastases and fatal outcome (25%)
>10 cm	≤5 mitoses/50 HPF	Highly malignant	Metastases and fatal outcome (50–90%)



Fig. 20.41: Large polypoidal and ulcerated circumferential tumor mass of diffuse large B cell lymphoma is seen in small intestine (arrows).

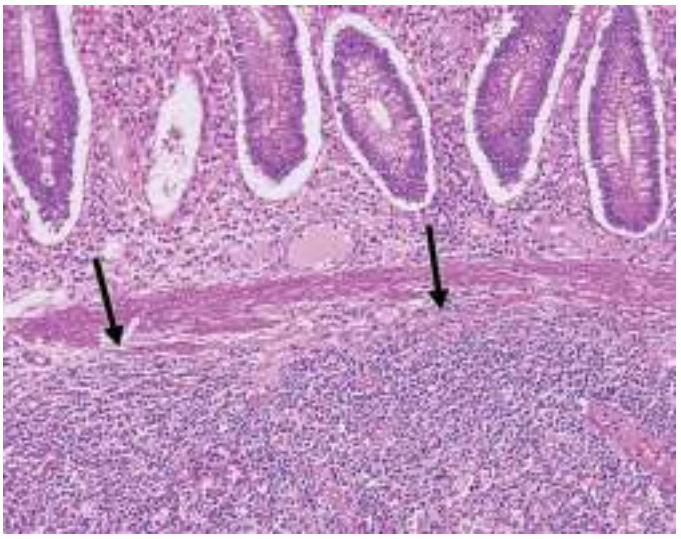


Fig. 20.42: Diffuse large B cell lymphoma. Tumor is infiltrating intestinal mucosa and composed of sheets of large atypical lymphoid cells (arrows) (400X).

DISORDERS OF LARGE INTESTINE

ANATOMY AND PHYSIOLOGY

Large intestine measures 1.5–1.8 meters in length extending from ileocecal valve to dentate line of the anal canal. It consists of right and left colon. Right colon is subdivided into cecum, ascending colon and proximal transverse colon.

- Left colon consists of distal transverse colon, descending colon, sigmoid colon and rectum. Rectum measures 10.5–12 cm in length.
- Transverse colon always has mesentery. Mesentery is seen in ascending colon in 12% and descending colon in 22% of cases.

BLOOD SUPPLY

Superior mesenteric artery supplies blood to the right colon as far as distal transverse colon. Inferior mesenteric artery supplies blood to left colon and rectum. Remainder of the rectum and anal canal are supplied by middle rectal arteries and branches of the pudendal arteries.

HISTOLOGY

Large intestine consists of mucosa, submucosa, muscular coat and serous coat. Mucosa of colon is lined by simple columnar epithelium with thin brush border and numerous goblet cells. The crypts of Lieberkühn are straight and branched lined by goblet cells. At the base of the crypts endocrine and undifferentiated cells are present.

- Mucosa absorbs some ions, soluble compounds and maintains water balance. It secretes mucus, which lubricates colon and protects mucosa. Mucosa breaks down undigested carbohydrates, proteins, and amino acids into products that can be expelled in feces or absorbed and detoxified by liver.
- Large intestine synthesizes certain vitamins of B complex and vitamin K. Muscular layer moves contents along length of colon into sigmoid colon and rectum by contraction of circular and longitudinal muscles.

NEUROMUSCULAR DISORDERS

HIRSCHSPRUNG'S DISEASE

Hirschsprung's disease, also referred as congenital megacolon, is characterized by congenital absence of ganglion cells usually in the submucosal and muscular coat of large bowel (distal sigmoid colon and rectum). It most often affects male infants in 80% of cases (Fig. 20.43).

Predisposing Factors

Hirschsprung's disease is associated with Down syndrome and Waardenburg syndrome. Waardenburg syndrome is characterized by congenital deafness, gut malrotation, gastric diverticulum, intestinal atresia, urogenital abnormalities and cardiovascular abnormalities. Acquired megacolon may be associated with Chagas' disease.

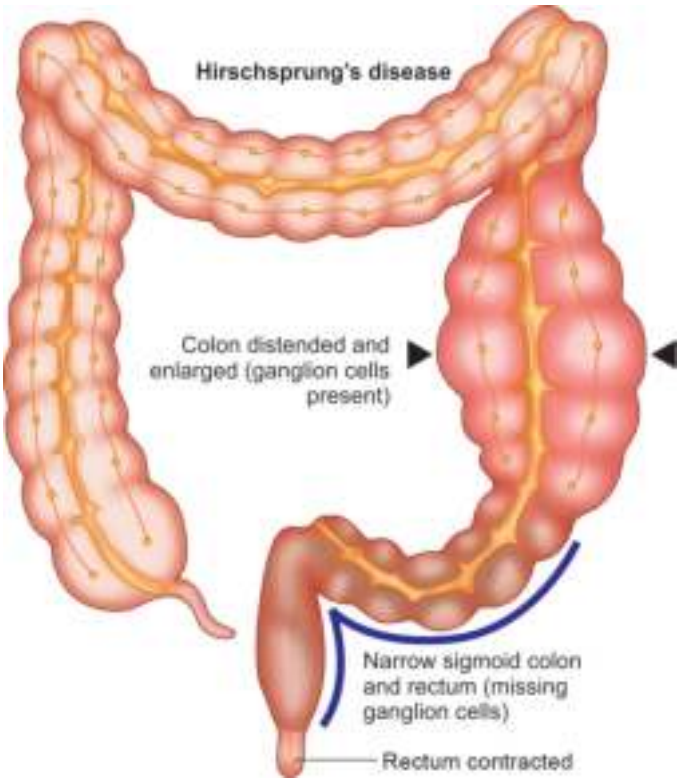


Fig. 20.43: Hirschsprung's disease. It shows narrow sigmoid colon absence of ganglion cells and dilated normal colon with presence of ganglion cells proximal to constricted segment.

Molecular Genetic Alterations

RET gene located on 10q11.2 is the most common gene mutation demonstrated in Hirschsprung's disease. Other gene mutations in this disorder include EDNRB gene (located on 13q22), SOX10 (located on 22q13), SIP1 gene (located on 2q22), PHOX2B gene (located on 4q12) and neuregulin gene (located on 8).

EDNRB gene present on surface of cells encodes protein that connects the neural crest cells to digestive tract. Interaction of endothelin 3 with endothelin receptor type B protein is essential for development of enteric neurons. Gene mutations in Hirschsprung's disease are given in Table 20.46.

Table 20.46 Gene mutations in Hirschsprung's disease

Gene Mutation	Located on Chromosome
RET gene	10q11.2
EDNRB gene	13q22
SIP1 gene	2q22
PHOX2B	4q12
Neuregulin gene	8

Pathogenesis

Marked dilation of the colon (ganglionic segment) occurs proximal to the stenotic rectum (aganglionic segment), with clinical signs of intestinal obstruction.

- **Physiologic state:** During intrauterine life, the neural crest cells migrate into upper alimentary tract and then follow caudally via vagus nerve fibers by 12 weeks of gestation. These ganglion cells are present in submucosa (Auerbach's plexus) and between muscular coats (Meissner's plexus). Ganglion cells are responsible for the peristaltic moment of the intestine in postnatal life.
- **Pathologic state:** Failure of migration of ganglion cells in the submucosal (Auerbach's plexus) and muscular coat (Meissner's plexus) in the large intestine, especially rectum results in Hirschsprung's disease. Immune-mediated neuronal necrosis has been suggested in the pathogenesis of Hirschsprung's disease, that shows aberrant expression of MHC class II molecules in colon responsible for recurrence of disease after surgery.

Colon Sites Involved

Rectum is always involved and hence the preferred site for biopsy. Sigmoid colon and adjacent proximal colon are involved, but entire colonic involvement very rare. Aganglionic segment of colon is permanently contracted causing intestinal obstruction, which leads to dilatation of proximal normal (ganglionic) segment.

Classification

Based on length of large intestine in Hirschsprung's disease involved, infant has classic segment (<40 cm colon), long segment (colon involvement extending to small intestine), short segment (a few cm of colon involved), ultra-short (<2 cm colon involved) and zonal form (skip areas in colon).

Clinical Features

Newborn with Hirschsprung's disease develops delayed passage of meconium, severe constipation, vomiting and abdominal distension. There may be electrolyte imbalance, peritonitis, superimposed enterocolitis, toxic megacolon and perforation.

On clinical examination, anal canal and rectum are small devoid of stool with tight anal sphincter. Abdominal distension, radiograph and barium enema findings in Hirschsprung's disease are shown in Fig. 20.44A to C.

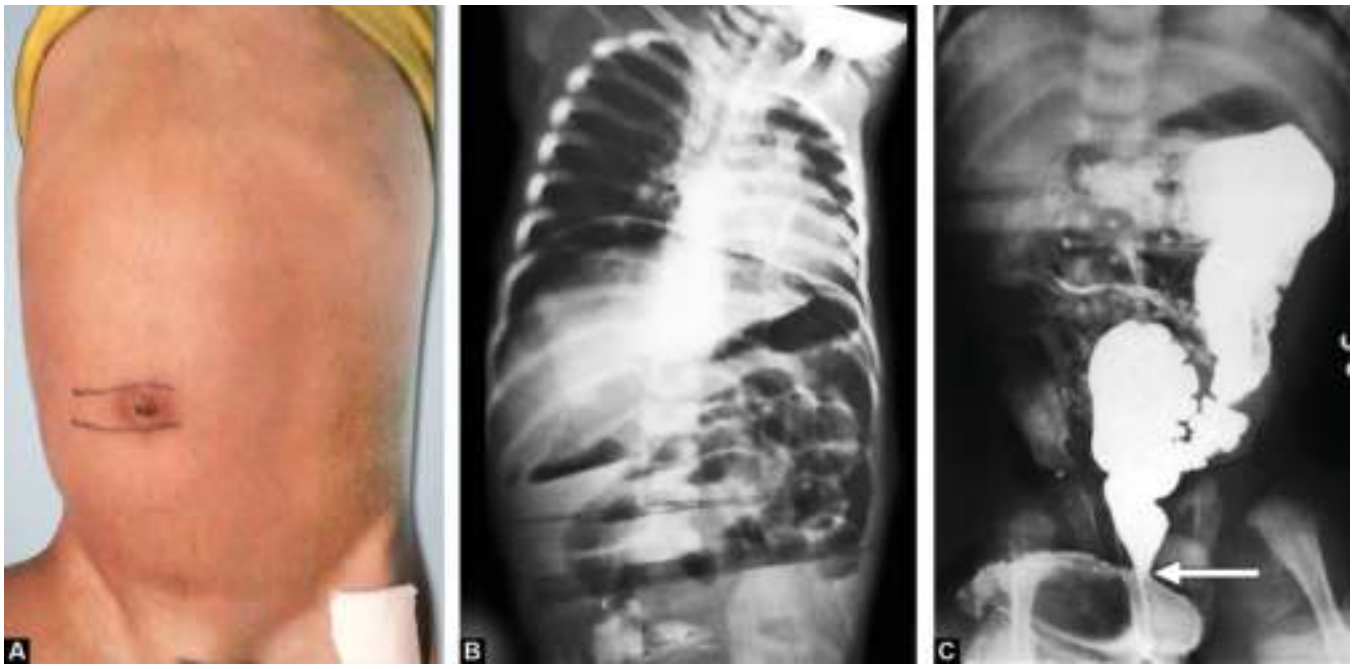


Fig. 20.44: Hirschsprung's disease. (A) Child shows abdominal distension, (B) abdominal radiograph shows dilated loops with multiple air fluid levels suggestive of intestinal obstruction, (C) barium enema shows cone-shaped transition zone at the rectosigmoid junction (arrow).

Surgical Pathology: Hirschsprung's Disease	
Gross Morphology	
▪ Sigmoid colon and rectum (aganglionic bowel) are narrowed due to absence of ganglion cells. ▪ Proximal segment of colon (ganglionic bowel) is dilated (Fig. 20.45).	
Light Microscopy	
Narrowed distal segment shows complete absence of ganglion cells in submucosa (Auerbach's plexus) and muscular coat (Meissner's plexus). It is accompanied by hypertrophy of nerves (Fig. 20.46).	
Immunohistochemistry Technique	
▪ Identification of ganglion cells may be difficult in neonates due to immaturity of the ganglion cells. ▪ Ganglion cells are positive for neuron-specific enolase (NSE). Hypertrophied nerve bundles are positive for S-100, and cathepsin D. ▪ Another promising marker is calretinin, a vitamin D-dependent calcium binding protein, absence of which has been seen in Hirschsprung's disease.	
Markers	**Expression**
▪ Neuron-specific enolase (for ganglion cells) ▪ S-100 protein (for nerve bundles) ▪ Cathepsin D (for nerve bundles) ▪ Calretinin (vitamin D-dependent calcium binding protein)	▪ Positive ▪ Positive ▪ Positive ▪ Positive

Laboratory Diagnosis

The diagnosis of Hirschsprung's disease is made clinically (chronic constipation in a young child) but must be confirmed by biopsy, which typically shows an absence of ganglion cells.

- **Rectal biopsy:** Rectum is always involved in Hirschsprung's disease and this is the preferred site for biopsy at least 2 cm above anal verge in infants and 3 cm above anal verge in children.

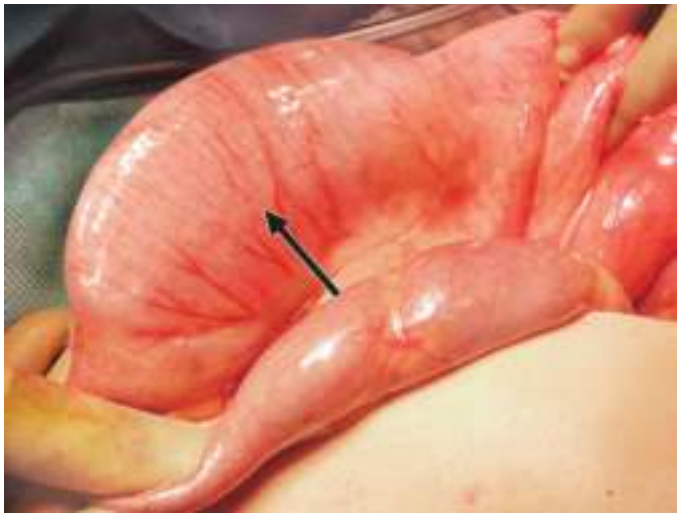


Fig. 20.45: Hirschsprung's disease. The baby of one week had been constipated since birth and exhibited signs of intestinal obstruction. Barium enema showed a narrowed lower rectal segment with dilated rectum above it. There were no ganglion cells in the narrowed segment (arrow). (*Courtesy:* Dr. Anusha S Bhatt.)

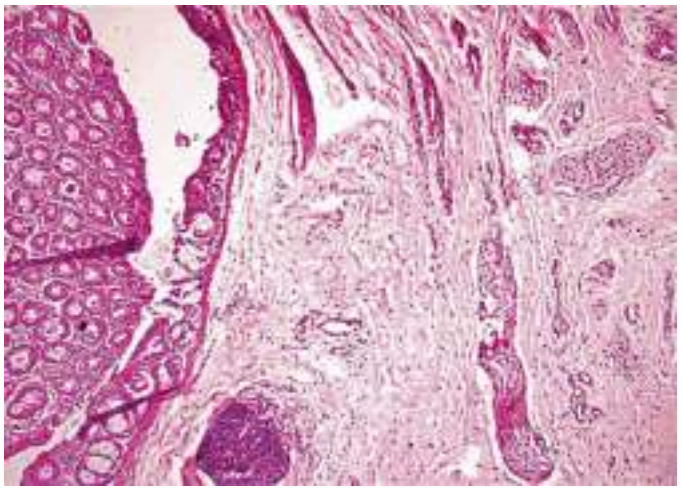


Fig. 20.46: Hirschsprung's disease. Punch biopsy from the rectosigmoid region shows increase in the number and size of nerve bundles in the submucosa with conspicuous absence of ganglion cells (100X).

- **Histologic examination:** Light microscopy shows absence of ganglion cells in the affected segment of intestine.
- **Imaging techniques:** Barium enema-contrast material flows into an unexpanded distal segment, then passes through a cone-shaped area and finally enters the dilated proximal bowel.
- **Electromanometry:** It is only useful to confirm the diagnosis in cases of ultra-short segment of Hirschsprung's disease.
- **Frozen section:** Acetylcholinesterase is used to demonstrate marked increase in thick nerve fibers in the wall of affected intestine. Ratio of acetylcholinesterase to butyryl cholinesterase is increased in Hirschsprung's disease.

Surgical Treatment

The part of the intestine that does not contain ganglion cells must be resected.

PSEUDO-OBSTRUCTION SYNDROME

Pseudo-obstruction syndrome is a heterogeneous group of neuromuscular disorders characterized by colonic inertia and constipation.

Diagnosis is really a challenge. Careful evaluation of nerves or muscle fibers of the muscularis propria on histopathologic examination, special stains and clinical correlation help in establishing diagnosis.

DIVERTICULAR DISORDERS

DIVERTICULOSIS

Diverticulum is pulsion (or false) herniation of pockets of mucosa and submucosa through the defects in weakened muscular propria throughout the colon along the antimesenteric taeniae coli, where arteries penetrate the muscular propria.

Locations

Diverticula are almost always multiple. Diverticulosis most often occurs in the sigmoid colon in 85% of cases. Sigmoid diverticular disease is the most common cause of hematochezia (bright red blood in stool) from lower intestinal tract and colovesical fistulas. Diverticulum disease of large intestine is shown in Fig. 20.47.

Pathogenesis

Diverticuli occur due to increased intraluminal pressure presumably due to low fiber content in diet especially in older persons. These diverticuli are pulsion diverticula that form two rows along the antimesenteric taeniae coli at the site, where arteries perforate the muscular propria. These are most common in men older than age 40 and in people who eat a low fiber diet.

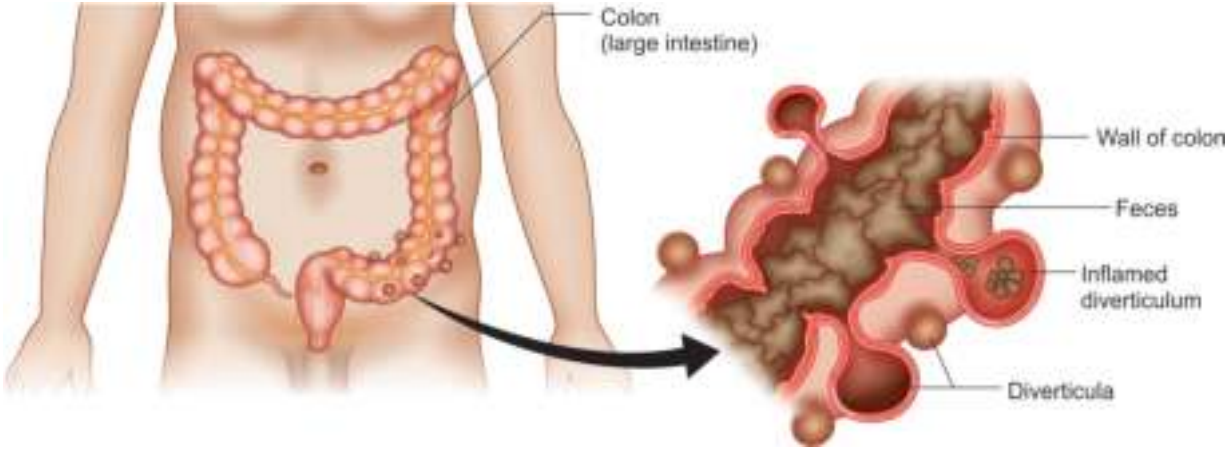


Fig. 20.47: Diverticulum disease of large intestine.

Clinical Features

Patient with diverticulosis most often becomes asymptomatic and will remain so unless diverticulitis develops.

- Diverticulitis results from the irritation caused by retained fecal material that obstructs the lumen of a diverticulum.
- Long-standing cases present with ribbon-like stools, intermittent diarrhea, abdominal distension, abdominal rigidity and pain, nausea, vomiting with diminishing or absent bowel sounds. Clinical features of diverticular disease of large intestine are given in Table 20.47.

Complications

Complications of diverticular disease include acute inflammation, perforation, fistula, stricture and bleeding. Complications of diverticular disease are given in Table 20.48.

Diagnosis

Diverticular disease is diagnosed by double contrast barium enema and colonoscopy. Fistulas are demonstrated by barium enema radiologic examination. Most diverticular abscesses are confirmed by ultrasonography.

Table 20.47 Clinical features of diverticular disease of large intestine

Disorder	Clinical Features
Diverticulosis	▪ Asymptomatic lesion ▪ Colicky abdominal pain ▪ Altered bowel habit
Acute diverticulitis	▪ Constant abdominal pain ▪ Fever ▪ Nausea and vomiting ▪ Bleeding ▪ Altered bowel habit ▪ Localized or generalized abdominal pain

Laparotomy is performed for generalized peritonitis to remove pus and feces.

Table 20.48 Complications of diverticular disease

Complications	Clinical Findings
Acute inflammation	▪ Abscess formation ▪ Peritonitis (local or generalized)
Perforation	▪ Local fecal peritonitis ▪ Generalized fecal peritonitis
Fistula	▪ Colovesical fistula ▪ Colovaginal fistula
Stricture	Stricture formation
Bleeding	Bleeding from diverticuli

Radionuclide imaging and arteriography are performed to localize the source of bleeding as well as angiodysplasia.

DIVERTICULITIS

Diverticulitis refers to inflammation of diverticula. It commonly affects elderly persons. In diverticulitis, retained undigested food and bacteria accumulate in the diverticular sac.

Pathogenesis

Colonic bacteria attack the thin walls of the diverticulum resulting in perforation, peritonitis (most serious complication), abscess formation, bowel obstruction, or hemorrhage. Occasionally, the inflamed colon segment may adhere to the bladder or other organs and cause a fistula.

Clinical Features

Patient presents with lower abdominal pain, tenderness, and bright red rectal bleeding (most frequent finding). Patient has other signs of acute inflammation such as fever and leukocytosis.

OBSTRUCTIVE DISORDERS

INTESTINAL OBSTRUCTION

Intestinal obstruction refers to partial or complete obstruction of the bowel lumen. Obstruction of small intestine is more serious. Most common cause of bowel obstruction is adhesions from previous surgery. It may also occur due to congenital bowel deformities. Bowel obstruction is of three kinds: intraluminal (e.g. tumor, foreign bodies), intramural and extraintestinal.

Pathology

In initial phase of bowel obstruction peristalsis temporarily increases in attempt to force contents past obstruction. Bowel distension impedes venous blood supply to bowel, halting normal absorptive processes.

- Bowel wall swells and becomes edematous as water, sodium, and potassium are secreted into intestine and not absorbed from it.
- Gas-forming bacteria collect above obstruction enhancing distension. Untreated patient develops severe hypovolemia and sepsis with fatal outcome.

Clinical Features

Patient suffering from bowel obstruction presents with colicky pain followed by a pain-free interval accompanied by constipation and inability to pass gas (flatus). Untreated complete obstruction of the large

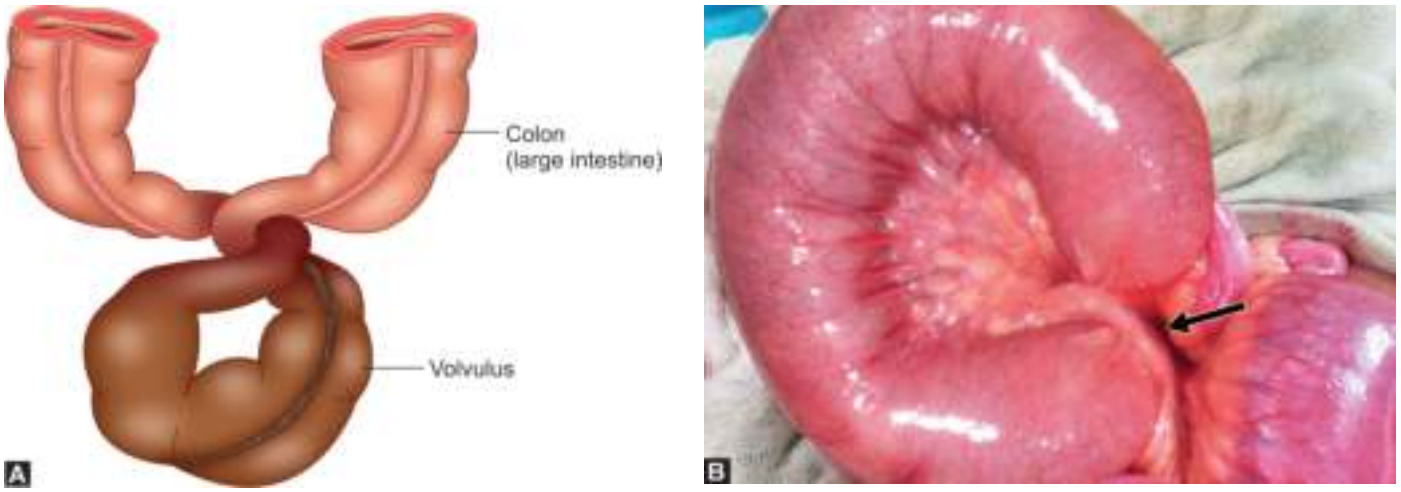


Fig. 20.48: (A) Volvulus shows twisting of a mobile segment of the intestine, (B) its mesenteric root (arrow).

bowel can cause death within hours due to shock and vascular collapse. Ultimately, bowel obstruction may lead to ischemia, necrosis, and death.

VOLVULUS

Volvulus is twisting of a mobile segment of the intestine on its mesenteric root. It occurs in sigmoid colon (most common site) and small intestine. The twist is counterclockwise in most cases. Volvulus is always a consequence of congenital anomaly.

Pathology

During intrauterine life, defective intestine rotation results in abnormal positions of the small intestine and colon, anomalous attachments, and bands. Kinking of bowel interrupts the blood supply resulting in bowel infarction, intestinal gangrene and obstruction. Volvulus shows twisting of a mobile segment of the intestine on its mesenteric root is shown in Fig. 20.48A and B.

INTUSSUSCEPTION

An intussusception is an invagination of one segment of proximal bowel (usually the terminal ileum) into the distal bowel (usually the cecum), causing intestinal obstruction. Colo-colic intussusception is relatively uncommon. The inner loop of the gut may become necrotic unless the invagination is everted surgically.

- Intussusception is most common cause of intestinal obstruction in children <2 years of age due to active peristalsis.
- In adults, intussusception occurs due to organic cause such as pedunculated polyps or malignant tumor growth. Small pedunculated tumors carried by peristalsis may pull forward the loop to which such a tumor is attached.

Clinical Features

Child presents with signs of obstruction (colicky pain) and rectal bleeding (currant jelly stools) from bowel infarction.

INFLAMMATORY BOWEL DISEASE

INFLAMMATORY BOWEL DISEASE: OVERVIEW

Inflammatory bowel disease (IBD) comprises two diseases: ulcerative colitis (most common) and Crohn's disease of unknown etiology, however psychological, infectious and autoimmune factors have been implicated.

- Ulcerative colitis and Crohn's disease share many features, but may differ from one another. The diagnosis is made by exclusion and correlating all the clinical, endoscopic, radiologic findings.
- Peak incidence of IBD is observed in the same age group (15–25 years). Females are more affected than males in 20–25 years of age group. There is family history of inflammatory bowel disease in 20% of cases. Features of inflammatory bowel disease are shown in Fig. 20.49A and B and pathogenesis of inflammatory bowel disease is shown in Fig. 20.50.
- Inflammatory bowel disease is associated with intestinal complications and extraintestinal manifestations.

Clinical Pearls: Inflammatory Bowel Disease (IBD)

- **Inflammatory bowel disease:** Ulcerative colitis and Crohn's disease can present with abdominal pain, bloody diarrhea, fever and weight loss. Involvement of colon can lead to colon cancer. Screening colonoscopy should be performed every 1–2 years after 8–10 years of colonic involvement.

- **Clinical features:** Patient suffering from ulcerative colitis most often presents with abdominal pain and bloody diarrhea. Crohn's disease presents with involvement of upper gastrointestinal tract, masses, skip lesions, fistulous tracts, obstruction, transmural granulomas, malabsorption of fats and vitamin B_{12}, perianal disease, cholesterol gallbladder stones. Extraintestinal manifestations of IBD are arthralgia, ocular lesions (iritis, uveitis), sclerosing cholangitis and skin lesions (pyoderma gangrenosum, erythema nodosum).
- **Laboratory findings:** Serum anti-*Saccharomyces cerevisiae* (ASCA) is positive in ulcerative colitis and negative in Crohn's disease. Serum antineutrophil cytoplasmic antibody (ANCA) is negative in ulcerative colitis and positive in Crohn's disease.
- **Treatment:** Steroids can be used to control acute exacerbations of inflammatory bowel disease. Ulcerative colitis is cured by colectomy. TNF inhibitors (e.g. adalimumab, certolizumab, etanercept, golimumab, infliximab) control Crohn's disease associated with fistula formation. TNF inhibitors reactivate tuberculosis. Patient should be screened for tuberculosis. Always screen the patients with a PPD prior to its use in fistulizing Crohn's disease. Azathioprine and 6-mercaptopurine are administered in severe disease with recurrent symptoms.

Locations

Ulcerative colitis begins in the rectum (friable red mucosa) and extends in continuity to involve part or

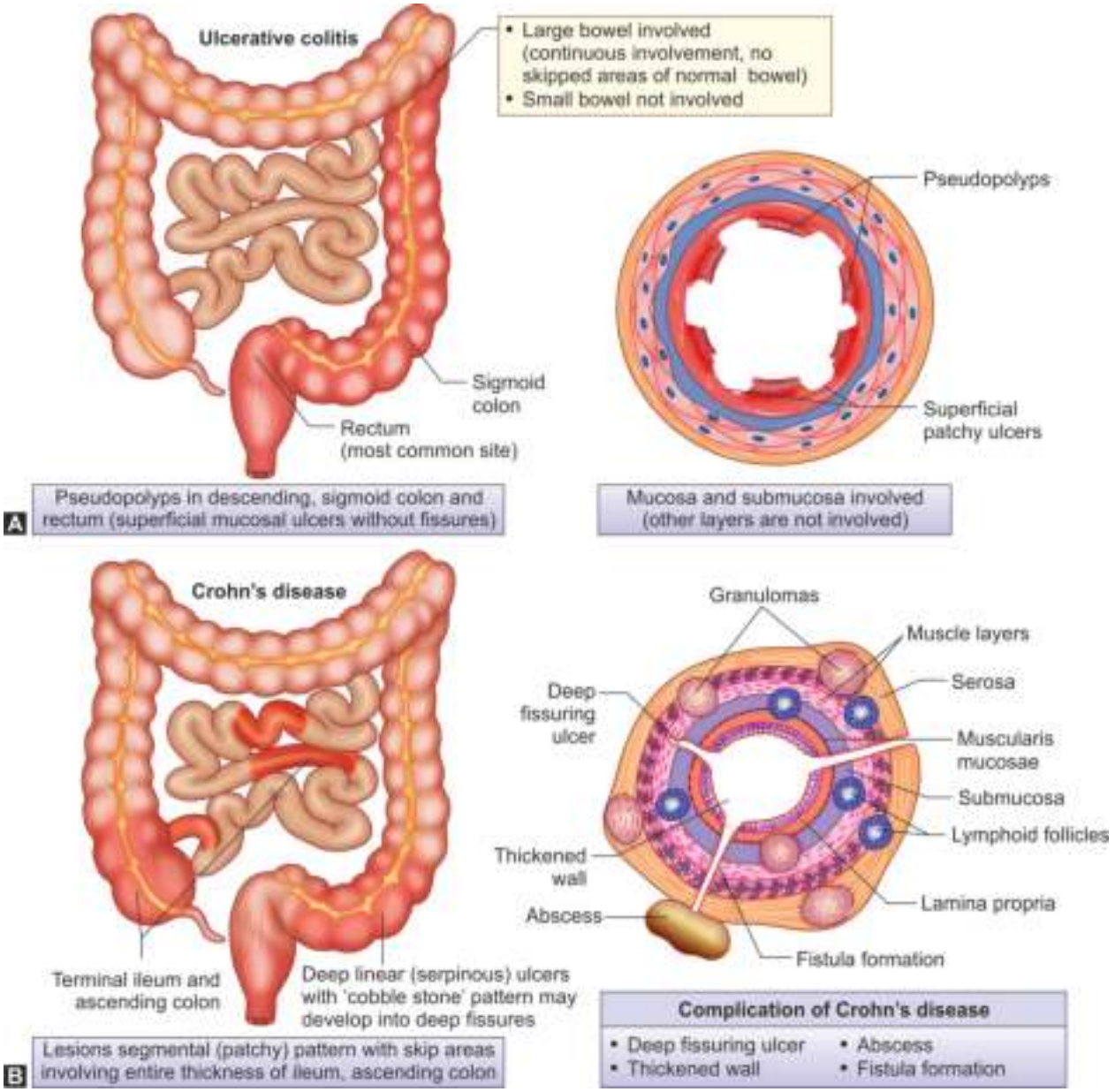


Fig. 20.49: Features of inflammatory bowel disease (IBD). (A) It shows ulcerative colitis, (B) it shows Crohn's disease.

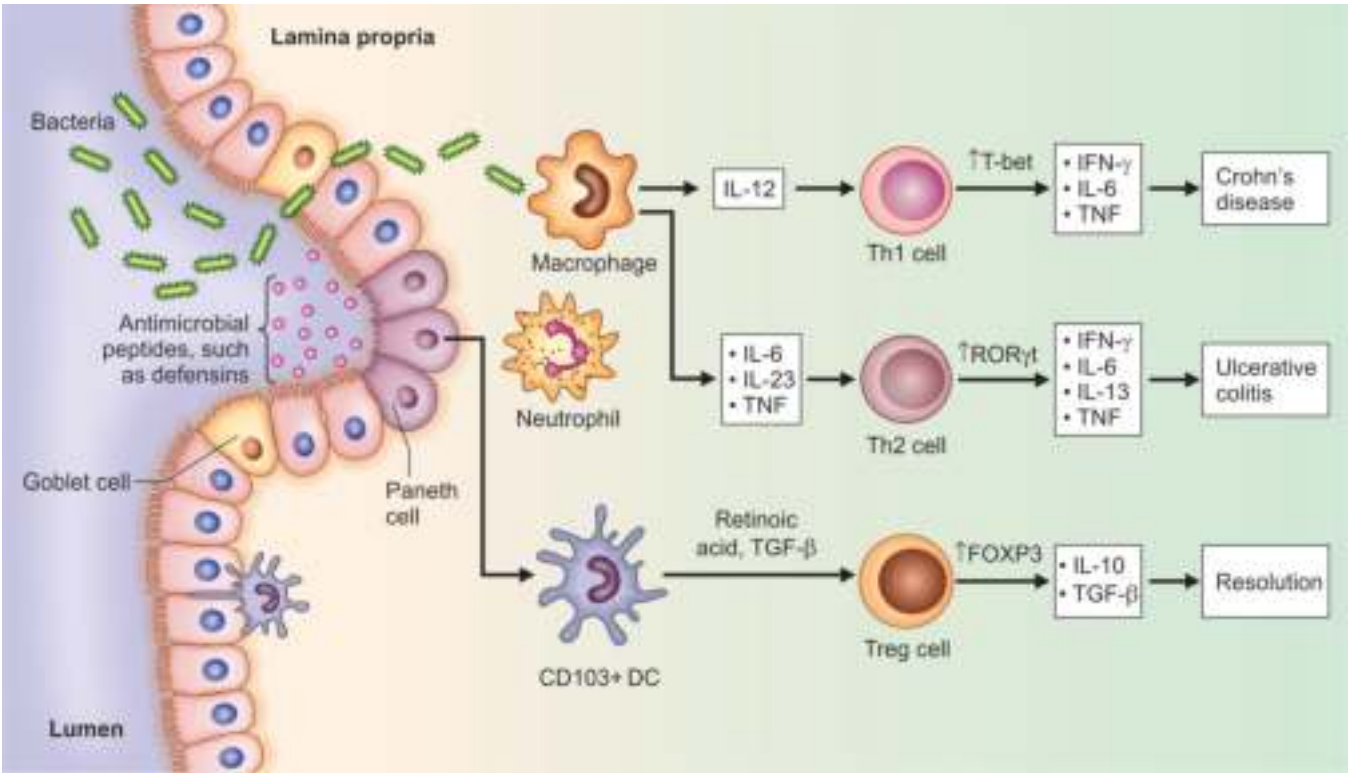


Fig. 20.50: Pathogenesis of inflammatory bowel disease.

the entire colon. On the other hand, Crohn's disease involves terminal ileum (30%), colon and small bowel (50%), colon alone (20%) having discontinuous/skipped involvement of the bowel.

Surgical Pathology: Inflammatory Bowel Disease

Gross Morphology

- In ulcerative colitis, mucosa and submucosa of the rectum, colon and appendix show ulcerations forming pseudopolyps. There is absence of fistulae formation. Anus is not commonly involved.
- Crohn's disease involves all layers of small or large intestine.

Light Microscopy

- Ulcerative colitis produces crypt abscesses due to accumulation of neutrophils resulting in rupture and crypt atrophy.
- Crohn's disease is associated with noncaseating granulomas in 50% of cases, lymphoid aggregates, and aphthoid mucosal ulcers. Involvement of the ileum also favors Crohn's disease.

Endoscopy

Ulcerative colitis produces ulceration of colon with pseudopolyp formation due to bulging of inflamed mucosa surrounded by areas of ulceration.

Crohn's disease produces linear ulcers, cobble stone appearance, luminal narrowing (string signs on barium studies), and fistula formation.

Complications

Ulcerative colitis most often results in colon adenocarcinoma in an inflammation-dysplasia-carcinoma sequence.

- On the other hand, conventional colorectal carcinoma develops in an adenoma-carcinoma sequence. HLA-B27 positive patient may develop ankylosing spondylitis, toxic megacolon and sclerosing cholangitis (increased risk with duration), HLA-B27 positive ankylosing spondylitis, toxic megacolon, and sclerosing pericholangitis.
- Crohn's disease may involve whole gastrointestinal tract from mouth to anus with formation of fissures and fistulae.

Extraintestinal Manifestations

Extraintestinal manifestations in patient with ulcerative colitis and Crohn's disease include uveitis, episcleritis, skin lesions, e.g. erythema nodosum, pyoderma gangrenosum and granulomatous inflammation; joint involvement, e.g. migratory polyarthritis, ankylosing spondylitis and sacroilitis; sclerosing cholangitis especially in ulcerative colitis; pulmonary lesion, e.g. bronchiectasis; and venous and arterial thromboembolism.

Clinical Features

Patient with ulcerative colitis presents with abdominal cramps, diarrhea with blood and mucus, rectal bleeding,

and tenesmus (ineffective and painful straining of stool).

On the other hand, patient with Crohn's disease presents with right lower quadrant colicky pain (obstruction) with diarrhea and anal bleeding. Differences between ulcerative colitis and Crohn's disease are given in Table 20.49.

ULCERATIVE COLITIS

Ulcerative colitis is chronic superficial inflammatory disease that affects the mucosa and submucosa of the colon and rectum resulting in mucosal ulcerations, pseudopolyps (mucosal remnants of previous severe ulceration) formation. Disease is of unknown etiology, which begins in the rectum and sigmoid colon, rarely

Table 20.49 Differences between ulcerative colitis and Crohn's disease

Character	Ulcerative Colitis	Crohn's Disease
Gross morphology		
Distribution	▪ Diffuse pattern (continuous without skip lesions) ▪ Sigmoid colon (common) ▪ Ileum involvement (<10%) ▪ Rectum always involved ▪ Upper GIT involvement (usually absent)	▪ Segmental (patchy) pattern with skip lesions ▪ Terminal ileum and ascending colon (common) ▪ Ileum involvement common ▪ Rectum always spared ▪ Upper GIT involvement (may be with lesions from mouth to anus)
Lumen of intestine	Dilated megacolon	Stenosis (string sign)
Depth of inflammation	Mucosal and submucosal layers (superficial lesions)	Transmural (entire thickness) involvement
Bowel wall thickness	Thin	Thickened or normal
Creeping mesenteric fat	Absent	Present (common finding)
Mucosal surface appearance	Friable, hemorrhagic, ulcerated and pseudopolyps (common finding)	Deep linear patchy ulceration; cobblestone-like appearance, but formation of pseudopolyps rare
Ulcers formation	Superficial mucosal ulcers without fissures	Deep linear ulcers with formation of deep fissures
Submucosa	Normal to reduction in width	Widened due to edema and lymphoid aggregates
Muscular layer	Usually spared except in cases of megacolon	Always involved showing presence of noncaseating granuloma in 60% cases
Serous layer	Normal (smooth)	Inflammation present
Complications		
Ulceration of mouth and stomach	Absent	May be present in 1–5% cases
Fibrous strictures	Never	Common due to fibrosis
Fistula formation and sinus tracts	Extremely rare	Internal and external fistulas in 10% cases
Toxic megacolon	Present (hypotonic and distended bowel)	Absent
Perianal abscess and fistula	Absent	Present in 50% of cases
Malignant change	May occur infrequently in disease of more than 10 years duration	Present in 1% cases
Type of malignancy	Carcinoma is more common than lymphoma	Lymphoma is more common than carcinoma
Light microscopy		
Crypt abscess	Present in mucosa	Absent in mucosa
Inflammatory cells	Nonspecific acute and chronic inflammatory cells (e.g. lymphocytes, plasma cells, neutrophils, eosinophils and mast cells) confined to mucosa and submucosa	Chronic inflammatory cells (e.g. lymphocytes, plasma cells and macrophages) forming lymphoid aggregates are seen involving all layers of intestine (transmural)
Well-formed granulomas	Absent	Most often present in 60% cases in muscular coat
Transmural lymphoid aggregates	Absent	Common

Contd...

Table 20.49 Differences between ulcerative colitis and Crohn's disease (*Contd...*)

Character	Ulcerative Colitis	Crohn's Disease
Immunologic features		
Lymphocytes	CD4+, Th2	CD4+, Th1
Cytokines	IL-4, IL-5, IL-13 and TGF-β	IL-12, TNF-α and IFN-γ
ANCA-P antibodies	Positive in most cases (70%)	Positive in a few cases
Clinical features		
Age group	Young adults	Young adults
Gender predilection	No gender predilection	Women > men
Clinical findings	Recurrent left-sided abdominal cramping with bloody diarrhea and mucus	Recurrent right lower quadrant colicky pain (obstruction with diarrhea)
Primary sclerosing cholangitis	More common	Less common
Antibodies to *Saccharomyces cerevisiae*	Negative	Positive

affects terminal ileum. The disease cycles between exacerbation and remission.

Age Group

Ulcerative colitis occurs primarily in young adults (15–30 years) and then 55–65 years of age.

Clinical Features

Patient presents with recurrent bloody diarrhea with mucus (hallmark), abdominal cramping, tenesmus (ineffective and painful straining of stool), weight loss, anorexia, nausea, vomiting, and weakness.

Surgical Pathology: Ulcerative Colitis

Gross Morphology

- Ulcerative colitis is characterized by superficial ulcerations limited to mucosa and submucosa of large intestine.
- It begins in the rectum (friable red mucosa) and extends in continuity to involve part or the entire colon.
- Ulcerative colitis does not involve the mucosa of the anal transitional zone or the anal canal.
- Ulceration in colon is continuous in its distribution, so skip lesions are not encountered (Fig. 20.51A and B).

Light Microscopy

Colon mucosal biopsy in a patient with ulcerative colitis is performed for assessment of disease activity, surveillance of dysplasia and exclusion of other superimposed disease processes.

Colonic changes are confined to mucosa and submucosa in ulcerative colitis. Histologic changes depend on progression of disease (Fig. 20.52A and B).

- **Abscesses formation:** Rectum and colon show extensive ulceration of mucosa and submucosa. Mucosa shows hyperemia resulting in mucosal friability. Exudative inflammation leads to crypt abscesses formation in colon. Polymorphonuclear cells are particularly evident as aggregates within distended crypts (crypt abscess) of Lieberkühn resulting in rupture and atrophy. Initially, the colon's mucosal surface becomes dark, red, and velvety.
- **Ulcers formation:** Abscesses form and coalesce into ulcers. The ulcers are irregular in outline involving mucosa and submucosa, but in severe cases there is extension into the main muscular coat and perforation is likely. The ulcers expand and coalesce involving large areas. Ulcerations are continuous unlike Crohn's disease. Sloughing of mucosa results into bloody mucus filled stools.
- **Healing stage:** The intestinal wall becomes thickened and fibrosed. As granulation tissue replaces the muscle layer, the colon narrows, shortens, and loses its characteristic pouches (haustral folds). Colon shows formation of pseudopolyps due to mucosal remnants of previous severe ulceration.

Complications

HLA-B27 positive persons develop ankylosing spondylitis, toxic megacolon, and sclerosing pericholangitis in ulcerative colitis cases. Presence of ulcerative colitis-associated dysplasia is usually indication for colectomy. Complications of ulcerative colitis are given in Table 20.50.

- **Acute complications of ulcerative colitis:** These include toxic megacolon (medical emergency-marked dilation of colon), perforation, hemorrhage, and dehydration due to electrolytes imbalance.
- **Chronic complications of ulcerative colitis:** Patient may develop anemia (bloody diarrhea) and colon adenocarcinoma (increased risk with duration 5–10% per decade of pancolitis). HLA-B27 positive persons develop ankylosing spondylitis, and sclerosing pericholangitis.

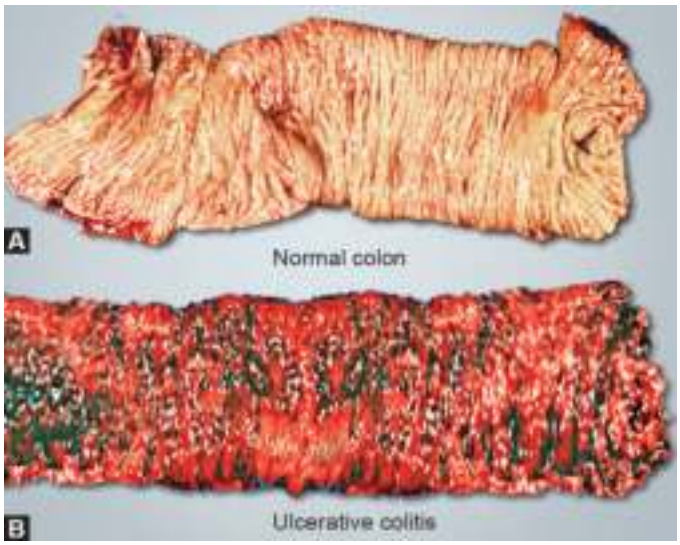


Fig. 20.51: Ulcerative colitis. (A) Normal colon, (B) ulcerative colitis with pseudopolyps formation as a result of intense inflammation of mucosa and submucosa. Inflammation begins in the rectum involving sigmoid colon and extends upward and around to the ascending colon.

Laboratory Diagnosis

The diagnosis of ulcerative colitis is made by exclusion and correlating all the clinical, endoscopic, radiologic, and pathologic findings.

Table 20.50 Complications of ulcerative colitis

Complications	Common Findings
Blood loss	▪ Acute hemorrhage ▪ Chronic hemorrhage leading to anemia
Electrolyte disturbances	Due to severe diarrhea in acute phase
Toxic megacolon	May develop insidiously
Colorectal carcinoma	Increased risk of colorectal carcinoma, if patient develops disease in childhood, with clinically severe attack, total involvement of the colon and continuous rather than intermittent symptoms
Skin	Erythema nodosum (subcutaneous inflammation)
Liver	▪ Pericholangitis (inflammation around bile ducts) ▪ Sclerosing cholangitis (fibrous constriction and obliteration of bile ducts) ▪ Cholangiocarcinoma ▪ Chronic active hepatitis
Eye	▪ Iritis ▪ Uveitis ▪ Episcleritis
Joints	Increased incidence of ankylosing spondylitis

CROHN'S DISEASE

Crohn's disease is one of the main entities in inflammatory bowel disease and is characterized by transmural chronic inflammation of terminal ileum (most common), ascending and sigmoid colon (20%). Disease may involve lymph nodes and supporting membranes. Presence of noncaseating granulomas in ileum favors Crohn's disease. It can affect different regions of the bowel, stomach, or intestines. There are five different types of Crohn's disease, each affecting different parts of the gastrointestinal tract: ileocolitis, ileitis, gastroduodenal, jejunoileitis and Crohn's colitis.

Age Group

Crohn's disease affects men and women equally in 20–40 years of age group, although no age group is exempted. It also tends to run in families up to 20% of patients. It occurs most frequently in people of Jewish

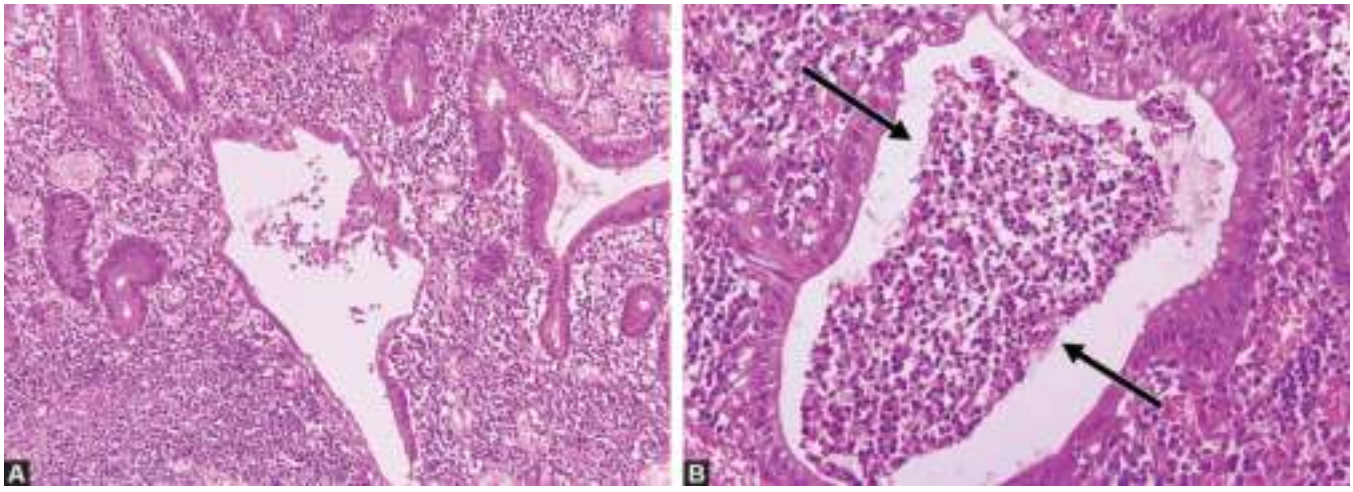


Fig. 20.52A and B: Ulcerative colitis. The intense inflammation of ulcerative colitis is confined primarily to the mucosa and submucosa. The colonic mucosal epithelium demonstrates loss of goblet cells. Crypt abscesses are a histologic finding more typical with ulcerative colitis (arrows) (400X).

descent. Neoplastic transformation is much less frequent in Crohn's disease than in ulcerative colitis.

Clinical Features

Patient presents with generalized abdominal pain especially in right lower quadrant, diarrhea, fever, malabsorption, weight loss, intestinal obstruction due to fibrous stricture, and pain or drainage near or around the anus due to inflammation and fistulas between loops of intestine and between the intestine, urinary bladder, vagina, and skin.

Pathology Pearls: Molecular Pathogenesis of Crohn's Disease

- Modern theory on the pathogenesis of Crohn's disease has proven that gut microbiota (ecological communities of commensal, symbiotic and pathogenetic microorganisms) and environmental factors lead to abnormal immune response in genetically predisposed patients.
- Genome-wide association analysis in patients with Crohn's disease worldwide demonstrated several genetic mutations that increase the risk for Crohn's disease.
- Gut microbiota is considered a compulsory and an essential part in the pathogenesis of Crohn's disease. Intestinal dysmicrobism with excessive amounts of different bacterial strains can be demonstrated in all patients of inflammatory bowel disease. The discovery of *Escherichia coli* entero-invasive on resection biopsies in patients with Crohn's disease now increases the likelihood of antimicrobial or vaccination-type treatments.
- Recent studies on Crohn's disease has been focused on intestinal immunology such as molecular activation pathways and targeted therapy.
- Leukocyte adhesion inhibitors block the leukocyte homing mechanism and prevent cellular immune response.
- Clinical trials are going on treatment of Crohn's disease with anti-integrin antibodies, chemokine receptor antagonists and SMAD4 antisense oligonucleotides. MicroRNAs are disease biomarkers of Crohn's disease.
- Chronic inflammation involves all layers of the intestinal wall (transmural involvement) in Crohn's disease described as under:
  - **Cobblestone appearance of mucosa:** Lymphatic channels of submucosa blocked by enlarged lymph nodes result in edema linear mucosal ulcers oriented along long axis of intestine. Elevation of surviving mucosa between the ulcers gives nodular cobblestone appearance. Submucosal edema, abscesses, later fibrosis and fissures are also seen.
  - **String sign:** Fibrosis of intestinal wall causes thickening and narrowing of the lumen. Intestinal lumen shows narrowing is demonstrated as 'string sign' on barium studies.
  - **Noncaseating epithelioid cell granulomas:** These are seen in 50% of cases.
  - **Skipping lesions:** Crohn's disease has skipping lesions (ulcerations) on the mucosa while ulcerative colitis has continuous lesions.
  - **Oval elevated patches in mucosa:** Peyer's patches of small intestine are also inflamed, exhibiting oval elevated patches.
  - **Inflammation of serous coat:** Serous coat of intestine is inflamed, that gets adhered to other intestinal loop.
  - **Strictures:** Strictures are formed in the involved bowel due to thickening, shortening and narrowing.
  - **Fistulous tract:** Narrow fissures formed may penetrate deep and forms fistulous tract with adjacent loop of gut.

Surgical Pathology: Crohn's Disease

Gross Morphology

- Wall of the gut shows thickening due to inflammation, fibrosis and hypertrophy of the muscular coat giving rubbery appearance and stricture formation.
- Crohn's disease produces skipped lesions. Linear mucosal ulcers are oriented along long axis. Mucosa between the ulcers gives nodular cobblestone appearance. Narrow fissures formed may penetrate deep and form fistulous tract with adjacent loop of gut (Fig. 20.53A and B).

Light Microscopy

- Histologic examination shows transmural inflammation composed of lymphocytes, plasma cells and histiocytes.
- Submucosa and subserosa of the gut show noncaseating granulomas, and lymphoid aggregates, which may also be seen in regional lymph nodes (Fig. 20.54).

Comparison of histologic features useful in differential diagnosis of Crohn's disease and intestinal tuberculosis is given in Table 20.51.

COLITIS: OTHER CAUSES

PSEUDOMEMBRANOUS COLITIS

Pseudomembranous colitis is potentially fatal acute mucosal reaction of colon to toxins produced by *Clostridium difficile*. Most cases are associated with prior administration of antibiotics resulting in loss of normal intestinal bacterial flora. There is formation of pseudomembrane composed of superficial plaque-like irregular grayish mucosal fibrinous exudates. Rectosigmoid colon is the most common site of pseudomembranous colitis.

Pathogenesis

Administration of broad-spectrum antibiotics suppresses the normal flora of intestine and causes overgrowth of *Clostridium difficile*.

- *Clostridium difficile* exotoxin causes acute inflammation of colonic mucosa. Increased vascular permeability leads to exudation of proteins especially fibrinogen, which gets polymerized to insoluble fibrin.

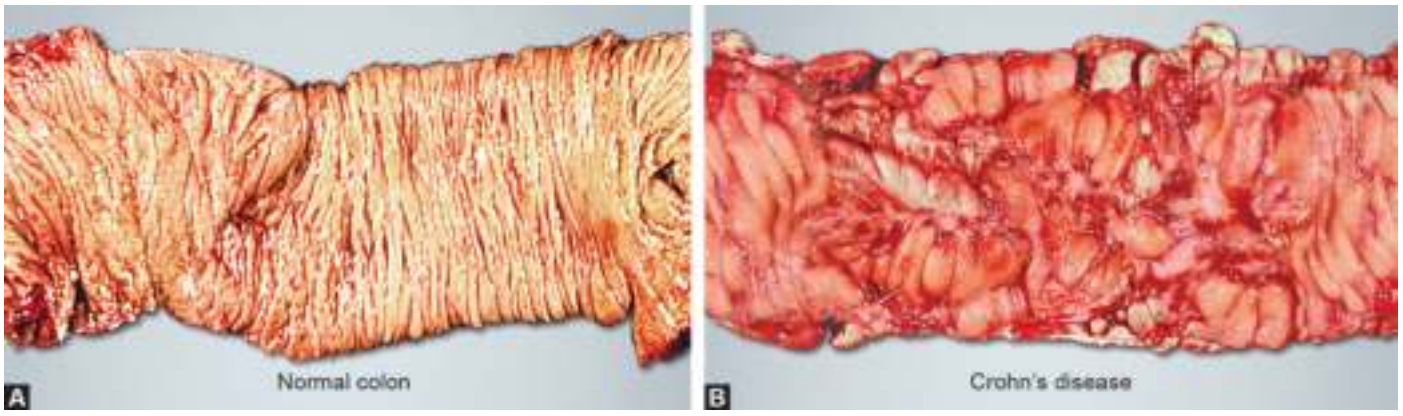


Fig. 20.53: Crohn's disease of the terminal ileum. It shows thickening of the wall and irregular nodular 'cobblestone' appearance of the mucosal surface. The patient presented with recurrent attacks of lower abdominal pain and vomiting for 6 weeks. A mass was palpable in the right iliac fossa.

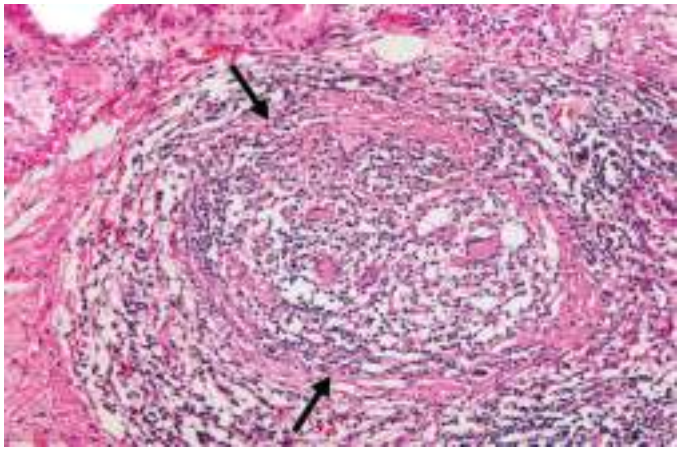


Fig. 20.54: Crohn's disease. It shows transmural inflammation with lymphocytes, epithelioid cell granulomas and giant cells (100X). Special stains for organisms were negative (arrows) (100X).

- Fibrin admixed with mucus from the colonic glands and necrotic tissue forms pseudomembrane attached loosely to the remnants of mucosa beneath it. Forceful removal of pseudomembrane leaves behind bleeding ulcerations.

Clinical Features

Patient presents with diarrhea, fever, and lower abdominal tenderness occurring in patients on broad-spectrum antibiotic therapy.

Therapeutic Correlation

Specific administration of antibiotics (vancomycin, metronidazole), hydration and discontinuation of antibiotics are effective measures to eradicate *Clostridium difficile*.

Diagnostic Approach

Lesions can be demonstrated by endoscopy. *Clostridium difficile* toxin is demonstrated in the stool.

AMOEBIC COLITIS

Amoebic colitis is caused by *Entamoeba histolytica* transmitted by fecal–oral route. The cyst of parasite

Table 20.51 Comparison of histologic features useful in differential diagnosis of Crohn's disease and intestinal tuberculosis

Histologic Features	Crohn's Disease	Intestinal Tuberculosis
Caseating epithelioid cell granulomas	Absent	Frequent
Confluent epithelioid cell granulomas	Absent	Frequent
Numerous granulomas	Rare	Common
Prominent lymphoid cuff	Uncommon	Frequent
Ulcers in intestine (both aphthous and deep)	Uncommon	Common
Architectural distortion	Common	Common
Changes of chronicity unassociated with sites of granulomatous inflammation	Common	Absent
Multiple sites of intestine involvement	Common	Common
Mucosal cobblestone appearance	Common	Uncommon
Fistula formation	Common	Uncommon
Anal/perianal disease	Common	Rare

invades colonic mucosa (intestinal amoebiasis) or by hematogenous route reaches the liver, lung and brain resulting in extraintestinal amoebiasis (amoebic abscess).

Pathogenesis

Cecum or ascending colon are most common sites of amoebic ulcers.

- Cysts of *Entamoeba histolytica* ingested become small trophozoites in the alkaline medium of intestine. Small trophozoites develop into adult trophozoites, move to cecum and become highly invasive pathogen. These trophozoites cause lysis the host tissue. These are converted to cysts.
- Direct extension of the liver abscess through the diaphragm into the right lobe of lung.
- Patient may present with ameobic dysentery and low-grade fever. Comparison of typhoid fever, bacillary dysentery and intestinal amoebiasis is shown in Table 20.52.

Surgical Pathology: Amoebic Colitis

Gross Morphology

- Amoebic ulcers are most often seen in cecum or ascending colon. Less common sites of involvement include ascending colon, sigmoid colon, rectum and appendix.
- Ulcers range from pinpoint-sized to flask-shaped in cecum, which give reddish brown hue resembling anchovy paste (Fig. 20.55).

Light Microscopy

- *Entamoeba histolytica* is demonstrated in the base and at the margins of ulcers, chiefly in the submucosa. Some red blood cells are phagocytosed by the trophozoites termed erythrophagocytes.
- Intestine biopsy shows large number of trophozoites of *Entamoeba histolytica* is shown in Fig. 20.56.

CHOLERA

Vibrio cholerae without causing mucosal inflammation (small bowel, colon) secretes toxin that activates the adenyl cyclic AMP dependent water pump in the cell membrane. The intestinal cells secrete chloride accompanied by water into the lumen producing secretory diarrhea.

RADIATION COLITIS

Radiation colitis occurs as a result of radiation therapy given in prostate and cervical cancers. Radiotherapy produces lesions ranging from reversible injury of the intestinal mucosa to chronic inflammation, ulceration, and fibrosis of the intestine.

Table 20.52 Comparison of typhoid fever, bacillary dysentery and intestinal amoebiasis

Causative Organisms	*Salmonella typhi*	*Clostridium difficile* Exotoxin	*Entamoeba histolytica*
Intestinal involvement	Lower ileum and cecum	Sigmoid and rectum	Cecum
Inflammation	Typhoid granuloma	Fibrinous pseudomembrane	Necrosis
Location of ulcers	Longitudinal axis of intestine	Map-like ulcer	Flask-shaped ulcers with reddish brown hue resembling anchovy paste
Clinical features	Continued fever, diarrhea, confusion and bradycardia	Bloody mucoid diarrhea and tenesmus	Diarrhea with bleeding



Fig. 20.55: Intestinal amoebiasis. Large bowel shows flask-shaped amoebic ulcers with reddish brown hue resembling anchovy paste (arrows).

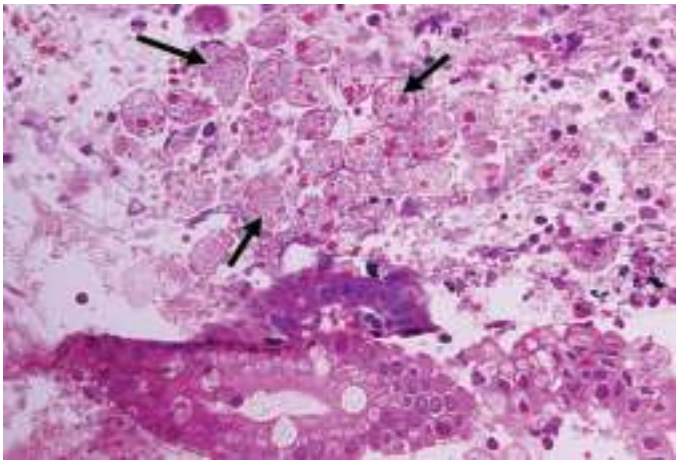


Fig. 20.56: Intestine biopsy shows large number of trophozoites of *Entamoeba histolytica* (arrows) (400X).

DIVERSION COLITIS

Diversion colitis refers to an inflammatory response in the blind segment, most often the rectum Hartmann pouch following ileostomy or colostomy formation. This process is caused by deficiency of short-chain fatty acids.

Pathologic findings include granular friable mucosa with marked lymphoid hyperplasia and cryptitis mimicking inflammatory bowel disease. Clinical history is most important to exclude other causes.

COLLAGENOUS COLITIS

Collagenous colitis refers to presence of thick collagen layer at the subepithelial region. Thickness of the collagen layer is variable. On endoscopy, colonic mucosa looks normal. Patient presents with chronic diarrhea more often in middle aged woman. There is presence of capillaries and inflammatory cells entrapped in collagenous fibers of colon. Evaluation requires well-oriented sections in difficult case. Masson's trichrome stain may be useful to demonstrate collagen fibers in the colon.

STERCORAL ULCER

Incomplete evacuation of the feces in debilitating disease or old persons may lead to fecal impaction. Fecal impaction causes pressure necrosis of the mucosa resulting in stercoral ulcer. Complications of stercoral ulcer include rectal bleeding and perforation.

VASCULAR DISORDERS OF COLON

LARGE BOWEL INFARCTION/ISCHEMIC BOWEL DISEASE

Large bowel has arterial blood supply from more than one artery. Splenic flexure and rectosigmoid junction are the most common sites of infarcts in colon. These are less vascularized areas known as **watershed regions**.

Locations

Ischemic bowel disease occurs in the watershed zones, i.e. splenic flexure between superior and inferior mesenteric arteries. Less commonly, ischemic bowel disease occurs in rectosigmoid junction (Sudeck's point) due to its presence distal to the last collateral arterial connection with proximal arteries.

Large bowel infarction with watershed zones is shown in Fig. 20.57. Causes of large bowel ischemia are given in Table 20.53.

Pathology

Arterial insufficiency of colon results in mucosal, mural, or transmural infarction involving the wall of the colon. In transmural infarctions, the bowel undergoes hemorrhagic infarction (danger of perforation) with mucosal ulcerations and pseudomembrane formation. Acute ischemic injury of the large bowel is given in Table 20.54.

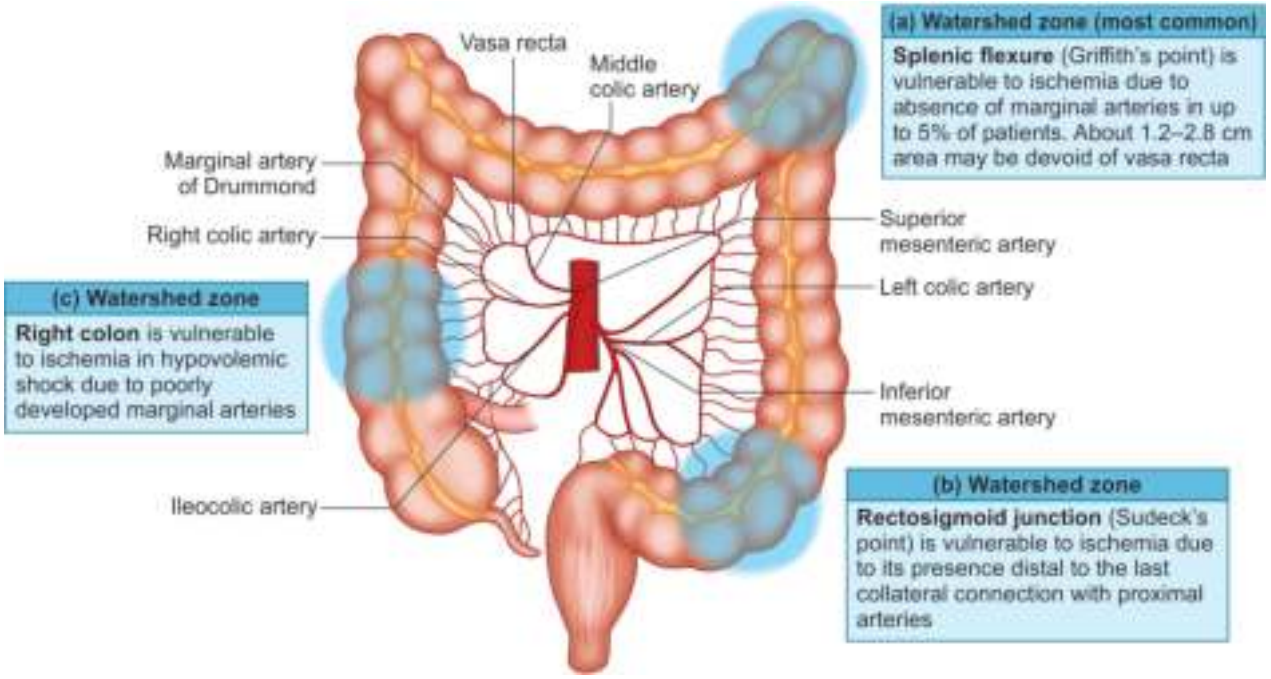


Fig. 20.57: Large bowel infarction with watershed zones.

Table 20.53 Causes of large bowel ischemia

Categories	Disorders
Acute vascular occlusion	Thrombosis or embolism
Nonocclusive mesenteric ischemia	Necrotizing enterocolitis
Blood vessel diseases	Vasculitides and vasculopathies
Vascular compression	▪ Volvulus ▪ Intussusception ▪ Celiac axis compression
Hypercoagulable states	▪ Pancreatic carcinoma ▪ Myeloproliferative syndromes ▪ Antiphospholipid antibodies (APAs) ▪ Hyperhomocysteinemia ▪ Heparin-induced thrombocytopenia
Drugs	Chemotherapeutic agents
Infections	▪ *Clostridium difficile* ▪ *Escherichia coli* ▪ Staphylococcal enterocolitis ▪ Cytomegalovirus ▪ *Aspergillus flavus* ▪ *Candida* species

Mucosal ischemic colitis is caused by Clostridium difficile, Escherichia coli, nonsteroidal anti-inflammatory drugs, Crohn's disease, radiation and collagen colitis.

Surgical Pathology: Ischemic Bowel Disease

Gross Morphology

Morphology of ischemic bowel disease is variable depending on duration and severity of vascular compromise. Ischemic bowel segment shows dusky appearance (Fig. 20.58).

Light Microscopy

- Histologic examination in acute ischemic colitis shows sloughing of surface epithelium. The blood vessels are congested.
- There is presence of acute inflammatory cells in the wall. Chronic bowel ischemia shows atrophic surface epithelium and fibrotic lamina propria (Fig. 20.59).

Clinical Features

Patient with ischemic bowel disease presents with pain in splenic flexure region and bloody diarrhea. Repair occurs by fibrosis that may eventuate in ischemic strictures.

ANGIODYSPLASIA OF COLORECTAL REGION

Angiodysplasia occurs due to dilation of mucosal and submucosal venules of cecum or ascending colon and rectum in elderly persons. Mesenteric angiography is a diagnostic tool.



Fig. 20.58: Ischemic bowel disease. It shows dusky blackish discoloration.

Table 20.54 Acute ischemic injury of the large bowel

Stage of Ischemic Injury	Gross Morphology	Histologic Features	Prognosis
Mucosal necrosis	Mucosa shows congestion, hemorrhage and small shallow ulcers	Intestine shows mucosal and crypts disruption, hemorrhage, edema and neutrophilic infiltration	Changes are usually reversible on restoration of vascular supply
Mural necrosis	Mucosa shows marked congestion, hemorrhage and ulcers; serosa shows congestion	Mucosa, submucosa and inner muscular layer shows necrosis and hemorrhage; neutrophilic infiltrate in the inner muscular layer	Mural necrosis progresses to transmural necrosis; healing leads to submucosal scar and stricture formation
Transmural necrosis	Intestine shows gangrenous, flaccid, dilated intestine with fibrinous exudate; perforation	Muscular layer shows congestion necrosis, edema and neutrophilic infiltrate; congestion of serosa	Transmural necrosis leads to intestinal perforation; surgical resection of massive gangrenous gut always indicated



Fig. 20.59: Ischemic bowel disease. It shows sloughing of epithelium, congested blood vessels and acute inflammatory cells (100X).

Clinical Features

Patient presents with unexplained lower bowel bleeding (bright red blood in stool known as hematochezia). It is associated with aortic stenosis and von Willebrand's disease due to unknown etiology. It is the second most common cause of hematochezia after diverticulosis. Causes of gastrointestinal hemorrhage are given in Table 20.55.

Table 20.55 Causes of gastrointestinal hemorrhage

Age Group	Disorders
Children	▪ Meckel's diverticulum ▪ Juvenile polyp ▪ Ulcerative colitis ▪ Crohn's disease
Adults	▪ Ulcerative colitis ▪ Crohn's disease ▪ Adenomatous polyp ▪ Gastric carcinoma ▪ Colorectal carcinoma ▪ Arteriovenous malformations ▪ Hereditary telangiectasia ▪ Nonsteroidal anti-inflammatory drugs ▪ Infective colitis ▪ Hemorrhoids ▪ Solitary rectal ulcer ▪ Anal fissure
Elderly persons	▪ Diverticular disease ▪ Angiodysplasia ▪ Adenomatous polyp ▪ Gastric carcinoma ▪ Colorectal carcinoma ▪ Ischemic colitis ▪ Ulcerative colitis ▪ Crohn's disease ▪ Radiation proctitis

NEOPLASTIC DISORDERS OF LARGE INTESTINE

Polyp is a gross description of any circumscribed mass of tissue that develops on the inside wall of a hollow organ and projects above surrounding mucosa. The polyp by itself has no clinical significance. Polyps may be neoplastic, inflammatory, or hamartomatous. Histologic examination of the excised polyp establishes the nature and its clinical significance. Hyperplastic polyp, pedunculated tubular adenoma and sessile villus adenoma in colorectal region are shown in Fig. 20.60.

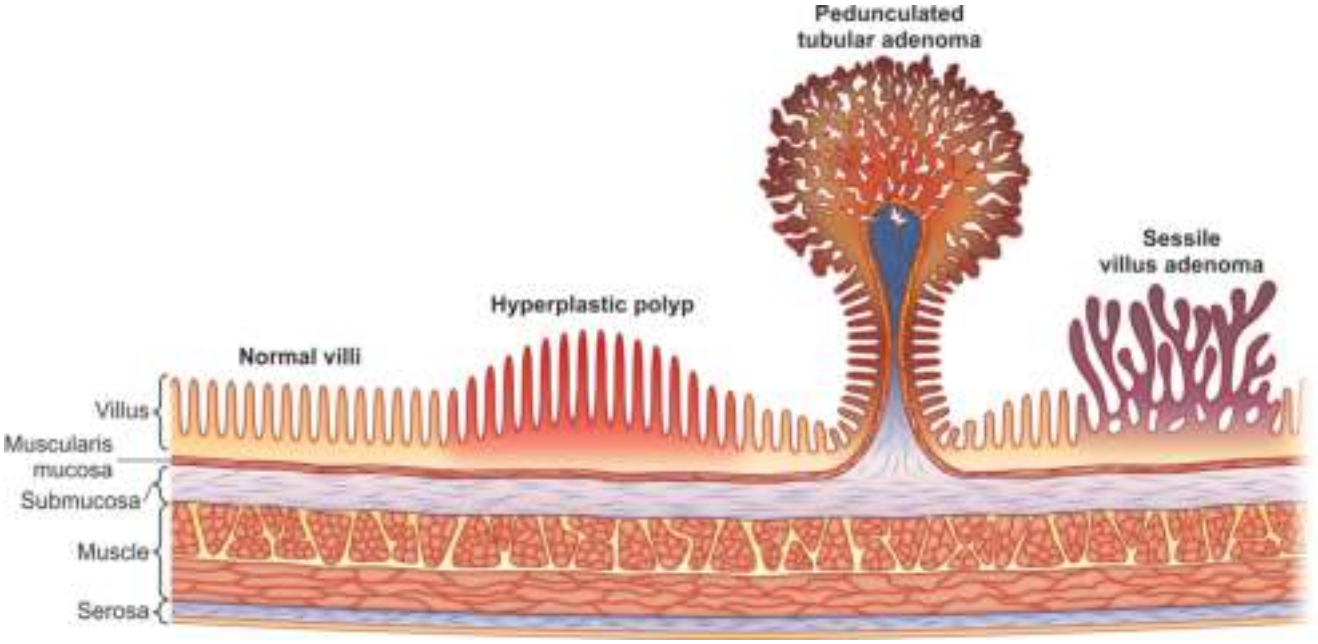


Fig. 20.60: Hyperplastic polyp, pedunculated tubular adenoma and sessile villus adenoma in colorectal region.

Table 20.56 Revised (2019) WHO classification of tumors of the colon and rectum

Benign Epithelial Tumors and Precursor Lesions	Malignant Epithelial Tumors
Serrated dysplasia, low-grade	**Adenocarcinoma, NOS (70–80%)** ▪ Serrated adenocarcinoma (10%) ▪ Mucinous (colloid) adenocarcinoma (8–10%) ▪ Signet ring cell carcinoma (2%) ▪ Medullary carcinoma (1%) ▪ Adenoma-like adenocarcinoma ▪ Micropapillary adenocarcinoma ▪ Poorly cohesive carcinoma ▪ Adenosquamous carcinoma ▪ Carcinoma, undifferentiated, NOS ▪ Carcinoma with sarcomatoid component
Serrated dysplasia, high-grade ▪ Hyperplastic polyp, microvesicular type ▪ Hyperplastic polyp, goblet cell ▪ Hyperplastic polyp, mucin type	
Adenomatous polyp, low-grade dysplasia	
Adenomatous polyp, high-grade ▪ Tubular adenoma, low-grade ▪ Tubular adenoma, high-grade ▪ Villus adenoma, low-grade ▪ Villus adenoma, high-grade ▪ Tubulovillus adenoma, low-grade ▪ Tubulovillus adenoma, high-grade ▪ Advanced adenoma	**Neuroendocrine tumor, NOS** ▪ Neuroendocrine tumor, grade 1 ▪ Neuroendocrine tumor, grade 2 ▪ Neuroendocrine tumor, grade 3 ▪ L cell tumor ▪ Glucagon-like peptide-producing tumor ▪ PP/PYY-producing tumor ▪ Enterochromaffin-like cell carcinoid ▪ Serotonin-producing tumor
Glandular intraepithelial neoplasia, low-grade	**Neuroendocrine carcinoma, NOS** ▪ Large cell neuroendocrine carcinoma ▪ Small cell neuroendocrine carcinoma
Glandular intraepithelial neoplasia, high-grade	Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN)

Term NOS, not otherwise specified. Adapted from 2019 WHO classification of tumors of digestive system.

Large intestine harbors most malignant lesions. Colorectal carcinoma is currently the most common tumor, which affects both men and women with peak in 7th decade. Rectum is the most common site for adenocarcinoma followed by sigmoid colon. Moderately differentiated adenocarcinoma is the most common histologic variant of adenocarcinoma of colon. Patient with colorectal carcinoma has varied clinical presentation depending on the primary location in the large intestine. Revised (2019) WHO classification of tumors of large intestine is given in Table 20.56.

Table 20.57 Serrated polyps of the colon and rectum

Hyperplastic polyps (HPs) in distal colon (70–85%)
Microvesicular hyperplastic polyps (MVHPs)
Goblet cell hyperplastic polyps (GCHPs)
Mucin-poor hyperplastic polyps (MPHPs)
Traditional serrated adenomas (TSAs)
Sessile serrated adenomas/polyps (SSA/Ps)
Sessile serrated adenomas/polyps with cytologic dysplasia

SERRATED POLYPS

Serrated polyps are the most common polyps in distal colon and rectum. Hyperplasia of colonic epithelium leads to formation of elongated glands with saw-tooth appearance on cross section.

- Serrated polyps consist of hyperplastic polyps, sessile serrated adenoma polyp with or without cytologic dysplasia, traditional serrated adenomas and serrated polyposis syndrome (Table 20.57).
- Microvesicular hyperplastic polyps (MVHPs), traditional serrated adenomas and serrated polyposis syndrome are believed to be precursor lesions for colorectal carcinomas evolving through BRAF (V600E) mutation and subsequent DNA methylation.
- According to WHO definition, hyperplastic polyposis is characterized by criteria: (a) presence of at least five histologically diagnosed serrated polyps proximal to the sigmoid colon, out of which two are larger than 10 mm; or (b) presence of any number of serrated polyps proximal to the sigmoid colon in someone who has first-degree with serrated polyposis syndrome or (c) presence of more than serrated polyps of any size that are distributed throughout colorectal region.

Hyperplastic Polyps

Hyperplastic polyps have been subdivided into three types: microvesicular hyperplastic polyps (MVHPs), goblet cell hyperplastic polyps (GCHPs) and mucin-poor hyperplastic polyps (MPHPs). Vast majority of hyperplastic polyps are of microvesicular type. Incidence of K-RAS mutations, BRAF (V600E) mutations have been demonstrated in all three subtypes of hyperplastic polyps in colon and rectum.

Surgical Pathology: Hyperplastic Polyps in Colon and Rectum

Gross Morphology

On gross examination, hyperplastic polyps in colon and rectum tend to be small, dome-shaped (convex) structure. Color is paler than the surrounding mucosa. These polyps often sit on the crest of the mucosal folds. Occasionally, hyperplastic polyps may be pedunculated and grossly mimic adenomas.

Light Microscopy

Hyperplastic polyps have been subdivided into three types: microvesicular hyperplastic polyps, goblet cell hyperplastic polyps and mucin-poor hyperplastic polyps.

- **Microvesicular hyperplastic polyps (MVHPs):** In MVHP, the epithelial cells are an admixture of goblet cells and columnar cells with apical microvesicular mucin vacuoles. The goblet cells are decreased in numbers compared with normal mucosa. MVHP has a large proliferative zone that occupies basal half of the mucosa. Rarely, eosinophilic absorptive cells are demonstrated. The cells at the crypts base appear crowded, with somewhat hyperchromatic nuclei. These polyps should not be confused with an adenoma.
- **Goblet cell hyperplastic polyps (GCHPs):** Goblet cell hyperplastic polyps are observed in the distal colon and rectum. The mucosa is thickened. Subepithelial basement membrane and goblet cells are quite prominent.
- **Mucin-poor hyperplastic polyps (MPHPs):** Mucin-poor hyperplastic polyps are uncommon. Columnar cells and mucin are depleted. These histologic changes mimic those of epithelial regeneration and similar to seen in prolapse.

Traditional Serrated Polyps

Traditional serrated polyps tend to be pedunculated/polypoidal lesions. At low magnification, traditional serrated polyps have complex serrated architecture, hence often misdiagnosed as villus adenomas. These polyps composed of uniform population of penicillate cells with bright eosinophilic cytoplasm, elongated nuclei and small nucleoli. These lesions may undergo dysplastic change and progression to malignancy.

Sessile Serrated Adenoma/Polyp with Dysplasia

Sessile serrated adenomas/polyps with dysplasia have been characterized by combinations of classic adenoma, hyperplastic polyp, sessile serrated adenoma/polyp and traditional serrated adenoma.

PEUTZ-JEGHERS SYNDROME

Peutz-Jeghers syndrome is an autosomal dominant disorder due to LKB1/STK11 gene mutation in 75% of affecting young children of 10–15 years of age. It is characterized by multiple hamartomatous polyps in gastrointestinal tract, i.e. small intestine (most common), stomach and colon in decreasing frequency; melanotic pigmented macules (1–5 mm) of lips, perioral skin, hands and genitalia. There is increased risk of cancers of gastrointestinal tract, thyroid, breast, lung, pancreas, gonads, and urinary bladder in Peutz-Jeghers syndrome. On histologic examination, polyp is composed of branching network of smooth muscle fibers continuous with the muscularis mucosa that support irregularly arranged glands of the polyp.

Diagnostic Criteria

Diagnostic criteria for Peutz-Jeghers syndrome include (a) two or more histologically confirmed polyps in Peutz-Jeghers syndrome, (b) any number of polyps in Peutz-Jeghers syndrome, (c) characteristic, prominent mucocutaneous pigmentation with a family history of Peutz-Jeghers syndrome, and (d) any number of polyps in Peutz-Jeghers syndrome. Uncommon tumors associated with Peutz-Jeghers syndrome are ovarian sex cord tumors and uterine cervical adenoma/adenocarcinoma; and Sertoli cell tumor in males.

Molecular Genetic Alterations

Genetic basis for the Peutz-Jeghers syndrome is a germline mutation in the LKB1/STK11 gene located on chromosome 19. STK11 is a serine-threonine kinase, which participates in proliferation in the mammalian target of rapamycin (mTOR) signaling pathways. Clinical trials are going on mTOR rapamycin inhibitor.

Clinical Features

Most patients are diagnosed in their second decade. Clinical presenting symptoms of hamartomatous polyps depend on location and size of polyps. Small polyps can cause obstruction and abdominal pain, whereas polyps in rectosigmoid region present with bloody stools or prolapse protrusion or prolapse of the polyps.

Pathologic Features

Hamartomatous polyps in Peutz-Jeghers syndrome vary in size from <1 to >3.5 cm. These may be sessile or pedunculated. Basic architecture of polyp is that of glandular epithelium with 'Christmas tree'-like appearance resting on branching smooth muscle framework that originates from the muscularis

mucosa. The glandular epithelium of polyp resembles the normal epithelium of the intestine. Diagnostic difficulty occurs in polyps with ischemic changes often secondary to intussusception. Areas of adenomatous and carcinomatous transformation within these hamartomatous polyps have been demonstrated in 5–10% of cases.

COWDEN SYNDROME

Cowden syndrome is nonhereditary disorder, which affects >50 years of age. It is characterized by hamartomatous polyps in colon. There is presence of crypt dilatation and edema in nonpolypoidal mucosa. There is increased risk of benign and malignant tumors of thyroid and breast in Cowden syndrome.

JUVENILE RECTAL POLYP

Juvenile rectal polyp is a solitary hamartomatous pedunculated polyp also known as retention polyp, which affects children in first two decades of life, most often in <5 years of age. It is usually solitary and localized chiefly in the rectum. Approximately 40% of the cases are autosomal dominant, which have risk for development of cancers of stomach, small intestine and colon.

Diagnostic Criteria

Juvenile polyp may be associated with the juvenile polyposis syndrome. The diagnostic criteria for polyposis syndrome include: (a) presence of >5 juvenile polyps in the small intestine and rectum, (b) presence of juvenile polyps in throughout the gastrointestinal tract, and (c) presence of any number of polyps in a patient with a family history of juvenile polyposis. Juvenile polyps may be present throughout gastrointestinal tract in infants, which may present with diarrhea, anemia and hypoalbuminia.

Molecular Genetic Alterations

Gene mutations of BMPR1 (10q22) and SMAD4/DPC (18q21) have been demonstrated in juvenile rectal polyps. Associated intestinal and extraintestinal defects (e.g. malrotation of gut, mesenteric lymphangioma, Fallot tetralogy, coarctation of aorta, hydrocephalus) have been reported in these cases.

Surgical Pathology: Juvenile Rectal Polyp

Gross Morphology

- Juvenile rectal polyp is mainly pedunculated, rarely sessile, and usually <3 cm in diameter.
- Juvenile rectal polyp smooth glistening and gelatinous surface with reddish to tan discoloration.
- On cut section, juvenile rectal polyp is filled with cystic-filled spaces.

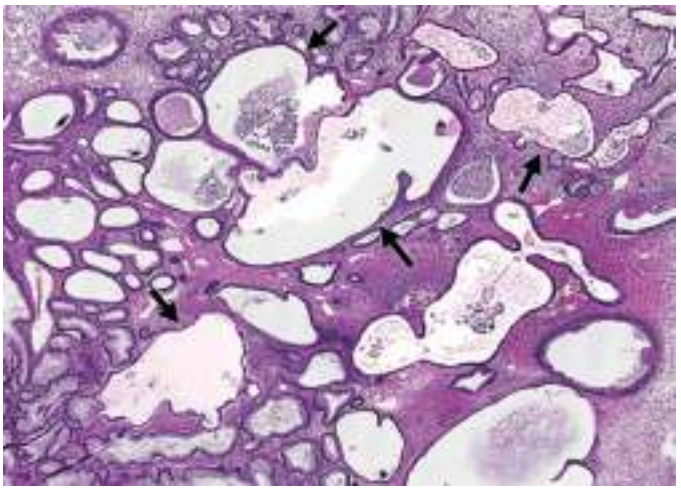


Fig. 20.61: Juvenile rectal polyp shows mucus-filled cystic glands and edematous, usually inflamed stroma (arrows) (100X).

Light Microscopy

- Juvenile polyp is composed of mucus-filled cystic, dilated and torturous glands and in edematous and inflamed stroma.
- The glands are composed of well-formed mucus-secreting cells that may become flattened and attenuated. The stroma usually contains acute and chronic inflammatory cells and granulation tissue.
- When dilated and tortuous glands rupture into the stroma leading to a foreign body giant cell reaction.
- Occasionally, osseous metaplasia may be demonstrated in juvenile polyp (Fig. 20.61).

FAMILIAL JUVENILE POLYPOSIS

Familial juvenile polyposis is an autosomal dominant disorder characterized by multiple juvenile polyps in the gastrointestinal tract. Familial juvenile polyps may undergo malignant transformation.

INFLAMMATORY POLYPS

Inflammatory polyps may be secondary to inflammatory disorders of the intestine. These are demonstrated in the setting of ulcerative colitis (10–20%), Crohn's disease, ischemic disease, amoebiasis, schistosomiasis and adjacent to ulcers and at surgical anastomosis sites.

- Inflammatory polyps may be rounded nodules comprising caps of granulation tissue and remnants of mucosa in the setting of chronic inflammatory bowel disease. Rectal mucosa is most common site.
- The entities of inflammatory polyps secondary to mucosal prolapse are inflammatory cap polyps, inflammatory myoglandular polyps and diverticular polyps.
- Inflammatory polyps are limited to the rectosigmoid region and distal colon. Inflammatory myoglandular polyps are mainly located in the distal colon and

Table 20.58 Differences between tubular and villus adenomas in colorectal regions

Parameters	Tubular Adenoma	Villus Adenoma
Definition	Consisting of >75% tubular appearance	Consisting of >50% villus appearance
Occurrence	90–95% of total adenomas	<1% of total adenomas
Location	▪ Colon (90%) ▪ Rectosigmoid region (50%)	▪ Rectum ▪ Rectosigmoid region
Age group	Earlier age group	Elderly persons
Gross morphology	Small, pedunculated	Large sessile velvet-like appearance projecting above normal mucosa
Light microscopy	Stalk composed of fibrovascular tissue covered by benign mucin secreting mucosal cells	Frond-like villus structures covered by dysplastic and disordered columnar epithelial cells
Risk of malignancy	Uncommon	Relatively common

rarely in ileum, that may cause intussusception. Diverticular polyps occur in the background of diverticular disease.

CRONKHITE-CANADA SYNDROME

Cronkhite-Canada syndrome is a nonhereditary disorder affecting >50 years of age. It is characterized by multiple hamartomatous polyps in stomach, small intestine and colorectal region associated with protein losing enteropathy alopecia, nail atrophy and cutaneous hypopigmentation.

- Patient presents with diarrhea, weight loss, abdominal pain, anorexia and weakness, alopecia, nail changes and hyperpigmentation of the skin.
- Histologic features of these polyps are identical to juvenile polyps with the exception of intervening stroma. Polyp is composed of tortuous and cystically dilated glands and present in the nonpolypoidal intervening mucosa.

NEOPLASTIC POLYPS

Adenomatous polyps are true neoplasms arising from the mucosal epithelium. These polyps are more common in men than women.

- Neoplastic polyps are distributed throughout the large intestine, i.e. right colon (40%), left colon (40%) and rectosigmoid region (20%). These are rarely seen in small intestine.
- Neoplastic polyps are subdivided into tubular adenomas (most common raspberry on a stalk), villus adenomas, tubulovillus (mixture of tubular and villus); and sessile serrated adenomas (serrated epithelium lining the crypts). Neoplastic polyps may be pedunculated (attached by stem) or sessile (broad base attachment). Differences between tubular and villus adenomas in colorectal regions are given in Table 20.58.
- All adenomas are precancerous lesions. The risk for malignant transformation parallels the percentage of the villus component (finger-like projections) in the polyp and size >2 cm increases the risk for developing colon carcinoma (e.g. rectosigmoid region) in villus adenoma. Patients are most often asymptomatic, but rectal bleeding may occur. Risk of colon carcinoma in neoplastic polyps is given in Table 20.59.

Table 20.59 Risk of colon carcinoma in neoplastic polyps

Neoplastic Polyp	Size of Neoplastic Polyp		
	(<1 cm)	(1–2 cm)	(>2 cm)
Tubular adenoma	1%	10.2%	34.7%
Tubulovillus adenoma	3.9%	7.4%	45.8%
Villus adenoma	9.5%	10.3%	52.8%

Risk of development of colon carcinoma is higher in villus adenoma.

Tubular Adenomas

Tubular adenomas are most common benign neoplastic polyps in the colon (90%) and rectosigmoid region (50%). Tubular adenomas may undergo malignant transformation to colon adenocarcinoma in 1–3% of cases. Risk for development of colon adenocarcinoma is proportional to the increasing size and number of polyps. Most tubular adenomas are excised through endoscopy in 99% of cases.

Surgical Pathology: Tubular Adenomas

Gross Morphology

Tubular adenomas are usually multiple and pedunculated attached to the large intestine by a stalk. These may be sessile. These are spherical in shape with shaggy surface. These are generally <2 cm in size with reddish or darker in color than the surrounding mucosa (Fig. 20.62A and B).

Light Microscopy

- Tubular adenoma is composed of closely packed tubular glands forming the head of the polyp lined by mucus-secreting columnar epithelium.
- More than 80% of tumor cells form glandular and tubular arrangement (Fig. 20.63).

Villus Adenomas

Villus adenomas account for 10% of neoplastic polyps in rectum and rectosigmoid region. These are >2 cm in size in diameter with velvety surface showing finger-like villus structures and high potential for malignant change. Large villus adenomas may secrete copious electrolyte-rich mucus, resulting in decreased serum potassium level and acute renal failure. Approximately 50% of all villus adenomas undergo malignant transformation to colon adenocarcinoma.

Surgical Pathology: Villus Adenomas

Gross Morphology

- Villus adenomas are solitary, sessile with broad base measuring ≥1–5 cm in diameter. These are most often located in the rectum extending over a wide area as thick, carpet-like growth.
- Finger-like projections (villi) protrude from the base of the polyps into the lumen of the intestine.

Light Microscopy

Villus adenomas are composed of villi (i.e. finger-like protrusions lined with columnar epithelium). More than 80% of tumor cells form villus projections (Fig. 20.64).

Tubulovillus Adenomas

Tubulovillus adenomas in large bowel constitute 15% of adenomatous polyps, which represent an intermediate

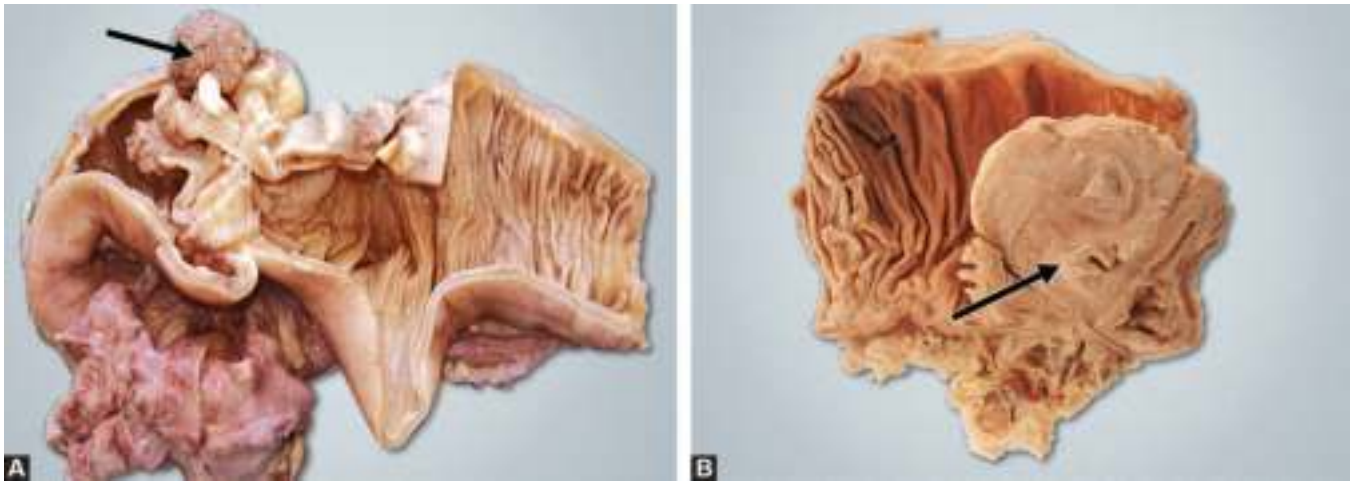


Fig. 20.62A and B: Tubular adenoma in large intestine shows pedunculated polyp (arrows). (*Courtesy:* Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

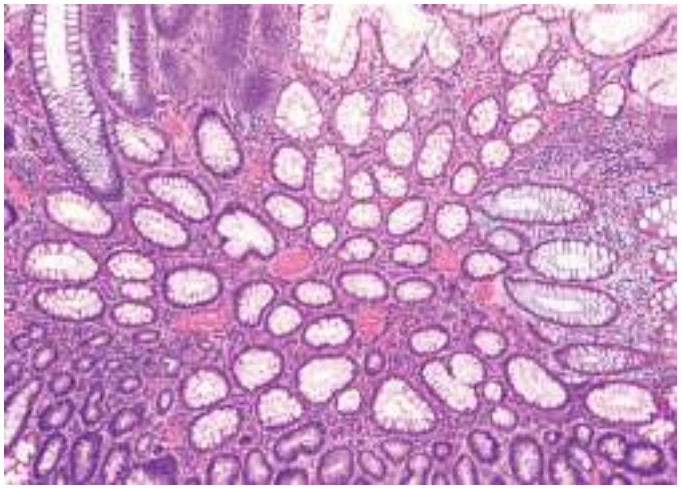


Fig. 20.63: Tubular adenoma in colon. It shows closely packed tubular glands lined by mucus-secreting columnar epithelium (100X).

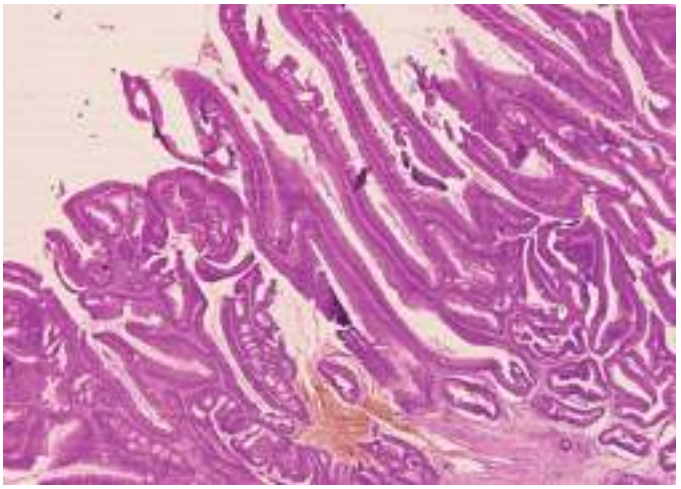


Fig. 20.64: Villus adenoma in colon. It shows finger-like protrusions lined with columnar epithelium (100X).

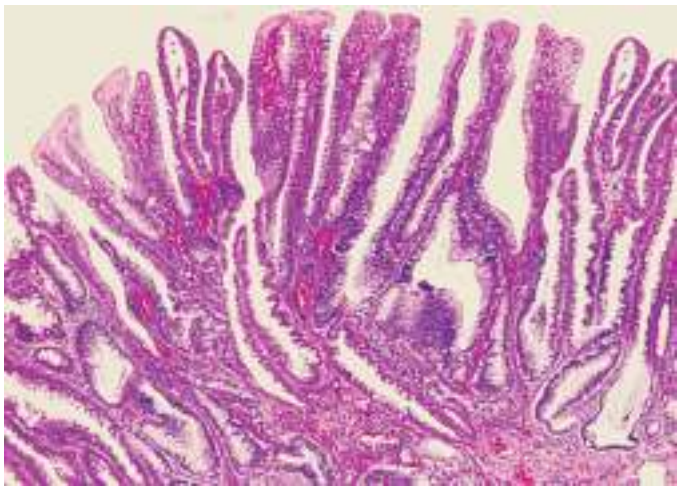


Fig. 20.65: Tubulovillus adenoma in colon. It shows tubular and villus components (100X).

step between tubular and villus adenomas in all respects. If the tubular adenoma component constitutes >40% of the total tumor mass or 20–80% villus component, it is known as tubulovillus adenoma. Tubulovillus adenoma shows tubular and villus components (Fig. 20.65).

Sessile Serrated Adenomas

Sessile serrated adenomas in colon resemble hyperplastic polyps, but unlike hyperplastic polyps, these may undergo malignant transformation. Sessile serrated adenomas most often occur in right colon and demonstrate microsatellite instability. Sessile serrated adenomas can be very subtle and are easily missed on endoscopy.

MULTIPLE POLYPOSIS SYNDROMES

Multiple polyposis syndromes are associated with a greatly increased risk for malignant transformation.

FAMILIAL ADENOMATOUS POLYPOSIS COLI

Familial adenomatous polyposis coli (FAP) is an autosomal dominant disorder associated with inactivation of the APC gene (adenomatous polyposis coli gene) located on chromosome 5q21.

- FAP is characterized by development of >100 tubular adenoma polyps (500–2500) in colon and rectum, particularly in the rectosigmoid region.
- Polyps are not present at birth. These appear in childhood and become clinically evident in second decade of life. Familial adenomatous coli with multiple polyps covering entire mucosa is shown in Fig. 20.66.

Molecular Genetic Alterations

Progression from adenoma to colon adenocarcinoma involves a series of steps in familial adenomatous



Fig. 20.66: Familial adenomatous coli. It shows multiple polyps covering virtually the whole of the mucosal surface of the colon >100 tubular adenoma polyps (ranging 500 to 2500).

polyposis coil. Patient with familial adenomatous polyposis coli has mutation in the APC tumor suppressor gene located on long arm of chromosome 5.

Further new mutations occur in 30–50% of patients, which include mutation of p53 tumor suppressor gene on chromosome 17 and point mutation of RAS oncogene.

Malignant Potential

The risk of malignant transformation approaches 100%. The risk of malignancy is related to the size of adenoma, amount of villus component, grade of epithelial dysplasia and family history.

Dysplastic foci in adenoma of FAP show stratification, hyperchromatic nuclei, mitotic figures in upper crypts and surface epithelium.

Management

By the age of 40 years, all the patients with FAP develop colon adenocarcinoma hence need prophylactic total colectomy.

Mortality in FAP

Noncolonic causes of death in patient with familial adenomatous polyposis (FAP) are pancreatic carcinoma, central nervous system tumors and complications of therapy.

GARDNER'S SYNDROME

Gardner's syndrome is a variant of familial polyposis syndrome due to APC gene mutation. It is an autosomal dominant disorder, which affects children 10–15 years of age.

Gardner's syndrome is associated with numerous adenomatous polyps in colon along with osteomas in the mandible and abdominal desmoid.

TURCOT'S SYNDROME

Turcot's syndrome is an autosomal dominant disorder due to APC gene mutation (5q21). It is characterized by multiple adenomatous polyps in colon with increased

risk for colorectal carcinoma and brain cancers (medulloblastoma or glioblastoma multiforme). Turcot's syndrome may be associated with familial adenomatous polyposis or Lynch syndrome.

Molecular Genetic Alterations

Molecular basis of Turcot's syndrome is either due to mutation of APC gene located on long arm of chromosome 5q21 or mutation in one of the mismatch repair genes associated with Lynch syndrome (MLH1 and PMS2). Medulloblastoma occurs due to mutation of APC gene and glioblastoma multiforme due to mutation of mismatch repair genes.

JUVENILE POLYPOSIS COLI

Juvenile polyposis coli is an autosomal dominant syndrome, which occurs due to mutation of PTEN gene. Patient develops >6 colorectal polyps.

TUBEROUS SCLEROSIS

Tuberous sclerosis is caused by mutation in the TSC1 or TSC gene, which encodes hamartin and tuberin proteins, respectively. Tuberous sclerosis is associated with multiple hamartomas in rectum, brain (as cortical tubers, subependymal hamartomas), face, and kidneys (renal angiomyolipoma in 80% cases).

MALIGNANT TUMORS OF COLON

HEREDITARY NONPOLYPOSIS COLON CANCER

Hereditary nonpolyposis colon cancer (HNPCC) is also known as Lynch syndrome. There is increased risk for development of carcinoma of endometrium (most common), right colon carcinoma, ovary, urothelium, brain and stomach in these patients.

Muir-Torre syndrome is an autosomal dominant syndrome that is thought to be subtype of HNPCC. Persons are prone to develop cancers of the colon, genitourinary tract tumors and skin (keratoacanthoma and sebaceous tumors).

Molecular Genetic Alterations

All hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome) occurs due to germline gene mutations in one of the MMRs DNA mismatch repair gene, i.e. MSH2, MSH6, MLH1, MLH3 and PMS2 genes. There may be mutation of TACSTD regulatory gene. These gene mutations cannot correct errors in the nucleotide pairing. Microsatellite instability (MSI) is demonstrated in 90% of the tumors that develop in these patients.

- Sessile serrated adenoma is the precursor lesion for HNPCC or Lynch syndrome.
- BRAF and K-RAS gene mutations never occur in HNPCC or Lynch syndrome.
- Sporadic colorectal carcinoma is usually caused by hypermethylation of the MLH1 promoter in 15% of cases.
- Microsatellite instability results from dysfunction of DNA mismatch repair genes, i.e. MSH2, MSH6, MLH1, MLH3 and PMS2. These genes are detected by immunohistochemistry, PCR-based genotyping and analysis of mononucleotide repeats (BAT25, BAT26) and dinucleotide repeats (D5S346, D2123, D17S250).

Location

HNPCC is frequently seen on the right-sided colon with expansile growth and mucin production.

Surgical Pathology: Hereditary Nonpolyposis Colon Cancer

Light Microscopy

- Histologic examination reveals mucinous or medullary features of colon carcinoma, infiltrated by lymphocytes.
- Crohn's-like lymphocytic reaction is demonstrated.

Management

Extensive clinical history and screening, i.e. genetic testing for microsatellite instability and expression of mismatch repair genes by immunohistochemistry technique for development of HNPCC is required in the surgical management of these patients.

COLORECTAL CARCINOMA

Colorectal carcinoma is the most common tumor, which affects both men and women. Most colorectal carcinomas develop as sporadic carcinomas in 99% of cases. Several hereditary cancer syndromes and other polyposis syndromes causing colorectal carcinoma are given in Table 20.60. Hereditary nonpolyposis colorectal cancer (HNPCC) represents 3–4% of colorectal carcinoma. Familial adenomatous polyposis (FAP) and remaining cancer syndrome represent are responsible for colorectal carcinoma <1% of cases.

Risk Factors

Risk factors for colorectal carcinoma are familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome, inflammatory bowel disease (ulcerative colitis, Crohn's disease), history of colorectal cancer in first degree relatives, dietary factors (consumption of red meat, animal fat, saturated fat, refined carbohydrate, low fiber content), obesity, tobacco smoking, chronic alcoholism and excessive consumption of nonsteroidal anti-inflammatory drugs.

Table 20.60 Several hereditary cancer syndromes and other polyposis syndromes causing colorectal carcinoma

Polyposis Syndrome	Inheritance	Clinical Features	Gene and Location	Gene Product Function	Risk for Colorectal Carcinoma
Familial adenomatous polyposis (FAP) and variants	Autosomal dominant	>100 colonic and rectal adenomas (ranging 500–2500), nearly 100% lifetime risk for colorectal carcinoma by age of 40 years	APC gene	▪ MUTYH gene (normally codes for MYH glycosylase involved in repair) ▪ Growth inhibitory β-catenin sequestration; targeting of β-catenin for destruction	100%
Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome	Autosomal dominant	70–80% lifetime risk for development of colorectal carcinoma; predisposition for extracolonic malignancies including endometrial carcinoma	▪ MLH1 ▪ MSH2 ▪ PMS2 ▪ MSH6 ▪ MLH3 ▪ BER-Ep4	DNA mismatch repair	10–50%
Juvenile polyposis syndrome	Autosomal dominant	Hamartomatous polyps in gastrointestinal tract, increased risk for development of colorectal carcinoma	SMAD4 (18q21.1)	▪ BMPR1A (bone morphogenetic protein receptor, type 1A on 19q23.2) ▪ TGF-β signaling	65%
MUTYH-associated polyposis	Autosomal recessive	Multiple polyps >100 in colorectal regions	MUTYH	Protein regulates MYH glycosylase enzyme	60–70%
Peutz-Jeghers syndrome	Autosomal dominant	Hamartomatous polyps in with or without adenomatous component in small intestine, large intestine and stomach in decreasing frequency, skin macules, and predisposition for extracolonic malignancies of thyroid, breast, lung, pancreas, gonads and urinary bladder	STK11/ LKB1 tumor suppressor gene	Serine threonine kinase	40%
Polymerase proofreading-associated polyposis	Autosomal dominant	Multiple adenomas in colon	▪ POLD1 ▪ POLE	Exonuclease proofreading domain	30–70%
Cowden syndrome and Bannayan-Ruvalcaba-Riley (BRR) syndrome	Autosomal dominant	Hamartomatous small, sessile polyps in gastrointestinal tract, no predisposition for colorectal cancer	PTEN (phosphatase and tensin homolog) on chromosome 10q23	Protein phosphatase and tensin homolog	10%
Gardener syndrome	Autosomal dominant	>100 colonic adenomas, congenital hypertrophy of the retinal pigment epithelium (CHRPE), osteomas in mandible, abdominal desmoid tumors	APC gene	Growth inhibitory: β-catenin sequestration; targeting of β-catenin for destruction	Unknown risk frequency
Turcot syndrome	Autosomal dominant	Familial adenomatous polyposis (>100 colonic adenomas) medulloblastoma or glioblastoma multiforme	APC gene	Growth inhibitory β-catenin sequestration; targeting of β-catenin for destruction	Unknown risk frequency
Attenuated familial adenomatous polyposis	Autosomal dominant	>15 but <100 colonic adenomas, >90% by age of 70 years	▪ APC gene ▪ MYH ▪ MUTYH	Growth inhibitory β-catenin sequestration; targeting of β-catenin for destruction	Unknown risk frequency

Contd...

Table 20.60 Several hereditary cancer syndromes and other polyposis syndromes causing colorectal carcinoma (*Contd...*)

Polyposis Syndrome	Inheritance	Clinical Features	Gene and Location	Gene Product Function	Risk for Colorectal Carcinoma
MYH-associated polyposis	Autosomal recessive	Familial adenomatous polyposis-like clinical presentation, and demonstration of APC gene mutation	MYH gene	DNA damage repair	Unknown risk frequency
Muir-Torre syndrome	Autosomal dominant	HNPCC syndrome features, sebaceous tumors and extrahepatic malignancies	▪ MLH1 ▪ MSH2 ▪ PMS2 ▪ MSH6 ▪ MLH3 ▪ BER-Ep4	DNA mismatch repair	Unknown risk frequency
Familial colorectal cancer type X (FCCTX)	Hereditary nonpolyposis disorder	Microsatellite stable disorder, predisposition to colorectal cancer in 50–90% by age of 70 years, no risk for extracolonic cancers	Unknown	No evidence of DNA mismatch repair deficiency	Unknown risk frequency
NTHL1-associated polyposis	Autosomal recessive	Multiple polyps in colorectal regions	NTHL1	Protein regulates DNA glycosylase 1 enzyme	Unknown risk frequency
Constitutional mismatch repair deficiency syndrome	Autosomal recessive	Multiple polyps in colorectal regions	▪ MLH1 ▪ MSH2 ▪ MSH6 ▪ PMS2	DNA mismatch repair	Unknown risk frequency
Hereditary mixed polyposis syndrome	Autosomal dominant	Multiple polyps in colorectal regions	GREM1	Protein encodes BMP (bone morphogenic protein)	Unknown risk frequency
MSH3-associated polyposis	Autosomal recessive	Multiple polyps in colon	MSH3	DNA mismatch repair	Unknown risk frequency
AXIN2-associated polyposis	Autosomal dominant	Multiple polyps in colon and stomach; tooth agenesis	AXIN2 (17q23-q24)	Wnt pathway inhibitor	Unknown risk frequency
Serrated polyposis	Unknown	Multiple polyps in distal colon and rectum	Unknown	Unknown	Unknown risk frequency

- Consumption of red meat, animal fat and saturated fat enhance bile acid synthesis, that get converted by gut bacteria to powerful carcinogenic agent.
- Consumption of diet low in fiber content decreases the stool bulk, increases fecal transit time and alters gut bacterial flora resulting in production of toxic oxidative products and then development of colorectal carcinoma. Consumption of diet rich in refined carbohydrate produces potential carcinogens. Low intake of vitamins A, C and B decrease oxygen-derived free radical scavenger activity and increase for colorectal carcinoma.
- High-fiber content in diet is protective, e.g. bran (the hard-outer layer of whole cereals such as oats, wheat, rice, and corn), fruits (apricot rich in calcium, potassium, phosphorus, iron, vitamin A and figs), nuts (coconuts, almonds) and boiled pea. Risk for colorectal carcinoma increases in immunocompromised state. Risk factors for colorectal carcinoma are given in Table 20.61.

Molecular Pathogenesis

In the sporadic colorectal carcinoma pathway, adenomas are characterized in the early stages by low-grade epithelial dysplasia precede the development of high-grade dysplasia, which may then progress to invasive colorectal carcinoma. There are two main types of precursor lesions for colorectal carcinoma: conventional

Table 20.61 Risk factors for colorectal carcinoma

Elderly persons
Familial adenomatous polyposis
Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome
Inflammatory bowel disease ▪ Ulcerative colitis ▪ Crohn's disease
History of colorectal cancer in first degree relatives
Dietary factors ▪ Red meat intake ▪ Saturated fat intake ▪ Animal fat intake ▪ Refined carbohydrate intake
Obesity
Tobacco smoking
Nonsteroidal anti-inflammatory drugs
Chronic alcoholism
Excessive consumption of nonsteroidal anti-inflammatory drugs

adenomas or adenomatous lesions (most common) and serrated polyps (second most common).

- The epithelium lining conventional colonic adenomas display cytologic features of dysplasia. In inflammatory bowel disease-associated neoplasia, the background colonic mucosa demonstrates variable degrees of chronic colitis, and eventually leads to low-grade epithelial dysplasia, which in turn may progress to high-grade dysplasia and invasive colon adenocarcinoma.
- The morphologic features of the neoplastic lesions significantly overlap in sporadic colorectal carcinoma and inflammatory bowel disease-associated neoplasia, but the inflammatory environment that characterizes chronic colitis dictates a number of different molecular mechanisms of neoplastic development and progression to colorectal carcinoma.
- Colorectal carcinoma that develops through the serrated pathway originates from the serrated polyps demonstrates epithelial dysplasia. Poorly differentiated colorectal carcinoma that develops through the microsatellite instability pathway demonstrates prominent intraluminal lymphocytes. The presence of microsatellite instability in the tumor DNA is analyzed by the microsatellite instability marker BAT25.
- Early detection of colonic adenomas and colorectal carcinoma in average-risk persons is done by performing flexible sigmoidoscopy, colonoscopy, double-contrast barium enema, CT colonography every 5–10 years. Tests that primarily detect colorectal carcinoma include annual examination of guaiac-based fecal occult blood test and fecal immunochemical test. Histologic examination of the biopsy is the gold standard for diagnosis.

Table 20.62 Distribution of colorectal carcinoma in decreasing frequency

Location	Frequency
Rectosigmoid region	38%
Sigmoid colon	20%
Descending colon	10%
Cecum	8%
Hepatic flexure colon	8%
Transverse colon	5%
Ascending colon	3%
Anal canal	3%

Locations

Rectosigmoid is the most common site for colon carcinoma (38% of cases), sigmoid colon (20% of cases), descending colon (10% of cases), cecum (8% of cases), hepatic flexure (8% of cases), transverse colon (5% of cases), ascending colon (3% of cases), and anal region (3% of cases). Left-sided colon carcinoma most likely to obstruct lumen, while right-sided colon carcinoma more likely to bleed. Distribution of colorectal carcinoma in decreasing frequency is given in Table 20.62.

Pathology Pearls: Recent Advances of Colorectal Carcinoma Stem Cell (CRCSC) Biology

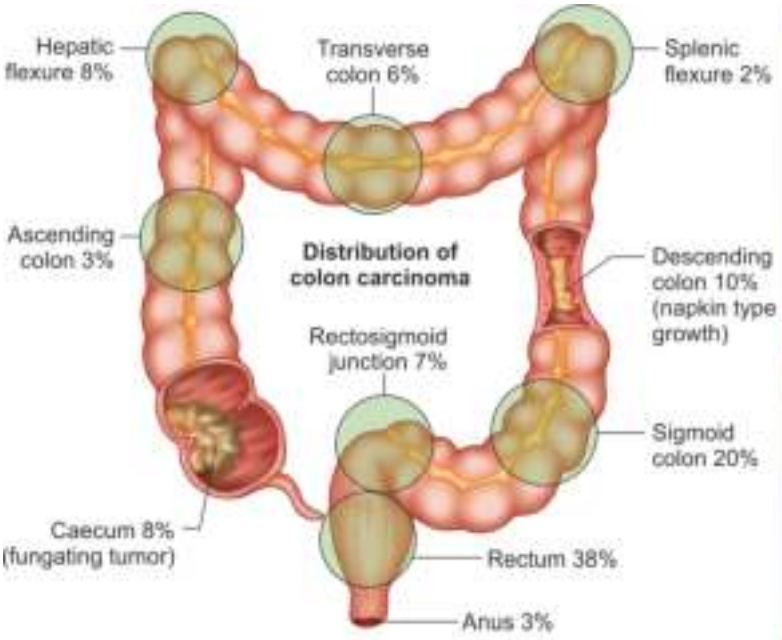
- Stem cell line includes totipotent stem cell in single cell embryo, embryonic stem cells derived from inner cell mass of the blastocyst (pluripotent) in 5–7 days embryo, cord blood stem cells (multipotent), placental stem cells (multipotent), and adult stem cells (multipotent) in infants and adults.
- Totipotent cells in single cell embryo have the potential to become any type in the adult body and any cell of the extraembryonic membranes (e.g. placenta).
- Pluripotent stem cells have the potential to make any differentiated cell in the body except placenta, which is derived from trophoblast (Fig. 20.67).
- Colorectal carcinoma demonstrates a variety of stem cells at various stages of differentiation within the tumor with enhanced self-renewal properties that drive long-term tumor growth, differentiation, chemotherapy resistance, relapse and metastasis.
- The maintenance of the stem cells and activation differentiation potential are fundamentally orchesterized by microenvironment stem cell niche-related cellular and humoral signaling pathways.
- Colorectal carcinomas can give rise to multiple lineages within the neoplasm. The resulting cellular plasticity contributes to tumor heterogeneity; hence it poses great difficulty in cancer therapy. Various functional clones of colorectal carcinoma stem cells can be present within a single tumor, further contributing to tumor heterogeneity. Colorectal carcinoma

stem cell markers, regulators and signaling pathways serve as important therapeutic targets. Colorectal carcinoma stem cells specific agents can be combined with chemotherapy to target both colorectal carcinoma stem cells and bulk tumor cells.

- Panel of immunomarkers used to analyze colorectal carcinoma includes CD11/Proml, Lgr5, CD44, Bmi1, CD166, CD26, ALDH1, EphB2 and Dclk1. Characteristics of colorectal carcinoma stem cells (CRCSCs) are given in Table 20.63.

Molecular Genetic Alterations

Colorectal carcinoma is a slow-growing tumor. It develops through a set of anatomic changes progressing from normal mucosa to adenomatous polyp carcinoma *in situ*, to invasive carcinoma to metastatic tumor. Combination of genetic and epigenetic abnormalities are observed in the development of colorectal carcinoma.



Risk factors: Colorectal carcinoma

- Age >50 years
- High fat, low fiber diet
- Ulcerative colitis and Crohn's disease
- Familial adenomatous polyposis (FAP)
- Hereditary nonpolyposis colorectal cancer (HNPCC)
- Adenomas (villous, tubular and tubulovillous)
- Peutz-Jeghers syndrome
- Family history of colorectal cancer

Immunohistochemistry: Colorectal carcinoma

Tumor markers	Expression
• Carcinoembryonic antigen (CEA)	Positive
• CK20	Positive
• CDX2 (transcription factor)	Positive
• SAT2 (transcription factor)	Positive
• p53	Positive
• β-Catenin	Positive
• CK7	Focally positive

Serum tumor markers used to detect colorectal carcinoma

Category	Tumor marker	Cancers	Category	Tumor markers	Cancers
Oncofetal proteins	Carcinoembryonic antigen (CEA)	• **Colon carcinoma** • NSGCT of testis • Gastric carcinoma • Pancreatic carcinoma • Lung carcinoma • Breast carcinoma	Mucins and glycoproteins	CA 19-9	• **Colon carcinoma** • Pancreatic carcinoma
				CA 72-4	• **Colon carcinoma** • Gastric carcinoma

Fig. 20.67: Colorectal carcinoma. Figure shows distribution, risk factors, serum tumor markers and immunohistochemistry.

Table 20.63 Characteristics of colorectal carcinoma stem cells (CRCSCs)

Categories	Comments
Properties of colorectal carcinoma stem cells (CRCSCs)	
Self-renewal	Self-renewal is a process by which stem cells divide and proliferate with maintenance of undifferentiated state throughout life
Differentiation	Stem cell differentiation involves the changes of a cell to more specialized cells by succession of alterations in cell morphology, membrane potential, metabolic activity and responsiveness to certain signals
Heterogeneity	Stem cell heterogeneity including genetic and nongenetic mechanisms refer to biological differences amongst normal and stem cells originated within the same tissue
Plasticity	Stem cell from one tissue may be able to give rise to cell types of completely different tissue, a phenomenon known as plasticity, e.g. hematopoietic stem cells developing into cardiac muscle and hepatocytes producing insulin

Contd...

Table 20.63 Characteristics of colorectal carcinoma stem cells (CRCSCs) (*Contd...*)

Categories	Comments
Therapy resistance	Cancer stem cells, a subset of cells within the colorectal carcinoma with the potential for self-renewal, differentiation and tumorigenicity, are thought to the major cause of cancer therapy failure
Metastasis	Cancer stem cells have potential for invasiveness and metastasis of colorectal carcinoma. Epithelial–mesenchymal transition (EMT) is a highly conserved cellular process that transforms epithelial cells into mesenchymal cells. Recent studies show that there is a link between EMT and colon carcinoma stem cells formation, which might have implications in tumor progression and therapeutic resistance
CRCSCs microenvironment	
Hypoxia: HIF1a	A major regulator of cellular adaptation to hypoxia is the hypoxia-inducible factor (HIF) family of transcription factors, which play important roles in angiogenesis, stem cell maintenance, metabolic reprogram mining resistance to apoptosis, autocrine growth factor signaling in colorectal carcinoma
Endothelium: Jagged 1	Endothelial cells promote colorectal carcinoma stem cell phenotype through a soluble form of Jagged 1. Without cell to cell contact, endothelial cells synthesize soluble factor Jagged 1, via ADAM17 proteolytic activity, that lead to NOTCH activation in colorectal carcinoma cells in a paracrine manner
HGF	Tumor derived human growth hormone (HGH) has been implicated in colorectal carcinoma development and progression. However, HGH role in colorectal carcinoma is yet to be established
CRCSCs and metastasis	
CD44	HNRNPLL, a newly identified colorectal carcinoma metastasis suppressor gene modulates alternative splicing of CD44 during epithelial–mesenchymal transition. CD44 mutation has been associated with colorectal metastasis
CD26	CD26, dipeptidyl peptidase IV is a multifunctional cell surface glycoprotein, that participates in colorectal cancer progression, tumor cell invasion and metastasis
EMT	Epithelial–mesenchymal transition (EMT) is a highly conserved cellular process that transforms epithelial cells into mesenchymal cells. Recent studies show that there is a link between EMT and carcinoma stem cells formation, which might have implications in tumor progression and therapeutic resistance
DICER1	Impaired function of DICER1 function causes upregulation of colon cancer stem cells and distant metastasis
MiR-146a-5p	MiR-146a-5p functions as tumor suppressor in various cancers. Epigenetic changes play an important role in the development of colorectal carcinoma. Overexpression of UHRF1, as an epigenetic regulator downregulates MiR-146a-5p tumor suppressor resulting in invasion and distant metastasis
HGF	Tumor derived human growth hormone (HGH) has been implicated in cancer development and progression
SDF-1	There has evidence showing that CXCR-4 chemokine receptor and its ligand, stromal cell derived factor 1 (SDF-1) plays an important role in colon carcinoma progression and metastasis
CCL9	BCL-9 promotes tumor progression by conferring enhanced proliferative, metastatic, and angiogenic properties of cancer stem cells in colon carcinoma
CRCSC signaling pathways	
Wnt/β-catenin pathway	Recent advances in high-throughput sequences revealed recurrent mutation in Wnt signaling pathway mutations in intestinal stem cell in addition to β-catenin in colorectal carcinoma (Table 20.64)
NOTCH miRNA-34a pathway	NOTCH signaling pathway promotes serrated neoplasia pathway in colorectal carcinoma through epigenetic modifications of EPHB2 and EPHB4
Micro-RNA-34a pathway	Micro-RNA-34a signaling pathway is tumor suppressor. Its dysregulation has been implicated in colorectal carcinoma
Hedgehog pathway	Hedgehog signaling pathway normally plays an important role in the embryogenesis and somatic development. Abnormal activation of this pathway has been linked to colorectal cancer
TGF-β pathway	TGF-β signaling pathway normally participates in cell proliferation, differentiation, migration and apoptosis. It is one of the most commonly altered cellular pathways in colorectal carcinoma (30%) and other human cancers
p53 pathway	The p53 signaling pathway is the most commonly mutated gene in colorectal cancer in proximal colon carcinoma (34%), distal colorectal carcinoma (42%) and other cancers. The p53 gene mutation plays an important role in the adenoma–carcinoma transition during tumor pathologic process

Contd...

Table 20.63 Characteristics of colorectal carcinoma stem cells (CRCSCs) (*Contd...*)

Categories	Comments
NF-κB pathway	The nuclear factor-kappa B (NF-κB) signaling pathway is a regulator of immune response and inflammation that has been implicated in the carcinogenic process of colorectal carcinoma
ID1/ID3 pathway	Activation of ID1/ID3 pathway governs colon carcinoma-initiating cell-renewal through cell-cycle restriction driven by the cell-cycle inhibitor. Additionally, silencing ID1/ID3 signaling pathway increases sensitivity of colon carcinoma-initiating cell to the chemotherapeutic agents
P13K pathway	Alterations of the P13K pathway are common in colorectal carcinoma, and the activation of the pathway either through PIK3A and PIK3CB, mutation or amplification of AKT, loss of function of PTEN and hyperactivation of mTORC1 could be contributory to the malignant transformation of benign lesions. One of the important current drug targets is the phosphatidylinositol 3-kinase (P13K) pathway to treat colorectal carcinoma

Table 20.64 The Wnt/β-catenin pathway and familial adenomatous polyposis linked to colorectal carcinoma

Signaling of the Wnt/β-catenin pathway is mediated through the ligands of Frizzled family of receptors, and the coreceptor LRP5 or LRP6. In the absence of the ligand, the Wnt pathway is inactive through the negative regulation of the downstream effector β-catenin
When β-catenin present in sufficient quantity in the nucleus, it stimulates transcription of target genes (c-Jun, c-Myc, cyclin D1)
Lack in Wnt/β-catenin signaling through the Frizzled receptor results in sequestration of β-catenin in a multiprotein complex including APC. Auxin and other proteins which exert a negative effect both through the cytoplasmic sequestration of β-catenin and by targeting it for destruction by proteasome through phosphorylation
In the presence of Wnt ligand, the Frizzled receptor and LRP5/LRP6 form a complex, which results in the recruitment and sequestration of Auxin at the cell surface, thereby inhibiting the kinase activity of APC complex. This results in the stabilization and nuclear translocation of β-catenin, thus resulting in transcription of target genes (c-Jun, c-Myc, cyclin D1)
In the setting of an APC gene mutation, the multiprotein APC/Auxin/β-catenin complex is disrupted, most commonly due to truncation of the APC protein in domains responsible for protein-protein interaction. This results in stabilization and nuclear transcription of β-catenin with the net effect of constitutively active transcription of growth-promoting genes and otherwise under tight regulation. Patient develops familial adenomatous polyposis with >500–2500 polyps in the colon linked to colorectal carcinoma

- Two distinct pathways have been described such as APC/β-catenin pathway and classic adenoma-carcinoma sequence with microsatellite pathway. Both pathways involve stepwise accumulation of multiple mutations. The Wnt/β-catenin pathway and familial adenomatous polyposis are given in Table 20.64.
- Oncogenes, their mode of activation and associated with colon carcinoma are given in Table 20.65. Tumor suppressor gene mutations associated with colon carcinoma are given in Table 20.66. Morphologic and molecular changes in the adenoma-colon carcinoma sequence are shown in Fig. 20.68.

APC/β-Catenin Signaling Pathway

The APC/β-catenin signaling pathway is essential for intestinal homeostasis. It is aberrantly activated in most colorectal carcinomas through a mutation of tumor suppressor APC gene mapped on chromosome 5q21. APC protein is an essential component of cytoplasmic protein complex that targets β-catenin is for destruction. β-Catenin is a multifunctional protein, which maintains intestinal homeostasis.

Table 20.65 Oncogenes, their mode of activation and associated with colon carcinoma (signal transduction proteins)

Category	Proto-oncogene	Mode of Activation	Associated Malignancies
GTP-binding (G) proteins	K-RAS gene	Point mutation	▪ *Colon carcinoma* ▪ Lung carcinoma ▪ Pancreas carcinoma
RAS signal transduction	BRAF gene	▪ Point mutation ▪ Translocation	▪ *Colon carcinoma* ▪ Melanoma ▪ Leukemia

Table 20.66 Tumor suppressor genes associated with colon carcinoma

Gene	Protein	Function	Familial Tumors	Sporadic Cancers
▪ SMADA-2 ▪ SMADA-4	▪ SMADA-2 protein SMADA-4 protein	▪ Both inhibit TGF-β signaling pathway. These repress CDK4 and MYC ▪ These also induce expression of CDK inhibitor	Juvenile polyposis	▪ *Colon carcinoma* ▪ Pancreas carcinoma
RB gene	▪ Retinoblastoma protein (locus 13p14) ▪ Mutation by deletion and nonsense	It codes for pRB protein, master brake on cell cycle by inhibiting nuclear transcription and G1 to S transition during cell cycle	▪ Retinoblastoma ▪ Osteosarcoma	▪ Retinoblastoma ▪ Osteosarcoma ▪ Breast carcinoma ▪ *Colon carcinoma* ▪ Prostate carcinoma ▪ Urinary bladder carcinoma ▪ Lung carcinoma
Angiogenesis inhibitors				
STK11	Liver kinase B1 (LKB1) or STK11	It activates AMPK family of kinases. It suppresses cell growth when cell nutrients and energy levels are low	*Peutz-Jeghers syndrome:* Cancers of GIT, pancreas	Diverse carcinomas in 5–20% cases

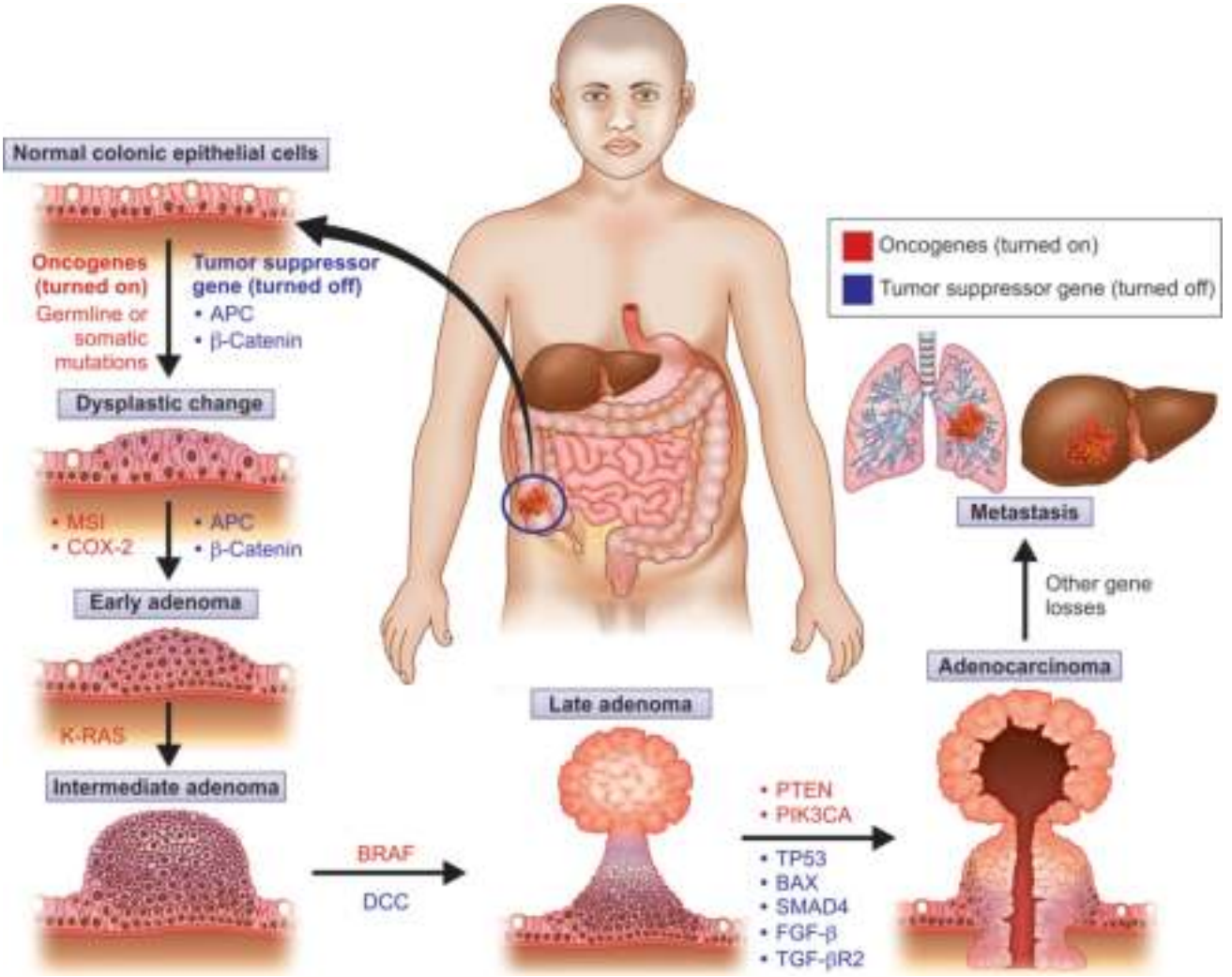


Fig. 20.68: Morphologic and molecular changes in the adenoma-colon carcinoma sequence. It is postulated by Knudson's hypothesis that loss of normal copy of tumor suppressor APC gene occurs early in life (first hit). This is the first hit. Loss of intact copy of APC gene follows (second hit). Other gene mutations include K-RAS, losses at 18q21 involving SMAD2 and SMADA4 and inactivation of tumor suppressor p53 gene. It leads to the emergence of colorectal carcinoma and metastases, in which additional gene mutations occur.

Classic Adenoma-Colon Carcinoma Sequence with Microsatellite Instability Signaling Pathway

Classic adenoma-carcinoma sequence with microsatellite pathway is associated with defects in DNA mismatch repair. It accounts for 80% of sporadic colorectal carcinomas.

- **First hit (normal mucosa to mucosa alteration):** Gene mutations in the development of colon carcinoma may be inherited (germline) or acquired (somatic).
  - Mutations of tumor suppressor APC gene (5q21) and mismatch repair genes such as MSH2 at 2p22 participate in alteration of normal mucosa.
  - APC is a key negative regular of β-catenin, a component of Wnt signaling pathway.
  - Mutation of APC gene has been demonstrated in sporadic cancers (colon, stomach and pancreas) and familial adenomatous polyposis.
  - Under physiologic state, APC gene prevents nuclear transcription by degrading β-catenin, an activator of nuclear transcription.
  - With loss of function of APC, accumulation of β-catenin activates transcription of genes encoding MYC and cyclin D1 leading to cell proliferation.
  - Additional mutations of K-RAS have been observed in adenomas and invasive colorectal carcinomas.
- **Second hit (altered mucosa to adenoma):** Methylation abnormalities, inactivation of normal alleles of APC, and MSH2 transform altered mucosa to adenoma.
- **Adenoma to colonic malignant transformation:** K-RAS proto-oncogene is located on chromosome 12p—a signal transducing protein. Point mutation of K-RAS has been identified in colon carcinoma in 50% of cases. There is as well as homozygous loss of additional cancer suppressor genes (DCC at 18p21, p53 at 17p13) participate in transformation of adenoma to colon carcinoma.
- **Microsatellite instability (MSI) mechanism:** Additional mutations and gross chromosomal alterations such as loss of DNA repair genes mutated TGF-β receptor gene and defective BAX gene produce marked changes in colon carcinoma growth.

Neoplastic progression is also associated with mutations of tumor suppressor genes encoding SMADA2 and SMADA4, which are effectors of TGF signaling. Under physiologic state, TGF signaling inhibits the cell cycle.

- **Defects in DNA mismatch repair genes:** DNA mismatch repair genes include principally MLH1, MSH2, MSH6, and PMS2. Normally, DNA repair genes correct errors in nucleotide pairing.
  - Mutations of DNA mismatch repair genes cannot correct errors in nucleotide pairing. There is increased risk of development of carcinoma of right colon, known as hereditary nonpolyposis colon cancer (HNPCC or Lynch syndrome). It is often preceded by serrated adenomas, rather than tubular adenomas seen in the traditional APC tumor pathway.
  - Extensive history of screening of early development of colorectal carcinoma is required in the management of these patients. Sporadic mismatch repair defects may also occur and are usually related to abnormal methylation.
- **TP53 tumor suppressor gene mutation:** TP53 mutation is associated with colon carcinoma. It is a gatekeeper tumor suppressor gene located on chromosome 17. The p53 gene product is a nuclear binding protein that halts cell cycle in G1 by inhibiting nuclear transcription factor, until the mutation is repaired.

Clinical Correlation

Left-sided colon carcinoma is most likely to obstruct lumen, while right-sided colon carcinoma is more likely to bleed. Many colorectal carcinomas are within the reach of the finger (during digital rectal examination) or the rectosigmoidoscope. Patient presents with occult bleeding, change in bowel habits, loss of weight, loss of appetite and anemia.

Clinical manifestations according to location of carcinoma in large bowel are given in Table 20.67. Differences between right-sided colon carcinoma and left-sided colorectal carcinomas are given in Table 20.68.

Table 20.67 Clinical manifestations according to location of carcinoma in large bowel

Symptoms	Right-sided Colon Carcinoma (Frequency %)	Left-sided Colon Carcinoma (Frequency %)	Carcinoma Rectum (Frequency %)
Pain abdomen	80	60	5
Mass abdomen	70	40	0
Rectal bleeding	20	20	60
Change in bowel habit	40	60	80
Weight loss	50	15	25
Vomiting	30	10	0
Obstruction	5	20	5

Table 20.68 Differences between right-sided colon carcinoma and left-sided colorectal carcinomas

Characteristics	Right-sided Colon Carcinoma	Left-sided Colorectal Carcinoma
Location of growth	Cecum and ascending colon	Descending colon and rectosigmoid region
Growth pattern	Usually fungating or polypoid large cauliflower-like, soft friable growth projecting into the lumen (rarely napkin-ring type)	Napkin-ring configuration as a result of fibrosis. It leads to obstruction of lumen
Invasion of growth	More invasive growth	Less invasive growth
Clinical correlation	Silent lesion and diagnosed later	Obstructive lesion, so diagnosed early
Histologic features	Similar	Similar
Involvement of other structures	More common such as mesentery, lymph nodes, distant metastases	Less common such as mesentery, lymph nodes, distant metastases
Prognosis	Good	Poor

Right-sided Colon Carcinoma

- Adenocarcinoma of ascending and transverse colon tend to be exophytic, polypoidal growth, or crater-like ulcerations with raised margins, and commonly present with bleeding per rectum. Right-sided colon carcinoma is more invasive and metastasizes early as compared to left-sided lesions.
- Patient presents with black, tarry stools, rectal bleeding, anemia, abdominal aching, pressure, or dull cramps, weakness, fatigue, exertional dyspnea, diarrhea, constipation, anorexia, weight loss and vomiting.

Left-sided Colorectal Carcinoma

- Adenocarcinoma of the descending colon, rectum or sigmoid colon often presents as a circumferential annular/napkin ring configuration mass narrowing the intestinal lumen. It produces early obstruction of colon and rectum lumen.
- Patient presents with black, tarry stools or rectal bleeding, intermittent abdominal fullness or cramping; rectal pressure, constipation, diarrhea or ribbon-like stool, and dark or bright red blood in stool; mucus in or on stool.

Surgical Pathology: Colorectal Adenocarcinoma

Gross Morphology

- Colorectal adenocarcinomas show wide range of gross appearances including fungating intraluminal growth, ulcerated growth with heaped up edges and circumferential growth.
- Tumor is firm with grey white appearance.
- Approximately 10% are mucinous adenocarcinomas (Figs 20.69 and 20.70).

Light Microscopy

- Classic colorectal adenocarcinoma is composed of irregularly distributed tubular structures in a desmoplastic stroma. There is presence of complex cribriform architecture and intraluminal necrosis. It shows abundant pool of mucin in 50% of the tumor area.
- Signet ring adenocarcinoma is rare in colon and composed of cells with abundant intracytoplasmic vacuoles arranged in diffuse infiltrative cords, nests and sheets (Fig. 20.71).

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in conventional colon adenocarcinoma (microsatellite stable) includes CEA, CK7, CK20, CDX2 (specific marker associated with poor prognosis), SAT, β-catenin and villin. TTF-1, PAX2 and WT1 markers are negative in conventional colon adenocarcinoma.

Markers	Expression
Carcinoembryonic antigen (CEA)	Positive
Cytokeratin 7 (CK7)	Positive
Cytokeratin 20 (CK20)	Positive
CDX2 (transcription factor)	Positive
SAT (transcription factor)	Positive
β-Catenin	Positive
p53	Positive
Villin	Positive
TTF-1	Negative
PAX2	Negative
WT1	Negative

Mode of Spread

Right-sided colon carcinoma is more invasive that metastasizes early as compared to left-sided colon carcinoma.

- **Lymphatic route:** Colorectal carcinoma invades lymphatic channels and initially involves the lymph nodes immediately underlying the tumor.
- **Hematogenous route:** Blood-borne metastases from colorectal carcinoma is usually seen in the liver and then lungs. Solitary liver metastasis may be treated by wedge resection giving months to years of symptom-free remission. Multiple liver metastases are not resectable. These patients may respond to chemotherapy for months/years.

Prognostic Factors

Poor prognostic factors of colorectal carcinoma include high tumor stage, poor differentiation of colon

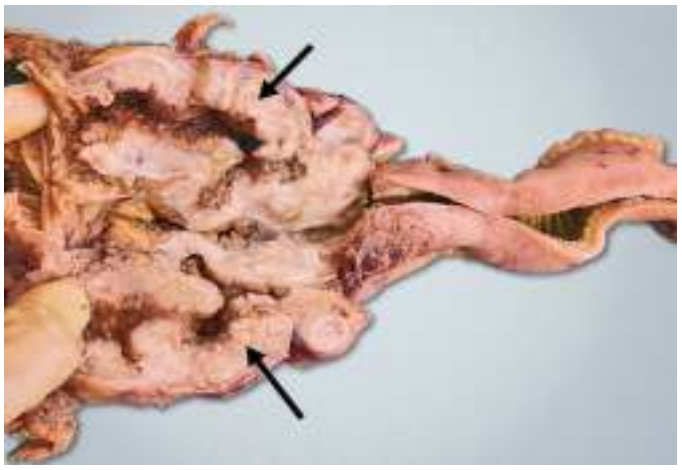


Fig. 20.69: Carcinoma in cecum. Tumor shows fungating intraluminal growth. Tumor is firm with gray white appearance. Cut surface is gray white (arrows). (*Courtesy:* Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)



Fig. 20.70: Carcinoma in rectum. Tumor shows fungating and ulcerated intraluminal growth. Tumor is firm with grey white appearance. Cut surface is gray white with areas of necrosis (arrow).

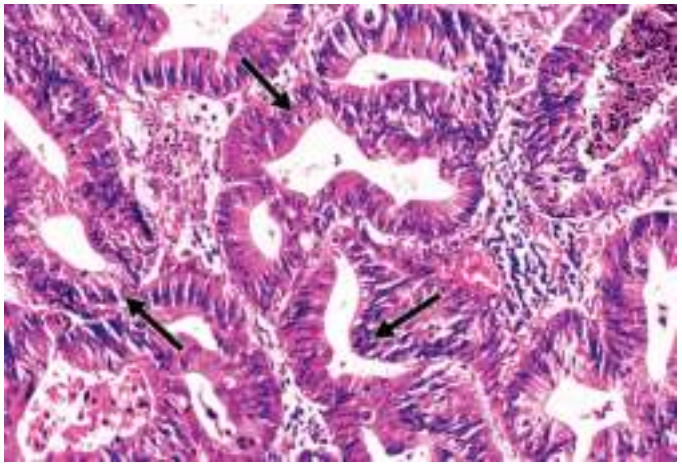


Fig. 20.71: Classic colorectal adenocarcinoma. Tumor is composed of irregularly distributed tubular structures in a desmoplastic stroma (arrows) (400X).

Table 20.69 Molecular genetic markers associated with good prognosis in colorectal carcinoma

Low recurrence score is analyzed by oncotype Dx colon cancer assay (current validated marker)
Low risk analyzed by color print (current validated marker)
No deletion of chromosomes 18q (DDC or SMAD4 genes in this region) is analyzed by cytogenetic study
No deletion of chromosomes 17p and 8 is analyzed by cytogenetic study
Absence of TP53 gene mutation
Loss of CDKN1B (p27) expression
High-frequency of mismatch repair status (deficient microsatellite instability) analyzed by immunohistochemistry
Absence of K-RAS and N-RAS gene mutation is associated with good prognosis. RAS gene mutations are demonstrated in 40–50% of colorectal carcinomas. Anti-EGFR therapy is ineffective in these colorectal carcinomas with RAS gene mutations
Absence of BRAF gene mutation is associated with good prognosis. BRAF gene mutation is demonstrated in colorectal carcinomas. Anti-EGFR therapy is ineffective in these colorectal carcinomas with RAS gene mutations. Anti-EGFR therapy is ineffective in these colorectal carcinomas with BRAF gene mutations

carcinoma, lymphovascular and/or perineural invasion and age group more than 40 years. By convention, signet ring cell carcinoma, small cell carcinoma and undifferentiated carcinoma are high-grade neoplasms associated with poor prognosis.

Complications

Complications of colorectal carcinoma include obstruction, hemorrhage, perforation and infection. Molecular genetic markers associated with good prognosis in colorectal carcinoma are given in Table 20.69.

Clinical Pearls: Diagnostic Modalities of Colon Carcinoma

- **Serum tumor markers:** Serum carcinoembryonic antigen (CEA), CA 19-9 and CA 72-4 levels are increased in colon carcinoma. Serum tumor markers used to detect colorectal carcinoma are given in Table 20.70.
- **Digital rectal examination:** Digital rectal examination is carried out to feel the growth in the rectum and anal canal.
- **Barium enema:** Barium enema with or without contrast media is used to visualize the local deformities of intestinal topography.
- **Sigmoidoscopy:** Sigmoidoscopy rigid or flexible fiber optic type is performed to visualize rectal tumors or routine screening.
- **Colonoscopy:** Colonoscopy is used to visualize the colon for detection of early polypoidal tumors during preoperative period and recurrences after surgical resection. Multiple biopsies may be obtained for histologic examination.
- **Computed tomography (CT):** Computed tomography is performed to stage disease and detection of metastases.

- **Magnetic resonance imaging (MRI):** Magnetic resonance imaging is performed to detect metastatic disease.
- **Transrectal ultrasonography:** Transrectal tomography is an excellent choice for staging rectal carcinoma.
- **Laparotomy:** Laparotomy is performed to detect metastases to abdominal regions especially liver and omentum, that often remains undetected by current imaging techniques.

Therapeutic Response

Epidermal growth factor receptor (EGFR) inhibitor drugs, e.g. cetuximab and panitumumab are effective thereby for EGFR positive colorectal carcinoma especially when the cancer stem cells are negative for both BRAF and K-RAS gene mutations.

Prognosis

The grade and stage of the tumor (Astler-Coller system) determine the prognosis of colorectal carcinoma. Location of the tumor, extent of bowel involvement, histologic grading and presence or absence of metastasis. The overall 5-year survival regardless of the stage is 35%.

Table 20.70 Serum tumor markers used to detect colorectal carcinoma

Category	Tumor Markers	Examples	
Oncofetal proteins	Carcinoembryonic antigen (CEA)	▪ *Colon carcinoma* ▪ Nonseminomatous germ cell testicular tumor ▪ Stomach carcinoma	▪ Pancreatic carcinoma ▪ Lung carcinoma ▪ Breast carcinoma
Mucins and glycoproteins	▪ CA 19-9 ▪ CA 72-4	▪ *Colon carcinoma* ▪ *Colon carcinoma*	▪ Pancreatic carcinoma ▪ Gastric carcinoma

DISORDERS OF APPENDIX

ANATOMY AND HISTOLOGY

The appendix is a tubular structure extending from the cecum. It has an average length 7–10 cm. It is lined by colonic-type mucosa, surrounded by submucosa and muscular coats.

- In children and young adults, the mucosa contains numerous lymphoid follicles. In the elderly persons, the lumen often shows fibrous obliteration.
- Appendix fills with food and empties regularly into the cecum. Because appendix empties insufficiently due to its small lumen, therefore, it is prone to obstruction and vulnerable to infection.

INFLAMMATORY DISORDERS

ACUTE APPENDICITIS

Acute appendicitis is bacterial infection associated with proximal obstruction of the lumen. It is a common cause of acute abdomen. It is most frequent in adolescents and young adults and has no sex predilection.

Etiopathogenesis

Appendix becomes inflamed and edematous as a result of lumen occlusion by fecolith or carcinoid tumor. It leads to increase in intraluminal pressure, ischemic mucosal injury, and secondary bacterial infection. Other causes of appendix lumen occlusion include submucosal lymphoid hyperplasia in the course of viral infection, pinworms, carcinoid tumor of appendix and barium from X-ray studies.

Clinical Features

Patient with acute appendicitis presents with pain in periumbilical region (central abdomen) radiating to right iliac quadrant. Systemic symptoms include fever, nausea and vomiting. Rebound tenderness may be demonstrated by pushing the abdominal wall at McBurney's point.

- Atypical presentation is encountered if the appendix is in retroperitoneal or intrapelvic location.
- Abdominal mass in the right lower quadrant may be palpated following a rupture of appendix and formation of a periappendiceal abscess. Pathologic disorders confused with acute appendicitis are given in Table 20.71.

Surgical Pathology: Acute Appendicitis

Gross Morphology

- External surface of appendix is congested appendix and distal half covered by purulent exudate.
- The lumen contains a purulent exudate and often a fecolith (Fig. 20.72).

Light Microscopy

- Diagnostic criteria of acute appendicitis include infiltration of muscular coat of appendix by polymorphonuclear infiltration.
- Acute inflammatory infiltrate extends from the mucosa through the full thickness of the appendiceal wall (Fig. 20.73).

Complications

If untreated by surgical resection, acute appendicitis most often leads to perforation or periappendiceal abscess, or both. Transmural necrotizing inflammation of the appendix results in peritonitis. Patient may develop septicemia resulting in pylephlebitis (inflamed thrombosis of the portal vein), liver abscess and subphrenic abscess.

Laboratory Diagnosis

Peripheral smear examination shows leukocytosis with increased neutrophil count. Ultrasound and X-ray are performed in atypical cases of appendicitis prior to surgery.

Table 20.71 Pathologic disorders confused with acute appendicitis

Mesenteric lymphadenitis
Acute salpingitis
Acute cholecystitis
Ectopic pregnancy
Ruptured ovarian follicle
Torsion ovarian cyst
Inflammatory bowel disease
Acute pyelonephritis and urinary tract infection



Fig. 20.72: Acute appendicitis. It shows congestion on the external surface of inflamed appendix.

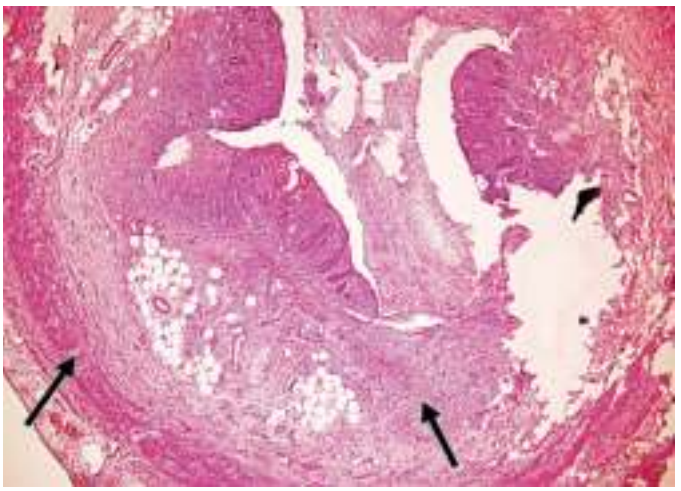


Fig. 20.73: Diagnostic criteria of acute appendicitis. It shows neutrophilic infiltration in the muscular coat (arrows) (100X).

Therapeutic Correlation

The inflamed appendix in acute appendicitis should be surgically removed because of possible devastating complications of perforation or abscess.

Precautions Prior to Surgery

Yersinia enterocolitica can cause mesenteric granulomatous lymphadenitis in children. Patient presents with pain in the right lower quadrant (pseudoappendicitis), diarrhea, reactive arthritis, erythema nodosum, and septicemia. Infected children frequently undergo laparotomy due to a mistaken diagnosis of appendicitis.

MUCOCELE OF APPENDIX

Mucocele of appendix refers to a dilated mucus-filled appendix and is based on the gross and microscopic appearance of appendix.

Pathogenesis

Mucocele of appendix occurs due to chronic obstruction and mucinous cystadenoma or mucinous cystadenocarcinoma.

Complications

Rupture of a neoplastic mucocele may seed the peritoneal cavity with mucus-secreting tumor cells, a condition referred to as pseudomyxoma peritonei.

NEOPLASTIC DISORDERS OF APPENDIX

CARCINOID TUMOR

Carcinoids are neuroendocrine tumors of low-grade malignancy. These are most commonly located in the submucosa of the intestines (e.g. appendix, terminal

ileum, and rectum). The most common appendiceal neoplasm is neuroendocrine (carcinoid) tumor, which is usually detected as an incidental finding. Tip of the appendix is the most common site. Common benign and malignant tumors of the appendix are given in Table 20.72.

Clinical Course

Clinical course of carcinoid tumor of appendix depends on size of the neoplasm.

- **Carcinoid tumor of appendix <2 cm:** It is considered benign. It rarely metastasizes. Perineural or lymphovascular is not indicative of malignant potential.
- **Carcinoid tumor of appendix >2 cm:** It invades mesoappendix and metastasizes to regional lymph nodes, liver, lungs and bones in 66% of cases.

Table 20.72 Common benign and malignant tumors of appendix

Benign Tumors of Appendix	
Adenoma	
▪ Villus adenoma	▪ Tubulovillus adenoma
▪ Tubular adenoma	▪ Cystadenoma
Serrated lesions	
▪ Hyperplastic polyp	▪ Traditional serrated adenoma
▪ Sessile serrated adenoma	
Carcinoid tumor (neuroendocrine tumor)	
Malignant Tumors of Appendix	
Mucinous adenocarcinoma	
Neuroendocrine carcinoma	
Goblet cell carcinoid	
Mixed adenoneuroendocrine carcinoma (also known as adenocarcinoma ex-goblet cell carcinoid)	

DISORDERS OF ANUS AND PERIANAL REGION

ANATOMY AND HISTOLOGY

Anal canal is present at the terminal end of gastrointestinal tract. It measures 3–4 cm in length. The mucosal lining the upper region of the anal canal is extension of rectal mucosa. The mucosa lining the middle region of the anal canal is known as transition zone measuring 0.5–1 cm above the dentate line has features of both metaplastic squamous mucosa and urothelium.

The mucosa of distal portion of anal canal extending from the dentate line to the anal verge, is composed of specialized nonkeratinizing squamous epithelium with melanocytes. Important disorders of anorectal region are shown in Fig. 20.74.

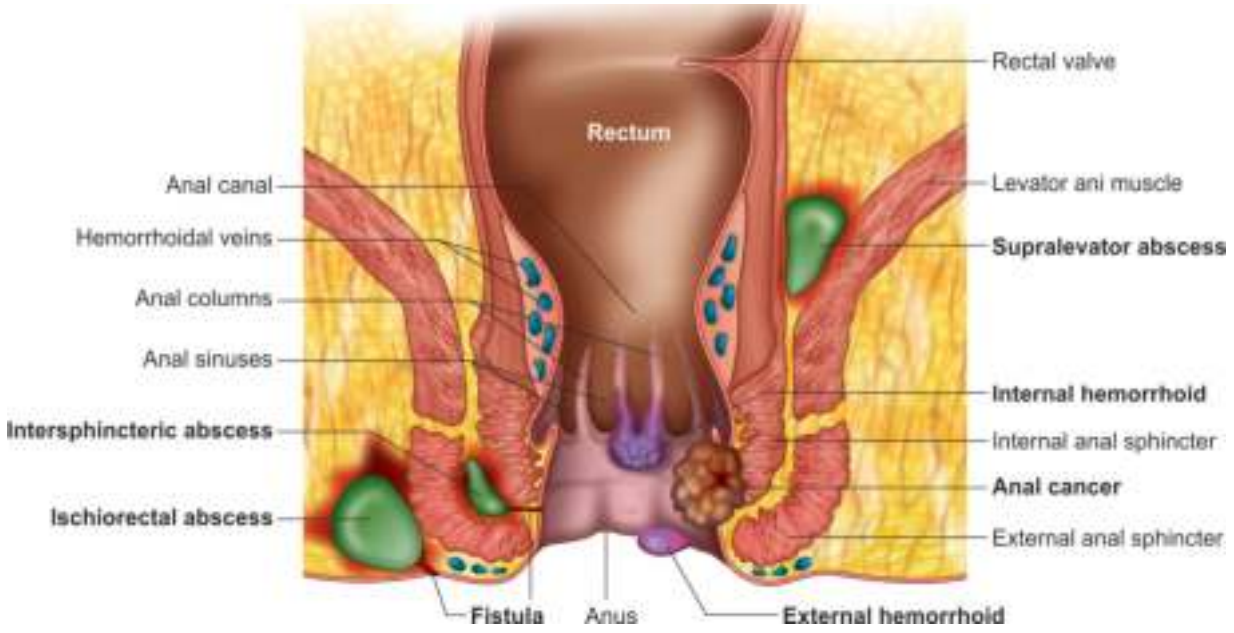


Fig. 20.74: Anatomy of anal canal and important disorders. The upper portion of the anal canal consists of colorectal-like columnar epithelium. The middle portion of the anal canal above dentate line is lined by transitional epithelium, which is squamous metaplasia of the columnar epithelium. The lower portion of the anal canal is lined by nonkeratinized stratified squamous epithelium devoid of epidermal appendages (hair follicles, apocrine glands and sweat glands). Beyond the anal verge, perianal skin is hair-bearing keratinizing squamous epithelium.

VASCULAR DISORDERS OF ANAL CANAL

HEMORRHOIDS

Hemorrhoids are painless dilated venous plexuses in the anal canal. All hemorrhoids result from increased portal venous pressure.

- Precipitating factors for hemorrhoids include chronic constipation, straining at stool, pregnancy, obesity, chronic liver disease and low fiber diet.
- Hemorrhoids should not be diagnosed unless prolapse or bleeding per rectum is a dominant symptom. Classification of hemorrhoids is given in Table 20.73. Investigation of rectal bleeding is given in Table 20.74.

External Hemorrhoids

External hemorrhoids originate from below the anorectal junction. It is covered by anal skin. It occurs as a result of dilation of inferior hemorrhoidal veins.

- These are predisposed by a low fiber diet. When thrombosed, these contain clotted blood.
- These become painful, swollen, shiny blue and itchy resulting in bleeding during defecation. Resolution of external hemorrhoids leaves a painless flabby skin sac around the anal orifice.

Table 20.73 Classification of hemorrhoids

Degree	Features
First degree	Bleeding
Second degree	Prolapse and reducing spontaneously
Third degree	Prolapse needing pushing back
Fourth degree	Prolapse permanent

Treatment of hemorrhoids is based on symptoms rather than appearances.

Table 20.74 Investigation of rectal bleeding

Symptoms (Bright Red Blood)	Diagnostic Tools
Bright red blood seen only on paper	▪ Inspection of stool ▪ Rectal examination ▪ Proctoscopy ▪ Rigid sigmoidoscopy
Bright red blood drippling into pain	▪ Proctoscopy ▪ Rigid sigmoidoscopy
Bright red blood mixed with motion	▪ Proctoscopy ▪ Rigid sigmoidoscopy ▪ Flexible sigmoidoscopy
Dark red blood	▪ Proctoscopy ▪ Rigid sigmoidoscopy ▪ Flexible sigmoidoscopy ▪ Barium meal
Anyone over 40 years of age and family history of colorectal anaplasia	Flexible sigmoidoscopy

Internal Hemorrhoids

Internal hemorrhoids originate from dilatation of superior hemorrhoidal veins above the anorectal junction and are covered by mucous membrane.

When the person performs Valsalva maneuver, internal hemorrhoids appear as red mucosal mass may protrude from anus. Internal hemorrhoids are not palpable.

NON-NEOPLASTIC DISORDERS OF ANAL CANAL

PILONIDAL SINUS

Pilonidal sinus is hair containing cyst or sinus located in the midline over the coccyx or lower sacrum.

- On clinical examination, it opens up as a dimple with visible hair and possibly, an erythematous halo.
- Pilonidal sinus may appear as a palpable cyst. When pilonidal sinus is enlarged, it has a palpable sinus tract. Although it is congenital disorder, yet the lesion is diagnosed between the ages of 15 or 30 years.

ANORECTAL FISTULA

Chronic inflammation in anorectal region creates an abnormal passage from inner anus or rectum out to skin surface surrounding anus, which is known as anorectal fistula. It most often occurs due to local abscess. Patient presents with serosanguineous or purulent discharge from red raised tract opening anorectal fistula on applying pressure. Bidigital palpation may reveal an indurated cord.

ANAL FISSURE

Anal fissure is a longitudinal tear in the superficial mucosa at the anal margin. More than 90% of anal fissures are located in the posterior midline area, which are accompanied by hyperplastic papule known as sentinel tag. Anal fissure most often results from trauma due to passage of hard stool or irritable diarrheal stool.

Patient presents with pain during and after bowel action, minor bleeding, itching, constipation, and painful spasm of sphincter.

RECTAL MUCOSAL PROLAPSE

The rectal mucosa protruding through the anus is known as rectal mucosal prolapse, which appears as moist red doughnut with radiating lines.

- **Incomplete rectal mucosal prolapse:** When prolapse is incomplete, only the mucosa is bulging.
- **Complete rectal mucosal prolapse:** When complete rectal mucosal prolapse occurs, which includes the anal sphincter following Valsalva maneuver such as straining at stool or exercise.

Predisposing Factors

Predisposing factors for rectal mucosal prolapse include constipation, excessive straining at defecation, deep pouch of Douglas, redundant sigmoid colon, decreased sigmoid colon, decreased anal canal sphincter tone and reduced pelvic support.

Other conditions causing rectal mucosal prolapse are large hemorrhoids, prolapsing rectal tumor, anal warts and polyps.

Surgical Pathology: Rectal Mucosal Prolapse

Light Microscopy

Histologic examination shows mucosal surface erosion, epithelial hyperplasia, mixed inflammatory infiltrates and lamina propria fibromuscular hyperplasia.

Clinical Features

Patient presents with rectal mucosal prolapse related symptoms, i.e. defecation, mucus discharge, bleeding, and incontinence.

PRURITUS ANI

Pruritus ani is characterized by sensation of itch, burning or pain. It is more common in men than women.

Etiopathogenesis

Pruritus ani is idiopathic in 50% of cases. Anorectal causes of pruritus ani include fissures, fistula, papilloma, skin tags, hemorrhoids and prolapse. Dermatological causes are psoriasis, lichen planus, Paget's disease, candidiasis and thread worms. Dietary factors implicated in pruritus ani include coffee, tea, spices, pork, alcohol, tomatoes and drugs (e.g. antibiotics, laxatives, colchicine, and quinidine).

Clinical Features

Patient presents with slight itch in perianal skin that may progress to severe itch especially during summer.

Management

Secondary causes of pruritus ani should be identified and treated. Patient should be advised about personal hygiene.

NEOPLASTIC DISORDERS OF ANAL CANAL

Anal cancer is relatively uncommon. It accounts for about 4% of anorectal malignancies. Various anal neoplasms include squamous cell carcinoma, malignant melanoma, lymphoma and anal gland adenocarcinoma. Overall anal cancers are more common in women. Lesions at the anal margins are more common in men. Revised (2019) WHO classification of tumors of the anal canal is given in Table 20.75.

Table 20.75 Revised (2019) WHO classification of tumors of the anal canal

Benign Epithelial Tumors and Precursor Lesions
▪ Squamous intraepithelial neoplasia, low-grade
▪ Squamous intraepithelial neoplasia, high-grade
Malignant Epithelial Tumors
▪ Squamous cell carcinoma, NOS
• Verrucous squamous cell carcinoma
▪ Adenocarcinoma, NOS
Neuroendocrine Tumors
▪ Neuroendocrine tumor, NOS
• Neuroendocrine tumor, grade 1
• Neuroendocrine tumor, grade 2
• Neuroendocrine tumor, grade 3
▪ Neuroendocrine carcinoma, NOS
• Large cell neuroendocrine carcinoma
• Small neuroendocrine tumor carcinoma
Mixed Neuroendocrine–Non-neuroendocrine Neoplasm (MiNEN)

Term NOS, not otherwise specified.
Adapted from 2019 WHO classification of tumors of the anal canal.

ANAL CARCINOMA

Anal carcinoma arises at or above the dentate line and not visible at the anus. Anal carcinoma arising below the dentate line is visible at the anus. Anal carcinoma behaves like basal cell carcinoma and has favorable prognosis.

Etiopathogenesis

There is increased incidence of anal cancer among male homosexuals. Approximately 50% of anal cancers are associated with human papillomavirus infection.

Clinical Features

Patient presents with bleeding, pain, swelling and ulceration around anus. As the anal cancer advances, patient suffers worsening pain, disturbed bowel habit, urinary incontinence and rectovaginal fistula.

- Approximately, 50% of anal cancers are diagnosed before the malignancy has spread beyond primary site.
- About 13–25% are diagnosed after the anal cancer has spread to inguinal lymph nodes and 10% to distant organs. Delay in presentation and diagnosis cause direct as well as distant metastases.
- Early anal cancers may be confused with warts, fissures, hemorrhoids leading to delayed diagnosis.

Epidermoid Carcinoma

Epidermoid carcinoma accounts for about 80% of anal cancers. The tumor is most frequently encountered in the anus at anorectal junction in the male homosexual population. HPV types 16 and 18 are linked to epidermoid carcinoma.

Surgical Pathology: Anal Carcinoma
Gross Morphology
Anal cancers appear as ulcers or proliferative growth with ulcerated areas.
Light Microscopy
On histologic examination, tumor varies predominantly well-differentiated keratinizing large cell carcinoma at the anal margin to less differentiated nonkeratinizing to small cell carcinoma in the upper anal canal (Fig. 20.75).

Basaloid Carcinoma

Basaloid carcinoma occurs at the squamocolumnar junction or transitional zone, sometimes called cloacogenic carcinoma.

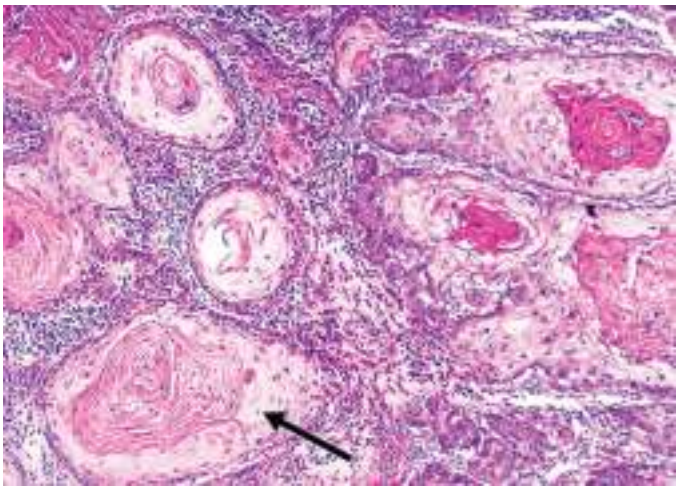


Fig. 20.75: Well-differentiated squamous cell carcinoma of anus (arrow) (400X).

DISORDERS OF PERITONEUM

ANATOMY AND HISTOLOGY

Serosal membranes are derived from the mesoderm, which form the visceral and parietal surfaces of pleural, pericardial and peritoneal cavities including tunica vaginalis. On histologic examination, serous membranes are lined by flat mesothelial cells resting on a basement membrane, below which lies a poorly delimited connective tissue layer.

NON-NEOPLASTIC DISORDERS OF PERITONEUM

ACUTE PERITONITIS

Acute peritonitis is caused due to perforation of gastric, biliary, intestine or pancreas. Spontaneous bacterial peritonitis most often occurs in children, immunocompromised persons or patients with cirrhosis.

GRANULOMATOUS SEROSITIS

Granulomatous serositis presents as small nodules in the setting of disseminated cancers. It may be caused by infections, noninfectious causes, autoimmune and meconium induced. Causes of granulomatous serositis are given in Table 20.76.

Table 20.76 Causes of granulomatous serositis

Categories	Disorders
Infectious causes	▪ Fungal infections (histoplasmosis, Cryptococcus) ▪ Parasites (Schistosomiasis, Echinococcus or Ascaris)
Noninfectious causes	▪ Sutures or starch ▪ Implants of keratin derived from mature cystic teratoma, endometrioid adenocarcinoma with squamous differentiation, squamous cell carcinoma of cervix
Autoimmune disorders	Crohn's disease or sarcoidosis
Meconium	Meconium in neonate inducing peritonitis

METAPLASIA OF PERITONEUM

Metaplasia of peritoneum most often occurs in women in the abdomen and pelvic region due to its close embryologic relationship with Müllerian ducts. Its pathogenesis is discussed below.

- **Endometriosis:** When endometriosis develops in the wall of intestine, ureter or urinary bladder, the woman presents with signs and symptoms resembling malignancy. On histologic examination, endometriosis lesion is composed of endometrial glands with stroma, dense fibrosis and adhesions. Endometriosis can induce metaplasia of peritoneum.
- **Endocervicosis:** Endocervicosis comprises benign glands resembling endocervical type of epithelium leading to metaplasia of peritoneum.
- **Ectopic decidual metaplasia:** Ectopic decidual metaplasia of peritoneum occurs in pregnant women. There is presence of small gray white nodules on the peritoneal surfaces.

- **Walthard nests:** Walthard nests appear as yellow-white nodules on the serosal surface of fallopian tubes. These may undergo cystic change. Histologic examination reveals transitional metaplasia of peritoneum.
- **Disseminated peritoneal leiomyomatosis:** It is uncommon multifocal proliferation of smooth muscle-like cells thought to represent hormone-induced metaplasia of peritoneum.

FIBROSIS OF PERITONEUM

Reactive peritoneal fibrosis occurs in the setting of recurrent peritonitis often associated with long-term peritoneal dialysis, cirrhosis, or surgery.

Sclerosing peritonitis occurs as a result of hyperplasia of submesothelial mesenchymal cells covering small intestine, diaphragm, liver, spleen in the setting of peritoneal dialysis, autoimmune disorders and carcinoid tumors.

EOSINOPHILIC PERITONITIS

Eosinophilic peritonitis occurs in the settings of childhood atopy, autoimmune collagenous disorders, hypereosinophilic syndrome, lymphomas and metastatic carcinoma.

NEOPLASTIC DISORDERS OF PERITONEUM

PRIMARY NEOPLASMS

WHO classification of tumors of peritoneum is given in Table 20.77.

Well-differentiated Papillary Mesothelioma

Well-differentiated papillary mesothelioma is a low-grade tumor associated with asbestos exposure. It most often occurs in women during reproductive period in 80% of cases. Clinical course is indolent.

Surgical Pathology: Well-differentiated Papillary Mesothelioma

Gross Morphology

Tumor is solitary or multifocal <2 cm in diameter. Cut surface is gray white exhibiting nodules and papillary structures.

Light Microscopy

Tumor is composed of papillary fronds with fibrovascular core covered by single layer of bland cuboidal to flattened mesothelial cells.

Immunohistochemistry Technique

Panel of immunomarkers is used to analyze expression in diffuse malignant mesothelioma on histologic sections.

Markers	Expression
▪ Calretinin	▪ Positive
▪ Thrombomodulin	▪ Positive
▪ WT1	▪ Positive
▪ Cytokeratin 5/6	▪ Positive

MOC-31, Ber-EP4, CA19-9 and CD15 (LeuM1) markers are negative in diffuse malignant mesothelioma. These are used to distinguish peritoneal epithelioid mesothelioma from peritoneal and ovarian serous carcinomas.

Multicystic Mesothelioma

Multicystic mesothelioma is a low-grade neoplasm also known as multicystic peritoneal inclusion cyst. On histologic examination, multilocular cysts are lined by mesothelium with bland cytologic features harboring areas of conventional malignant mesothelioma.

Diffuse Malignant Mesothelioma

Diffuse malignant mesothelioma of peritoneum is a rare neoplasm. On histologic examination, tumor resembles ectopic decidual reaction.

Table 20.77 WHO classification of tumors of peritoneum

Categories	Tumors
Mesothelial tumors	▪ Well differentiated papillary mesothelioma (low-grade tumor associated with asbestos exposure) ▪ Multicystic mesothelioma (low-grade tumor also known as multilocular peritoneal inclusion cyst) ▪ Diffuse malignant mesothelioma ▪ Adenomatoid tumor
Smooth muscle cell tumors	Leiomyomatosis peritonealis disseminata
Tumors of uncertain origin	Desmoplastic small round cell tumor
Epithelial tumors	▪ Primary peritoneal adenocarcinoma ▪ Primary peritoneal borderline tumor

SECONDARY NEOPLASMS

Secondary peritoneal metastasis is most common malignant process caused by invading cancer stem cells (CSCs) from malignant tumors involving ovary, colorectal region, stomach, breast and lung including melanoma. Metastasis from ovarian, gastric, and colorectal malignancies is associated with increased risk of recurrence and mortality. Primary tumors metastasizing to the peritoneum are given in Table 20.78.

Pseudomyxoma Peritonei

Pseudomyxoma peritonei (PMP) is a clinical term used to designate diffuse intra-abdominal gelatinous ascites with jelly-like mucinous implants on peritoneal surfaces from mucinous neoplasm of appendix, stomach, ovary, pancreas, and hepatobiliary system.

Table 20.78 Metastases to the peritoneal serosal surface

Primary Tumors Metastasizing to the Peritoneal Serosal Surface
Ovarian carcinoma
Cervical carcinoma
Breast carcinoma
Biliary tract carcinoma
Upper gastrointestinal tract carcinoma
Sarcomas of female reproductive tract
Endometrial carcinoma
Fallopian tube carcinoma
Pancreatic carcinoma
Lung carcinoma
Lower gastrointestinal tract carcinoma

GASTROINTESTINAL TRACT DISORDERS: DIAGNOSTIC APPROACH

Distribution of carcinomas in upper gastrointestinal tract is shown in Fig. 20.76. *Helicobacter pylori*-induced gastrointestinal disorders are shown in Fig. 20.77. Appendix disorders include acute appendicitis and carcinoid tumor. Important disorders of large bowel are shown in Fig. 20.78. Symptoms of colorectal disorders are given in Table 20.79. Differential diagnosis of lower abdominal mass is given in Table 20.80.

Large bowel disorders include hyperplastic polyps, neoplastic polyps, neoplasms, inflammatory bowel disease, diverticula, infectious colitis, drug-induced colitis and irritable bowel syndrome. Investigations of colorectal disorders include detailed clinical history, abdominal examination, anorectal examination, digital examination, proctoscopy, sigmoidoscopy, colonoscopy, barium enema, imaging techniques and feces examination.

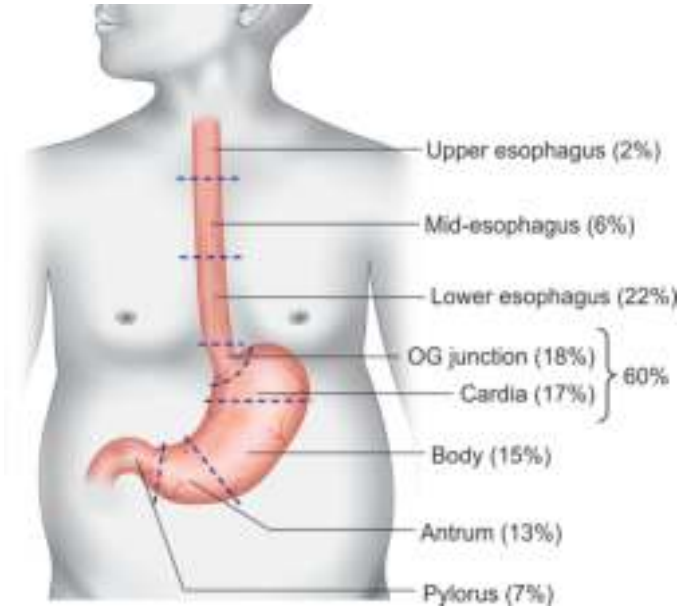


Fig. 20.76: Distribution of carcinomas in upper gastrointestinal tract.

- **Abdominal examination:** Abdomen should be inspected for visible peristalsis and distension. Palpable sigmoid colon is observed in constipated patients. Palpable liver may disclose metastatic deposits from colorectal carcinoma.
- **Anorectal examination:** Rectum and anus are readily accessible. Anorectal examination can detect pruritus ani, perianal warts, perianal abscess, perianal hematoma, prolapsing hemorrhoids, thrombosed hemorrhoids, anal fistula, anal fissures, anal cancer, rectal prolapse, rectocele and fecal soiling of perineum.
- **Digital examination:** Digital examination helps in diagnosing skin tags and Crohn's disease. On straining, prolapsing hemorrhoids, rectal prolapse and tumors are detected.
- **Proctoscopy:** Fiberoptic proctoscopy helps in visualization of rectal prolapsing mucosa, hemorrhoids, dentate line and anal epithelium.
- **Flexible sigmoidoscopy:** Flexible sigmoidoscopy permits examination up to 60 cm from the anal margin. It is performed without sedation in <10 minutes. Approximately, 70% of colorectal carcinomas are within reach of flexible sigmoidoscope.
- **Colonoscopy:** Patient should take a low residual diet followed by clear fluids for 48 hours and strong purgative before colonoscopy. Colonoscopy is more

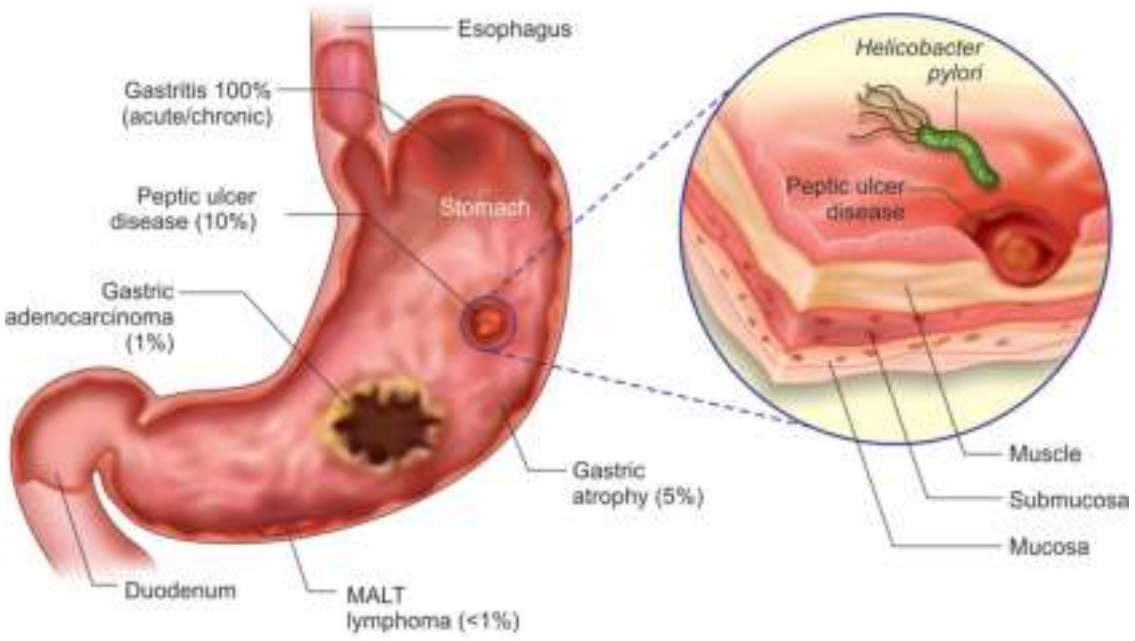


Fig. 20.77: *Helicobacter pylori*-induced gastrointestinal disorders.

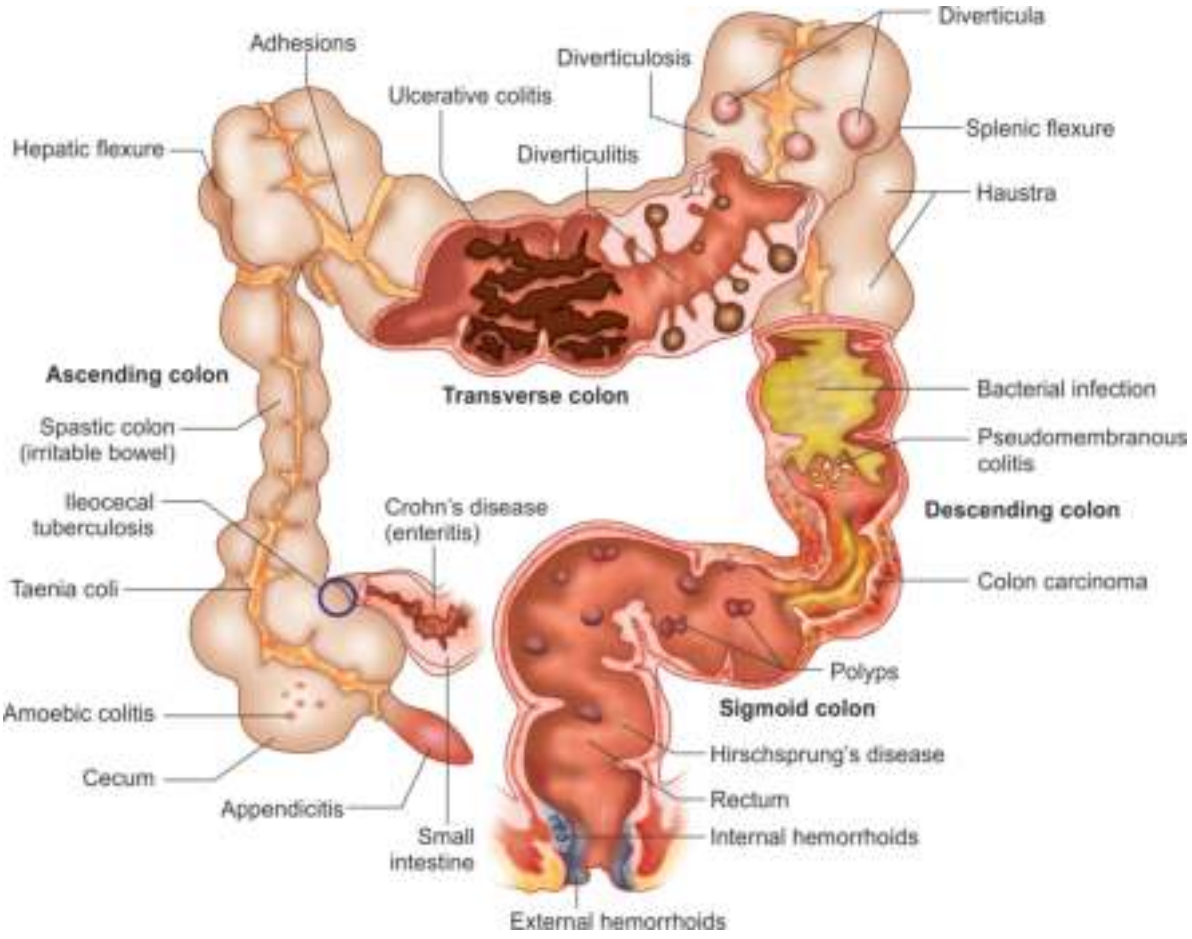


Fig. 20.78: Important disorders of large bowel.

Table 20.79 Symptoms of colorectal disorders

Parameters	Symptoms
Nonspecific symptoms	▪ Malaise ▪ Vomiting ▪ Weight loss
Abdominal pain	▪ Constant pain or colic ▪ Distension ▪ Borborygmi
Bowel habits	▪ Altered frequency ▪ Constipation or diarrhea ▪ Bleeding (bright red or mixed with mucus, dark or altered)
Anal and perianal	▪ Pruritus ▪ Pain related to defecation ▪ Prolapse ▪ Incontinence ▪ Discharge ▪ Tenesmus ▪ Swelling (painful or painless)

Table 20.80 Differential diagnosis of lower abdominal mass

Location	Disorders
Right iliac fossa	▪ Carcinoma of cecum ▪ Crohn's disease ▪ Appendicular abscess ▪ Ileocecal tuberculosis ▪ Actinomycosis ▪ Intussusception
Left iliac fossa	▪ Carcinoma of sigmoid colon ▪ Diverticular disease
Either iliac fossa	▪ Ovarian mass ▪ Ectopic pregnancy ▪ Transplanted kidney

sensitive for detecting fine mucosal abnormalities, biopsy sampling and therapeutic procedures such as polypectomy.

- **Double contrast barium enema:** In double contrast barium enema, air is pushed after evacuation of barium, which discloses gross anatomy, extracolonic abnormalities such as fistulas.
- **Imaging modalities:** Imaging techniques used to detect colorectal disorders include ultrasonography, CT scan and MRI scan.
  - Ultrasonography: It may demonstrate clinically inapparent metastases in patients with colorectal carcinoma.
  - CT and MRI scans: Preoperative computed tomography and magnetic resonance scanning give more accurate information in detection of local extent of rectal carcinoma and hepatic metastases than ultrasonography. MRI scanning is useful in the assessment of complex anal fistulas.
- **Feces examination:** Detection of occult blood in the feces is important in the investigation of patients with unexplained iron deficiency anemia.
  - Occult blood: Feces of patient smeared on a filter paper is impregnated with guaiac acid and then added hydrogen peroxide. Hematin from hemoglobin in the feces catalyzes the oxidation of guaiac acid giving characteristic color change to blue. Immunologic tests for human hemoglobin may prove to be more sensitive.
  - 24 hours fecal fat and D-xylose tests: Diagnostic modalities include 24 hours fecal fat excretion. D-xylose test, anti-tissue transglutaminase circulating autoantibodies and intestinal biopsy. The autoantibody test may also be used for screening prior to definitive diagnosis by biopsy.
  - Culture and sensitivity: It is done if infection is suspected such as *Entamoeba histolytica* and toxins of *Clostridium difficile* in patients with pseudomembranous colitis.
- ***Helicobacter pylori* tests:** In the advanced stage of *Helicobacter pylori*-induced gastrointestinal tract disorders; urea breath test or antibody test for *Helicobacter pylori* must be performed.
- **Serum tumor markers:** Serum carcinoembryonic antigen (CEA), CA 19-9 and CA 72-4 levels are increased in colon carcinoma.
- **Sampling of tissue biopsy:** Tissue biopsy is obtained by several procedures:
  - Endoscopic biopsy: Small fragments of mucosal tissue are obtained for histologic examination.
  - Suction biopsy: It makes possible to obtain submucosa for study.
  - Polypectomy: The specimen is bisected or serial sections depending on size of polypoid mass.
  - Endoscopic mucosal resection: Mucosal resections are obtained by this method.

Table 20.81 Panel of immunohistochemical markers applied in Barrett's esophageal intestinal metaplasia at the gastroduodenal junction and tumors of gastrointestinal tract

Immunohistochemical Markers	Expression
Barrett's esophageal intestinal metaplasia at the gastroduodenal junction	
Cytokeratin 7 (CK7) surface and deep region	Positive
Cytokeratin 20 (CK20) surface region only	Positive
DAS-1	Positive
MUC1	Positive
MUC2	Positive
Gastric adenocarcinoma (intestinal type)	
Cytokeratins (CKs)	Positive
Epithelial membrane antigen (EMA)	Positive
Carcinoembryonic antigen (CEA)	Positive
CDX2	Positive (specific marker)
Colorectal adenocarcinoma	
Carcinoembryonic antigen (CEA)	Positive
Cytokeratin 7 (CK7)	Positive
Cytokeratin 20 (CK20)	Positive
CDX2 (transcription factor)	Positive (specific marker associated with poor prognosis)
SAT (transcription factor)	Positive
β-Catenin	Positive
p53	Positive
Villin	Positive
Gastrointestinal stromal tumors	
c-KIT (CD117) encoding tyrosine kinase	Positive (strong staining in membranous, cytoplasmic or perinuclear globular patterns in 90%)
DOG1	Positive (most specific marker)
PDGFA	Positive
CD34	Positive (75%)
Smooth muscle actin	Positive (40%)
Protein kinase c-theta	Positive
Vimentin	Positive
Smooth muscle actin encoded by ACTA2 gene	Positive
Desmin	Positive (5%)
S-100 protein	Negative
Gastrointestinal carcinoid (neuroendocrine) tumors	
Synaptophysin	Positive (cytoplasmic staining)
Chromogranin A	Positive (cytoplasmic staining)
Neuron-specific enolase (NSE)	Positive (cytoplasmic staining)
CD56 (neuronal cell adhesion molecule—NCAM) neuroendocrine marker	Positive (membranous staining)
Cytokeratins (AE1/AE3 and CAM 5.2 epithelial marker)	Positive (cytoplasmic staining)
CDX2	Positive (50%)

Contd...

Table 20.81 Panel of immunohistochemical markers applied in Barrett's esophageal intestinal metaplasia at the gastroduodenal junction and tumors of gastrointestinal tract (*Contd...*)

Immunohistochemical Markers	Expression
Gastrointestinal tract mucosa-associated lymphoid tissue (MALT) lymphoma	
CD20	Positive
CD43	Positive
IgM > IgA > IgG	Positive
IgD	Negative
CD5	Negative
CD10	Negative
BCL-16	Negative
Cyclin D1	Negative

There is no definite immunohistochemical marker for gastric MALT lymphoma. Panel of immunohistochemical markers are appile to assess architecture of infiltration lymphoid cells, immunophenotype and chain restriction.

- Bowel resection: Portion of bowel resection is obtained to study various gastrointestinal disorders. Serosal surface is examined to study involvement by tumor. Bowel wall is inspected for tumor involvement, perforation and adhesion.

  Mesentery and soft tissue are dissected to obtain lymph nodes. A minimum of 12 lymph nodes is required for establishing staging criteria.

- **Immunohistochemistry technique:** Immunohistochemistry has now become the most powerful and widely used ancillary diagnostic tool in surgical pathology, especially in the diagnosis of undifferentiated tumors. Panel of immunohistochemical markers applied in Barrett's esophageal intestinal metaplasia at the gastroduodenal junction and tumors of gastrointestinal tract is given in Table 20.81.

CHAPTER

21

Liver, Gallbladder and Exocrine Pancreas

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

DISORDERS OF LIVER
- Anatomy and physiology
- Hepatocellular disease

JAUNDICE AND CHOLESTASIS
- Jaundice and cholestasis: overview
- Hereditary hyperbilirubinemia
- Neonatal cholestasis
- Jaundice: differential diagnosis

HEPATITIS
- Hepatitis: overview
- Viral hepatitis: acute/chronic viral hepatitis
- Hepatotropic viruses
- Autoimmune hepatitis (lupoid hepatitis)

BACTERIAL INFECTIONS AND PARASITIC INFESTATIONS OF LIVER
- Bacterial liver infections
- Parasitic liver infections
- Ascending cholangitis and liver abscess
- Drugs and toxins induced hepatocellular injury
  - Acetaminophen induced hepatocellular injury
  - Carbon tetrachloride induced hepatocellular injury

LIVER CIRRHOSIS AND PORTAL HYPERTENSION
- Liver cirrhosis
  - Etiologic classification
  - Morphologic classification
  - Pathophysiology
- Portal hypertension

METABOLIC LIVER DISORDERS
- Alcoholic liver disease
- Nonalcoholic fatty liver disease
- Hemochromatosis
- Wilson disease
- α_1-Antitrypsin deficiency
- Glycogen storage disease type 4
- Niemann-Pick disease and Gaucher's disease
- Amyloidosis of liver
- Indian childhood cirrhosis
- Acute fatty liver of pregnancy
- Reye syndrome

BILE DUCT DISORDERS
- Bile duct disorders: overview
  - Primary and secondary biliary cirrhosis
- Primary sclerosing cholangitis

VASCULAR DISORDERS OF LIVER
- Impairment of hepatic venous drainage (chronic venous congestion of liver)
- Noncirrhotic portal hypertension
- Miscellaneous intrahepatic vascular conditions

NEOPLASTIC DISORDERS OF LIVER
- Primary benign tumors
- Hepatocellular nodules
- Primary malignant tumors
- Metastatic tumors (liver metastases)

DISORDERS OF GALLBLADDER
- Gallbladder: overview
- Non-neoplastic disorders
- Cholelithiasis (gallbladder stone disease)
- Common neoplasms and precursor lesions of gallbladder
  - Gallbladder adenoma
  - Gallbladder adenocarcinoma
  - Klatskin tumor
  - Biliary adenocarcinoma
  - Extrahepatic bile duct adenocarcinoma

DISORDERS OF EXOCRINE PANCREAS
- Exocrine pancreas: structure
  - Anatomy and physiology
- Pancreatitis: overview
  - Acute pancreatitis
  - Chronic pancreatitis
  - Autoimmune pancreatitis
- Cystic fibrosis of pancreas
- Exocrine and endocrine pancreatic tumors
- Islet cell tumors
- Metastatic pancreatic tumors

DISORDERS OF LIVER

ANATOMY AND PHYSIOLOGY

NORMAL STRUCTURE

The liver and pancreas are derived as glandular outgrowths of the primitive foregut. The liver is the largest parenchymal organ weighing about 1000–1500 g. It lies just below the diaphragm. The right lobe liver is larger than the left lobe. The falciform ligament is the rough dividing line between the two lobes. External surface is smooth and brown. Cut surface is brown.

Anatomy of the liver and its relationship to the gallbladder, pancreas and duodenum is shown in Fig. 21.1.

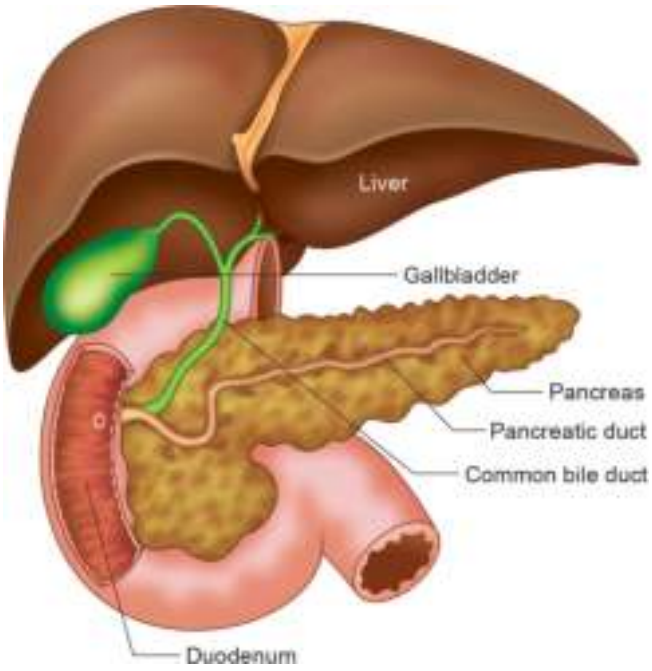


Fig. 21.1: Anatomy of the liver and its relationship to the gallbladder, pancreas and duodenum.

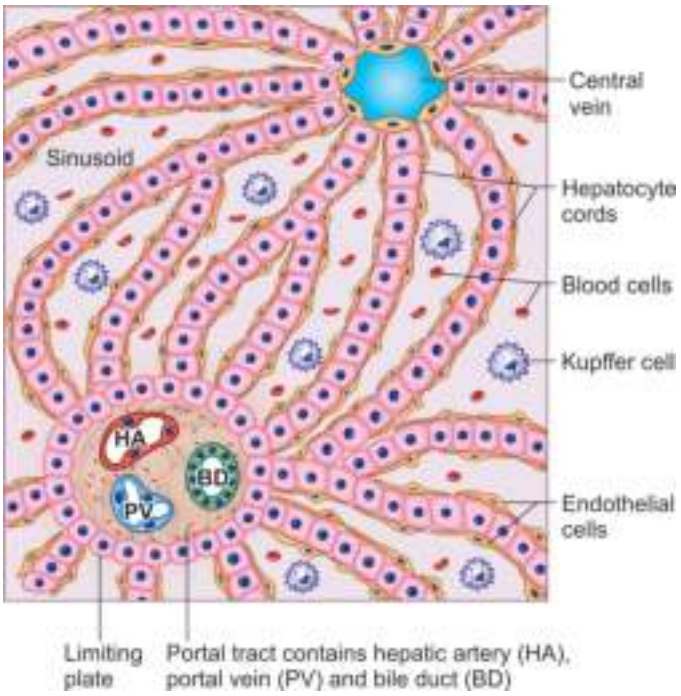


Fig. 21.2: Schematic representation of the normal liver lobule. The portal tract contains branches of the hepatic artery, portal vein, and interlobular bile duct. The liver cell plates converge to the terminal hepatic venule.

Liver Acinus (Lobule)

Liver is divided histologically into hexagonal or polyhedral lobules. The center of the liver lobule (acinus) has central vein. Portal triads are present at the periphery of the liver lobule. Portal triad consists a branch of hepatic artery, portal vein and bile duct (Figs 21.2 and 21.3).

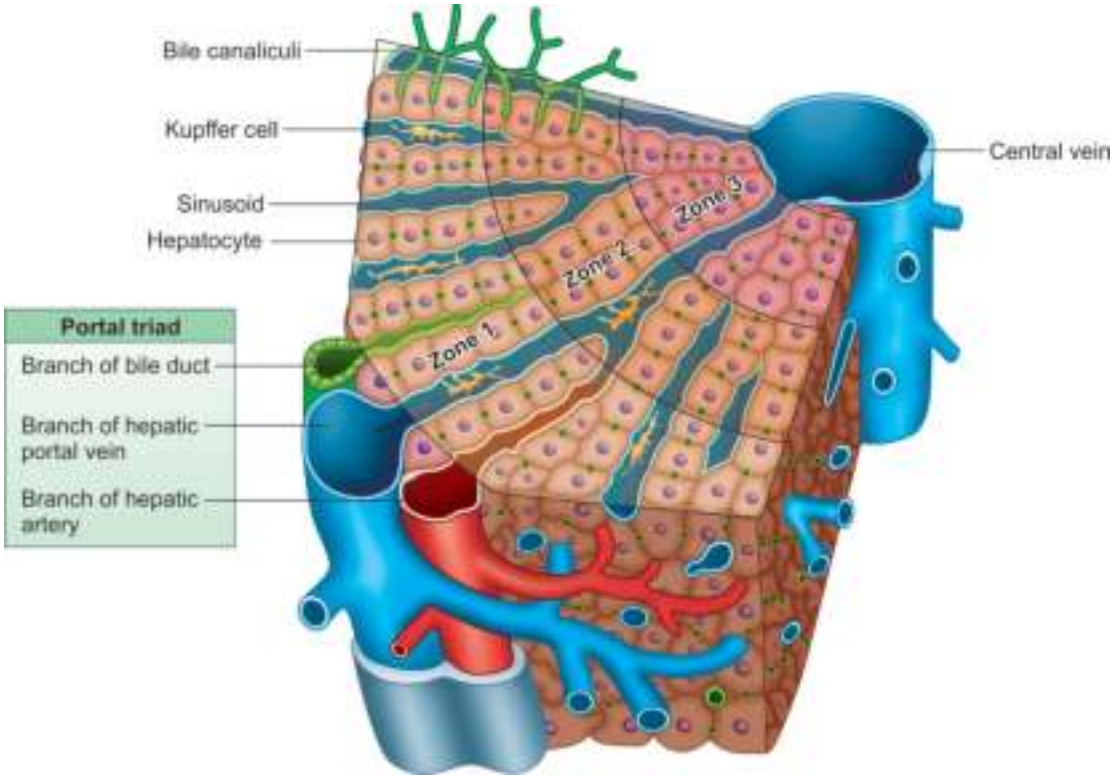


Fig. 21.3: Liver lobule shows cords of hepatocytes, sinusoids and bile ducts in the liver. Blood flows in sinusoids towards the center of the lobule (terminal hepatic vein) and bile in opposite direction draining in the bile ducts.

Portal Triad

Portal triad consists of branch of bile duct, portal vein and hepatic artery surrounded by fibrocollagenous tissue. The area surrounding central vein is known as centrilobular area. The area surrounding portal triad is known as periportal area.

Hepatocytes

Hepatocytes radiate from the portal tracts towards the terminal hepatic venules. Hepatocytes are arranged in one-cell thick columns. Sinusoid is present between each of these columns.

Limiting Plates

Portal tract is bound by a row of hepatocytes known as limiting plate. The liver cell plates are two-cell thick in babies and small children. By attaining the age of 5 years the normal one-cell thick pattern is established. Destruction of limiting plates in hepatitis is known as piecemeal necrosis.

FUNCTIONAL ZONES OF LIVER ACINUS (LOBULE)

Functionally, the liver can be divided into three zones between the portal triad and central vein; based on supply of oxygen and nutrients. Lobular and acinar regions of liver terminology are given in Table 21.1.

Pathology Pearls: Functional Zones of Liver Acinus

Zone 1 of Liver Acinus

- Zone 1 of liver acinus encircles the portal tracts (periportal zone). It receives richest oxygen and nutrients from hepaticartery.
- Zone 1 of liver acinus hepatocytes are specialized for oxidative liver functions such as gluconeogenesis, synthesis of glycogen from lactate, β-oxidation of fatty acids, catabolism of amino acids, urea synthesis, cholesterol synthesis, secretion of bile salts and acid; and sodium-dependent bile acid uptake.

Zone 2 of Liver Acinus

- Zone 2 of liver acinus is located between zone 1 and zone 3. It has moderate supply of oxygen and nutrients.

Zone 3 of Liver Acinus

- Zone 3 of liver acinus is located in most distant region from the portal tract around central vein (perivenular zone). It has poor supply of oxygen and nutrients.
- Zone 3 hepatocytes are important for glycolysis, synthesis of glycogen from glucose, lipogenesis, cytochrome P-450 based drug and toxins detoxification, removal of ammonia from blood by glutamine, ketogenesis, secretion of bile salts and acid; and sodium-dependent bile acid uptake.
- There is increased phagocytic activity of Kupffer cells. Necrosis of hepatocytes in Zone 3 of liver acinus associated with lymphocytic infiltration should raise concern for autoimmune hepatitis, liver transplant rejection and hepatocellular injury induced by alcohol and drugs.

Table 21.1 Lobular and acinar regions of liver terminology

Lobular	Acinar	Description
Periportal region	Zone 1	Area around the portal tract
Midzonal region	Zone 2	Parenchyma between central venule and portal tract
Centrilobular, central, centrizonal region	Zone 3	Area around central venule

Hepatic Vasculature

Near the hilum, portal tract contains portal vein, branch of hepatic artery and interlobular bile ducts. Liver receives blood supply from portal vein and hepatic artery. The liver cell plates converge to the terminal hepatic venule (Fig. 21.4).

Portal Vein

Liver receives 70–75% of blood flow from portal vein. Portal vein is formed by joining of the superior mesenteric vein and splenic vein. Normal portal vein pressure is 5–10 mm Hg.

Hepatic Artery

Liver receives 25–30% of oxygenated blood flow from hepatic artery. Hepatic artery occlusion does not cause necrosis of hepatocytes except in transplanted liver.

Hepatic Vein

Hepatic vein drains blood from the liver to the inferior vena cava. Hepatic abnormalities include disturbance of blood flow and portal hypertension.

Hepatic Sinusoids

Liver Sinusoids

The liver sinusoids are lined by endothelial cells. Underneath the endothelium, there is a row of hepatocytes. Sinusoidal endothelium lacks tight junctions between endothelial cells and basement membrane of the endothelium.

This anatomical arrangement permits free access of plasma from sinusoidal blood to the hepatocytes, thus facilitating two-way exchange of molecules between hepatocytes and blood.

Clinical Pearls: Diseases that can Affect Sinusoidal Blood Flow

- Sickle cell disease
- Cirrhosis
- Metastatic deposits in liver
- Chemotherapy
- Cytotoxic drug in preparation of bone marrow transplantation.

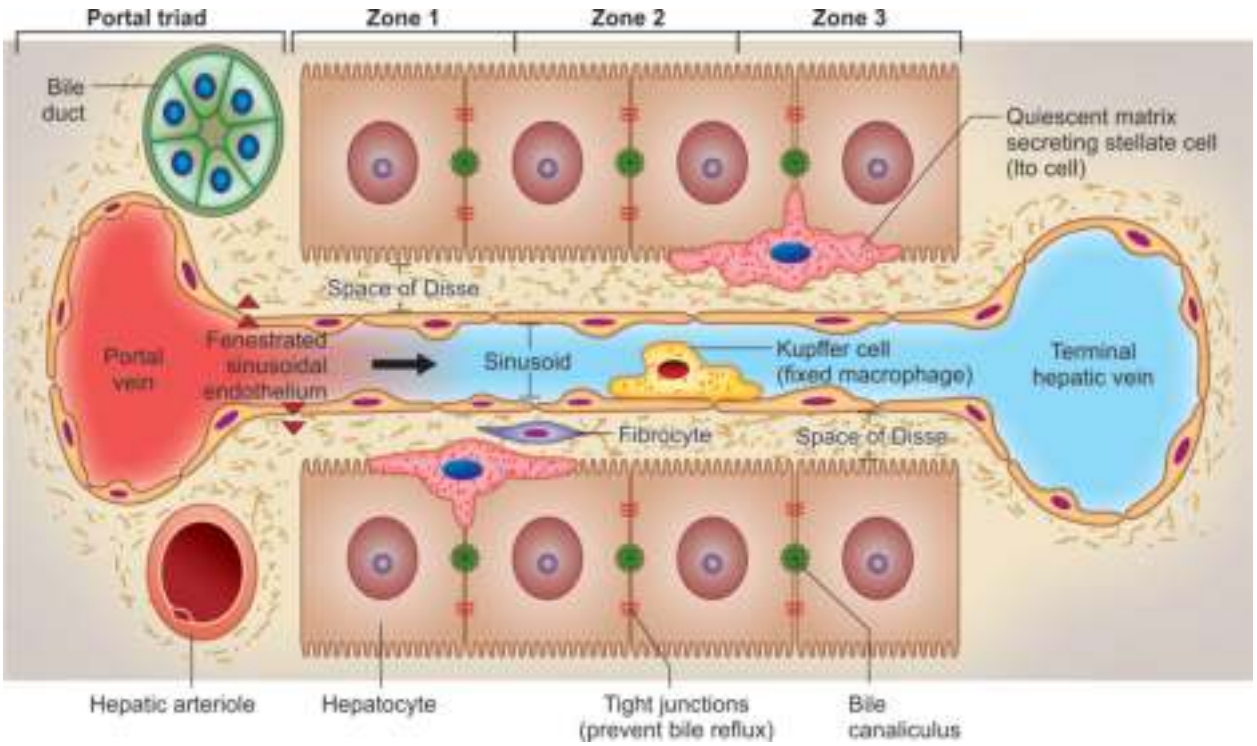


Fig. 21.4: The hepatic sinusoid and perisinusoidal space. Tight junction between hepatocytes prevents escape of bile.

Space of Disse

The space between the endothelium and hepatocytes is known as '**space of Disse**'. It contains collagen fibers especially type 3, proteoglycans and some fibronectin. Space of Disse consists of Kupffer cells and perisinusoidal stellate cells (Ito cells).

Kupffer Cells

Kupffer cells are adherent to sinusoidal endothelial cells inside sinusoid, which function as fixed tissue macrophages and participate in the phagocytosis of gut-derived bacteria. The Kupffer cell population is greatest in zone 1 of the liver acinus. Kupffer cells help in metabolism of vitamin A.

Perisinusoidal Stellate Cells (Ito Cells)

Perisinusoidal stellate cells (Ito cells) are in close contact with the liver cells, which may extend into small recesses between hepatocytes. Most of them contain vitamin A. These cells have myofibroblastic properties and participate in synthesis of collagen fibers during inflammation.

Bile Canaliculi

Bile canaliculi are present in between hepatocytes. Bile from bile canaliculi is drained into the bile ducts in portal triad and ultimately into the common bile duct. The canalicular microvilli are surrounded by a meshwork of contractile proteins, which maintain tone within the canalicular system. From the canaliculi, the bile flows into the periductules known as **canals of Hering**.

PHYSIOLOGY

Liver participates in excretion of substances, detoxification of substances, synthesis of vitamin K-dependent clotting factors (II, VII, IX, X), proteins, metabolism of carbohydrates, lipids and proteins; and storage of iron. Functions of the liver are given in Table 21.2. Functions of the liver and clinical manifestations due to altered function are given in Table 21.3.

Carbohydrate Metabolism

Liver participates in conversion of carbohydrates and proteins into triglycerides and fatty acids. It regulates blood glucose concentration by glycogenesis, glycogenolysis and gluconeogenesis. Hepatic pathway of carbohydrate metabolism is shown in Fig. 21.5.

Protein Metabolism

Liver participates in synthesis of plasma proteins (albumin, α and β globulins and fibrinogen), vitamin K-dependent coagulation factors (II, VII, IX, X), and nonessential amino acids. It also synthesizes α_1-antitrypsin and ceruloplasmin (Fig. 21.6).

- Decreased synthesis of albumin in liver leads to edema.

Table 21.2 Functions of the liver

Parameters	Liver functions
Lipid metabolism	▪ Oxidation of triglycerides into the fatty acids, further conversion into ketone bodies for fuel, glucose and cholesterol ▪ Synthesis and excretion of lipoproteins into the blood ▪ Synthesis of cholesterol and phospholipid ▪ Synthesis of bile acids ▪ Synthesis and excretion of cholesterol and bile acids
Carbohydrate metabolism	▪ Conversion of carbohydrates and proteins into triglycerides and fatty acids ▪ Regulation of blood glucose by glycogenesis, glycogenolysis and gluconeogenesis
Protein metabolism	▪ Synthesis of plasma proteins including albumin, α- and β-globulins ▪ Synthesis of nonessential amino acids ▪ Synthesis of coagulation factors (II, VII, IX, X)
Waste management, detoxification and drug metabolism	Detoxification of metabolic waste products (e.g. deamination of amino acids and production of urea)
Storage	Storage of vitamins, iron, carbohydrate (glycogen) and lipids
Intermediate metabolism	Clearance and excretion of drugs, toxins, metabolic byproducts (bilirubin, ammonia and estrogen hormone)
Secretion	Synthesis and secretion of bile

Bile juice contains many of the products of the above processes, i.e. bile salts, bilirubin, cholesterol, phospholipid, amino acids, steroids, enzymes, vitamins and porphyrins.

Table 21.3 Functions of the liver and clinical manifestations due to altered functions

Metabolism	Liver Functions	Clinical Manifestations due to Altered Function
Carbohydrate metabolism	Glycogen storage and synthesis of glucose derived from amino acids, lactic acid and glycerol	Hypoglycemia due to impaired glycogenolysis and gluconeogenesis. Abnormal glucose tolerance due to impairment of uptake and release of glucose by the liver
Fat metabolism	▪ Lipoproteins synthesis ▪ Conversion of proteins and carbohydrates to fat; synthesis, recycling and elimination of cholesterol; formation of ketone bodies from fatty acids	▪ Impaired synthesis of lipoproteins ▪ Altered serum cholesterol level
Proteins metabolism	▪ Deamination of proteins and synthesis of urea from ammonia ▪ Synthesis of plasma proteins ▪ Synthesis of coagulation factors (fibrinogen, prothrombin, factors V, VII, IX and X) ▪ Synthesis of bile salts ▪ Elimination of bilirubin by hepatocytes	▪ Increased blood ammonia level ▪ Decreased serum proteins especially albumin resulting in edema (transudate) ▪ Bleeding tendencies due to decreased synthesis of coagulation factors ▪ Malabsorption of fats and fat-soluble vitamins ▪ Increased serum bilirubin and jaundice
Metabolism of steroid hormones	▪ Sex hormones ▪ Glucocorticoids ▪ Aldosterone	▪ Gonadal dysfunction including gynecomastia in male ▪ Increased cortisol concentration (Cushing's syndrome) ▪ Manifestations of hyperaldosteronism (sodium retention and hypokalemia)
Metabolism of drugs	Drugs metabolism taking place in liver	Decreased metabolism of drug and its binding due to decreased synthesis of albumin
Vitamins and minerals	Storage of vitamins and minerals	Clinical manifestations of fat-soluble vitamins due to deficiency of storage in liver
Filtration and removal of bacteria	Liver Kupffer cells filter and remove bacteria	Increased exposure of coelomic cavities

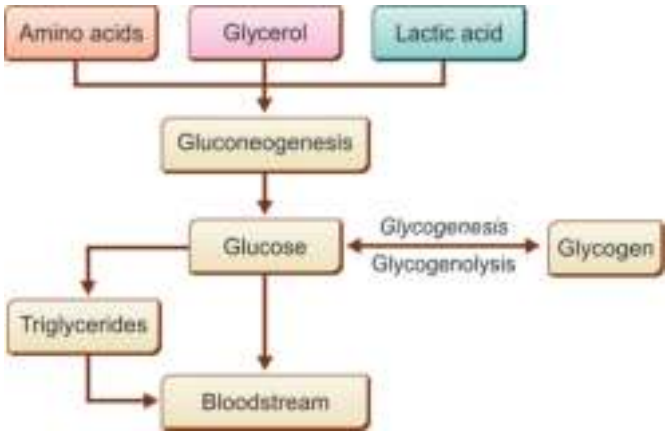


Fig. 21.5: Hepatic pathway of carbohydrate metabolism (storage and synthesis of glucose and conversion of glucose to fatty acids).

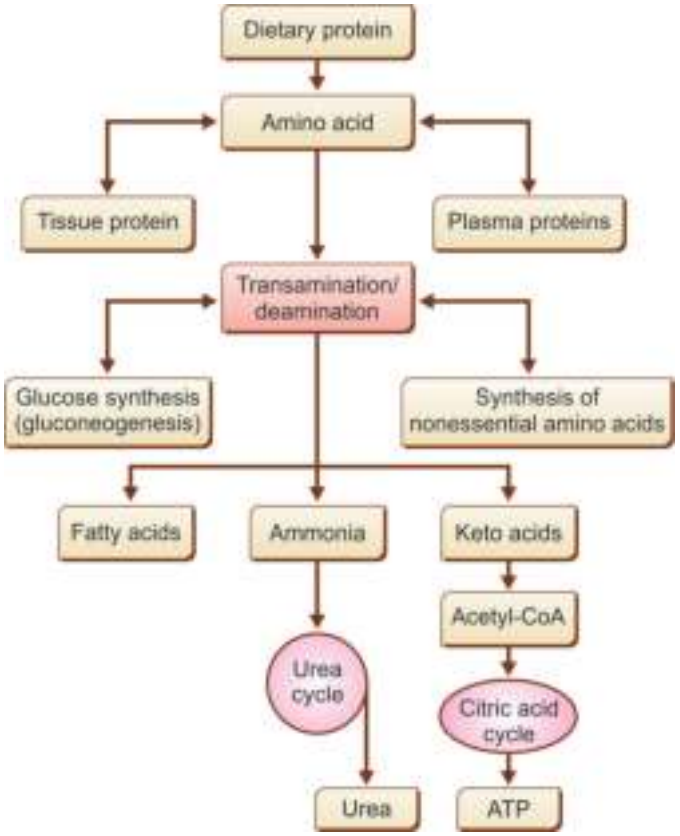


Fig. 21.6: Hepatic pathway of protein metabolism shows conversion of amino acids to proteins, nucleic acids, keto acids and glucose. The urea cycle converts ammonia generated by the deamination of amino acids to urea (acetyl-CoA, ATP and adenosine triphosphate).

- Decreased synthesis of fibrinogen and vitamin K-dependent coagulation factors (II, VII, IX, X) leads to coagulation disorders.
- The α_1-antitrypsin deficiency causes **panacinar emphysema** and **cirrhosis**.
- Deficiency of copper transporting ceruloplasmin protein results in **Wilson's disease**.

Lipid Metabolism

Liver oxidizes triglycerides into the fatty acids, which can be further metabolized into ketone bodies for fuel, glucose and cholesterol. Liver synthesizes and excretes lipoprotein into the blood. It participates in synthesis and excretion of cholesterol, phospholipid and bile acids. Hepatic lipid metabolism is shown in Fig. 21.7.

Intermediate Metabolism of Substances

Liver participates in the clearance and excretion of drugs, toxins metabolic byproducts (bilirubin, ammonia and estrogen hormone).

Detoxification of Metabolic Waste Products

Liver participates in detoxification of metabolic waste products (e.g. deamination of amino acids and production of urea).

Storage Function

Liver stores fat soluble vitamins (A, E, D and K), iron, carbohydrate (glycogen) and fat.

Secretion of Bile Juice

Liver participates in synthesis and secretion of bile. Bile juice contains water (95%), bile salts, bilirubin, phospholipid, cholesterol, amino acids, steroids, enzymes, vitamins and porphyrins.

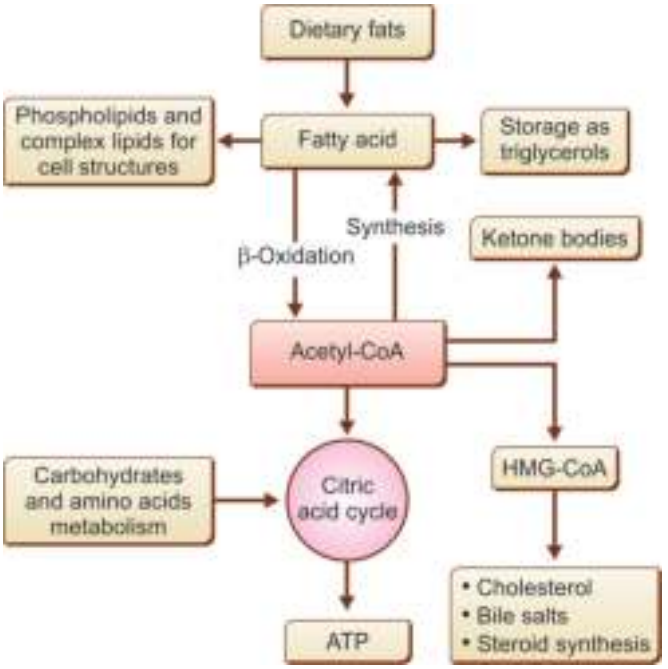


Fig. 21.7: Hepatic lipid metabolism shows β-oxidation breaking fatty acids into two-carbon acetyl-CoA units, which are used in the citric acid cycle to generate ATP or synthesis of cholesterol of keto acids leading to their release into blood for utilization by the tissues for energy purpose.

HEPATOCELLULAR DISEASE

Acute hepatocellular disease persists for <6 months. Chronic hepatocellular disease persists for >6 months. Causes of hepatocellular disease are given in Table 21.4. Enzymes indicative of liver plasma membrane integrity are given in Table 21.5.

PATTERNS OF LIVER NECROSIS

Focal Hepatic Necrosis

Focal necrosis of liver involves single cell or small group of cells in any area of lobules. But all liver lobules are not affected. Focal liver necrosis is caused by viral hepatitis, toxic agents and bacteria.

Table 21.4 Causes of hepatocellular disease

Duration of Hepatocellular Disease	Causes
Acute hepatocellular disease persists for <6 months	▪ Common causes: HBA, HBV, HBC, alcoholic hepatitis, toxic injury, ischemic injury ▪ Less common causes: HDV, HEV, CMV, EBV, Herpes virus, autoimmune hepatitis, Wilson disease, liver disease of pregnancy
Chronic hepatocellular disease persists for >6 months	▪ Common causes: HBV, HCV, alcoholic liver disease, nonalcoholic fatty liver (NAFL), drug toxicity, autoimmune hepatitis ▪ Inborn errors of metabolism: Hemochromatosis, α_1-antitrypsin deficiency, Wilson disease, glycogen storage disease, Gaucher's disease

In hepatocellular disease, the relative elevation of ALT and AST exceed the relative elevation in alkaline phosphatase (ALP).

Surgical Pathology: Focal Necrosis of Liver

Light Microscopy

- Histologic examination of liver biopsy shows Councilman's bodies (apoptotic bodies) in viral hepatitis.
- Kupffer cells hyperplasia is seen around necrotic cells.

Zonal Hepatic Necrosis

Zonal liver necrosis is characterized by involvement of identical region of all hepatic lobules. There are three types of zonal necrosis.

- **Centrilobular necrosis:** It most often occurs due to cardiac failure, hypotensive shock, viral hepatitis, carbon tetrachloride toxicity and chloroform toxicity.
- **Midzonal necrosis:** It is seen in yellow fever.
- **Periportal necrosis:** It occurs due to phosphorus poison and eclampsia of pregnancy.

Submassive Hepatic Necrosis

An entire hepatic lobule shows necrosis involving 26–75% of liver parenchyma.

Massive (Fulminant) Hepatic Necrosis

Fulminant acute liver failure is also known as *massive hepatic necrosis* involving >75% of liver parenchyma due to hepatotropic viruses, drugs, toxins and chemicals. Fulminant acute hepatitis may progress to hepatic encephalopathy within 2–3 weeks of onset of jaundice. Causes of fulminant hepatitis are given in Table 21.6. Clinical features of fulminant acute liver failure are given in Tables 21.7A and B.

Table 21.5 Enzymes indicative of liver plasma membrane integrity

Indicative of Hepatocellular Disease	Indicative of Biliary Tract Disease
Alanine aminotransferase (ALT)	Alkaline phosphatase (ALP)
Aspartate aminotransferase (AST)	Gamma-glutamyltransferase (GGT)
Lactate dehydrogenase (LDH)	5'-Nucleotidase (5'-NT)

Biliary tract disease produces relatively greater increase in alkaline phosphatase (ALP) than increase in ALT, AST or LDH. Alkaline phosphatase (ALP) is associated with plasma membrane of hepatocytes adjacent to the biliary tract.
Enzymes indicative of hepatocellular disease are alanine aminotransferase (AST). Alkaline phosphatase (ALP) elevations relate to biliary tract disease.

Table 21.6 Etiology of massive (fulminant) hepatic necrosis

Categories	Examples
Hepatotropic viruses	HBV, HDV, HEV (20% in pregnant women) and HCV (rare)
Therapeutic drugs	Acetaminophen, methyldopa, isoniazid, halothane, and antidepressants
Toxins	Myotoxin
Chemicals	Carbon tetrachloride and phosphorus

Table 21.7A Clinical features of fulminant acute liver failure

Fulminant Liver Failure	Clinical Manifestations
Hepatic encephalopathy	▪ Grade I: Mild confusion, euphoria or depression ▪ Grade II: Drowsy and semi-comatose ▪ Grade III: Stupor (marked confusion but rousable) ▪ Grade IV: Coma (unrousable)
Jaundice	Yellow discoloration of eyes
Bleeding tendency	Oozing from gastrointestinal tract
Asterixis (flapping tremors)	Demonstrated with the arms outstretched and hands dorsiflexed reflecting uremia

Table 21.7B Clinical features of liver disease

Clinical Signs and Symptoms	Mechanism	Clinical Features
Jaundice	Failure of uptake, conjugation and excretion of bilirubin	Yellow discoloration of tissues (sclera) due to deposition of bilirubin pigment
Bleeding manifestations	Failure of synthesis of vitamin K-dependent coagulation factors (II, VII, IX, X)	Easy bruising and prolonged clotting time of blood
Edema (transudate)	Failure of synthesis of albumin causing decreased oncotic pressure exerted by plasma proteins	Swelling in the dependent part of body due to accumulation of fluid (water) in extracellular compartment
Ascites	Portal hypertension and decreased hepatic synthesis of albumin	Accumulation of fluid in peritoneal cavity (ascites), demonstrated by shifting dullness
Gynecomastia	Failure to detoxify endogenous or exogenous estrogens	Enlargement of bilateral breasts
Hepatic encephalopathy	Occurs due to failure to detoxify ammonia and excitatory amino acids derived from protein breakdown	Altered consciousness, lack of coordination, coma and flapping tremors
Hematemesis and melena	Portal hypertension	Blood loss in vomitus and stool

Clinical Pearls: Liver Transplantation

Liver transplantation has emerged as an established and well-accepted therapeutic option for patients with acute and chronic hepatocellular failure and hepatocellular carcinoma. The indication for liver transplantation should be assessed independently of etiology. Aim of liver transplantation is to prolong patient's life and improve quality of life.

- **Specific indications:** Specific indications of liver transplantation include acute hepatic fulminant failure, chronic hepatic failure, hepatocellular carcinoma, cirrhosis, extrahepatic biliary atresia, inborn errors of metabolism including α-antitrypsin deficiency, sclerosing cholangitis, and hepatic vein thrombosis (Budd-Chiari syndrome).
- **Prognosis of liver transplantation:** Acute hepatic fulminant failure continues to be associated with high mortality rate and emergency liver transplantation is often the only life-saving. Survival after liver transplantation for acute fulminant hepatocellular failure has been reported to less favorable survival for patients with chronic liver disease.
- **Liver transplantation evaluation processes:** Liver transplantation evaluation processes include hematology evaluation, laboratory biochemical investigations (serum bilirubin, AST, ALT, prothrombin time and international normalized ratio, liver function tests, renal function tests, thyroid profile, ceruloplasmin, viral hepatotropic viruses and autoantibodies), hepatic imaging (sonography with Doppler, MS—CT/dynamic MRT), cardiopulmonary evaluation, and pathogens evaluation (CMV, EBV, Mycobacterium tubercle bacilli).
- **Absolute contraindications:** Absolute contraindications for hepatic transplantation include severe cardiopulmonary disease, severe pulmonary arterial hypertension (mean pulmonary artery pressure (PAP) $\geq$25 mm Hg in right heart), hepatocellular carcinoma with extrahepatic metastasis and current extrahepatic malignancies.
- **Complications:** Most common complications include acute graft rejection, vascular thrombosis, biliary leakage or stricture, infections, malignancy and adverse effects of immunosuppressive drugs. Graft-versus-host disease causes damage to interlobular ducts of liver.

Clinical Features

Patient of massive liver necrosis presents with jaundice, bleeding, confusion and disorientation. Marked elevation of ALT and AST are characteristic findings. Serum bilirubin rises rapidly. There is prolonged prothrombin time and international normalized ratio (PT–INR).

Surgical Pathology: Massive Hepatic Necrosis

Gross Morphology

- Liver is soft flabby shrunken in size one-third of its normal weight (500 g) with wrinkled Glisson's capsule.
- There is presence of patches of greenish bile stained areas.
- Cut surface shows areas of necrosis and patches of bile stain.

Light Microscopy

- Liver biopsy reveals necrotic liver lobules, leaving a collapsed collagenous and reticulin framework.
- Necrosis and collapse of reticulin framework are responsible for shrinkage of liver.
- The portal tracts appear to be close to each other.
- There may be slight infiltration by chronic inflammatory cells in the portal areas (Fig. 21.8).

Prognosis

Mortality rate is very high in >80% of massive hepatic necrosis cases. These patients die unless an emergency liver transplantation is performed. Rest of the patients recover due to regeneration of hepatocytes.

Hepatic Necrosis: Histologic Patterns

Liver cell injuries may occur in isolated or group of hepatocytes, periportal piecemeal necrosis and bridging necrosis described as under.

- **Single cell or group of cells necrosis of hepatocytes:** This pattern of necrosis of hepatocytes is seen around terminal hepatic venule. Necrosis of hepatocytes occurs due to viral hepatitis, toxins or obstruction to venous drainage of liver or congestive heart failure (Fig. 21.9).
- **Periportal piecemeal necrosis of hepatocytes:** Periportal piecemeal necrosis of hepatocytes is seen around portal tract. It occurs in chronic active hepatitis, autoimmune hepatitis and active cirrhosis (Fig. 21.10).

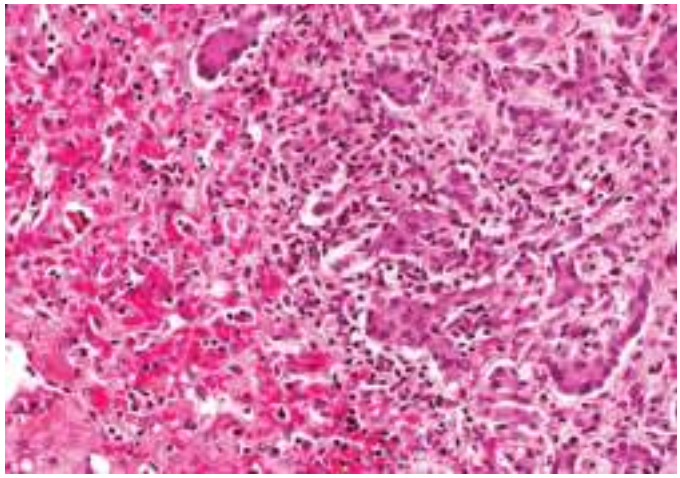


Fig. 21.8: Massive liver necrosis (acute liver failure). There is extensive hepatocyte necrosis seen here in a case of acetaminophen overdose. The hepatocytes at the right are dead, and those at the left are dying. This pattern can be seen with a variety of hepatotoxins. Acute liver failure leads to hepatic encephalopathy (400X).

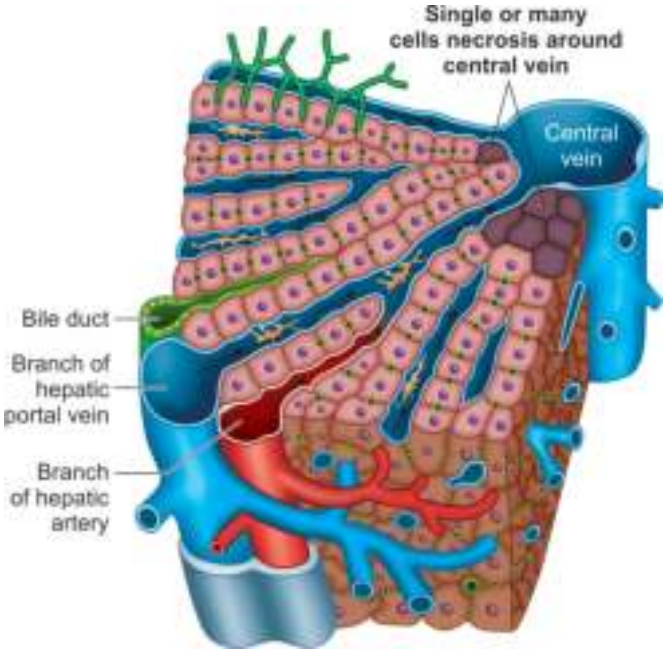


Fig. 21.9: Single cell or group of liver cells necrosis around terminal hepatic venule. This pattern of necrosis of hepatocytes occurs due to viral hepatitis, toxins or obstruction to venous drainage of liver or congestive heart failure.

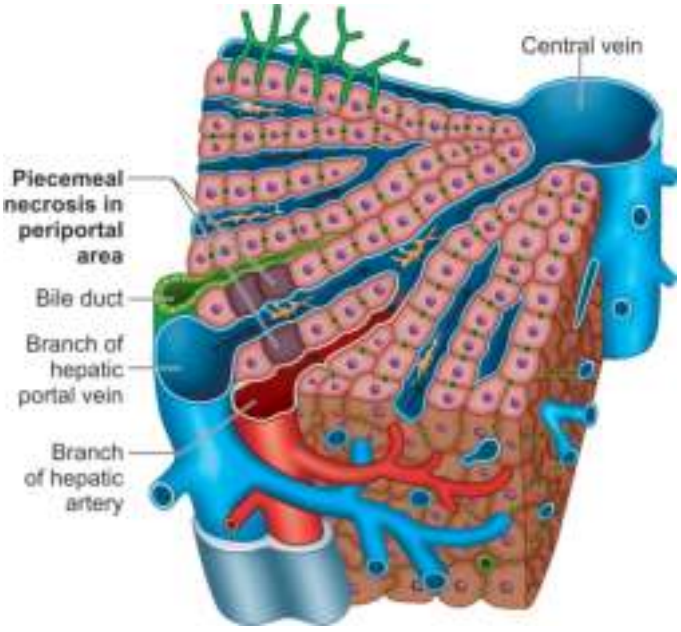


Fig. 21.10: Piecemeal necrosis around periportal area. It occurs in chronic active hepatitis, autoimmune hepatitis and active cirrhosis.

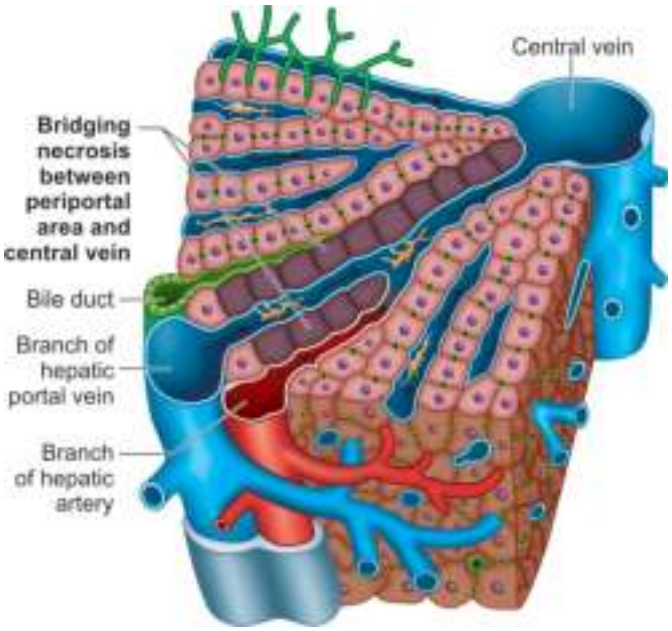


Fig. 21.11: Bridging necrosis of hepatocytes between portal triad and terminal hepatic venule. It occurs in severe viral infection.

- **Bridging necrosis of hepatocytes:** Bridging necrosis of hepatocytes occurs between portal tract and terminal hepatic venule in severe viral infection (Fig. 21.11).

CLINICAL FEATURES

Patient with liver disease shows various clinical manifestations described as under.

Jaundice

Jaundice refers to yellow discoloration of tissues (sclera) due to deposition of bilirubin pigment as a result of raised serum bilirubin concentration.

Bleeding Tendencies

Patient presents with easy bruising and prolonged clotting time of blood due to failure of synthesis of vitamin K-dependent coagulation factors (II, VII, IX, X).

Edema Legs

Patient presents with edema in dependent parts of lower limb due to decreased synthesis of albumin.

Ascites

Patient presents with ascites due to portal hypertension and decreased albumin synthesis by liver.

Gynecomastia

Patient presents with bilateral gynecomastia due to failure of detoxification of estrogens.

Hepatic Encephalopathy

Patient with hepatic encephalopathy presents with altered consciousness, lack of coordination, coma and flapping tremors due to failure of detoxification of ammonia and excitatory amino acids derived from protein breakdown.

Blood Loss in Vomitus and Melena

Patient with portal hypertension presents with bleeding from esophageal varices and melena.

LABORATORY DIAGNOSIS OF LIVER DISORDERS

Liver function tests frequently give an idea of the likely cause of liver disease. Routine and special laboratory tests in evaluation of liver disorders are given in Table 21.8. Laboratory investigations in liver disorders are given in Table 21.9. Diagnostic utility of serum

Table 21.8 Routine and special laboratory tests in evaluation of liver disorders

Liver Function Tests	Reason for Abnormal Results	Liver Disorders
AST (aspartate transaminase)	High value due to hepatocellular injury	Acute and chronic hepatic disorders
ALT (alanine transferase)	High value due to hepatocellular injury	Acute and chronic hepatic disorders
Alkaline phosphatase	High value due to cholestatic injury, bile duct injury, infiltrative processes	Biliary tract disorders, liver transplantation, granulomatous hepatitis, primary and secondary liver tumors
Total bilirubin and direct bilirubin	High value due to functional hepatocellular injury, cholestatic injury, bile duct injury	Acute hepatitis, chronic hepatitis, Dubin-Johnson syndrome, Gilbert syndrome, advanced liver disease with hepatocellular failure
Serum albumin	Values parallel synthetic function of hepatocytes	Cirrhosis (hepatocytes failure to synthesize albumin)
Serum globulins	Values parallel hepatocellular injury	Cirrhosis and autoimmune hepatitis (serum globulins increased level)
PT–INR	High value due to hepatocellular dysfunction	Severe hepatitis, cirrhosis (high value of PT–INR)
Blood test: γ-glutamyl transferase	High value due to injury to biliary ducts	Biliary tract disorders

Contd...

Table 21.8 Routine and special laboratory tests in evaluation of liver disorders (*Contd...*)

Liver Function Tests	Reason for Abnormal Results	Liver Disorders
Blood test: 5-nucleosidase	High value due to injury to biliary ducts	Biliary tract disorders
Blood test: lactic dehydrogenase (LDH)	High value due to hepatocellular injury	Liver cell ischemia and drug-induced hepatocellular injury
Blood test: α_1-antitrypsin	Value varies, increased in inflammatory diseases, decreased in inherited disorder	Infections (high value) and α_1-antitrypsin deficiency (low value)
Blood test: α-fetoproteins (AFP)	High value in liver cell neoplasms, increased liver cell regeneration	Hepatocellular carcinoma
Serum iron	High value due to increased iron uptake by hepatocytes; and hemolytic process	Hemochromatosis, hemolytic anemia and ineffective hematopoiesis
Serum ceruloplasmin	Low value in copper storage diorders, copper deficiency	Wilson disease, Menkes disease, complete deficiency of ceruloplasmin
Hepatic tissue iron quantitation	High value due to deposition of iron in tissues in primary and secondary iron deposition	Primary or secondary hemochromatosis
Hepatic tissue copper quantitation	High value due to deposition of copper in tissues	Wilson disease and Indian childhood cirrhosis

Table 21.9 Laboratory investigations in liver disorders

Liver Disorders	Laboratory Investigations	Interpretation
Hepatocellular failure	▪ Serum albumin ▪ Prothrombin time (normal <15 seconds) measures coagulation factors II, V, VII, X	↓ ↓
Hepatocellular injury	▪ Alanine aminotransferase (ALT) normal <40 IU/L (hepatocellular enzyme) ▪ Aspartate aminotransferase (AST) normal <40 IU/L (hepatocellular enzyme) ▪ LDH (hepatocellular enzyme) ▪ γ-Glutamyl transferase (γ-GT) normal <50 IU/L (plasma membrane enzyme from damage to bile canaliculi) ▪ 5'-Nucleotidase (plasma membrane enzyme from damage to bile canaliculi) ▪ Serum alkaline phosphatase (plasma membrane enzyme from damage to bile canaliculi)	↑ ↑ ↑ ↑ ↑ ↑
Hepatocellular injury, biliary obstruction, liver failure, hemolysis, congenital hyperbilirubinemia	Serum bilirubin (normal 5–12 µmol/L)	↑
Hepatitis A infection	IgM anti-HAV antibody	↑
Hepatitis B infection or carrier state	HBsAg	↑
Chronic active hepatitis (HBV infection)	HBeAg	↑
HCV exposure	Anti-HCV antibody	↑
HCV-induced hepatitis	HCV RNA	↑
Alcoholic cirrhosis	IgA and γ-glutamyl transferase	↑
Autoimmune hepatitis	IgG, anti-smooth muscle antibody and antinuclear factor	↑
Primary biliary cirrhosis	▪ IgM, anti-mitochondrial antibody ▪ Antismooth muscle antibody ▪ Anti-nuclear antiboty ▪ Bile canalicular antibody	↑ ↑ ↑ ↑
Wilson's disease	Ceruloplasmin	↓
Hemochromatosis	Ferritin	↑
Hepatocellular carcinoma	α-Fetoproteins (normally undetectable)	↑
α_1-Antitrypsin deficiency	α_1-Antitrypsin	↓

Table 21.10 Diagnostic utility of serum autoantibodies in liver disorders

Autoantibodies	Result	Liver Disorders
Antimitochondrial antibody	Present	▪ Primary biliary cirrhosis (80–90%) ▪ Chronic active hepatitis (10–25%)
Anti-smooth muscle antibody	Present	▪ Chronic active hepatitis (20–60%) ▪ Primary biliary cirrhosis (10–30%) ▪ Alcoholic liver disease (10–15%)
Antinuclear antibody	Present	▪ Chronic active hepatitis (20–50%) ▪ Primary biliary cirrhosis (15–40%) ▪ Drug-induced chronic active hepatitis (10–30%)
Anti-DNA antibody	Present	All types of liver disorders (30–60%)
Hepatocyte membrane antibody	Present	▪ Chronic active hepatitis (40%) ▪ Cirrhosis (60%) ▪ HBsAg negative acute hepatitis (15%)
Bile canalicular antibody	Present	▪ Chronic active hepatitis (20–40%) ▪ Primary biliary cirrhosis (20–40%)

autoantibodies in liver disorders are given in Table 21.10. Certain patterns of abnormalities suggest a particular type of liver disease, but none is specific to establish diagnosis.

Serum Alkaline Phosphatase

Alkaline phosphatase enzyme is located on the cell membrane of biliary canaliculi.

- Marked increase in level of serum alkaline phosphatase indicates disease of the biliary system (intrahepatic cholestasis, extrahepatic biliary obstruction, or primary biliary cirrhosis).
- Moderate increased levels of alkaline phosphatase are less useful in preceding cause of liver disease.

Serum Transaminases

Transaminases are enzymes located in the hepatocyte cytoplasm.

- Marked increase in level of serum transaminases reflects active necrosis of hepatocytes from any cause. ALT (alanine aminotransferase) is more specific for the liver than AST (aspartate aminotransferase).
- Mild increase in serum transaminases may be seen in cholestasis, as well as following administration of drugs such as anticonvulsants.
- Elevated glutamyl transpeptidase level is a sensitive index of hepatocellular damage.

Serum Conjugated and Unconjugated Bilirubin

Bilirubin is predominantly derived from hemoglobin in the normal turnover of red blood cells, and to a lesser extent, from myoglobin in skeletal muscle.

- Serum bilirubin analysis is useful to classify hyperbilirubinemia as predominantly unconjugated or conjugated. When the ratio of conjugated to total bilirubin is 0.4 or greater, predominantly conjugated hyperbilirubinemia is present.
- Biliary duct obstruction can be intrahepatic or extrahepatic. The most common cause of extrahepatic tract obstruction after the neonatal period is cholelithiasis.
- Recommended ranges for bilirubin, types of hyperbilirubinemia, unconjugated hyperbilirubinemia without hemolysis, unconjugated hyperbilirubinemia with hemolysis and commonly used methods to measure bilirubin are given in Tables 21.11 to 21.15.

Serum Albumin

Serum albumin reflects synthetic properties of the liver. Because of its half-life, low levels of albumin reflect long-standing liver disease, particularly cirrhosis.

Serum Ceruloplasmin

Ceruloplasmin levels are reduced in Wilson's disease. In Wilson's disease mutation of ATP7B gene located on chromosome 13 leads to deficiency of a copper transporting ATPase resulting in decreased copper transport into bile and impairs its incorporation into ceruloplasmin.

Table 21.11 Recommended normal ranges for bilirubin

Analyte	Bilirubin Range
Total bilirubin	0.2–1.0 mg/dl
Conjugated bilirubin	0.0–0.2 mg/dl

Table 21.12 Types of hyperbilirubinemia

Hyperbilirubinemia	Characteristics	Congenital Causes	Other Causes	Excretion of Bilirubin in Urine
Conjugated bilirubin (direct)	Water soluble, loosely bound to albumin	▪ Dubin-Johnson syndrome ▪ Rotor syndrome	▪ Hepatocellular damage ▪ Gallbladder stones ▪ Pancreatic cancer ▪ Biliary cirrhosis ▪ Biliary atresia	Present
Unconjugated bilirubin (indirect)	Insoluble, tightly bound to albumin	▪ Gilbert syndrome ▪ Crigler-Najjar syndrome type 1 (severe) and type 2 (mild)	▪ Hemolytic anemia ▪ Physiological jaundice of the newborn ▪ Hepatocellular disease	Absent

Table 21.13 Unconjugated hyperbilirubinemia without hemolysis in various age groups

Newborns
Mild to moderate and transient unconjugated hyperbilirubinemia
Physiological jaundice
Breast milk jaundice
Persistent unconjugated hyperbilirubinemia
Crigler-Najjar syndrome type 1 (severe) and type 2 (mild)
Children and Adults
Gilbert syndrome

- Accumulated copper causes toxic injury to organs by oxygen-derived free radicals, which displace metals from metalloenzymes.
- Decreased serum ceruloplasmin, increased hepatic copper (>250 µg/1 g dry weight of liver) and increased urinary copper are consistently seen in Wilson's disease.

Serum Transferrin

Serum transferrin level is increased in hemochromatosis.

Prothrombin Time–INR Test

Serum coagulation (clotting) abnormalities are commonly seen in liver disease due to failure of hepatic synthesis of

Table 21.14 Unconjugated hyperbilirubinemia with hemolysis

Schistocytes Present in Peripheral Blood	
Microangiopathic hemolytic anemia	Rule out disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS)
Artificial cardiac valve	History of cardiac valve replacement
Autoimmune hemolytic anemia	Perform Coomb's test
Schistocytes Absent in Peripheral Blood	
Hemolysis in bone marrow	Rule out vitamin B_{12} deficiency
Red blood cell membrane defects	Examine peripheral blood smear for spherocytes, elliptocytes and stomatocytes
Red blood cell enzyme defects	Examine peripheral blood smear for bite cells in G6PD deficiency
Hemoglobinopathies	Perform hemoglobin electrophoresis

Table 21.15 Commonly used methods to measure bilirubin

Method	Principle	Comments
Total bilirubin	Bilirubin + Diazotized sulfanilic acid + Accelerator → Azodipyroles (reddish purple)	Classic diazo reaction described by Ehrlich; accelerator is caffeine and sodium benzoate. All fractions of bilirubin react with this method
Direct bilirubin	Bilirubin + Diazotized sulfanilic acid → Azodipyrole (reddish purple)	Classic diazo reaction minus accelerator, only conjugated and δ-bilirubin reacts with this method
Indirect bilirubin	Indirect bilirubin = Total Bilirubin – Direct bilirubin	Calculation done

vitamin K-dependent clotting factors. This is a feature of liver failure, as consequence of the end-stage of chronic liver disease such as cirrhosis. The one-step prothrombin time and international normalized ratio is a sensitive index of vitamin K-dependent clotting factors (II, VII, IX and X).

Serum Autoantibodies

Several liver diseases are associated with abnormal immunological parameters (autoantibodies).

- Primary biliary cirrhosis is associated with anti-mitochondrial autoantibodies and elevated serum IgM levels.
- Autoimmune chronic hepatitis is associated with anti-smooth muscle antibodies.

Table 21.16 Indications of liver biopsy

Persistent abnormal liver function tests
Jaundice of unclear origin
Portal hypertension and ascites
Grading and staging of chronic hepatitis
Grading and staging of chronic biliary diseases
Confirmation of fatty liver and grading and staging of steatohepatitis
Fever of unknown origin
Hereditary liver diseases
Metabolic liver diseases
Abnormal serum iron study
Diagnosis of space-occupying lesion in liver
Evaluation of transplanted liver
Unexplained splenomegaly

Imaging Studies

Imaging studies are valuable in patients with jaundice or tumors and space occupying lesions as part of pre-operative assessment.

Liver Biopsy

Percutaneous Vim Silverman needle used for liver biopsy is usually performed to diagnose liver diseases. Blind biopsy of right side of the liver is used to assess probable diffuse disease processes. CT or ultrasound-guided needle biopsy may be used to sample discrete lesions.

- Some liver diseases are diagnosed by considering clinical features, blood tests (LFT), serological tests for viral hepatitis and urine analysis.
- Indications of liver biopsy methods, complications and routine and special histochemical stains; immuno-histochemical stains and molecular techniques used in interpretation of liver biopsy are given in Tables 21.16 to 21.19.

Table 21.17 Complications of liver biopsy

Pain abdomen (>50%) subsides in 1–2 days
Hemorrhage from punctured site (less frequent)
Hemoperitoneum
Subcapsular hematoma in liver
Hemobilia (bleeding in biliary tree)
Bile leakage with peritonitis associated with severe obstruction of the large bile ducts
Lacerations of gallbladder, kidney, pancreas and other internal organs
Pneumothorax right side
Arteriovenous fistula

Table 21.18 Liver biopsy methods

Liver Biopsy Methods	Technique	Advantages	Comments
Percutaneous liver biopsy	Cutting needle (Vim Silverman, trucut), suction needle (Jamshidi, Klatskin, Menghini)	Most common, least invasive technique for histologic evaluation	Puncture or lacerations of adjacent organs by inexperienced
Transjugular liver biopsy	Catheter through the internal jugular vein	Liver biopsy in patients with severe coagulation disorders, fulminant hepatocellular failure, massive ascites or severe obesity	Smaller and fragmented biopsy specimens
Laparoscopic (open liver biopsy)	Laparoscopic open biopsy is taken	Provides largest liver biopsy specimens in cirrhosis, chronic liver diseases such as viral hepatitis, primary sclerosing cholangitis and nodular regenerative hyperplasia	Most invasive and expensive technique
Computed tomography or ultrasound-guided liver biopsy	CT- or ultrasound-guided liver biopsy performed	<1 mm bore needle used for fine needle aspiration under CT or ultrasound guided performed to visualize hepatic lesion and to avoid intersecting blood vessels	Reduced risk for hemorrhage of cirrhotic patient compared with transcutaneous biopsy

Table 21.19 Routine and special stains; immunohistochemical stains and molecular techniques used in interpretation of liver biopsy

Stain	Histologic Features	Clinical Correlation
Routine and special stains		
Hematoxylin and eosin	Routine assessment of liver histology	Liver architecture
PAS (periodic acid–Schiff) stain	▪ Glycogen ▪ Neutral polysaccharides	▪ Glycogen storage disease ▪ Gaucher disease ▪ Niemann-Pick disease
PAS (periodic acid–Schiff) stain after diastase digestion	▪ Lipochrome pigment in perivenular hepatocytes ▪ α_1-Antitrypsin inclusions in portal hepatocytes ▪ Lysosomal activity of Kupffer cells in portal tract region ▪ Basement membrane of interlobular ducts ▪ Fungi	▪ Elderly persons ▪ α_1-antitrypsin deficiency (homozygous or heterozygous state) ▪ Areas of cell necrosis in acute and chronic hepatitis ▪ Biliary disorders ▪ Diagnosis of fungal infection
Masson trichrome stain	▪ Collagen fibers ▪ Hepatocytes lobular confluent necrosis	▪ Chronic liver disease ▪ Fulminant hepatitis (severe massive necrosis of hepatocytes)
Von Gieson stain	Elastic fibers of large- and medium-sized blood vessels	Fibrointimal thickening, vascular occlusion, non-cirrhotic portal fibrosis and allograft vascular rejection
Reticulin stain	▪ Assessment of basic hepatic cord sinusoid reticulin, collagen III framework, liver architecture, thickness of hepatic plates, ducts and ductules ▪ Fibrosis ▪ Confluent necrosis of condensed fibers	▪ Evaluation of hepatic cords thickness (normal thickness of hepatic cord 1–2 cells; while in hepatocellular carcinoma thickness of hepatic cord >2–3 cells); reticulin is decreased or absent in hepatocellular carcinoma ▪ Chronic liver disease ▪ Fulminant hepatitis (severe massive necrosis of hepatocytes)
Perls Prussian blue	Hemosiderin	Primary hemochromatosis (iron deposition in hepatocytes), hemolytic anemia (iron deposition in Kupffer cells); and blood transfusion reactions (iron deposition in Kupffer cells)
Fouchet stain and Hell's bilirubin stain	Demonstration of bile	Cholestatic liver disease
Serius red stain	Direct staining of mature collagen type 1 fibers	Fibrosis in chronic liver disease
Congo red stain	Amyloid	Amyloid gives salmon-pink under LM and green birefringence under polarized microscope
Grocott's methenamine silver (GMS) stain	Fungi, microorganisms and parasites	Candida, Aspergillus, Cryptococcus, *Pneumocystis jerovecii*
Ziehl-Neelsen stain	Mycobacterium tubercle bacillus	Mycobacterium tubercle bacillus (human strain) and *Mycobacterium avium-intracellulare* complex
Rubeanic acid and rhodamine stains	Copper	Wilson disease
Orcein (Shikata) and Victoria blue stains	▪ Copper binding protein (granular staining) ▪ Hepatitis B surface antigen (diffuse cytoplasmic stain) ▪ Elastic fibers	▪ Indian childhood cirrhosis and primary biliary cirrhosis ▪ Chronic viral hepatitis type B ▪ Differentiation of old and new fibrosis
Warthin-Starry stain	Spirochetes (*Treponema pallidum*)	Syphilis
Oil red O and Sudan black stains	Neutral fats on frozen sections	Fatty liver disease
Phosphotungstic acid hematoxylin (PTAH) stain	Fibrin deposits in sinusoids and blood vessels	Toxemia of pregnancy, humoral allograft rejection, Q-fever and HELLP syndrome

Contd...

Table 21.19 Routine and special stains; immunohistochemical stains and molecular techniques used in interpretation of liver biopsy (*Contd...*)

Stain	Histologic Features	Clinical Correlation
Immunohistochemical stains		
HBsAg	Cytoplasmic staining of hepatocytes	Chronic viral hepatitis (HBV)
HBcAg	Nuclear staining of hepatocytes	Chronic viral hepatitis (HBV)
HCV	Cytoplasmic staining of hepatocytes	Chronic viral hepatitis (HCV)
Delta antigen	Nuclear staining of hepatocytes	Chronic viral hepatitis delta virus
Ubiquitin	Mallory-Denk bodies	Active fatty liver (alcoholic or nonalcoholic), chronic biliary tract disease
Cytokeratins 7,19	Epithelium of bile duct and ductules	Cytokeratin staining absent due to loss of interlobular bile duct in primary biliary disease and chronic allograft rejection
α-Antitrypsin	Presence of intracytoplasmic inclusions in periportal area	α-Antitrypsin deficiency
Cytomegalovirus (CMV)	Presence of CMV giving nuclear staining in hepatocytes	Cytomegalovirus (CMV) infection (active or latent)
Molecular techniques		
Polymerase chain reaction (PCR)	Demonstration of viruses and other infectious agents; and gene mutations	▪ Chronic viral hepatitis (HBV) ▪ Chronic viral hepatitis (HCV) ▪ HFE gene in hemochromatosis
Gene microarray analysis	Gene mutation	HFE gene in hemochromatosis
Epstein-Barr virus encoded RNA (EBER) probe	Epstein-Barr virus	Lymphoma and post-transplant lymphoproliferative disorders

Purpose of Liver Biopsy

Liver biopsy is examined to diagnose disease, its severity and progression. Prior to liver biopsy, PT–INR estimation is essential to distinguish between hepatocellular jaundice and obstructive jaundice.

Laboratory Investigations

Prior to liver biopsy, screening laboratory studies must be carried out 24–48 hours in advance such as complete blood counts, PT–INR, activated partial thromboplastin time (APTT), bleeding time and cross-matching for possible transfusion.

- After liver biopsy, pulse, blood pressure and abdominal girth should be monitored to rule out bleeding.
- Liver biopsy in a case of viral hepatitis is performed to confirm the disease, ascertain etiology, staging fibrosis and rule out superimposed disease process.
- For most chronic liver disease, a minimum of 1.5 cm in length and 6 portal tracts is usually sufficient to analyze liver biopsy specimen.

JAUNDICE AND CHOLESTASIS

JAUNDICE AND CHOLESTASIS: OVERVIEW

Jaundice is also called icterus. Clinical jaundice appears when serum bilirubin exceeds 2.5 mg/dl. It causes yellow discoloration of sclerae, skin, and mucous membranes due to accumulation of bilirubin, the catabolic product of the heme moiety of hemoglobin. It is most often associated with hepatocellular disease, biliary obstruction, or hemolytic anemia.

BILIRUBIN METABOLISM

After life span of RBCs 100–120 days, these cells are destroyed by reticuloendothelial system and liberate hemoglobin, which dissociates to form heme and globin. Simplified pathway of bilirubin metabolism is shown in Fig. 21.12. Pathophysiology of jaundice is shown in Fig. 21.13.

- Heme consists of porphyrin and iron. Globin goes to amino acid pool. Iron goes to iron storage

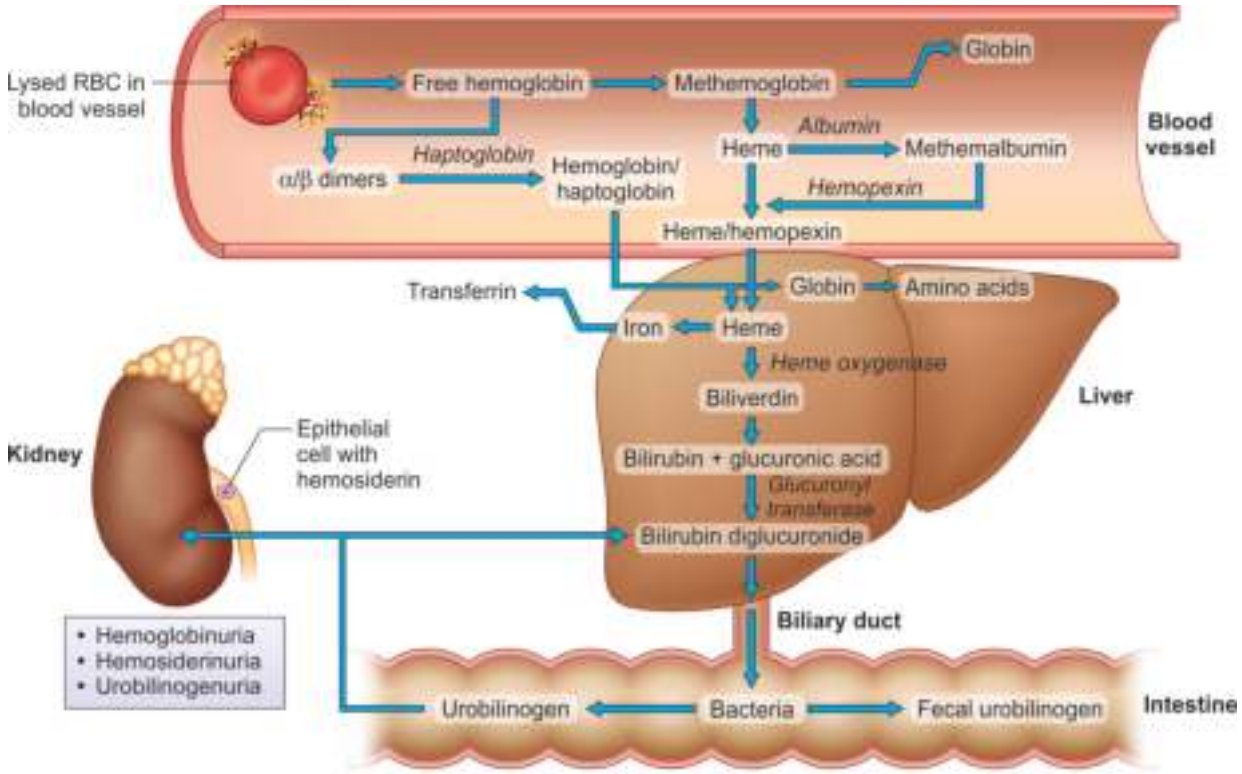


Fig. 21.12: Simplified pathway of bilirubin metabolism. Hemolytic anemia leads to increased biliary excretion of bilirubin. Obstruction of biliary passages will cause regurgitation of conjugated, water-soluble bilirubin into the blood which is then excreted in the urine. Hepatocellular damage in hepatitis will cause impaired biliary excretion of urobilinogen and conjugated bilirubin; these are excreted in the urine, causing it to darken. The enterohepatic circulation returns *cholic acid* and *chenodeoxycholic acid* to the liver; this enhances bile secretion.

sites, e.g. bone marrow, spleen and liver for reutilization.

- Porphyrin is converted into biliverdin and then unconjugated bilirubin (water insoluble), which goes to liver to form conjugated bilirubin by glucuronyl transferase. Conjugated bilirubin enters bile duct and enters small intestine and excreted in feces.
- Part of bilirubin in intestine is absorbed and goes to liver, which is called enterohepatic circulation.
- Rest of conjugated bilirubin is excreted as stercobilinogen in feces. Total normal bilirubin level is 0.3–1.0 mg/dl. Differences between direct and indirect bilirubin are given in Table 21.20. Deranged bilirubin metabolism in various disorders is given in Table 21.21.

PHYSIOLOGICAL JAUNDICE OF NEWBORN

Physiological jaundice of newborn is also called neonatal jaundice. It is common but clinically insignificant characterized by unconjugated hyperbilirubinemia observed during the first week of life.

- Transient jaundice results from both increased bilirubin production and relative deficiency of enzyme glucuronyl transferase in the immature liver especially in premature infants. It is known as physiological jaundice of newborn.
- It must be distinguished from neonatal cholestasis due to extrahepatic biliary atresia, α_1-antitrypsin deficiency and cytomegalovirus infection.

CHOLESTASIS

Failure to drain bile juice leads to the appearance in the plasma of increased concentrations of all constituents of bile such as bilirubin, alkaline phosphatase, IgA and less often cholesterol.

Causes of cholestasis are given in Table 21.22. Cholestasis due to intrahepatic and extrahepatic causes is given in Table 21.23.

Secondary Changes in Liver Resulting from Cholestasis

- **Scattered liver cell necrosis:** Increased concentration of bile causes scattered liver cell injury.
- **Cellular debris in macrophages:** Macrophages contain bile pigment and cellular debris.
- **Periportal fibrosis and proliferation of bile ductules:** These histologic findings are nonspecific.
- **Feathery degeneration of hepatocytes:** Hepatocytes are swollen due to accumulation of water and bile pigment.

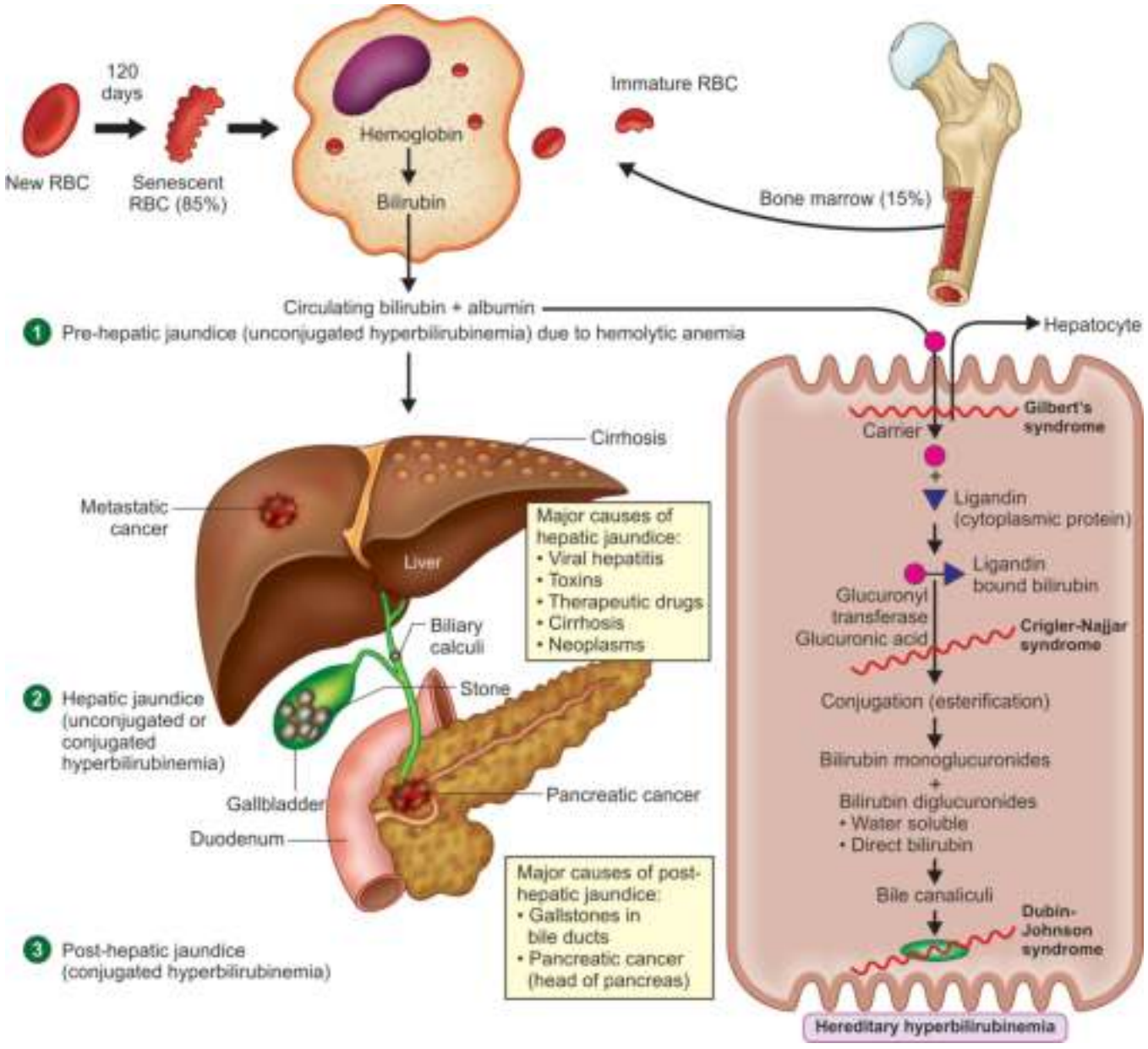


Fig. 21.13: Pathophysiology of jaundice.

Table 21.20 Differences between direct bilirubin and indirect bilirubin

Direct Bilirubin	Indirect Bilirubin
Normal range (0–0.2 mg/dl)	Normal range (0.3–0.7 mg/dl)
Conjugated bilirubin (direct reacting)	Unconjugated bilirubin (indirect reacting)
Water soluble	Lipophilic and water insoluble
Loosely bound to albumin	Tightly bound to albumin
Easily filtered by glomeruli and excreted in urine	Not filtered by glomeruli, hence not excreted in urine
Unable to cross blood–brain barrier	Able to cross blood–brain barrier and damages brain of the newborn (kernicterus) with erythroblastosis fetalis

Total serum bilirubin normal range (0.3–10. mg/dl).

- **Formation of bile lakes:** Long-standing cholestasis may cause hepatocellular injury. Extravasated bile pigments form brownish-yellow masses admixed with cellular debris.

Table 21.21 Deranged bilirubin metabolism in various disorders

Pathophysiology of Hyperbilirubinemia	Causes
Prehepatic causes (increased bilirubin production)	▪ Hemolytic anemia ▪ Aortic aneurysm rupture ▪ Massive intestinal hemorrhage ▪ Blood resorption from large hematoma
Hepatic causes (decreased uptake of bilirubin)	▪ Gilbert's syndrome* ▪ Diffuse hepatocellular injury by HBV and HCV ▪ Chloramphenicol drug ▪ Flavaspidic drug used in tapeworm infestations (drug inhibiting uptake of bilirubin by binding to the ligand) ▪ Contrast media used for cholecystography
Hepatic impaired conjugation of bilirubin (unconjugated hyperbilirubinemia)	▪ Neonatal jaundice ▪ Crigler-Najjar syndrome (types 1 and 2)* ▪ Gilbert's syndrome ▪ Hepatic impaired excretion of bilirubin (conjugated hyperbilirubinemia) ▪ Dubin-Johnson syndrome* ▪ Rotor's syndrome* ▪ Hepatitis ▪ Pregnancy associated cholestasis (recurrent jaundice of pregnancy) ▪ Drugs causing intrahepatic cholestasis such as oral contraceptives and anabolic hormones ▪ Alcoholic cirrhosis ▪ Primary biliary cirrhosis ▪ Hepatic infiltration ▪ Sepsis
Posthepatic obstruction of bilirubin outflow in extra-hepatic biliary passages (conjugated hyperbilirubinemia)	▪ Primary sclerosing cholangitis ▪ Gallstones ▪ Cholangiocarcinoma ▪ Portahepatitis lymph nodes ▪ Biliary atresia ▪ Surgical-induced biliary stenosis ▪ Carcinoma of head of pancreas ▪ Carcinoma of ampulla of Vater ▪ Pancreatitis

**Gilbert's syndrome, Crigler-Najjar syndrome, Dubin-Johnson syndrome, and Rotor's syndrome are examples of hereditary hyperbilirubinemia.*

Table 21.22 Causes of cholestasis

Pathophysiology of Cholestasis	Causes
Extrahepatic mechanical obstruction	▪ Primary sclerosing cholangitis ▪ Gallstones ▪ Cholangiocarcinoma ▪ Portahepatitis lymph nodes ▪ Biliary atresia ▪ Surgical induced biliary stenosis ▪ Carcinoma of head of pancreas ▪ Carcinoma of ampulla of Vater ▪ Pancreatitis
Intrahepatic mechanical obstruction	▪ Granulomas ▪ Metastatic neoplasms in liver ▪ Cystic fibrosis

Contd...

Table 21.22 Causes of cholestasis (*Contd...*)

Pathophysiology of Cholestasis	Causes
Mechanical obstruction in infancy	Biliary atresia (extrahepatic or intrahepatic)
Intrahepatic cholestasis without obstruction	▪ Certain infections ▪ α-Antitrypsin deficiency ▪ Part of paraneoplastic syndromes in renal cell carcinoma ▪ Pregnancy ▪ Viral hepatitis ▪ Hepatotoxic drugs ▪ Idiopathic recurrent cholestasis ▪ Familial progressive cholestasis (Byler's disease) ▪ Primary biliary cirrhosis ▪ Other types of cirrhosis

Table 21.23 Cholestasis due to extrahepatic and intrahepatic causes

Liver Biopsy (LM)	Extrahepatic Cholestasis	Intrahepatic Cholestasis
Cholestasis	Severe degree	Mild degree
Bile pigments present	▪ Ducts and ductules ▪ Rupture of bile canaliculi leading to bile lake formation	Hepatocytes and Kupffer cells
Acute inflammation (PMN cells) in bile ducts	Present surrounding bile duct and canaliculi	Absent surrounding bile duct and canaliculi
Feathery degeneration of hepatocytes	Unremarkable	Marked

RECURRENT JAUNDICE DURING PREGNANCY

Recurrent jundice most often occurs in the third trimester of pregnancy, after seven months of gestation. Biochemical findings mimic extrahepatic biliary obstruction. Jaundice clears after delivery, which may recur with subsequent pregnancy. Etiology is related to increased levels of estrogens. Complications include stillbirths, prematurity and fetal distress.

HEREDITARY HYPERBILIRUBINEMIA

Under physiologic state, after a span of 100–120 days, red blood cells release unconjugated bilirubin. Hepatocytes participate in conjugation of bilirubin and its excretion in biliary ducts.

- Three steps in bilirubin metabolism include: uptake of unconjugated bilirubin by hepatocytes, conjugation of bilirubin by glucuronyl transferase enzyme in hepatocytes and excretion of conjugated bilirubin in biliary ducts. Defects at any level may result in congenital hyperbilirubinemia.
- Examples are Gilbert's syndrome, Crigler-Najjar syndrome, Dubin-Johnson syndrome and Rotor syndrome. Comparison of various types of hereditary hyperbilirubinemias is given in Table 21.24.

Clinical Pearls: Hereditary Hyperbilirubinemia

- **Gilbert syndrome:** It is an autosomal or recessive disorder that results in an unconjugated hyperbilirubinemia due to a partial deficiency of glucuronosyltransferase enzyme used to metabolize bilirubin.
- **Crigler-Najjar syndrome:** It is an autosomal disorder characterized by the absence of bilirubin conjugating enzymes in the liver.
  - Crigler-Najjar syndrome type 1 is an autosomal recessive disorder characterized by complete absence of glucuronosyltransferase enzyme leading to elevated levels of unconjugated bilirubin, kernicterus and death within the first 18 months of life.
  - Crigler-Najjar syndrome type 2 is an autosomal dominant disorder, less severe, nonfatal form of disease with partial defect in the conjugating glucuronosyltransferase enzyme.
- **Dubin-Johnson syndrome:** It is an autosomal recessive disorder that results from a defect in glucuronide transport proteins which carry bilirubin from the liver into the biliary system. Patient presents with conjugated hyperbilirubinemia, hepatomegaly, and dark pigmentation of the liver. Staining for melanin is negative.
- **Rotor syndrome:** It is a variant of Dubin-Johnson syndrome characterized by chronic conjugated hyperbilirubinemia and defective excretion of organic ions into the bile. It requires no treatment. It has excellent prognosis. Hepatocytes lack dark pigmentation.

Table 21.24 Comparison of various types of hereditary hyperbilirubinemias

Disorder	Inheritance	Bilirubin Metabolism Defects	Serum Bilirubin	Clinical Course	Prognosis
Unconjugated hyperbilirubinemia					
Gilbert's syndrome	Autosomal dominant	UDP-glucuronyl transferase activity decreased	<6 mg/dl	Episodic hyperbilirubinemia (marked jaundice)	Excellent
Crigler-Najjar syndrome type 1	Autosomal recessive	UDP-glucuronyl transferase activity absent	>20 mg/dl	Fatal outcome during neonatal period (mild jaundice)	Poor
Crigler-Najjar syndrome type 2	Autosomal dominant	UDP-glucuronyl transferase activity decreased	<20 mg/dl	Mild course (mild jaundice)	Good
Conjugated hyperbilirubinemia					
Dubin-Johnson syndrome	Autosomal recessive	Impaired bilirubin excretion due to mutation of canalicular multidrug resistance protein 2 (MRP2)	<5 mg/dl	Intermittent mild jaundice, mild hepatomegaly (brown-to-black in hepatocytes)	Excellent
Rotor's syndrome	Autosomal recessive	Decreased hepatic uptake and storage. Decreased biliary excretion	<6 mg/dl	Benign course (mild jaundice)	Good

GILBERT'S SYNDROME

Gilbert's syndrome is an autosomal dominant or recessive disorder characterized by mild, recurrent, unconjugated hyperbilirubinemia (<6 mg/dl) due to impaired clearance of bilirubin in the absence of significant liver disease. This disease is usually detected in later life. Aside from jaundice, patient is asymptomatic. No treatment is usually necessary.

- The cause is a combination of decreased bilirubin uptake by hepatocytes and reduced activity of glucuronyl transferase enzyme.
- Patient presents with episodic hyperbilirubinemia precipitated by stress, fatigue, alcohol use, or recurrent infection.

Surgical Pathology: Gilbert Syndrome

Light Microscopy

- The liver architecture appears normal except for mild non-specific accumulation of lipofuscin pigment in the centrilobular zones.
- There are other mild abnormalities, which can be seen in electron microscopy.

Clinical Pearls: Gilbert Syndrome

- Bilirubin has antioxidant property, and in persons with mildly increased bilirubin levels in Gilbert syndrome may offer a protective effect.
- Patients with Gilbert syndrome have a lower incidence of ischemic heart disease due to decreased production of advanced glycation end products, which prevents endothelial dysfunction and reduces progression of atherosclerosis.
- Patients with Gilbert syndrome have lower incidence of Hodgkin disease, endometrial carcinoma and cancer-related mortality.
- Patients with Gilbert syndrome are at higher risk for developing pigment stones in gallbladder (cholelithiasis) in the settings of hemolytic disorders such as β-thalassemia major, sickle cell disease and hereditary spherocytosis.

CRIGLER-NAJJAR SYNDROME

Crigler-Najjar syndrome is an autosomal dominant/recessive disorder characterized by unconjugated hyperbilirubinemia due to lack of glucuronyl transferase enzyme or its decreased activity in hepatocytes. Differences between Crigler-Najjar syndrome types 1 and 2 are given in Table 21.25. Crigler-Najjar syndrome has two variants described as under:

- **Crigler-Najjar syndrome type 1:** It is most severe form of autosomal recessive disorder. A total lack of glucuronyl transferase results in early death from kernicterus; damage to the basal ganglia and other parts of the central nervous system caused by unconjugated bilirubin.
- **Crigler-Najjar syndrome type 2:** It is less severe form of autosomal dominant disorder and compatible with life. It responds to phenobarbital therapy, which decreases the serum concentration of unconjugated bilirubin.

DUBIN-JOHNSON SYNDROME

Dubin-Johnson syndrome is an autosomal recessive familial disorder. Conjugated hyperbilirubinemia occurs due to defective bilirubin transport. Liver biopsy shows conspicuous deposition of melanin-like pigment.

Table 21.25 Differences between Crigler-Najjar syndrome types 1 and 2

Characteristics	Crigler-Najjar Syndrome	
	Type 1	Type 2
Inheritance	Autosomal recessive disorder	Autosomal dominant disorder
Glucuronyl transferase enzyme	Complete deficiency	Deficiency varying from one person to another
Kernicterus	Common	Rarely
Clinical course	Severe type	Mild type
Bilirubin	Unconjugated hyperbilirubinemia	Bile consisting of unconjugated as well as conjugated hyperbilirubinemia
Prognosis	Death during first year	Life is compatible depending on content of glucuronyl transferase enzyme
Response to phenobarbital therapy	No response	Responds well results in synthesis of glucuronyl transferase enzyme

Molecular Genetic Alterations

Dubin-Johnson syndrome occurs due to ABCC2 gene mutation that codes for canalicular multidrug resistance protein 2 (MRP2). MRP2 participates in ATP-dependent excretion of bilirubin mono- and diglucuronides across the canalicular membrane into the bile canaliculi.

Clinical Features

Patient presents with intermittent mild jaundice, hepatomegaly and nonspecific gastrointestinal symptoms.

Surgical Pathology: Dubin-Johnson Syndrome

Gross Morphology

Gross examination reveals blackish discoloration of liver due to accumulation of polymerized epinephrine metabolites.

Light Microscopy

- Liver architecture is normal and shows deposition of brown-to-black melanin-like pigment in hepatocytes and Kupffer cells mainly in centrilobular region (Fig. 21.14).
- Melanin-like pigment in hepatocytes feature differentiates from other congenital conjugated hyperbilirubinemia.

Histochemistry

The chemical nature of this pigment is not clear. The dark melanin-like pigment is demonstrated by Masson-Fontana stain.

Laboratory Investigations

Serum bilirubin level is raised between 2 and 5 mg/dl. Serum liver transaminases (ASI and ALT) and alkaline phosphatase are within normal limits. Bromsulphthalein excretion is a diagnostic test for Dubin-Johnson syndrome.

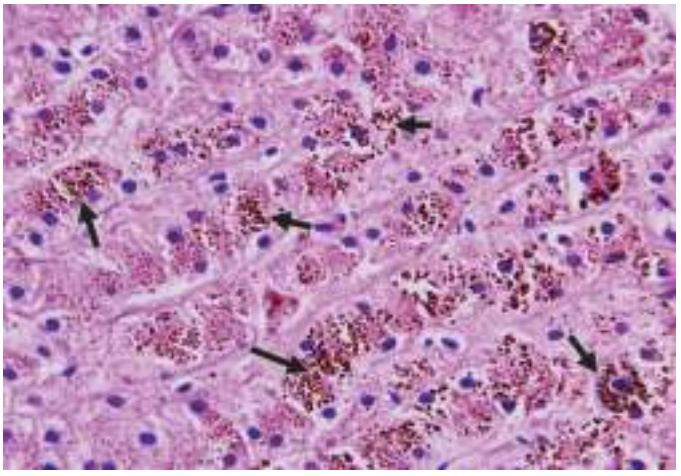


Fig. 21.14: Dubin-Johnson syndrome. Liver shows deposition of brown-to-black melanin-like pigment in hepatocytes and Kupffer cells mainly in centrilobular region (arrows) (400X).

ROTOR SYNDROME

Rotor's syndrome is a relatively benign disorder characterized by conjugated hyperbilirubinemia. It is similar to Dubin-Johnson syndrome, but abnormal melanin-like pigment is not present. Both Dubin-Johnson syndrome and Rotor syndrome cause conjugated hyperbilirubinemia (serum bilirubin ≤6 mg/dl).

NEONATAL CHOLESTASIS

Neonatal cholestasis occurs due to various disorders is given as follows (Table 21.26).

Table 21.26 Major causes of neonatal cholestasis

Categories	Etiological Agents
Bile duct obstruction	Extrahepatic biliary atresia/stenosis
Neonatal infection	Cytomegalovirus, bacterial sepsis, syphilis or urinary tract infection
Toxic substances	Drugs like acetaminophen, methyldopa, halothane, antidepressants and isoniazid
Metabolic disorders	Niemann-Pick disease, tyrosinemia, galactosemia, cystic fibrosis, α_1-antitrypsin deficiency or defective bile acid synthetic pathways
Miscellaneous disorders	Indian childhood cirrhosis, Alagille syndrome (paucity of bile ducts) or hypotensive shock
Idiopathic neonatal hepatitis	Unknown

EXTRAHEPATIC BILIARY ATRESIA/STENOSIS

Extrahepatic biliary atresia is defined as portal or total absence of permeable bile duct between porta hepatis and the duodenum. Cholestasis is total and permanent. Needle liver biopsy allows correct identification of the disorder.

- Extrahepatic biliary atresia is most common cause of pathologic infant jaundice; common reason for pediatric liver transplantation.
- Stenotic or atretic portions of extrahepatic biliary tree cause chronic extrahepatic large duct obstruction.
- Disorder may occur in two forms: (a) fetal/embryonic (congenital) and (b) perinatal/acquired. Acquired extrabiliary stenosis occurs as a result of progressive inflammation leading to obstruction of the biliary tree. It results in marked cholestasis, intrahepatic bile duct proliferation, fibrosis and cirrhosis. The liver becomes rocky hard.

Surgical Pathology: Extrahepatic Biliary Atresia/Stenosis

Light Microscopy

- Histologic examination of liver biopsy shows lobular cholestasis, numerous brown-green bile plugs, portal neutrophilic infiltrate, bile ductular proliferation with elongated and angulated ductules (Fig. 21.15).
- Later, fibrosis or cirrhosis occurs.

ALAGILLE SYNDROME

Alagille syndrome is an autosomal dominant disorder, which can affect the liver, heart and other organs. Alagille syndrome is characterized by abnormalities in the bile ducts leading to liver damage. Mutation in NOTCH signaling pathway is involved in the development of Alagille syndrome.

Patient presents with mild to severe symptoms such as jaundice, pruritus, xanthomas, congenital heart defects, butterfly shape of vertebral column and other bones demonstrated on radiographs.

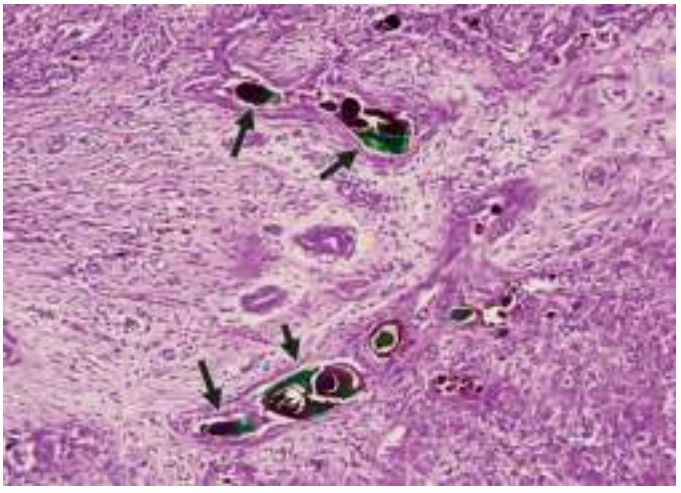


Fig. 21.15: Extrahepatic biliary atresia. Two months old infant presents with jaundice, pale stools, dark colored urine, and hepatomegaly. Microscopically, it shows numerous brown-green bile plugs, bile duct proliferation, and extensive fibrosis (arrows) (100X).

DRUG-INDUCED HEPATITIS

Various therapeutic drugs may cause drug-induced hepatitis, which include acetaminophen, methyldopa, halothane, antidepressants and isoniazid. Liver biopsy shows presence of eosinophils in the portal tracts, evidence of cholestasis, and sometimes granulomas.

CAROLI DISEASE

Caroli disease is an autosomal dominant trait disorder. Mutation of PKHD1 gene linked to ARPKD has been demonstrated in patient with Caroli disease. There is history of liver and kidney disease. It is characterized by segmental dilatation of larger intrahepatic ducts associated with bile inspissation and cholestasis.

It is frequently complicated by cholelithiasis and hepatic abscess. There is increased risk for development of cholangiocarcinoma.

NEONATAL HEPATITIS OF UNKNOWN ETIOLOGY

Neonatal hepatitis is of unknown etiology in 50% of cases. Approximately 30% of cases are assigned to α_1-antitrypsin deficiency alone. Most of the other known causes can be attributed to chromosomal abnormalities or intrauterine infections or biliary atresia.

Clinical Features

Neonatal hepatitis may cause jaundice during the first few weeks of life. Most infants with uncomplicated neonatal hepatitis eventually recover. Untreated biliary obstruction causes progressive fibrosis resulting in secondary biliary cirrhosis.

Surgical Pathology: Neonatal Hepatitis

Light Microscopy

- Liver biopsy in neonatal hepatitis shows presence of multinucleated giant cells (characteristic finding).
- Multinucleated giant cells contain as many as 40 nuclei and may appear detached from other cells in the liver plate.
- Hepatocytes may contain bile pigment due to prolonged cholestasis and hemosiderin (Fig. 21.16).

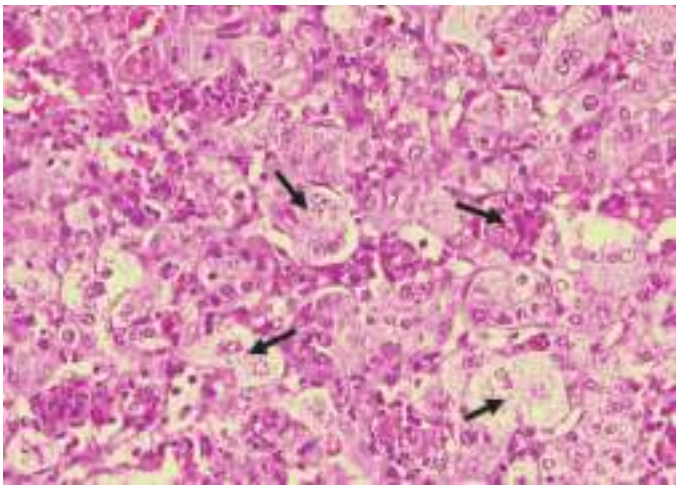


Fig. 21.16: Neonatal hepatitis. Seen here is the major differential diagnosis of biliary atresia: This is neonatal giant cell hepatitis. There is lobular disarray with focal hepatocyte necrosis, giant cell transformation, lymphocytic infiltration, Kupffer cell hyperplasia, and cholestasis (not seen here). Neonatal hepatitis may be idiopathic or of viral origin. Many neonates recover in a couple of months (arrows) (400X).

JAUNDICE: DIFFERENTIAL DIAGNOSIS

Differential diagnosis of jaundice (Table 21.27) and disorders causing type of hyperbilirubinemia is given in Table 21.28.

HEMOLYTIC JAUNDICE

Hemolytic jaundice occurs due to rapid destruction of red blood cells resulting in production of excess of bilirubin. Liver is not able to conjugate excess of bilirubin. Hemolytic disease of the newborn most commonly occurs with Rh blood group incompatibility between mother and fetus.

Clinical Features

Patient presents with jaundice due to excess of unconjugated bilirubin in blood. Hemolytic jaundice is seen in sickle cell disease, mismatched blood transfusions and some infections.

Urine Analysis

Urine analysis in hemolytic jaundice shows increased urobilinogen and absence of bilirubin (acholuria). Degree of urine urobilinogen increase is directly related to increased hemoglobin catabolism.

HEPATOCELLULAR JAUNDICE

Hepatocellular jaundice occurs due to hepatitis, congenital hyperbilirubinemia due to malfunction of glucuronyl transferase enzyme and hepatocellular

Table 21.27 Differential diagnosis of jaundice

Etiology	Serum	Urine	Stool Color
▪ Hemolytic anemia ▪ Hepatocellular jaundice (e.g. acute hepatitis)	▪ Unconjugated hyperbilirubinemia ▪ Mixed hyperbilirubinemia ▪ Increased transaminases	▪ Normal ▪ Dark	▪ Dark ▪ Pale
Extrahepatic obstructive jaundice (e.g. cholestasis)	▪ Conjugated hyperbilirubinemia ▪ Increased alkaline phosphatase ▪ Increased transaminases	Dark	Pale
Congenital hyperbilirubinemia (e.g. Gilbert's syndrome)	▪ Unconjugated or conjugated hyperbilirubinemia ▪ Other liver function tests: normal ▪ No evidence of hemolysis	Variable	Variable

Table 21.28 Disorders causing type of hyperbilirubinemia

Causes	Alkaline Phosphatase and AST/ALT	Hyperbilirubinemia
▪ Gallstone, carcinoma of common bile duct obstruct the bile flow ▪ Pancreatic head carcinoma ▪ Viral hepatitis induced hepatocellular damage	▪ Alkaline phosphatase level >AST/ALT occurs in obstructive jaundice ▪ Alkaline phosphatase level <AST/ALT	Direct (conjugated) bilirubin >15% of total bilirubin
▪ Physiological jaundice of newborn ▪ Hemolytic anemia ▪ Breast milk jaundice due to presence of β-glucuronidase in milk ▪ Diffuse hepatocellular damage due to cirrhosis, viruses and drugs ▪ Gilbert's syndrome ▪ Crigler-Najjar syndrome (types 1 and 2)	Not applicable	Indirect (unconjugated) bilirubin <15% of total bilirubin

carcinoma. Patient presents with jaundice due to excess of conjugated and unconjugated bilirubin in blood. Urine analysis may show normal or decreased urobilinogen.

EXTRAHEPATIC OBSTRUCTIVE JAUNDICE

Extrahepatic obstructive jaundice is related to mechanical obstruction of bile flow outside the liver due to gallstones or pancreatic tumors obstructing pancreatic biliary duct.

Clinical Features

Patient presents with jaundice due to excess of conjugated bilirubin in blood passage of clay-colored stools.

Urine Analysis

Urine analysis shows decreased urobilinogen. Serum ALT and AST enzyme levels are variable.

Blood Biochemistry

Alkaline phosphatase and cholesterol are increased.

HEPATITIS

HEPATITIS: OVERVIEW

Hepatitis refers to inflammation of the liver caused by viruses, autoimmune mechanisms, drugs, toxins or secondary to other systemic disorders.

- The known hepatotropic viruses primarily affecting hepatocytes include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis B-associated delta virus (HDV), hepatitis C virus (HCV), and hepatitis E virus (HEV). HAV and HEV transmit via fecal–oral route. HBV, HCV and HDV transmit via parenteral/sexual route.
- Viruses causing systemic disease can involve the liver, which include yellow fever virus (mid-zonal necrosis), Epstein-Barr virus, Ebola virus (mild hepatitis during the acute phase); cytomegalovirus (particularly in newborns and immunosuppressed persons); herpesviruses; and enteroviruses.
- HAV infection has direct cytopathic effect on hepatocytes. Hepatocellular injury occurs due to attack by CD8+ cytotoxic T cells and natural killer cells; and antibodies formed against viral antigens on infected hepatocytes.
- Extent of inflammation and hepatocellular necrosis in HBV infection depends on the immune response of the person.
  - Prompt immune response in HBV infection may cause hepatocellular injury to eliminate HBV. Marginal immune response in HBV infection fails to eliminate HBV, hence persistence of HBV antigens in hepatocytes results in chronic hepatitis or carrier state. Accelerated immune response in HBV infection can cause fulminant hepatitis.
- Infection with hepatotropic viruses may produce clinical manifestations of varying grades of severity ranging from mild jaundice, nausea and anorexia to fulminating illness (acute liver failure) associated with fatal outcome.
- Some cases may progress to cirrhosis with increased risk of development of hepatocellular carcinoma.
- Classification of hepatitis based on etiology, histologic activity (necrosis and inflammation) and degree of fibrosis (progression of disease) according to METAVIR scoring system is given in Table 21.29.
- Acute and chronic hepatitis may be caused by hepatotropic viruses (HAV, HBV, HCV, HDV and HEV). Acute hepatitis is characterized by jaundice, elevation of serum AST (aspartate transaminase) and ALT (alanine transferase), nausea and vomiting.
- Histologic features in acute hepatitis are evidence of hepatocellular injury and regeneration, chronic inflammatory infiltrate but **absence of fibrosis**.

Table 21.29 Classification of hepatitis based on etiology, histologic activity (necrosis and inflammation) and degree of fibrosis (progression of disease) according to METAVIR scoring system

Classification of Hepatitis Based on Etiology	
Hepatotropic viruses	HAV, HBV, HCV, HDV, HEV
Uncommon viruses	Cytomegalovirus and herpesvirus (immunocompromised state), yellow fever virus (mid-zonal necrosis), Epstein-Barr virus, rubella virus, Ebola virus, adenovirus, enterovirus
Parasites	*Entamoeba histolytica*, *Echinococcus granulosus*, *Schistosoma japonicum*, *Schistosoma mansoni*
Leptospira species	Weil's disease (jaundice, hemorrhagic fever and renal failure)
Autoimmune hepatitis	Nitrofurantoin, methyldopa, statins, methotrexate, oxyphenisatin acetate (laxative), α or β interferon, phenothiazines, infliximab
Classification of Hepatitis Based on Histologic Activity (Necrosis and Inflammation)	
Portal tract inflammation	Inflammation confined to portal tract region
Periportal tract inflammation	Inflammation extending from portal area to surrounding periportal tract region
Piecemeal necrosis (now known as interface necrosis)	Loss of hepatocytes between hepatic lobule and limiting plates of portal tracts demonstrated in viral hepatitis, autoimmune hepatitis and steatohepatitis
Bridging necrosis	Confluent necrosis of hepatocytes bridging adjacent central vein of hepatic lobules and limiting plates of portal tracts
Classification of Hepatitis Based on Activity Grade	
Grade 0	No activity in liver
Garde A1	Mild activity in liver
Garde A2	Moderate activity in liver
Grade A3	Severe activity in liver
Classification of Hepatitis Based on Degree of Fibrosis (Progression of Disease)	
Stage 0	No evidence of fibrosis in liver
Stage 1	Mild fibrosis confined to portal tracts
Stage 2	Moderate fibrosis in portal tract and periportal region
Stage 3	Severe fibrosis with bridging necrosis between central vein of hepatic lobules and limiting plates of portal tracts
Stage 4	Massive fibrosis (e.g. cirrhosis)

The METAVIR scoring system is a system used to assess the extent of fibrosis by histologic evaluation in a liver biopsy of patients with hepatitis. The grade indicates the activity or degree of inflammation while the stage represents the amount of fibrosis or scarring.

- Chronic hepatitis (chronic portal hepatitis, chronic lobular hepatitis, chronic active hepatitis) is caused by hepatotropic viruses such as HBV (10%), HCV (80%), and HDV (10%).
- Histologic features in chronic hepatitis are evidence of hepatocellular injury and regeneration, chronic inflammatory infiltrate but **presence of fibrosis**. Clinical course of the following viral hepatitis is given in Table 21.30. Differentiating Histologic features of acute and chronic viral hepatitis are given in Table 21.31. Histologic features of liver with specific hepatotropic viruses are given in Table 21.32.

Table 21.30 Clinical course of the following viral hepatitis

Clinical Patterns of Hepatitis	Hepatotropic Virus
Asymptomatic hepatitis	HAV (most often), HBV, HCV, HDV, HEV
Acute hepatitis without jaundice (anicteric hepatitis) or with jaundice (icteric jaundice)	HAV, HBV, HCV, HDV, HEV
Chronic hepatitis (chronic portal hepatitis previously known as chronic persistent hepatitis, chronic lobular hepatitis, chronic active hepatitis)	HBV (10%), HCV (80%), HDV (10%)
Chronic carrier state	HBV, HCV, HDV
Massive liver necrosis with acute hepatocellular failure	HAV, HBV, HCV, HDV, HEV

HAV and HEV transmit via fecal–oral route. HBV, HCV and HDV transmit via parenteral/sexual route.

Table 21.31 Differentiating histologic features of acute and chronic viral hepatitis

Hepatocellular Injury Parameters	Acute Viral Hepatitis	Chronic Viral Hepatitis
Hepatotropic viruses	▪ HAV (most often) ▪ HBV ▪ HCV ▪ HDV ▪ HEV	▪ NA ▪ 10% ▪ 80% ▪ 10% ▪ NA
Hallmark characteristics	▪ Prominent portal lymphocytic infiltrates, normal interlobular bile ducts ▪ Diffuse necro-inflammatory change in the perivenular zone in early stage of disease, apoptosis, Kupffer cell reaction ▪ Cholestasis (presence of bile plugs) in more severe cases ▪ Fibrosis in hepatic lobule absent	▪ Periportal interface inflammatory activity in active stage ▪ Lobular necro-inflammatory change irregularly distributed in active stage disease ▪ Cholestasis present ▪ Variable portal fibrosis with eventual bridging fibrosis between hepatic central vein and portal tract and development of cirrhosis
Liver architecture	Disrupted	Disrupted
Hepatic lobule involvement pattern	Centrilobular zone of liver	Throughout hepatic lobule
Ballooning swelling (degeneration) of hepatocytes	Present	Present
Regeneration of hepatocytes	Present	Present
Hepatocytes loss	Isolated hepatocytes or cluster of hepatocytes	Isolated hepatocytes or cluster of hepatocytes
Fibrosis in hepatic lobule	Absent	Present (portal and periportal fibrosis with bridging fibrosis between hepatic central vein and portal tract)
Spill over inflammation from portal tract to hepatic acinus	Uncommon	Common
Piecemeal necrosis	Piecemeal necrosis absent	Piecemeal necrosis present (hallmark of progressive disease)
Councilman hyaline bodies (apoptotic bodies) in sinusoids	Present	Absent
Ground glass appearance of hepatocytes	Absent	Present in HBV infection
Fatty change	Present in HCV infection	Present in HCV infection
Bile duct proliferation and lymphoid aggregates	Present	Present in HCV infection
Reactive changes in sinusoids	Kupffer cells laden with phagocytosed cellular debris; and chronic inflammatory cells infiltrate in sinusoids	Kupffer cells laden with phagocytosed cellular debris; and chronic inflammatory cells infiltrate in sinusoids
Cirrhosis	Absent	Progresses to cirrhosis
Hepatocellular carcinoma	Absent	Increased risk

Histologic features shared by acute and chronic viral hepatitis are hepatocellular injury, necrosis of hepatocytes, apoptosis of hepatocytes, regeneration hepatocytes and sinusoidal changes.

Table 21.32 Histologic features of liver with specific hepatotropic viruses

Hepatotropic Viruses	Acute Viral Hepatitis	Chronic Viral Hepatitis
HAV	Increase in plasma cells in portal region, periportal inflammation, cholestasis	No chronic viral hepatitis
HBV	Emperipolesis (presence of an intact cell within the cytoplasm of another cell)	Hepatocytes show ground glass appearance (endoplasmic reticulum synthesizing HBsAg) in 50–66% of cases, hepatocytes can show dysplasia in advanced disease
HBV + HDV	Microvesicular steatosis, hepatitis often with severe confluent hepatocellular necrosis	Microvesicular steatosis
HCV	Sinusoidal lymphocytosis, granulomatous necrosis	Portal lymphoid aggregates (≤50%), sinusoidal lymphocytosis, granulomatous necrosis, mild microvesicular steatosis in <25% of hepatocytes
HEV	Bile duct proliferation, bile duct ectasia, cholestasis with an acinar arrangement of hepatocytes	No chronic forms (rare exception is post-transplant cases)
HGV	Syncytial giant cells of hepatocytes, usually mild necroinflammatory change	No chronic viral hepatitis

VIRAL HEPATITIS

ACUTE VIRAL HEPATITIS

Incubation period of acute viral hepatitis (HAV, HBV, HCV, HDV, HEV, HGV) is the period from the time of infection and the first symptoms appear. Acute viral hepatitis has three phases: preicteric phase (prodromal phase), icteric phase (jaundice) and recovery phase (convalescence phase).

Preicteric Phase

The period of prodromal period and clinical symptoms vary with each of the hepatotropic virus.

- In acute hepatitis, patients may present with malaise, flu-like symptoms, anorexia, low-grade fever, arthralgia, myalgia with jaundice and passage of dark-colored urine and clay-colored stools in about 20% of cases most often paralleling the severity of the disease or specific hepatotropic virus involved. Right upper quadrant tenderness due to hepatomegaly may be present.
- Extrahepatic manifestations may also occur such as serum sickness manifestations (rash, urticaria, angioneurotic edema), polyarteritis nodosa (HBV infection), cryoglobulinemia, and renal disease (mild proteinuria, abnormal urinary sediments, rarely glomerulonephritis).
- Majority of cases with HCV infection may be asymptomatic or have vague non-specific symptoms.
- Patient with HAV infection may demonstrate hepatomegaly. Approximately 10% of patients with HBV infection show signs of serum sickness, characterized by skin rash, arthralgia, myalgia or mild proteinuria. Serum transaminases aspartate transaminase (AST) and alanine aminotransferase (ALT) increase steadily and attain peak before clinical jaundice appears. Peripheral blood smear reveals atypical lymphocytosis.

Icteric Phase (Jaundice)

The icteric phase is variable depending on the type of hepatitis. Patient develops itching, jaundice, abdominal pain and tenderness.

- Liver function tests demonstrate elevations of aspartate transaminase (AST) and alanine aminotransferase (ALT) usually >500 IU/L and most often over 1000 IU/L (ALT more specific for hepatocellular injury). Alkaline phosphatase value is usually normal or elevated about two times of normal values.
- Urine analysis shows darkening of urine due to increased excretion of bilirubin and urobilinogen. Jaundice is usually accompanied by improvement of constitutional symptoms.
- When hyperbilirubinemia and jaundice occur, the serum bilirubin value ranges from 5 to 20 mg/dl, depending on the degree of hepatotropic virus infection and necroinflammatory changes. Importantly, the PT–INR and serum albumin levels are generally normal in uncomplicated cases.

Recovery Phase

- Recovery phase is also known as convalescence phase. Symptoms are subsiding like return of appetite and resolution of jaundice.
- Recovery is the rule in the majority of cases. Liver function tests return to normal within few weeks after clinical presentation, although a prolonged clinical course lasting a few months can occur with acute HAV infection.

Table 21.33 Patterns of acute viral hepatitis

Pattern	Comments
Inflammatory-predominant pattern (with variable necrosis)	▪ Acute viral hepatitis ▪ Autoimmune hepatitis ▪ Wilson disease ▪ Celiac disease ▪ Idiosyncratic drug reaction
Cholestasis pattern (nonspecific)	▪ Acute viral hepatitis (HAV, HEV) ▪ Acute hepatitis (drug-induced)
Toxic pattern (direct hepatocellular injury)	▪ Halothane ▪ Acetaminophen ▪ Cocaine ▪ MDMA (3,4-methylenedioxy-N-methamphetamine) ▪ Organic solvents ▪ Toxic mushrooms ▪ Some herbal medications ▪ Herpes simplex virus ▪ Adenovirus ▪ Acute vascular injury/ischemia
Resolving pattern (nonspecific)	▪ Idiosyncratic drug reaction ▪ Systemic autoimmune disorders ▪ Systemic inflammatory disorders
Giant cell pattern (nonspecific in adults)	Autoimmune hepatitis
Mild nonspecific pattern	Early acute viral hepatitis versus biliary injury requires clinical and laboratory correlation

- In 1–2% of cases, fulminant clinical course can occur with any of the hepatotropic viruses.
- Pregnant women with HEV and patients with coexisting acute HBV and HDV coinfection present with jaundice, hepatic encephalopathy (lethargy, drowsiness and asterixis), ascites, gastrointestinal tract symptoms, renal failure and disseminated intravascular coagulation (DIC). Patterns of acute viral hepatitis are given in Table 21.33.

Pathology Pearls: Pathophysiology of Viral Hepatitis

The basic pathophysiologic processes of acute and chronic viral hepatitis are complex. The hepatotropic viruses first bind to the hepatocyte cell membranes.

- Prompt humoral antibody-mediated response clears the hepatotropic virus or inhibits penetration and viral replication. The hepatotropic viruses that penetrate into the hepatocytes may elicit an immune mediated response, resulting in viral clearance by way of necrosis of the infected hepatocytes and removal of infected hepatocytes by CD8+ cytotoxic T cells or inhibition of viral replication as a result of synthesis of anti-viral cytokines.
- Infection can persist with resultant hepatitis when the immune-mediated response is only partial/marginal (eliciting an active stage of chronic hepatitis) or totally absent (carrier state). Fibrogenesis from activation of stellate cells plays a crucial role in the pathogenesis of the ongoing disease.

Surgical Pathology: Acute Viral Hepatitis

Gross Morphology

- Acute viral hepatitis progressing to fulminant acute hepatocellular failure leads to reduction in size of the liver. Liver capsule shows wrinkling indicating rapid reduction of liver cell mass. Cut surface of liver exhibits red appearance. It may greenish due to cholestasis.
- In some cases, there is evidence of commencing regeneration of hepatocytes in the form of rounded yellowish areas scattered against the red background of necrotic liver.

Light Microscopy

Structural changes in acute viral hepatitis comprise evidence of hepatocellular necrosis in centrilobular zone, inflammation within the liver acini and portal tract connective tissue. Changes in the connective tissue framework most often occur as a result of extensive severe loss of hepatocytes. Acute viral hepatitis has two major features: lobular disarray and chronic inflammatory infiltrate. Ballooning degeneration of hepatocytes and Councilman bodies in sinusoids are prominent features. Morphologic features of acute viral hepatitis are shown in Figs 21.17 and 21.18 and Table 21.34.

- **Ballooning degeneration of hepatocytes:** Hepatocytes show degeneration with empty appearance and granules around nucleus, which correlates with intracellular edema and consequent dilatation of the endoplasmic reticulum, loss of ribosomes and swelling of the mitochondria. The cytoplasm

of hepatocytes does not bind eosin hence giving empty appearance. Ballooning degeneration may cause necrosis of some hepatocytes and this change may be reversible.

- **Biliary cholestasis:** Bile accumulates in the biliary canaliculi which causes feathery appearance of hepatocytes.
- **Fatty change:** Mild fatty change is seen in hepatocytes especially with HCV infection.
- **Hepatic lobular disarray:** Normal architecture of liver is disrupted.
- **Hepatocellular necrosis:** Isolated cell or clusters of hepatocytes may undergo irreversible cell necrosis. Apoptosis removes viral infected scattered hepatocytes. Apoptotic cells represent membrane-bound cellular chromatin remnants that are extruded into the hepatic sinusoids in cases of viral hepatitis, which are called 'Councilman bodies', which appear as eosinophilic stained structures in hematoxylin and eosin-stained sections seen under light microscope.
- **Bridging necrosis:** Severe hepatocellular injury may lead to bridging necrosis (portal-portal, central-central or portal-central).
- **Regenerative changes:** Hepatocytes may show regenerative changes. Sinusoidal cell reactive changes: Kupffer cells are prominent and increased in number in the sinusoids, which contain phagocytosed cellular debris, bile pigments and lipofuscin pigment. There is influx of lymphocytes, plasma cells and macrophages into sinusoids.
- **Inflammatory response with influx of mononuclear cells:** Portal tract is also the site of inflammation. Connective tissue of portal tract shows infiltration by lymphocytes, macrophages and plasma cells. Presence of neutrophils and eosinophils are not uncommon. Persistent inflammation and fibrosis is an unfavorable sign of chronic viral hepatitis progressing to post-necrotic cirrhosis. Spill over of chronic inflammatory cells (lymphocytes, macrophages and plasma cells) from portal tract into liver acinus causes liver cell necrosis. Inflammation is most severe in the perivenular region of acinar zone.
- **Changes in the connective tissue framework:** In majority of cases, there is insufficient hepatocellular necrosis exhibiting normal architecture of liver cell plates. In more severe cases with extensive hepatocellular necrosis exhibit bridging necrosis extending from central vein to portal tract. There is collapse of reticulin fiber framework in this area. Bridging necrosis is the most serious development, because there is increased risk of subsequent cirrhosis.

CHRONIC VIRAL HEPATITIS

Chronic viral hepatitis is defined as persistently abnormal liver function tests greater than six months after the acute viral hepatitis occurs with hepatotropic HBV and HCV.

- Hepatitis C causes chronic hepatitis in 80% of cases. Hepatitis B causes chronic hepatitis in 10% of cases. Liver may show mild, moderate, or severe injury depending on the extent of injury, inflammation and repair.
- Depending on clinical course, hepatotropic viruses may cause portal hepatitis (previously known as chronic persistent hepatitis), chronic lobular hepatitis and chronic active hepatitis.

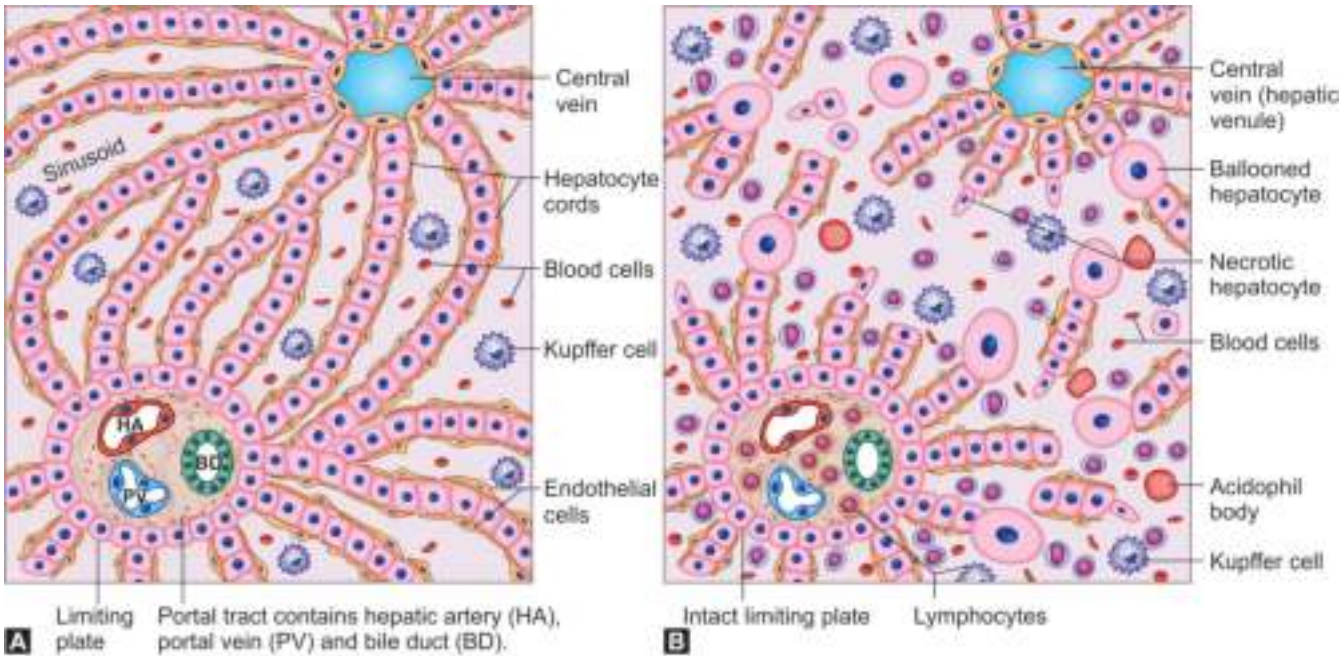


Fig. 21.17A and B: (A) Normal liver acinus contains a small portal tract at the center and terminal hepatic venules at the periphery, (B) acute viral hepatitis is diffuse inflammation caused by specific hepatotropic viruses. It shows spotty necrosis of hepatocytes (single/clusters), ballooning degeneration of hepatocytes, apoptosis of hepatocytes (Councilman bodies in sinusoids) and intrasinusoidal inflammation chiefly lymphocytes and a few macrophages.

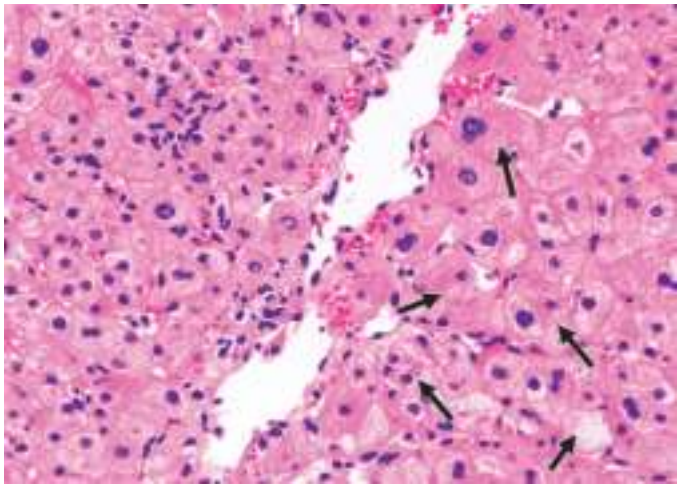


Fig. 21.18: Acute viral hepatitis. It shows spotty necrosis of hepatocytes, ballooning degeneration of hepatocytes, Councilman bodies in sinusoids and numerous lymphocytic infiltrate (arrows) (400X).

- Clinically, the patients are symptomatic in the early stages. The patients are diagnosed on the basis of abnormal liver function tests with appropriate viral serological analysis of chronic viral hepatitis.
- With time, patients develop advanced liver disease with portal hypertension manifested by splenomegaly, ascites, and/or esophageal varices.
- The serum aspartate transaminase (AST) and alanine aminotransferase (ALT) enzymes are usually elevated to various degrees but often fluctuate at times. Prolonged PT–INR and decreased serum albumin are indicators of advanced liver disease and hepatocellular impairment. In addition, marked elevations of serum aspartate transaminase (AST) and alanine aminotransferase (ALT) enzymes occur in patients with reactivation of chronic HBV infection.
- Advanced liver disease cases may demonstrate thrombocytopenia, leukopenia, low serum cholesterol values, and high ammonia level. Outcome of chronic viral hepatitis depends on anti-viral therapy, age of the patient and associated coexisting medical illness. Prognosis is poor in patients in human immunodeficiency virus (HIV) coinfection.

Portal Hepatitis

Portal hepatitis was previously known as chronic persistent hepatitis. It occurs only in HBV and HCV infections.

Light Microscopy

Liver biopsy in portal hepatitis shows following features (*refer* to Figs 21.19 and 21.20).

- Liver acinar architecture is preserved.
- Chronic inflammation is confined to portal tracts without damaging limiting plates. Connective tissue of portal tracts is infiltrated by lymphocytes, plasma cells and macrophages. Limiting plates are intact.
- Hepatocytes do not show evidence of patchy necrosis or piecemeal necrosis.

Table 21.34 Morphologic features of acute hepatitis

Morphologic Changes	Light Microscopy
Hepatocellular injury	▪ Liver cell swelling (ballooning degeneration) ▪ Cholestasis (bile plugs) ▪ Fatty change due to HCV
Hepatocellular necrosis	▪ Liver cell necrosis (isolated or clusters) ▪ Bridging necrosis in severe inflammation (portal-portal, central-central, portal-central) ▪ Lobular disarray (loss of normal architecture)
Hepatocytes regeneration	Proliferation of hepatocytes
Sinusoidal cell reactive changes	▪ Accumulation of phagocytosed cellular debris in Kupffer cells ▪ Influx of mononuclear cells in sinusoids
Portal tracts	▪ Mononuclear inflammatory infiltrate in portal tracts ▪ Spill over of inflammatory cells into adjoining parenchyma may show liver cell necrosis

Chronic Lobular Hepatitis

Chronic lobular hepatitis has been regarded by some authors as a variant of portal hepatitis.

Surgical Pathology: Chronic Lobular Hepatitis

Light Microscopy

- **Preservation of normal liver acinar architecture:** Liver acinar architecture is preserved in most part of liver. Collapse of the reticulin framework may be demonstrated in the region of the terminal hepatic venule. There may be clustering of Kupffer cells containing ceroid pigment. Ceroid pigment is partly oxidized lipid. These histologic findings suggest that loss of hepatocytes has taken place.
- **Evidence of patchy hepatocellular damage:** Hepatocytes show ballooning degeneration and acidophilic change. There is absence of piecemeal necrosis. Periportal fibrosis is minimal or absent. It rarely progresses to chronic active hepatitis and cirrhosis.

Chronic Active Hepatitis

Chronic active hepatitis is the most serious progressive liver disease characterized by a marked inflammatory response with concurrent evidence of long-standing

fibrosis disease that results in severe hepatocellular failure. Patient presents with nonspecific symptoms such as fatigue, anorexia and malaise. During carrier state, serum transaminase (AST and ALT) levels are normal; and histologic examination shows ground glass hepatocytes, but absence of inflammation.

Surgical Pathology: HBV Replication in Chronic Viral Hepatitis

Light Microscopy

Liver biopsy shows chronic inflammation in portal tracts, damaging the limiting plates and extending into liver lobule. There is presence of bridging necrosis and periportal fibrosis. Chronic viral hepatitis most often progresses to cirrhosis.

- **Ballooning degeneration of hepatocytes:** The hepatocytes show ballooning with scattered apoptotic cells. It depends on whether or not viral replication is occurring. Hepatocytes show ground glass appearance due to accumulation of HBsAg in the cytoplasm.
- **Biliary cholestasis:** Biliary cholestasis may be severe as a result of fibrosis or secondary to cirrhosis.
- **Kupffer cell hyperplasia:** Kupffer cells are also prominent and increased in number in the sinusoids, which contain phagocytosed cellular debris, bile pigments and lipofuscin pigment.
- **Piecemeal necrosis:** Hepatocellular necrosis in periportal region is known as piecemeal necrosis. It is observed in immunocompetent persons.
- **Bridging necrosis with periportal fibrosis:** In very severe cases, there may be presence of bridging necrosis with periportal fibrosis of connecting vascular structures of portal tract to terminal hepatic venules by thick band of collapsed reticulin. Collagen fibers are laid down to replace injured hepatocytes. Fibrous septa may extend into lobules. Coalescence of fibrous strands, associated with regeneration of hepatocytes may lead to cirrhosis.
- **Portal tract-chronic inflammation:** It is similar to that seen in portal hepatitis. Portal tracts are infiltrated by large number of lymphocytes, macrophages, and plasma cells. These inflammatory cells erode limiting plates and extend into lobular parenchyma. It is accompanied by periportal fibrosis. The expanded portal tracts often display proliferated bile ductules. Histology of portal hepatitis is shown in Figs 21.19 and 21.20.
- **Segmental bile duct lesions:** Both large and interlobular ducts undergo epithelial hyperplasia resulting in narrowing or occlusion of bile ducts. Histology of chronic active hepatitis is shown in Figs 21.21 and 21.22.

Pathology Pearls: Ground Glass Hepatocytes in Various Disorders

- HBV infection
- Lafora's disease (myoclonal epilepsy)
- Phenobarbital induced hypertrophy of smooth endoplasmic reticulum
- Glycogen storage disease type 4
- Multidrug intake

Viral Hepatitis: Biochemical Abnormalities

Biochemical abnormalities include serum enzymes, serum bilirubin, urine analysis and serological tests described as follows:

- **Serum transaminase enzymes:** There is increased level of transaminases (ALT and AST) three to five times. It may be over 1000 IU/L in severe hepatitis. Transaminases return to normal level over a period of several months. There is modest increase of alkaline phosphatase.
- **Serum bilirubin:** There is variable increase in serum bilirubin concentration.
- **Urine analysis:** Bilirubin is present in urine.
- **Serologic tests:** These must be performed to determine which virus has caused the disease. Serological markers in viral hepatitis are given in Table 21.35.

HEPATOTROPIC VIRUSES

HEPATITIS A VIRUS (HAV) INFECTION

Hepatitis A virus is single-stranded spherical unenveloped RNA icosahedral virus measuring 27 nm in diameter. HAV is inactivated by boiling for one minute or formaldehyde and chlorine. It is resistant to heat, acid and ether. HAV infection is usually a benign self-limiting disease, although it can cause acute fulminant hepatitis and death or need for liver transplantation in

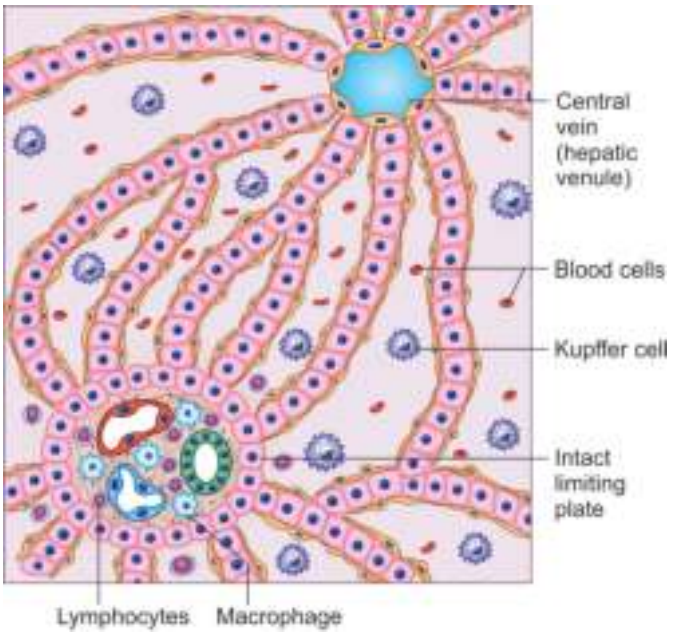


Fig. 21.19: Portal hepatitis (previously known as chronic persistent hepatitis). Chronic inflammation is confined to the portal tracts. Limiting plate is intact. Liver parenchyma appears normal.

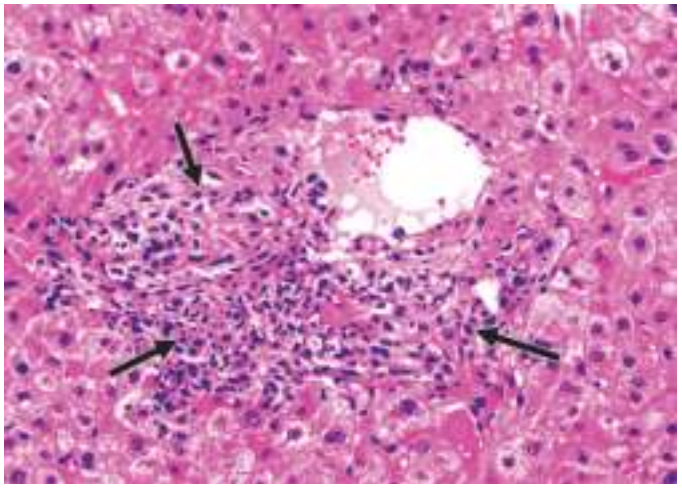


Fig. 21.20: Portal hepatitis. Chronic inflammation is confined to portal tracts. Lobular parenchyma appears normal. Hepatocytes show ballooning degeneration (arrows) (400X).

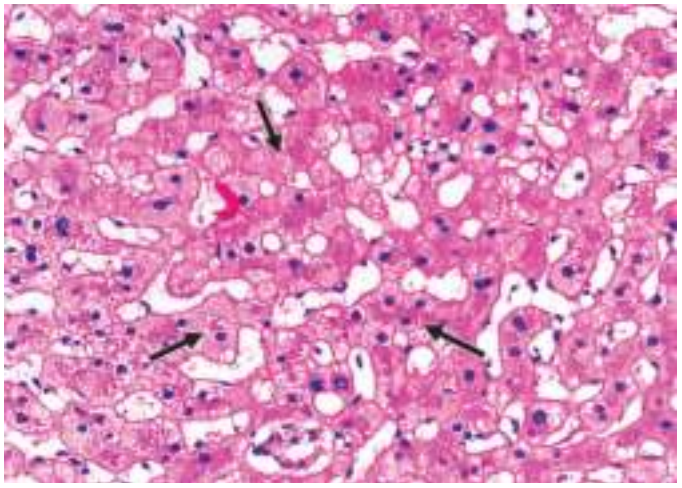


Fig. 21.22: Chronic active hepatitis. Ground-glass hepatocytes in chronic hepatitis. Smooth endoplasmic reticulum of hepatocytes distended with virions (HBsAg) gives ground-glass appearance of hepatocytes (arrows) (400X).

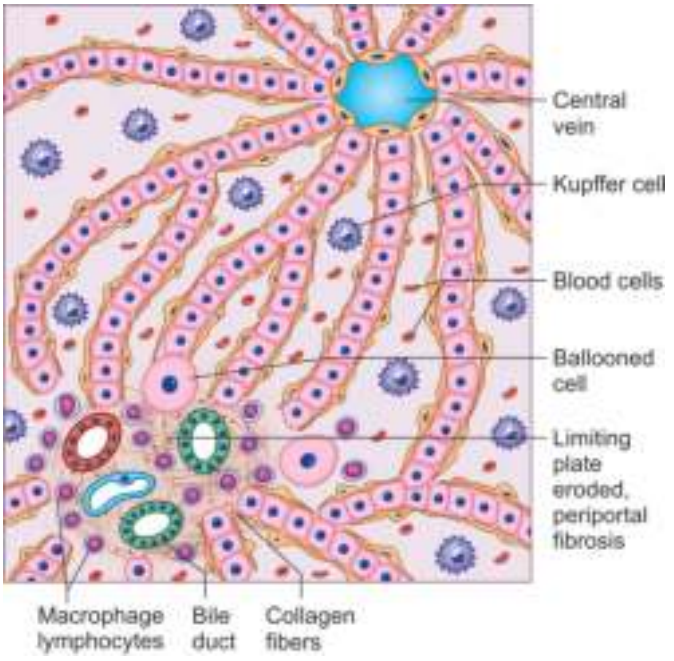


Fig. 21.21: Chronic active hepatitis is marked by severe chronic inflammation in the portal tracts. Periportal necrosis of hepatocytes is conspicuous, ballooned cells are present, and limiting plate is eroded. The inflammation extends into the lobular parenchyma and is accompanied by periportal fibrosis. The expanded portal tracts often display proliferated bile ductules and collagen fibers.

0.15–0.2% of cases. There is no carrier state or chronic state in HAV infection.

Etiology

Hepatitis A is contracted primarily by the **fecal–oral route**. Parenteral infection does not occur. Edible shellfish concentrate the virus in contaminated waters and may also transmit the infection especially in USA. Anal intercourse in homosexuals increases risk for hepatitis A. It has a brief incubation period of 15–45 days.

Pathogenesis

Hepatitis A virus replicates in the liver, and is excreted in the bile, and shed in the stool. There is only one antigenic type of HAV. The fecal shedding of HAV occurs during the first 2–3 weeks of the illness before and one week after onset of jaundice. Exposure to HAV gives lifelong immunity. Pathogenesis of HAV-induced hepatitis is shown in Fig. 21.23.

Age Group

The clinical presentation of symptoms is dependent upon age of the patients. Severity of symptoms is increasing in older age groups.

Children below 6 years most often remain asymptomatic. Older children and adults are symptomatic with clinical jaundice in 70% of cases. Symptoms usually last for approximately 2 months but can last longer.

Clinical Features

There are three phases of hepatitis induced by HAV—preicteric, icteric and recovery phases described as under:

- **Preicteric phase:** HAV is present in liver, bile, stool and blood. Patient is asymptomatic in this phase.
- **Icteric phase:** Patient presents with mild jaundice, malaise, nausea, anorexia and fever. Jaundice appears one week after the onset of symptoms with peak at around 10 days.
  - On clinical examination, liver may become enlarged and tender.

Table 21.35 Serological markers in viral hepatitis

Hepatotropic Hepatitis	Serology	Interpretation
HAV	▪ Anti-HAV IgG ▪ Anti-HAV IgM	▪ Past infection and lifelong immunity ▪ Acute HAV infection
HBV	▪ HBsAg ▪ HBeAg ▪ Anti-HBc IgG ▪ Anti-HBs antibody ▪ HBV-DNA qualitative ▪ HBV-DNA quantitative	▪ Acute and chronic HBV infection ▪ High degree of HBV infectivity and replication ▪ Past infection; acute and chronic HBV infection ▪ Past HBV infection and immunity; immunization ▪ Acute and chronic HBV infection ▪ Acute and chronic HBV infection; viral replication
HDV	▪ Anti-δ IgM and IgG ▪ HDV-RNA	▪ Acute HDV infection (high IgM and low IgG) ▪ Chronic HDV infection (high IgG and low to immeasurable IgM) ▪ Acute and chronic HDV infection
HCV	▪ Anti-HCV antibody ▪ HCV qualitative ▪ HCV quantitative	▪ Acute and chronic HCV infection ▪ Acute and chronic HCV infection ▪ Acute and chronic HCV infection; viral replication
HEV	▪ Anti-HEV IgM and IgG ▪ Anti-HEV IgG ▪ HEV-RNA	▪ Acute HEV infection (high IgM and low IgG) ▪ Past HEV infection and immunity ▪ Acute HEV infection
HGV	▪ Anti-HGV antibody ▪ HGV-RNA	▪ Acute and chronic HGV infection ▪ Acute and chronic HGV infection

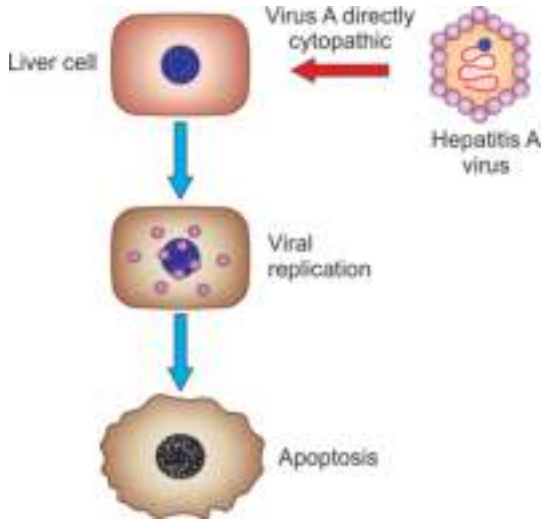


Fig. 21.23: Pathogenesis of HAV-induced hepatitis. HAV in contrast to HBV appears to be directly cytopathic.

- At this stage, liver function tests demonstrate elevations of aspartate transaminase (AST) and alanine aminotransferase (ALT) usually greater than 500 IU/L and most often over a 1000 IU/L, with ALT more specific for hepatocellular injury.
- Alkaline phosphatase value is usually normal or elevated about two times of normal values. When hyperbilirubinemia and jaundice occur, the serum bilirubin value ranges from 5 to 20 mg/dl, depending on the degree of HAV infection and necroinflammatory changes.
- Importantly, the PT–INR and serum albumin levels are generally normal in uncomplicated cases.
- Coagulation tests may be abnormal. One stage prothrombin time is sensitive indicator of severity of liver disease. HAV appears in stool 2–3 weeks before jaundice and one week after icteric phase.

- **Recovery phase:** Signs and symptoms in HAV infection generally disappear over a period of 3–8 weeks. Complete recovery occurs in most patients with mortality in <1%. Majority of patients recover without progressing to chronic hepatitis. HAV infection is not associated with cirrhosis or hepatocellular carcinoma. HAV infection has no carrier state.
- **Fulminant hepatitis:** Only a few patients may develop fulminant hepatitis with mortality in 0.1% of cases.

Serological Studies

The presence of IgM anti-HAV is indicative of acute hepatitis A, whereas IgG anti-HAV merely documents past infection indicating lifelong immunity (Fig. 21.24).

- **Anti-HAV of IgM antibody:** Initially, increased titer of anti-HAV of IgM antibody indicates active infection. It is a reliable diagnostic marker for acute HAV infection. Anti-A of IgM antibody appears in the blood, when patient becomes symptomatic. IgM antibody starts decreasing in a few months to one year.
- **IgG antibody:** Increased titer of anti-HAV of IgG antibody appears soon after IgM antibody in blood.

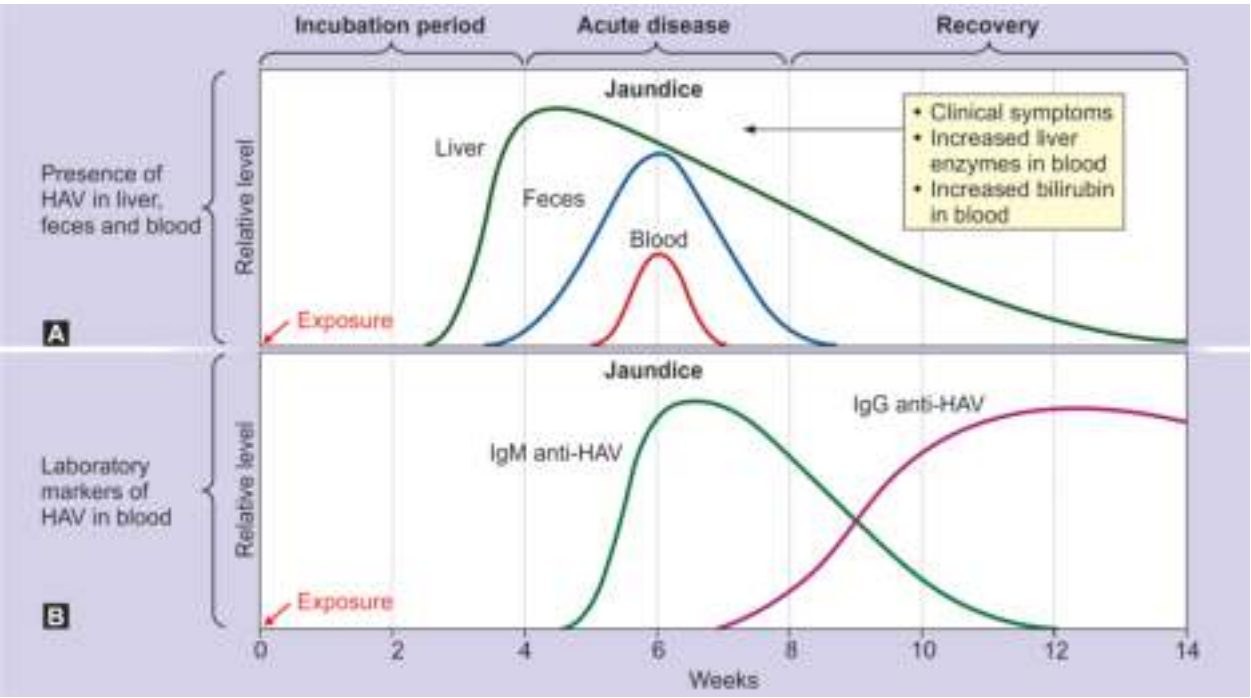


Fig. 21.24A and B: Serological studies in acute viral hepatitis due to HAV. The presence of IgM anti-HAV is indicative of acute hepatitis A, whereas IgG anti-HAV merely documents past infection indicating lifelong immunity. Presence of IgM antibodies coincides with a decline in fecal shedding of the virus.

It indicates exposure to HAV and recovery from infection or vaccination. It is a protective antibody giving lifelong immunity.

In patients with HAV infection, chronic hepatitis never occurs. There is lifelong immunity. Viral elimination is total without a carrier state.

Surgical Pathology: Acute Viral Hepatitis (HAV)
Light Microscopy
Histologic examination of liver biopsy in acute viral hepatitis (HAV) shows disarray of the liver cell plates accompanied by inflammatory infiltrate and liver cell necrosis.

Vaccination

The vaccine against HAV is available and highly effective. Vaccination is of a little benefit in prevention of hepatitis in people with known HAV exposure. It is intended to replace the use of immunoglobulin in people at high-risk for HAV.

HEPATITIS B VIRUS (HBV) INFECTION

HBV is complete virion measuring 42 nm in diameter known as **Dane particle**. It is hardy virus, which can withstand extremes of temperature and humidity. HBV consists of lipoprotein coat with central core (nucleocapsid).

- **Viral lipoprotein coat:** HBV consists of central core surrounded by lipoprotein coat containing the hepatitis B surface antigen (HBsAg).
- **Central core (nucleocapsid):** It is hexagonal in shape, which contains double-stranded circular DNA, DNA polymerase, hepatitis B core antigen (HBcAg), and hepatitis Be antigen (HBeAg).

  Presence of HBeAg in the serum indicates active viral replication, infectivity of serum and progression of liver cell damage (Figs 21.25 and 21.26).

Mode of Transmission

HBV is transmitted via transfusion of blood or blood products, hemodialysis, accidental needle-stick in health workers, intravenous drug abuse, homosexual activity, contact with body fluids by shedding of HBV in saliva, tears, and breast milk and transplacental transmission from mother to neonate.

Incubation Period

Incubation period of HBV infection is 4–26 weeks (average 60–90 days or 8 weeks).

Pathogenesis

HBV virions are not directly cytopathic, which integrate into the host DNA and induce hepatic inflammation leading to hepatocellular injury. Smooth endoplasmic reticulum of hepatocytes distended with HBV virions

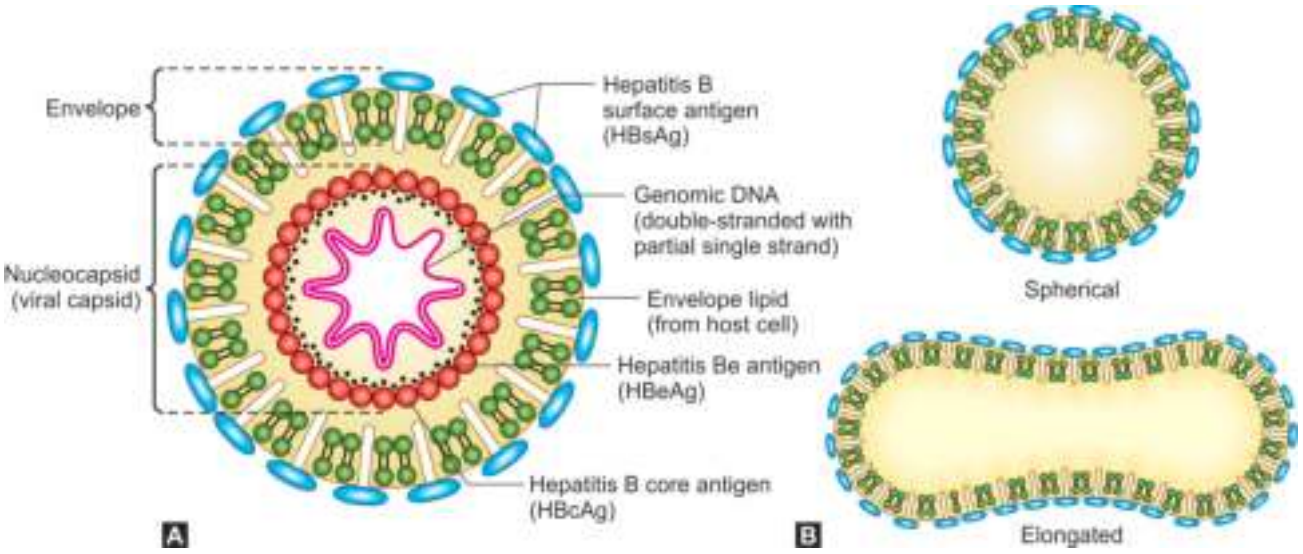


Fig. 21.25: Structure of hepatitis B virus. (A) Hepatitis B virus (Dane particles), (B) viral envelope particles containing HBsAg.

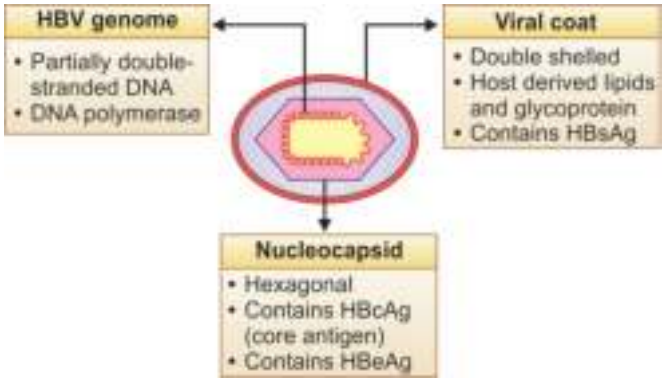


Fig. 21.26: Structure of hepatitis B virus shows HBV genome, viral coat and nucleocapsid.

gives ground glass appearance of hepatocytes. It indicates chronic carrier state.

Increased titers of HBeAg, HBV DNA and HBV DNA polymerase enzyme indicate active viral replication in blood and correlates with infectivity of the blood. Pathogenesis of HBV hepatitis is shown in Fig. 21.27.

- **Entry of HBV into the hepatocytes:** HBV entry into the hepatocytes may depend on polymerized human serum albumin (PHSA) serving as a linkage between the putative receptors of HBV and hepatocytes. Hepatitis B virus enters the host hepatocytes. HBV virion multiplication occurs in hepatocytes as a result of proliferation of HBV DNA.
- **Processing of HBV DNA:** HBV DNA of virus acts as the template for the formation of RNA, which is termed pregenomic. This term 'pregenomic' is given because RNA forms the template for the formation of viral DNA through the medium of virally encoded reverse transcriptase. It leads to formation of complete virions.

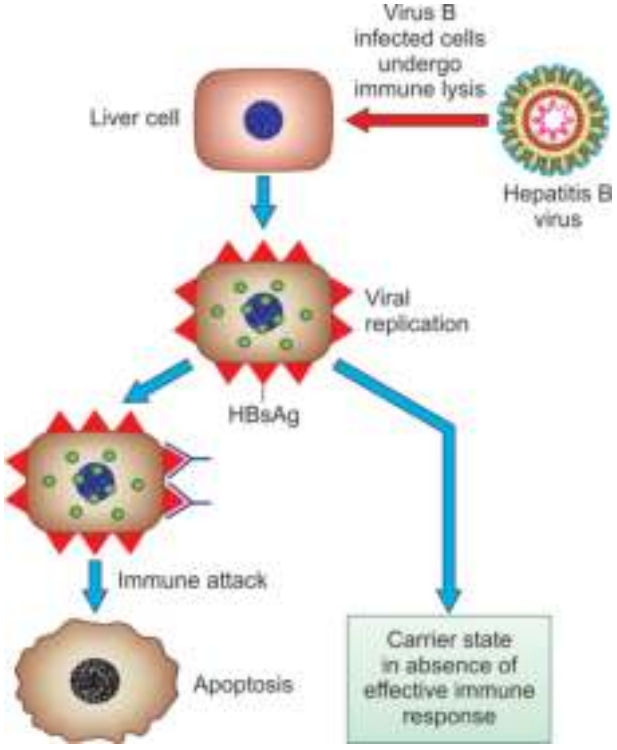


Fig. 21.27: Pathogenesis of HBV hepatitis. The HBV causes liver cell injury due to expression of HBsAg on surface of hepatocytes. The infected liver cells are eliminated by immune-mediated mechanism. Asymptomatic carrier state can ensure in the absence of specific immunity.

- **Integrative phase of HBV:** During integrative phase, virions are not produced; and there is absence of antigens and antibodies.

Hepatocellular Response to Virions

- **Weak hepatocellular response to CD8+ cytotoxic T cells:** There is formation of HBsAg and HBcAg,

which sensitize CD8+ cytotoxic T cells. Weak response of CD8+ cytotoxic T cells results in chronic hepatitis.

- **Strong hepatocellular response:** Strong response of CD8+ cytotoxic T cells causes hepatocellular injury. Excess of HBsAg is accumulated in smooth endoplasmic reticulum producing ground glass appearance of hepatocytes.

Extrahepatic Manifestations

HBsAg and anti-HBsAb form immune complex resulting in deposition in blood vessels, which activates complement system resulting in angioedema, arthritis, skin rashes, fever and glomerulonephritis.

Serological Assays in Acute HBV Infection

Serological assays may be employed to distinguish between acute and chronic hepatitis due to HBV infection.

- Most sensitive and specific commercial methods used in diagnosis of HBV infection are radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) by using specific antibodies against various HBV proteins. Both these methods can detect HBsAg as low as 0.25 ng/ml and anti-HBs antibodies.
- Polymerase chain reaction (PCR) is used to detect low concentration of HBV DNA present in both blood and tissue samples (Table 21.36 and Fig. 21.28A and B).

Table 21.36 Serological events in HBV infection

HBsAg	HBeAg	Anti-HBe	Anti-HBc	Anti-HBc IgM	Anti-HBs	Significance
+	+/–	+/–	+	+	–	Acute HBV infection
+	+	–	+	–/Weak positive	–	HBV carrier (high infectivity)
+	–	+	+	–	–	HBV carrier (low infectivity)
–	–	+/–	+	–	+	Past HBV infection
–	–	–	–	–	+	Past HBV immunization

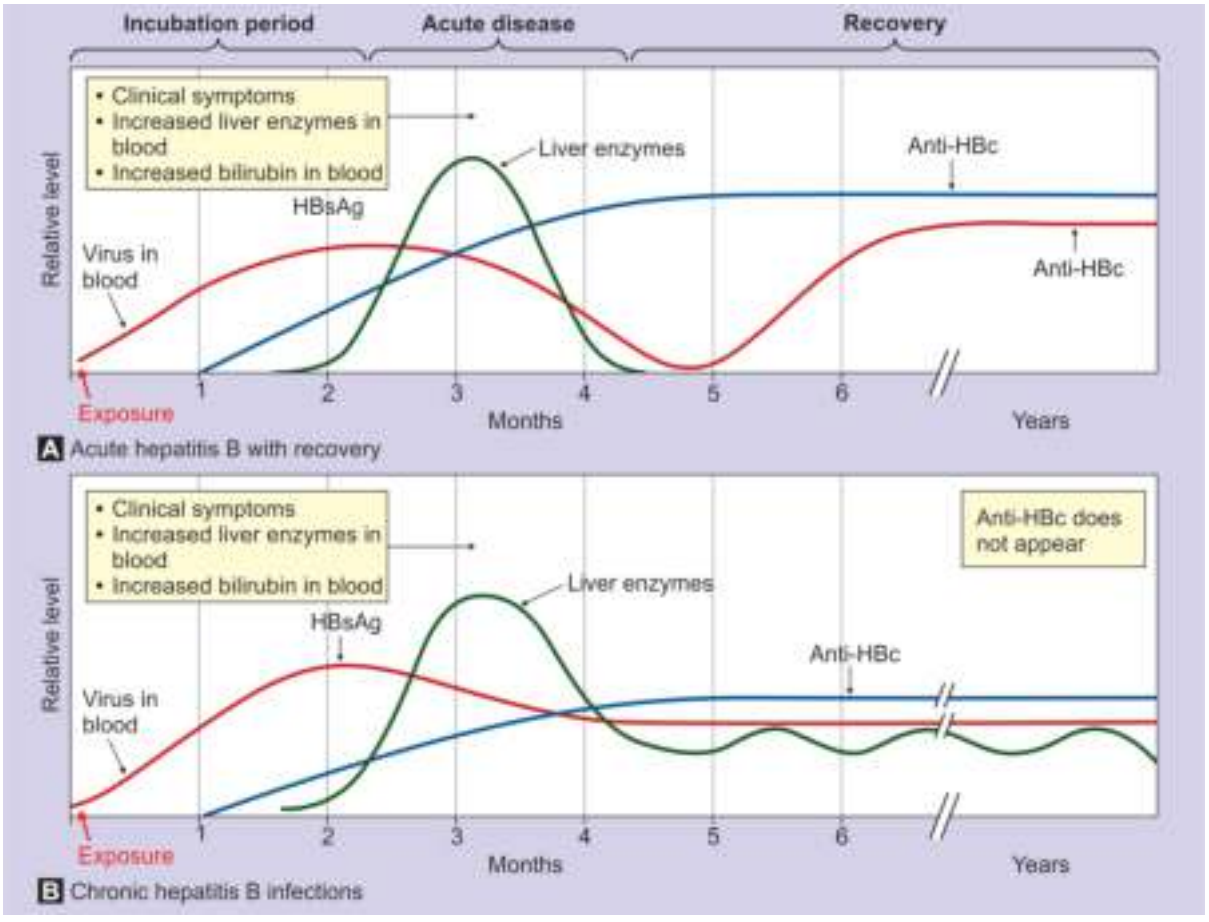


Fig. 21.28: Hepatitis B. (A) Clinical phases and serological markers. Acute infection is characterized by rapid appearance of viremia before clinical symptoms appear. There is disappearance of HBV from the blood and appearance of antibodies against hepatitis B surface antigen (HBsAg). (B) Chronic hepatitis is signaled by continuing onset of jaundice or presence of viremia.

- **HBsAg first marker in serum:** Hepatitis B surface antigen (HBsAg) is the first marker appearing in the serum and is demonstrated in the serum between 1 week and 2 months after exposure and before the appearance of clinical symptoms. It disappears from the blood during convalescence. Persistence of HBsAg is being used to differentiate acute from chronic viral hepatitis. HBsAg persists up to 4 months in acute hepatitis. Persistence of HBsAg >6 months defines chronic hepatitis.
- **HBeAg second marker in serum:** Presence of HBeAg in the serum indicates maximum infectivity and intense viral replication. As with surface antigen (HBsAg), 'e' antigen (HBeAg) appears before the onset of clinical manifestations and disappears from the serum in two weeks. As the 'e' antigen is cleared from the serum, the antibody to 'e' antigen appears in the serum. But there is a gap between disappearance of the 'e' antigen and demonstration of antibody.
- **Anti-HBc IgG and IgM markers in serum:** With the onset of clinical manifestations, anti-HBc IgG and IgM most often appear in the serum and remain in low concentration for life, which do not play significant role in viral elimination and rather serve as marker for infection. It is important to mention that HBcAg is not detectable in serum.
- **Anti-HBs antibody last marker in serum:** Last marker to appear in serum is anti-HBs antibody. It is measured if present in excess concentration. Anti-HBs antibody forms immune complexes with the circulating HBsAg. These antigen–antibody complexes may produce extrahepatic complications such as glomerulonephritis, necrotizing vasculitis and serum sickness like syndrome. The antibody to HBsAg participates in elimination of HBsAg.

There is a window period between the appearance of HBsAg and corresponding antibody. IgG anti-HBs is protective antibody to subsequent HBV infection. Its persistence indicates either active or passive immunization against HBV. Recombinant vaccine consists of HBsAg alone.

- **HBV DNA marker in serum:** The presence of viral DNA (HBV DNA) in the serum is the most certain indicator of hepatitis B virus (HBV) infection, which indicates viral burden and active replication. It evaluates recovery from infection after antiviral therapy. It helps in differentiation of inactive carrier state and chronic hepatitis in HBV infection. Serological markers and interpretation for hepatitis B virus infection are given in Table 21.37.

Hallmark of Chronicity of HBV Infection

Failure to clear HBsAg from serum after 6 months is the hallmark of chronicity due to HBV infection, which occurs in <5% of cases. Presence of HBeAg, HBV DNA or polymerase in the serum either singly or together indicates active HBV replication. In patients where there is no HBV replication, the continuing presence of HBsAg correlates with integration of the viral genome into the genome of host hepatocytes.

Clinical Course of HBV Infection

Majority of cases are subclinical (65%). Approximately 35% of cases develop acute fulminant viral hepatitis or chronic viral hepatitis. Approximately 10–30% cases of chronic hepatitis cases develop confluent hepatic necrosis, cirrhosis (<1%) and hepatocellular carcinoma.

Clinical course of hepatitis B infection is shown in Fig. 21.29. Differences between hepatitis induced by HAV and HBV are given in Table 21.38.

Table 21.37 Serological markers and interpretation for hepatitis B virus infection

Presence/Absence			Interpretation
HBsAg	Anti-HBsAb	Anti-HBcAb	
Positive	Negative	Negative	Early acute HBV infection
Positive	Positive or negative	Positive	Either acute or chronic HBV infection
Negative	Positive	Positive	Previous HBV infection and current immunity to the virus
Negative	Negative	Positive	Not clear interpretation either due to HBV infection in the remote past, low-level HBV infection, or false-positive/nonspecific reactions. If present, anti-HBs helps to validate anti-HBc reactivity
Negative	Negative	Negative	These results suggest that liver toxicity is due to some other agents other than HBV
Negative	Positive	Negative	These results are typical of a vaccinated individual

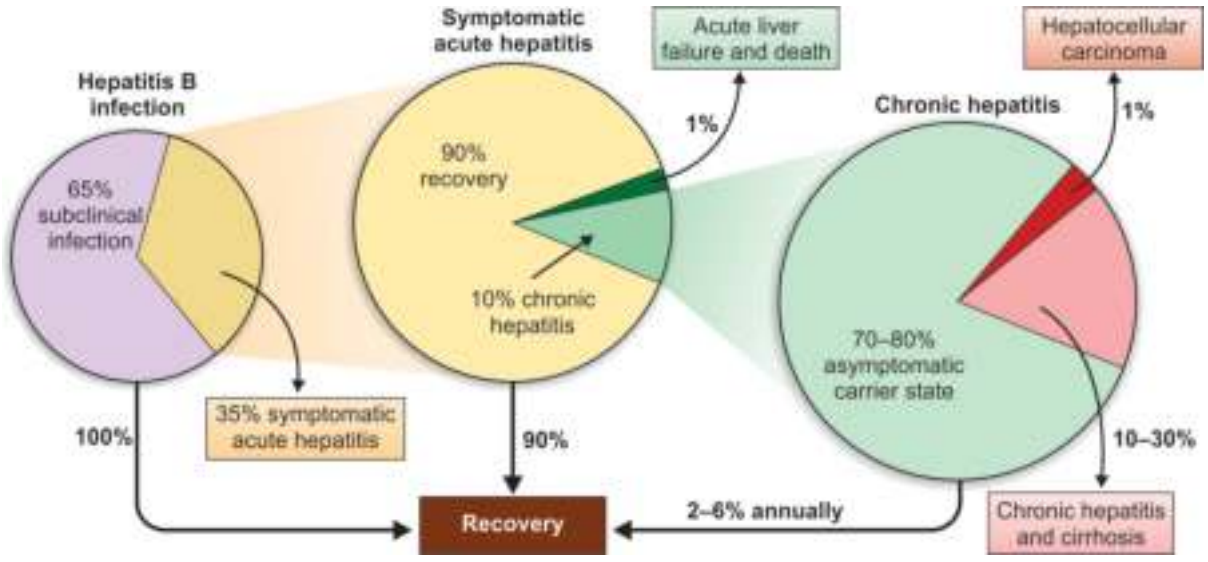


Fig. 21.29: Clinical course of hepatitis B infection. It has subclinical, acute hepatitis, chronic hepatitis or asymptomatic chronic carrier. Fulminant acute hepatitis with massive liver cell necrosis may be seen in 1% of cases. There is increased risk of development of cirrhosis and hepatocellular carcinoma.

Table 21.38 Differences between hepatitis induced by HAV and HBV

Parameters	Hepatitis A Infection	Hepatitis B Infection
Mode of transmission	Fecal–oral route	Parenteral route and close contact
Carrier state	Absent	Present
Chronic viral hepatitis	Absent	Present
Age	Children and adolescents	All age group
Viruses in stool and body secretions	Viruses are present in stool 2–3 weeks after onset of jaundice	Viruses are not excreted in stool. But these are present in body secretions
Viremia	Transient excretion	Viruses remain for prolonged period starting from incubation period, acute hepatitis to chronic hepatitis. Infection transmitted by such blood administration to other persons
Vertical transmission	Absent	Present
Fulminant viral hepatitis	Rare	More frequent
Risk for hepatocellular carcinoma	Absent	Present
Clinical course	Usually self-limited	Variable, it may be severe with fatal outcome

Pathology Pearls: Summary of Serological Studies in HBV-induced Hepatitis

- HBsAg is demonstrated in acute or chronic HBV-induced hepatitis.
- Demonstration of IgG anti-HBs and IgG anti-HBc indicates previous exposure to HBV.
- Demonstration of anti-HBs IgG indicates that the person is immunized.
- Anti-HBc IgM is the only serological marker demonstrated during window period.
- Window period is a period in which HBsAg and anti-HBsAb is not detectable in the serum, but anti-HBcAb and anti-HBeAb are the only markers of the disease. HbsAg, HBV DNA, and HBeAg are absent. It (anti-HBC-IgM) converts to anti-HBc IgG in 6 months.
- Qualitative marker of HBV replication is HBeAg.
- Quantitative (definite) marker of HBV replication is HBV DNA > HBV DNA polymerase.
- Inactive HBV carrier refers to HBeAg negative with normal ALT levels and undetectable or low level of HBV DNA (<2000 IU/ml).

Pathology Pearls: Precore or Basic Core Mutant HBV

- Patient with precore or basic core mutant HBV develops severe and progressive liver disease. There is increased risk for cirrhosis and hepatocellular carcinoma.
- Serological assays reveal HBeAg negative but positive anti-HBe. HBV DNA levels are >10,000 copies/ml.
- Blood biochemistry analysis reveals persistent or intermittent elevations in ALT (alanine aminotransferase) activity.

Clinical Manifestations of HBV Infection

- **Subclinical acute hepatitis:** Patient is asymptomatic or clinically unrecognized.
- **Acute icteric hepatitis:** HBsAg is the first marker of infection. It appears 2–8 weeks after exposure, and persists up to 4 months.
- **Fulminant acute hepatitis with massive hepatic necrosis:** It develops in <1% of clinically diagnosed HBV infections. Markedly elevation of ALT and AST are characteristic findings. Gross examination reveals soft flabby shrunken liver, and wrinkled Glisson's capsule. Histologic examination shows necrotic liver lobules, leaving a collapsed collagenous framework. These patients die unless an emergency liver transplantation is performed.
- **Chronic hepatitis:** Persistence of abnormal liver function tests and HBsAg in cytoplasm of hepatocytes for >6 months is considered chronic hepatitis.
  - Chronic carriers: These are otherwise healthy carriers. HBsAg persists longer than 6 months. There is absence of infective particles in 10–15% cases. Anti-HBc IgM converts to anti-HBc IgG.
  - Chronic active hepatitis: It occurs in 33% cases of chronic carriers. There is presence of infective particles. There is increased risk for post-necrotic cirrhosis (33%) and hepatocellular carcinoma (10%).

Surgical Pathology: Liver Biopsy in HBV Infection

Clinical indication for liver biopsy in HBV infection is to determine active viral hepatitis. In patient's serological or biochemical tests are inconclusive of clear picture of the disease activity.

Light Microscopy

- Liver biopsy in patient with chronic active hepatitis is performed to assess evidence of fibrosis and inflammatory grade of the disease.
- Liver shows infiltration of connective tissue of portal tracts by lymphocytes, plasma cells and macrophages.
- There is presence of fibrosis and piecemeal necrosis of the limiting plate of hepatocytes close to the portal tracts.
- Hepatocytes with HBsAg show cytoplasmic ground-glass appearance and sanded nuclei (Fig. 21.30).

Immunohistochemistry Technique

- Immunohistochemistry technique reveals HBsAg in cytoplasm. HBsAg is demonstrated by Shikata's orcein stain, Victoria blue and aldehyde fuscin stain.
- HBcAg is expressed in cytoplasm, membrane or nucleus and its presence indicates active HBV replication and infectivity of the virus.

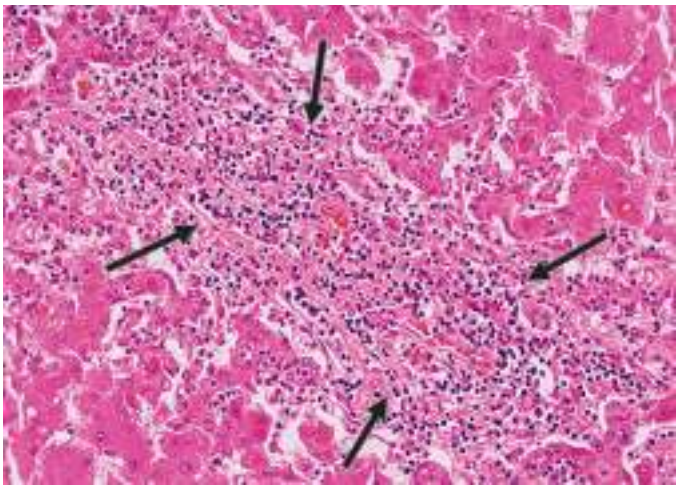


Fig. 21.30: Chronic active hepatitis. Chronic viral hepatitis leads to liver cell destruction. A mononuclear inflammatory cell infiltrate extends from portal areas and disrupts the limiting plate of hepatocytes which are undergoing necrosis, the so-called piecemeal necrosis of chronic active hepatitis. In this case, the hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (HBcAb) were positive (arrows) (400X).

HEPATITIS C VIRUS INFECTION

Hepatitis C virus (HCV) is 30–60 nm enveloped single-stranded RNA flavivirus. About 50% of affected persons develop biochemical evidence of the chronic liver disease.

- Approximately 10% of HCV infected cases develop chronic active viral hepatitis or cirrhosis.
- Coexistent conditions such as hepatitis B infection, alcoholism, hemochromatosis and α_1-antitrypsin deficiency adversely affect the clinical course of HCV infection.
- Factors associated with spontaneous clearing of HCV infection appear to include younger age, female sex, and certain histocompatibility genes. Outcome of untreated hepatitis C infection is shown in Fig. 21.31.

Incubation Period

Incubation period of HCV infection is similar to HBV, i.e. 4–26 weeks.

Mode of Transmission

Hepatitis C virus is transmitted by blood-borne parenteral route, sexual contact, transplacental infection of the fetus and co-infection with HBV described as under:

- **Blood and its products transfusion (parenteral route):** HCV is transmitted by blood-borne parenteral route with sharing of needles in drug addicts and transfusion of blood and its products. About 60–90% of intravenous drug users are infected with HCV.
- **Sexual contact:** HCV can also be transmitted by sexual intercourse with infected partner.

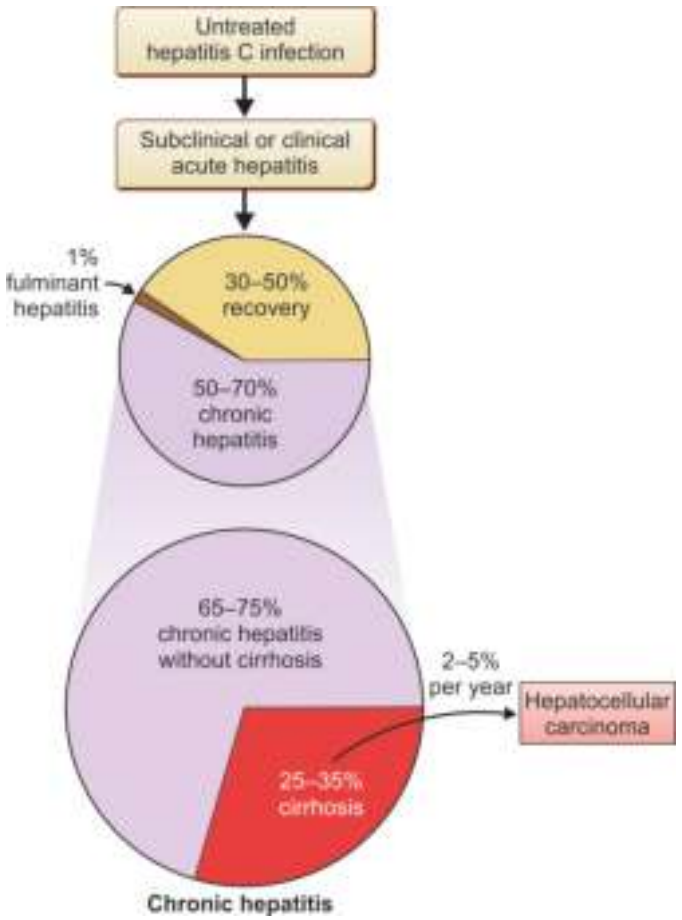


Fig. 21.31: Outcome of untreated hepatitis C infection.

- **Transplacental route:** Mother transmits HCV infection to fetus via transplacental route.
- **Co-infection:** Persons with HBV and HCV co-infection are also affected.

Clinical Features

Most people infected with HCV have a normal life span. It is persistent infection due to recurrent relapses as a rule in >85% of cases and usually mild. The most serious consequences of chronic HCV infection are progressive liver fibrosis leading to cirrhosis over 10–30, end-stage liver disease, and hepatocellular cancer.

Extrahepatic Manifestations

- **Cryoglobulinemia:** Immune response to HCV leads to cryoglobulinemia. IgM cryoglobulins precipitate at 4°C and dissolve again at 37°C. These are demonstrated in 35% of patients. Mixed cryoglobulinemia (IgM and IgG) is demonstrated in some of patients with hepatitis C infection. These cryoglobulins induce glomerular and vascular injury.
- **Membranous glomerulonephritis:** Membranous glomerulonephritis is also mediated by cryoglobulins. Patient may develop **nephrotic syndrome** characterized by massive proteinuria, hypoproteinemia, hyperlipidemia, lipiduria and generalized edema.
- **Raynaud phenomenon:** Vasculitis mediated by deposition of cryoglobulins in the blood vessel wall is associated with purpura termed Raynaud phenomenon.

Surgical Pathology: Liver Biopsy in HCV Infection

Light Microscopy

In addition to the features common to viral hepatitis, HCV infection may produce the following histologic changes:

- **Cytopathic changes in the hepatocytes:** Histologic examination shows scattered hepatocytes with intense degree of eosinophilia indicating damage to hepatocytes by HCV. There is no evidence of inflammation.
- **Presence of inflammatory cells within sinusoids:** The sinusoids show infiltration by lymphocytes and macrophages. The intensity of inflammation is out of proportion to the relatively small amount of hepatocytic damage.
- **Presence of inflammatory cells within portal tracts:** Connective tissue of hepatocytes may show lymphoid aggregates.
- **Evidence of intrahepatic bile ducts damage:** Presence of inflammatory infiltrate in bile duct epithelium causes destruction of intrahepatic bile duct in 30% of cases.
- **Focal fatty change:** Sublethal liver cell injury is seen in the form of small droplet fatty change (Fig. 21.32).

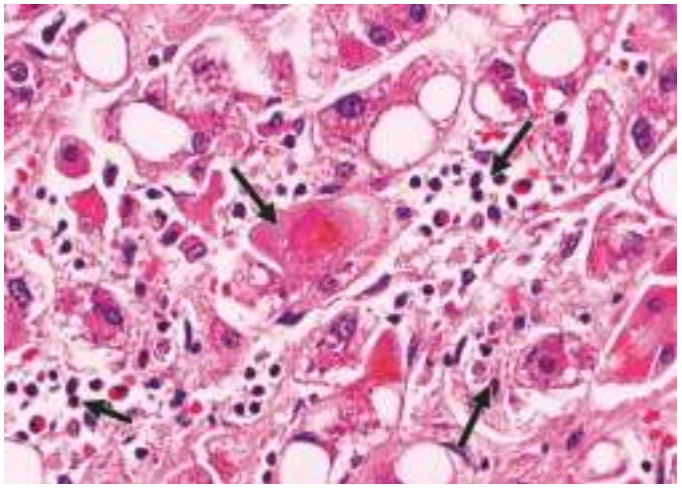


Fig. 21.32: Viral hepatitis C. This is a case of viral hepatitis C, in which half of cases leads to chronic liver disease. The extent of chronic hepatitis can be graded by the degree of activity (necrosis and inflammation) and staged by the degree of fibrosis. In this case, necrosis and inflammation are prominent, and there is some steatosis as well. Regardless of the grade or stage, the etiology of the hepatitis must be sought, for the treatment may depend upon knowing the cause, and chronic liver diseases of different etiologies may appear microscopically and grossly similar (arrows) (400X).

Pathology Pearls: Steatosis in HCV Infection

- Steatosis favors HCV genotype 3 replication, since lipid droplets in hepatocytes provide a scaffold for viral replication. It is most often associated with more inflammation activity and rapid fibrosis.
- Steatosis may represent superimposed disease processes, e.g. obesity, diabetes mellitus and alcoholism.

Serological Assays

Initially IgM anti-HCV antibody appears, which is followed by appearance of IgG anti-HCV antibodies. Chronic HCV infection shows episodic elevations in serum transaminases with persistent HCV RNA in blood.

- Anti-HCV IgG does not provide immunity due to antigenic variability and genome instability seen in HCV. Direct measurement of HCV in the serum remains the most accurate test for infection.
- The viral tests are highly sensitive and specific, but more costly than antibody tests.
  - **Screening with enzyme immunoassay:** Presence of anti-HCV IgG indicates infection or recovery. It is not a protective antibody.
  - **Confirmatory tests:** These include recombinant immunoblot assay (RIBA) and HCV RNA using polymerase chain reaction (Fig. 21.33A and B).

Treatment

α-Interferon is administered in HCV infected patients.

HEPATITIS D VIRUS INFECTION

Hepatitis D virus (HDV) is single-stranded spherical RNA virus containing delta protein antigen (HDAg), and surrounded by a proteinaceous coat of HBsAg. It requires co-infection or superinfection with HBV for replication. It is infective only when encapsulated by HBV. Incidence with HDV infection is especially high in intravenous drug users.

Pathology

Hepatitis D virus (HDV) has direct cytopathic effect on hepatocytes in contrast to HBV. HDV produces extensive hepatocellular injury. Liver shows microvesicular fatty change and many acidophilic '**Councilman bodies**' in sinusoids. Chronicity is very rare. Incubation period of HDV infection is 4–7 weeks in superinfection with HBV.

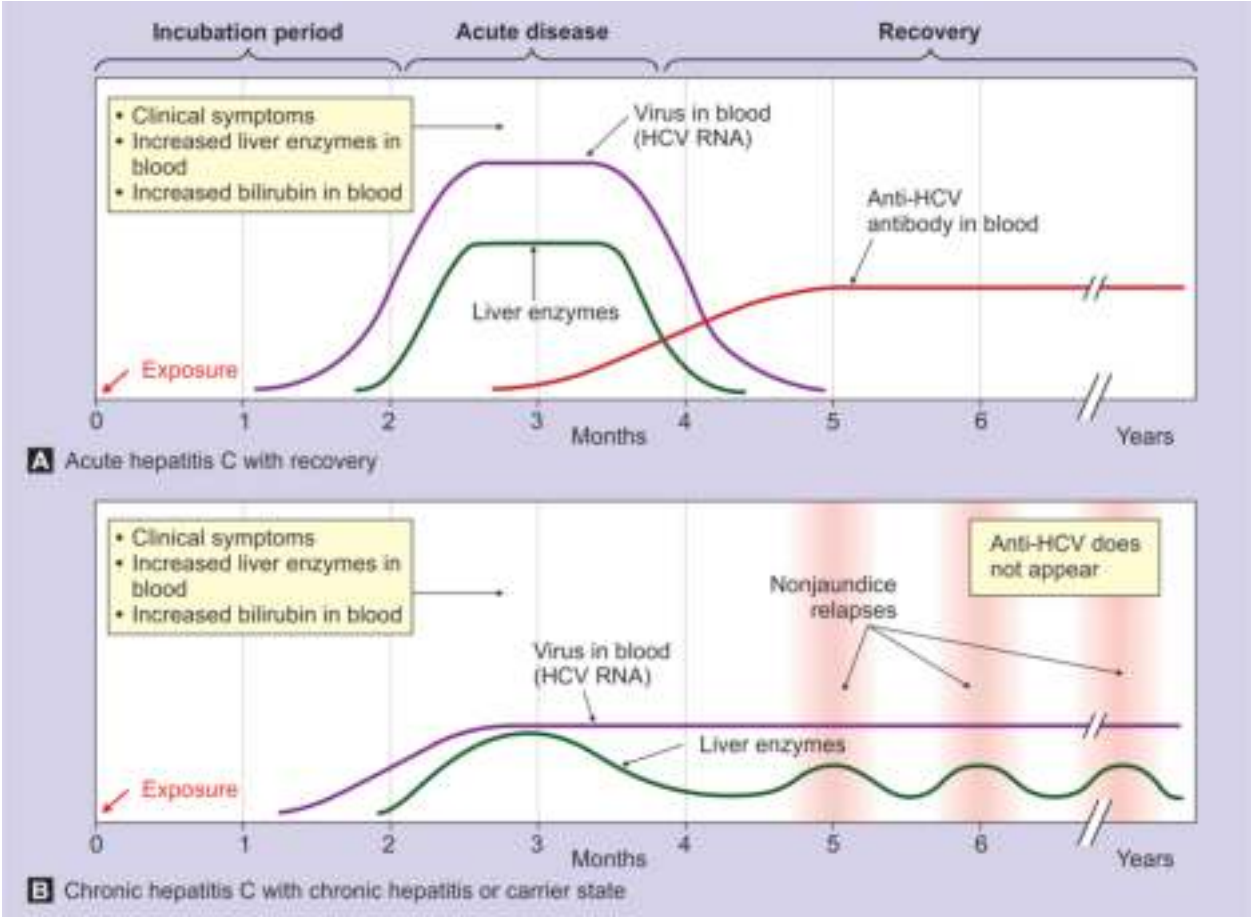


Fig. 21.33: Chronic hepatitis C. (A) Acute hepatitis C with recovery, (B) chronic hepatitis C with chronic hepatitis or carrier state.

Mode of Transmission

Mode of transmission of HDV can be acquired simultaneously with HBV (co-infection) or it may secondarily infect HBV carriers (superinfection). Both co-infection and superinfection may be followed by acute viral hepatitis (Table 21.39 and Fig. 21.34A and B).

- **Co-infection:** Person receiving infected blood transfusion with HBV and HDV develops acute hepatitis.
- **Superinfection:** Carriers of HBV develop superinfection with HDV.

Serological Studies

Anti-HDV IgM is most reliable marker of acute HDV infection. Anti-HBc IgM is also demonstrated. IgG is not a protective antibody.

HEPATITIS E VIRUS INFECTION

Hepatitis E virus (HEV) has now been cloned and sequenced. It is single-stranded RNA virus measuring 32–34 nm. HEV infection is contracted with recent travel to endemic areas, i.e. Africa, Asia, or Central America.

HEV infection resembles HAV infection, but it is more common in young adults rather than children. No vaccine has been developed for HEV prophylaxis.

Mode of Transmission

Hepatitis E virus is transmitted by fecal–oral route. Incubation period is 35–40 days.

Clinical Features

Like hepatitis A, hepatitis E usually presents as an acute self-limited illness with low mortality in 1–12% of cases. Patient presents with mild viral hepatitis with jaundice, fever and arthralgia that lasts about 6 weeks.

It does not progress to chronic liver disease. For unknown reasons, infection during pregnancy is associated with high mortality due to fulminant hepatic failure in 20% of cases.

Serological Assays in Hepatitis E Virus

Presence of anti-HBe IgM indicates active HEV infection. Anti-HEV IgG (protective antibody) indicates recovery.

Table 21.39 Co-infection and superinfection in relation to hepatitis D infection

Parameters	Co-infection	Superinfection
Status of patient before infection with HDV	HDV and HBV in healthy carrier	HDV infection in HBV carrier
Recovery from acute viral hepatitis with immunity	90%	10–15%
Risk of fulminant viral hepatitis and death	3–4%	7–10%
Risk of developing chronic viral hepatitis resulting in cirrhosis	Rare	80%

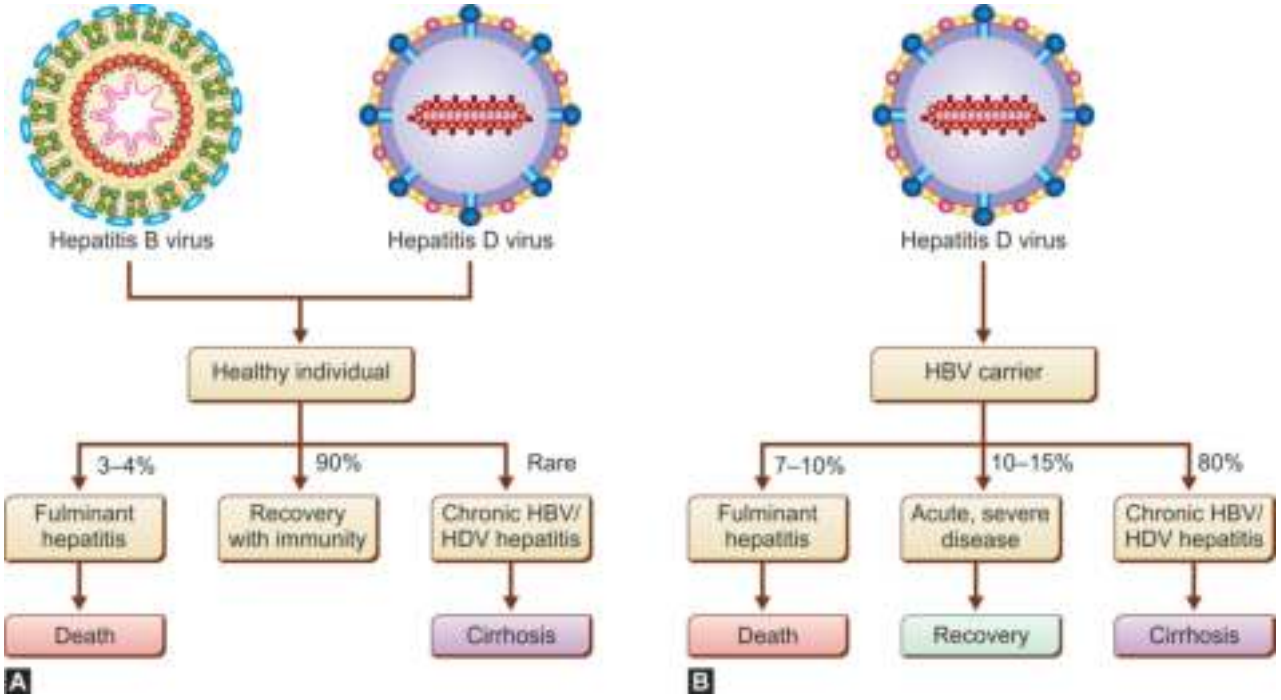


Fig. 21.34: (A) Co-infection, (B) superinfection in relation to hepatitis D infection.

HEPATITIS G VIRUS INFECTION

Hepatitis G virus (HGV) is a viral agent similar to HCV, which has been cloned and identified as HGV. Hepatitis G virus is a flavivirus transmitted parenterally by blood and found in 1–2% of blood donors and at a much higher rate among intravenous drug abusers. It does not lead to chronic viral hepatitis. It has no known relationship to hepatocellular carcinoma. Comparison of hepatotropic viruses is given in Table 21.40.

Table 21.40 Comparison of hepatotropic viruses

Characteristics	Hepatitis A (HAV)	Hepatitis B (HBV)	Hepatitis C (HCV)	Hepatitis D (HDV)	Hepatitis E (HEV)
Family	Picornavirus	Hepadnavirus	Flavivirus	Unknown	Calciviridae
Year of identification	1973	1965	1989	1977	1995
Genetic material	ssRNA	ssDNA	ssRNA	ssRNA	ssRNA
Morphology	Icosahedral capsid	Double shelled enveloped	Enveloped	Enveloped replication defective	Icosahedral unenveloped
Size of virus	27 nm	42 nm	30–60 nm	35 nm	32–24 nm
Transmission	▪ Fecal–oral route ▪ Male homosexuals	▪ Parenteral route ▪ Sexual contact ▪ Mother to fetus ▪ Breastfeeding	▪ Parenteral route ▪ Sexual contact	▪ Parenteral route ▪ Sexual contact	Fecal–oral route
Incubation period	2–6 weeks	4–26 weeks	2–26 weeks	4–7 weeks	2–8 weeks
Age group	Children and adolescents	Any age	Adults	Any age	Young adults
Onset	Acute onset	Insidious onset	Insidious onset	Insidious onset	Acute onset
Prevalence	Worldwide	Worldwide	Probably worldwide	Endemic region	Only in developing countries
Antigen	HAV (viremia transient)	▪ HbsAg ▪ HbcAg ▪ HbeAg	▪ HCV, C100-3 ▪ C-33c, and NS-5 –	▪ HbsAg ▪ HDV –	HEV – –
Antibodies	Anti-HAV	Anti-Hbs	Anti-HCV	Anti-HbsAg	Anti-HAV
Nature of illness	Mild	Occasionally severe	Moderate	Occasionally severe	Mild except during pregnancy
Fulminant hepatitis	0.1–0.4%	<1%	Rare	3–4%	0.3–3% (20% in pregnant women)
Carrier state	None	0.1–1%	0.2–0.4%	0.1–10%	No carrier state
Chronic hepatitis	Absent	5–10%	>70% cases	<5% (co-infection) and 80 (superinfection)	None
Cirrhosis	None	Yes	Yes	+/–	None
Hepatocellular carcinoma	No	Yes	Yes	Unknown	Unknown
Prognosis	Excellent	Poor	Moderate	Variable	Good
Associated findings	None	Serum sickness (5–10%), polyarteritis nodosa and membranous glomerulonephritis	Post-transfusion hepatitis, MPGN-1, and porphyria cutanea tarda	None	None
Specific prophylaxis	Immunoglobulin administration and vaccination	Immunoglobulin administration and vaccination	Nil	HBV vaccine	Nil

AUTOIMMUNE HEPATITIS (LUPOID HEPATITIS)

Autoimmune hepatitis is most common autoimmune chronic progressive disorder of liver especially in young women. It occurs due to reduction in number of CD8+ suppressor T cells. Women with autoimmune hepatitis show circulating antinuclear autoantibodies and elevated serum IgG concentration. But there are no serum markers to diagnose this disorder.

ETIOPATHOGENESIS

Autoimmune hepatitis is caused by viruses, drugs and in the settings of autoimmune disorders like rheumatoid arthritis, ulcerative colitis and Sjögren's syndrome. A pathogenetic role for immune-mediated mechanisms in autoimmune hepatitis is suggested by the presence of following:

- Positive LE cell phenomenon is demonstrated in 10% cases of systemic lupus erythematosus.
- Anti-nuclear antibodies are observed in >50% of cases.
- Smooth muscle cell autoantibodies are observed in 40–60% of cases.
- Autoantibody against receptor on cell membrane of hepatocytes is observed. Autoantibodies against microsomes are demonstrated.
- There is reduction in number of CD8+ suppressor T cells and their functional ability in autoimmune hepatitis.
- Heavy plasma cell infiltrate in liver is present.
- Rapid response to treatment with immunosuppressive agents has good response.

Classification

Autoimmune hepatitis is classified into two categories (types 1 and 2) based on the basis of autoantibodies (Table 21.41).

Table 21.41 Autoantibodies formed in autoimmune hepatitis types 1 and 2

Autoimmune Hepatitis Types	Autoantibodies
Autoimmune hepatitis type 1	▪ ANA (antinuclear antigen) most common ▪ SMA (smooth muscle actin antigen) most specific ▪ SLA (soluble liver antigen) ▪ LPA (soluble pancreas antigen)
Autoimmune hepatitis type 2	▪ LKM1 (liver, kidney microsome) ▪ LC-1 (liver cytosol 1 antigen)
Autoimmune hepatitis type 3	Antisoluble liver antigen

Clinical Features

Patient presents with jaundice, acne, amenorrhea and hepatosplenomegaly.

Surgical Pathology: Autoimmune Hepatitis

Light Microscopy

- Liver shows characteristic infiltration of plasma cells in periportal regions.
- Entire spectrum of hepatitis is demonstrated.

Prognosis

Untreated patient of autoimmune hepatitis may develop hepatocellular failure and cirrhosis having fatal outcome.

Management

Patients of autoimmune hepatitis are treated by immunosuppressive therapy. Liver transplantation is recommended for end-stage disease or history of recurrence.

BACTERIAL INFECTIONS AND PARASITIC INFESTATIONS OF LIVER

BACTERIAL LIVER INFECTIONS

Bacterial infections of the liver occur in many ways, in the setting of liver abscess, acute hepatitis, granulomatous hepatitis, hepatic tuberculosis, peritonitis, empyema, syphilitic hepatitis, and hepatic brucellosis. Right lobe of the liver is the most common sites of abscess formation and frequently observed in older patients or immunocompromised young individuals. Liver function test abnormalities and jaundice frequently accompany a variety of bacterial infections, especially sepsis. Bacterial infections can trigger acute decompensation of cirrhosis associated with development of organ dysfunction.

PYOGENIC LIVER ABSCESS

Pyogenic liver abscess is defined as a pus-filled mass in the liver caused by Staphylococci, Streptococci, and gram-negative enterobacteria, i.e. anaerobic inhabitants of the gastrointestinal tract. The bacteria gain access to the liver by direct extension from contiguous organs or through the portal vein or hepatic artery, bile ducts or by direct invasion or inoculation through the Glisson's capsule. Bacterial infections can originate in the biliary system, bacterial endocarditis, or pyelonephritis to produce pyogenic liver abscess. The routes of bacterial infection spread to liver are described as follows:

- **Arterial route of infection:** Bacteria reach the liver through hepatic artery during sepsis or in septic thromboemboli in bacterial endocarditis. Liver abscess may be caused by a variety of bacteria, such as Streptococci, Staphylococci, and gram-negative bacteria.
- **Portal vein infection:** Bacteria may originate in infected diverticula or mucosal abscesses. In pre-antibiotic era, bacterial appendicitis was a common cause of liver abscess; today it is uncommon.
- **Ascending biliary infection:** Bacteria ascending into the liver from the duodenum are most often gram-negative. Such infections usually occur in patients who have biliary duct stones or other diseases of biliary tract.
- **Direct invasion of liver:** Bacterial infection of the gallbladder may extend to liver transdiaphragmatic infection or direct entry of bacteria into liver from the infected peritoneum. It is a rare complication of severe infection and usually found in terminally ill patients or those who have had extensive surgery.
- **Direct inoculation of bacteria:** This type of infection is typically found in wounded people.

Surgical Pathology: Pyogenic Liver Abscess
Gross Morphology
Microabscesses are formed in the liver resulting in hepatomegaly (Fig. 21.35).
Light Microscopy
▪ On histologic examination, microabscess of liver contains numerous neutrophils. ▪ The beginning of an organizing abscess wall shows some pink fibrin (Fig. 21.36A and B).



Fig. 21.35: Liver abscesses. This patient died from gram-negative septicemia and the abscesses are a complication of this. The site of the original infection was not found (arrows).

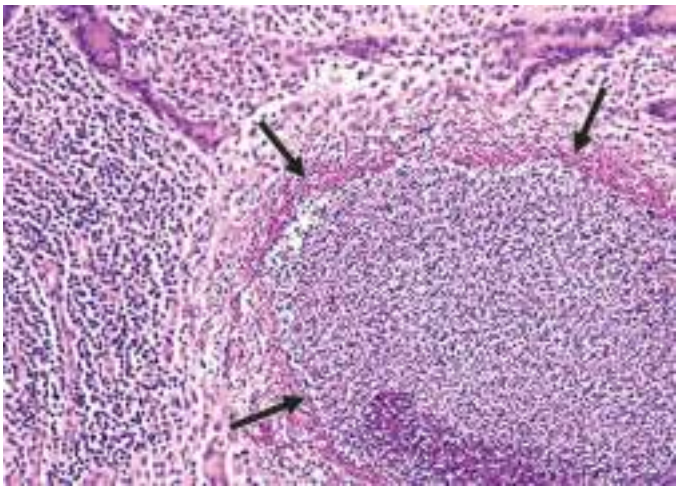


Fig. 21.36A: Pyogenic liver abscess. Microscopically, a microabscess of liver contains numerous neutrophils in the center. The beginning of an organizing abscess wall with some pink fibrin is seen here (arrows) (100X).

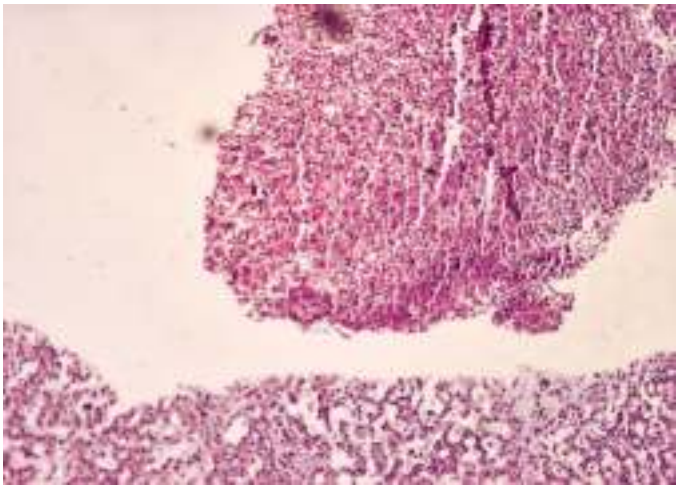


Fig. 21.36B: Pyogenic liver abscess. It is defined as pus-filled mass in the liver that can develop from injury to the liver of intra-abdominal infection disseminated from the portal vein. Histologic examination of pyogenic liver abscess reveals numerous neutrophils (400X).

GRANULOMATOUS LIVER ABSCESS

Granulomatous liver abscess occurs in the settings of tuberculosis, fungal infections and brucellosis. Tuberculous liver abscess most often occurs in developing countries. Tuberculosis is worldwide health problem caused by tubercle bacilli. It is transmitted by inhalation of droplets (most common), ingestion (infected milk) and skin inoculation (handling infected specimens). It may involve multiple systems.

Tubercle bacilli may disseminate via systemic circulation to seed distant organs such as bone marrow, liver, spleen, adrenal glands, retina, meninges, kidneys, fallopian tubes, and epididymis. Light microscopy of tuberculoses of liver is shown in Fig. 21.37.

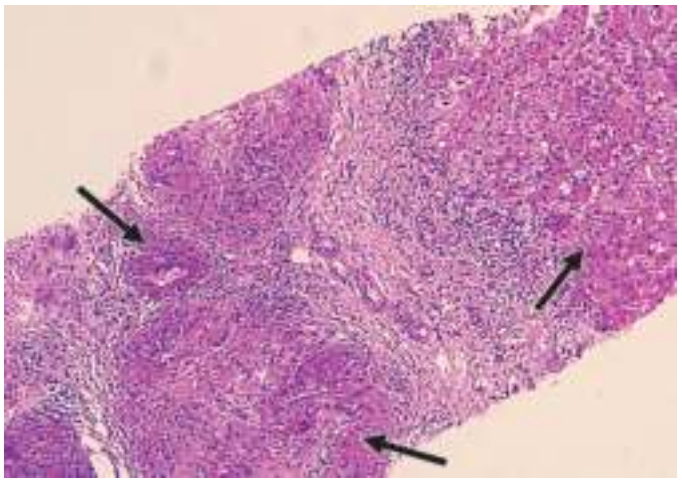


Fig. 21.37: Tuberculosis (TB) of liver. Liver biopsy is considered the most specific diagnostic test for hepatic TB. Caseating epithelioid cell granulomas are observed 50–85% of cases with miliary TB. Histologic examination shows multiple epithelioid cell granulomas (arrows) (100X).

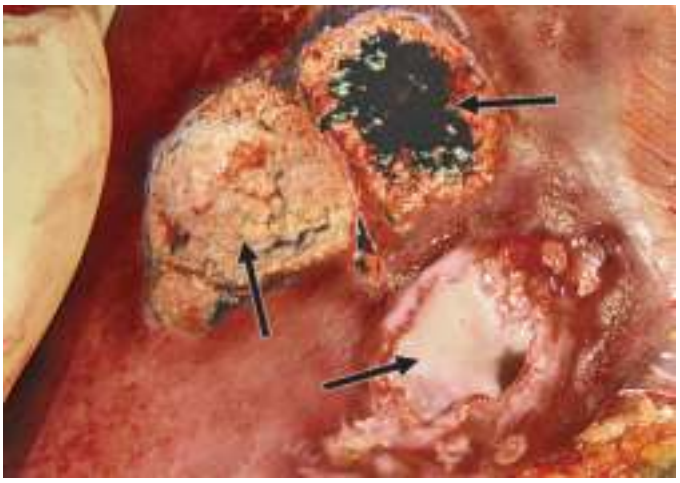


Fig. 21.38: Amoebic liver abscess. This is an amoebic abscess of liver. Abscesses may arise in liver when there is seeding of infection from the bowel, because the infectious agents are carried to the liver from the portal venous circulation (arrows).

PARASITIC LIVER INFECTIONS

Parasitic and helminthic infections are important in tropical countries. Such infections include malarial parasite, *Entamoeba histolytica, Leishmania donovani,* liver fluke (e.g. *Fasciola hepatica* and *Clonorchis sinensis*) and *Echinococcus granulosus*.

AMOEBIC LIVER ABSCESS

Amoebic liver abscess is the most common form of extraintestinal amoebiasis. It most often occurs in posterosuperior region of right lobe of liver.

Etiology

Amoebic liver abscess is caused by *Entamoeba histolytica*. Approximately, 30% of patients have a history of antecedent intestinal amoebiasis. It occurs most often in men than women and rare in children. Large amoebic abscess presses upon liver capsule resulting in pain.

Clinical Features

Patient presents with abrupt onset of fever and dull aching abdominal pain in the right upper quadrant or epigastrium, usually lasting <10 days duration. Jaundice is unusual.

Surgical Pathology: Amoebic Liver Abscess
Gross Morphology
Necrosed hepatocytes are admixed with blood resembling anchovy sauce (Fig. 21.38).
Light Microscopy
Abscess material from liver on histologic examination shows necrotic material with a few neutrophils with clusters of trophozoites of amoeba.

Laboratory Diagnosis

The diagnosis of amoebic liver abscess is most often made by radiological or ultrasound in conjunction with serological testing for antibodies to *Entamoeba histolytica*.

Complications

Amoebic liver abscess may be secondarily infected, which can extend and perforate into pleuropulmonary structures, subphrenic space, peritoneal cavity and pericardial sac. There may be dissemination of amoebic liver abscess to brain or kidney. Differences between amoebic and pyogenic liver abscesses are shown in Table 21.42.

HYDATID CYST IN LIVER

Hydatid cyst in liver is caused by *Echinococcus granulosus* and common in India. Right lobe of liver is the commonest site of hydatid cyst followed by lung. Accidental spillage during fine needle aspiration may cause anaphylactic shock.

- Hydatid disease is caused by larval or cystic stage of *Echinococcus granulosus* tapeworm or *Echinococcus multilocularis*. Adult parasites are found in dogs and sheep. Humans are infected after ingestion of eggs. The eggs hatch in the small intestine and their larvae pass to the liver by portal circulation. Most commonly the right lobe of liver is affected. However, any lobe of liver can be affected.
- Echinococcus multilocularis is less common. Cysts are multilocular containing thick pasty material, and surrounded by a fibrous wall. It has a more aggressive clinical course in comparison to *Echinococcus granulosus*.

Table 21.42 Differences between amoebic and pyogenic liver abscesses

Characteristics	Amoebic Liver Abscess	Pyogenic Liver Abscess
Causative organism	*Entamoeba histolytica*	*Escherichia coli*, Pseudomonas and Klebsiella
Liver lobe involved	Posterosuperior part of right lobe	Posterior part of right lobe
Abscess	Single abscess of variable size	Single or multiple abscesses
Gross morphology	The center of abscess has necrotic area containing reddish brown thick pus resembling anchovy or chocolate sauce. Abscess is surrounded by necrotic liver parenchyma	The center of abscess contains pus. Abscess is surrounded by fibrous capsule
Light microscopy	It shows leukocytes, RBCs, necrotic debris and *Entamoeba histolytica*	It shows infiltration by neutrophils
Septicemia	Absent	It may occur due to dissemination of pathogens
Jaundice	Rare	Always present

Surgical Pathology: Hydatid Cyst in Liver

Gross Morphology

Liver contains multiple hydatid cysts of various sizes. Color of the cysts is white of an boiled egg (Fig. 21.39).

Light Microscopy

- The hydatid cyst is filled with colorless fluid containing daughter cysts and brood capsules with scolices.
- The cyst wall shows an inner germinal layer (endocyst), outer chitinous (fibrous lamina) ectocyst layer surrounded by either granulation tissue or a fibrous capsule (pericyst).
- The adjacent liver shows eosinophilic infiltrate in portal tracts. The scolices having hooklets of 20–40 μm length (Fig. 21.40A and B).

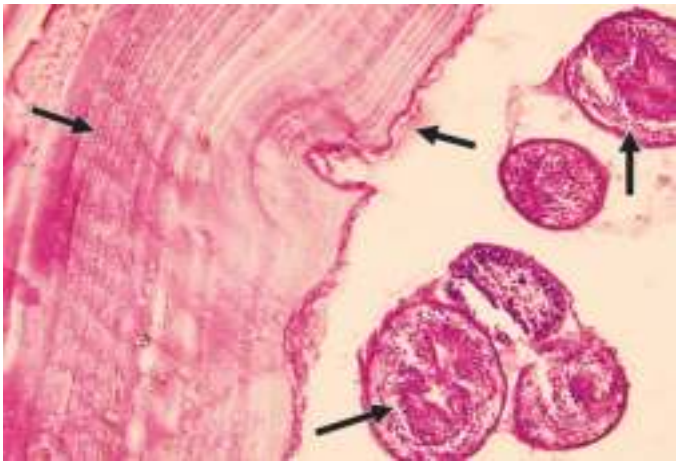


Fig. 21.40A: Hydatid cyst of liver. The cyst wall shows an inner germinal layer (endocyst), outer chitinous (fibrous lamina) ectocyst layer surrounded by either granulation tissue or a fibrous capsule (pericyst). Scolices having hooklets are also seen (arrows) (400X).



Fig. 21.39: Hydatid cyst of liver. Ingestion of eggs of *Echinococcus granulosus* in contaminated feces can infect humans. Hydatid cyst with multiple daughter cysts consists of outer thick fibrotic wall layer, middle layer of hyalinized, laminated, and acellular material, and inner layer of adult tapeworm heads (arrows)

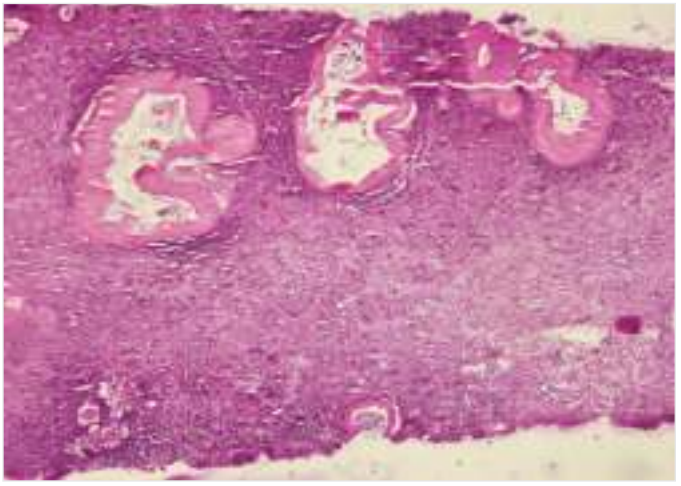


Fig. 21.40B: Hydatid cyst of liver. Disease is caused by *Echinococcus granulosus*. The principal complications are infection, biliary duct fistula and rupture into the peritoneum or chest. Diagnosis has become easier with advances in ultrasonography and computed tomography scanning. Histologic examination reveals multiple hydatid cysts within liver (400X).

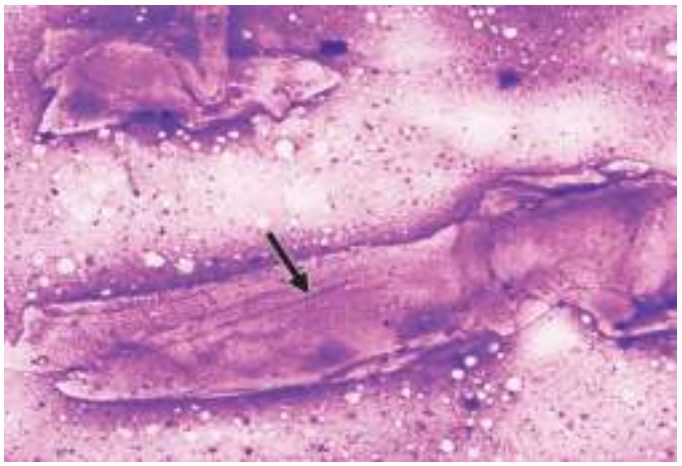


Fig. 21.41: FNAC of hydatid cyst of liver. Smear shows laminated membranous structures resembling ectocyst of hydatid cyst. Claw-like refractile hooklets are demonstrated in the background. PAS stain highlights the laminated membranous structures as magenta-colored structures and Gomori methenamine stain black. Hooklets are demonstrated by Ziehl-Neelsen stain as bright purple (arrow) (400X).

Cytologic Smear Examination

Cytologic smear shows laminated membranous structures resembling ectocyst of hydatid cyst. Claw-like refractile hooklets are demonstrated in the background (Fig. 21.41).

Periodic acid–Schiff (PAS) stain highlights the laminated membranous structures as magenta-colored structures and black hooklets by Gomori methenamine stain. Hooklets are demonstrated by Ziehl-Neelsen stain as bright purple.

Complications

Rupture of hydatid cyst into the peritoneal cavity results in fatal anaphylactic reaction. Rupture of cyst can involve into the gallbladder or through the diaphragm into the pleural space and lung.

HEPATOMEGALY DUE TO MALARIAL PARASITE

Hepatic involvement by malarial parasite causes hepatomegaly secondary to hypertrophy and hyperplasia of Kupffer cells.

SCHISTOSOMIASIS IN LIVER

Schistosomiasis in liver is caused by *Schistosoma mansoni* or *Schistosoma japonicum*. The adult worms lodge in the portal vein and its branches.

The eggs are highly antigenic and stimulate formation of granuloma, tissue destruction, scarring, and portal hypertension.

ASCENDING CHOLANGITIS AND LIVER ABSCESS

Ascending cholangitis after percutaneous ablation therapy of liver tumors often disseminates to liver resulting in liver abscess.

SUPPURATIVE THROMBOSIS OF PORTAL VEIN

Portal vein thrombosis most often occurs due to intra-abdominal sepsis following perforated appendix, peritonitis or diverticulitis. Infection from portal vein spreads to liver.

Patient presents with fever, rigors and abdominal pain in right upper quadrant. Liver functions are abnormal but clinical jaundice is uncommon. Portal vein thrombosis may cause portal hypertension.

DRUGS AND TOXINS INDUCED HEPATOCELLULAR INJURY

Exposure to drugs and industrial chemicals may cause acute hepatocellular injury. Chemicals act as systemic poisons and get converted to toxic intermediate leading to centrilobular hepatocellular injury. Drugs and toxic chemicals may affect one or more of the liver lobular structures such as hepatocytes, bile ductules, bile ducts, blood vessels and mononuclear phagocytes. Drugs and toxins causing hepatocellular injury are given in Table 21.43. Drugs and toxins affecting bile ductules and bile ducts are given in Table 21.44.

Table 21.43 Drugs and toxins causing hepatocellular injury

Pathological Findings	Mode of Action	Drugs and Toxins
Toxic hepatic coagulative necrosis in zone 3	Reactive metabolites	Paracetamol, carbon tetrachloride poisoning and mushroom *Amanita phalloides* poisonings
Cytosolic spotty necrosis producing intralobular inflammation	Formation of neoantigens as a result of hapten linkage with liver cell proteins	Halothane, erythromycin, isoniazid and α-methyldopa
Acute hepatitis like morphology with cholestasis	Immune-mediated mechanism	Sulphonylureas, phenothiazines and tricyclic antidepressants
Chronic hepatitis like morphology	Immune-mediated mechanism	Methotrexate, α-methyldopa, nitrofurantoin and oxyphenisatin

Contd...

Table 21.43 Drugs and toxins causing hepatocellular injury (*Contd...*)

Pathological Findings	Mode of Action	Drugs and Toxins
Macrovesicular fatty change	Due to decreased proteins synthesis, lack of apoprotein B-100, damage to endoplasmic reticulum, Golgi apparatus or plasma membrane of hepatocytes	Alcohol and methotrexate
Microvesicular fatty change	Reflecting impaired mitochondrial oxidation of fatty acids	Tetracycline, valproic acid (anticonvulsant), Reye's syndrome and poisoning by ingestion of unripe ackee fruit in Jamaica patients
Phospholipid accumulation in lysosomes	Due to inhibition of phospholipase by drugs	Amiodarone
Granulomatous hepatitis	Cell-mediated mechanism	Sulphonamide, allopurinol, carbamazepine, phenylbutazone and quinine
Cirrhosis	Direct toxicity	Ethanol, methotrexate and carbon tetrachloride
Hepatocellular adenoma, hepatocellular carcinoma	Carcinogenic agent	Estrogens and thorotrast contrast dye
Angiosarcoma	Carcinogenic agent	Vinyl chloride and inorganic arsenic

Table 21.44 Drugs and toxins affecting bile ductules and bile ducts

Pathological Findings	Mode of Action	Drugs and Toxins
Acute cholestasis	Estrogen binding with 17β-glucuronides causing cholestasis	Oral contraceptives, anabolic and androgenic steroids
Prolonged cholestasis with destruction of small bile ducts	Immune-mediated mechanisms	Chlorpromazine, paraquat poisoning and arsenic derivatives
Intrahepatic and extrahepatic stricture resembling sclerosing cholangitis	Mechanism unknown	Infusion of floxuridine into the hepatic artery in the treatment of liver metastases from colorectal carcinoma

Pathogenesis

Pathogenesis of hepatocellular injury by drugs occurs either by direct or idiosyncratic mechanisms.

Direct Hepatocellular Injury

Direct hepatocellular injury is dose-dependent. Drugs may produce intermediate metabolites and oxygen-derived free radicals and cause adverse effects on hepatocytes by peroxidation of cell membrane lipids leading to hepatocellular injury. Metabolic intermediates may also block the biochemical pathways by impairing mitochondrial oxidation of fatty acids. Therefore, hepatocytes show fatty change.

Idiosyncrasy Mechanism of Drugs

Idiosyncrasy mechanism of drugs causing hepatocellular injury is not dose-dependent.

- **Hypersensitivity:** Metabolites of isoniazid and halothane may cause hepatocellular injury by hypersensitivity reaction.
- **Autoantibody synthesis:** Autoantibody synthesized against drug produce adverse effects on cytochrome P450 enzyme.
- **Hapten formation:** Drug or its metabolite binds to cellular proteins and forms a hapten. This hapten acts as antigen leads to hepatocellular injury.

Surgical Pathology: Drug-induced Hepatocellular Injury

Light Microscopy

Drug-induced hepatocellular damage shows histologic changes, i.e. eosinophilic infiltrate, central lobular necrosis, noncaseating epithelioid cell granulomas, cholestasis and giant cell hepatitis.

ACETAMINOPHEN INDUCED HEPATOCELLULAR INJURY

Acetaminophen is an important analgesic recommended by the clinicians. Excessive consumption of acetaminophen is highly toxic to the liver. The toxic dose of acetaminophen after a single acute ingestion is in the range of 150 mg/kg in children and 7 g in adults. Drug toxicity should be suspected in all cases of acute hepatitis. Drug-induced hepatocellular injury can be either direct or indirect. Indirect hepatocellular injury is caused by metabolites and oxygen-derived free radicals that are produced as byproducts of xenobiotic metabolism.

Physiologic state

Normal dose of acetaminophen is rapidly absorbed from the stomach and small intestine and become conjugated in the liver to form nontoxic metabolite, then eliminated in the urine.

Pathologic state

In case of acute drug overdose, normal pathways of acetaminophen metabolism become saturated. Excess acetaminophen is then metabolized in the liver via the mixed function oxidase P450 system located in the smooth endoplasmic reticulum of the hepatocytes, yielding oxidative metabolites leading to centrilobular necrosis. Patient may later develop hepatocellular necrosis.

CARBON TETRACHLORIDE-INDUCED HEPATOCELLULAR INJURY

Carbon tetrachloride (CCl_4) is well-studied hepatotoxin. Immune reactions against a chemical or its metabolites also cause of indirect liver damage. Chemically-induced hepatocellular injury is classified as predictable when toxicity is immediate and dose-dependent and as unpredictable or idiosyncratic when toxicity occurs without explanation.

Exposure to industrial solvents, such as carbon tetrachloride, causes predictable toxic hepatocellular injury, characterized by centrilobular necrosis and elevated serum levels of transaminases (ALT and AST).

Pathogenesis

Carbon tetrachloride (CCl_4) is metabolized via the mixed function oxygenase (P450) system located in the smooth endoplasmic reticulum of the liver to a chloride ion and a highly reactive trichloromethyl free radical. Like the hydroxyl radical, this radical is a potent initiator of lipid peroxidation, which damages the plasma membrane and leads to hepatocellular necrosis.

Surgical Pathology: Carbon Tetrachloride (CCl_4)-induced Hepatocellular Injury

Light Microscopy

Acute, chemically-induced hepatocellular injury shows the entire spectrum of liver disease, from transient cholestasis to massive hepatic necrosis.

LIVER CIRRHOSIS AND PORTAL HYPERTENSION

LIVER CIRRHOSIS

Cirrhosis is end-stage liver disease with disruption of entire normal hepatic architecture, replaced by bridging fibrosis, presence of parenchymal regenerative nodules by proliferating hepatocytes, disruption of normal architecture of the entire liver with interference with its normal functioning and varying degree of portosystemic shunting.

- **Etiology:** Most common causes of cirrhosis are alcohol abuse, viral hepatitis (HCV, HBV), nonalcoholic steatosis, secondary biliary cirrhosis, hemochromatosis, drug (methotrexate), and chemical agent (carbon tetrachloride). Less common causes of cirrhosis are primary biliary cirrhosis, Wilson disease, autoimmune hepatitis, primary sclerosing cholangitis, and inborn errors of metabolism.
- **Complications:** Complications of liver cirrhosis are portal hypertension with subsequent sequelae, e.g. esophagogastric varices, ascites, hepatic encephalopathy, hepatocellular carcinoma, coagulopathy, jaundice, pruritus, hepatorenal syndrome and hepatobiliary syndrome. Liver cirrhosis is more prevalent among malnourished alcoholic people over 50 years of age.
- **Portal hypertension:** Portal hypertension is a vascular disorder of the liver characterized by increased resistance to portal blood flow at the level of sinusoids with the development of venous collaterals and ascites. It is classified based on site of portal venous obstruction: prehepatic, hepatic and post-hepatic.
  - Prehepatic portal hypertension occurs due to obstruction of portal and splenic vein secondary to thrombosis.
  - Intrahepatic portal hypertension is caused by vascular obstruction secondary to liver cirrhosis, metastatic tumors, or schistosomiasis.
  - Post-hepatic portal hypertension occurs due to venous chronic congestion secondary to constrictive pericarditis, tricuspid valvular insufficiency, congestive heart failure or hepatic vein occlusion (Budd-Chiari syndrome).
  - Portosystemic shunting is characterized by abnormal vascular connections between the hepatic portal vein and the systemic circulation. Clinical consequences of portosystemic shunt are esophagogastric varices, periumbilical collaterals (caput medusae), splenomegaly and hemorrhoids.

Pathology Pearls: Diagnostic Histologic Criteria for Cirrhosis

- Presence of bridging fibrosis in liver.
- Formation of parenchymal nodules due to proliferation of hepatocytes.
- Disruption of normal architecture of liver.

ETIOLOGIC CLASSIFICATION

Liver cirrhosis can be classified based to the etiology and morphologic appearance of liver in cirrhosis. Cirrhosis is classified according to etiology. Based on the etiology, cirrhosis is sub-classified as follows: viruses (HBV, HCV), toxins (alcohol abuse, drugs, i.e. acetaminophen, some antibiotics, antidepressants), nonalcoholic steatohepatitis, autoimmune hepatitis, cholestasis (primary biliary sclerosis, primary sclerosing cholangitis), vascular etiology (Budd-Chiari syndrome, sinusoidal obstruction syndrome, cardiac cirrhosis), and metabolic disorders (hemochromatosis, Wilson disease, α_1-antitrypsin deficiency). Etiological classification of cirrhosis is given in Table 21.45.

Alcoholic (Laennec or Nutritional) Cirrhosis

Alcoholic cirrhosis is an advanced stage of alcoholic liver disease that happens after years of drinking too much of alcohol. Alcoholic liver disease comprises liver steatosis, steatohepatitis, alcoholic cirrhosis. Liver shows micronodular pattern evolving in late stages to typical hobnail liver with large and irregular regenerative nodules.

Post-necrotic Cirrhosis

Post-necrotic cirrhosis is also called macronodular cirrhosis due to hepatitis B and C viruses, which involves areas of scarring in liver forming large and irregular nodules, that are >3 millimeters in diameter. Liver cirrhosis is an irreversible scarring of liver that can lead to hepatocellular failure and hepatocellular carcinoma more often than in other forms of liver cirrhosis. Clinicians usually prescribe antiviral drugs to prevent hepatotropic viruses (HBV and HCV) from causing more liver damage. One can prevent hepatitis B virus infection by getting vaccinated for hepatitis B vaccine.

Biliary Cirrhosis

Primary biliary cirrhosis is of probable autoimmune origin caused by anti-mitochondrial antibodies; obstructive jaundice. Secondary biliary cirrhosis is the result of long-standing extrahepatic biliary obstruction due to stone, stricture, pancreatitis or pancreatic carcinoma and congenital malformation.

Hemochromatosis-induced Cirrhosis

Primary hemochromatosis is familial defect in regulation of iron absorption resulting in massive accumulation of hemosiderin in hepatic and pancreatic parenchymal cells, myocardium, and other sites. Classic triad of bronze diabetes include diabetes mellitus, hemochromatosis, and skin pigmentation. Secondary hemochromatosis is caused by chronic iron overload of diverse etiology.

Wilson's Disease-induced Cirrhosis

Wilson's disease is an autosomal recessive disorder of copper metabolism, in which excess of copper is deposited in the liver, kidney tubular cells (amino aciduria and glycosuria), brain (basal ganglia especially the putamen of the lenticular nucleus impairing extrapyramidal motor functions), and 'Descemet membrane of the cornea' (Kayser-Fleischer ring). Ceruloplasmin levels are decreased in Wilson's disease secondary to copper deposition in various organs. Cirrhosis can be micronodular or macronodular in Wilson's disease.

Table 21.45 Etiological classification of cirrhosis

Common Causes of Cirrhosis	Rare Causes of Cirrhosis
Alcohol related liver disease (Laennec's cirrhosis, portal cirrhosis or hobnail cirrhosis)	Primary biliary cirrhosis (PBC)
HBV and HCV (most common in India)	Wilson's disease
Nonalcoholic liver disease	Autoimmune hepatitis
Secondary biliary cirrhosis (e.g. obstruction of the common bile duct by stone, stricture and pancreatitis or pancreatic carcinoma)	Primary sclerosing cholangitis
▪ Hemochromatosis ▪ Methotrexate drug-induced cirrhosis ▪ Carbon tetrachloride and ethanol induced cirrhosis	Inborn errors of metabolism (α_1-antitrypsin deficiency, galactosemia, glycogen storage disease, fructose intolerance and acid cholesterol ester hydrolase deficiency)

Inborn Errors of Metabolism Induced Cirrhosis

Persons with inborn errors of metabolism such as α_1-antitrypsin deficiency, galactose-1-phosphate uridyl-transferase deficiency (galactosemia) and glycogen storage disease are linked to development of liver cirrhosis. About 15% of adults with α_1-antitrypsin deficiency develop cirrhosis. α_1-Antitrypsin deficiency, an autosomal recessive disorder is the leading cause of liver disease. Most of the liver disease is associated with homozygosity for the Z mutant allele, the so-called PiZZ. It is a protein-folding disorder in which toxic insoluble ATZ proteins aggregate in endoplasmic reticulum of hepatocytes leading to endoplasmic reticulum stress, mitochondrial depolarization, and caspase cleavage, inflammation, fibrosis, liver cirrhosis, and increased risk for hepatocellular carcinoma.

MORPHOLOGIC CLASSIFICATION

Morphologic classification of cirrhosis is purely descriptive. It has no relevance to the clinical presentation and management of these patients. Based on the morphology, liver cirrhosis can be sub-classified as micronodular cirrhosis (uniform regenerating nodules <3 mm in diameter), macronodular cirrhosis (irregular regenerative nodules with a variation >3 mm in diameter), or mixed cirrhosis (when features of both micronodular and macronodular cirrhosis are present).

Micronodular Cirrhosis

Micronodular cirrhosis is characterized by regenerative nodules measuring <3 mm in size. Liver shows more irregular pattern. Micronodular cirrhosis occurs due to alcohol abuse, hemochromatosis, hepatic venous outflow obstruction, chronic biliary obstruction, jejunoileal bypass, and Indian childhood cirrhosis. Etiology, frequency, and light microscopic features in micronodular cirrhosis are given in Table 21.46.

Macronodular Cirrhosis

Macronodular cirrhosis is characterized by regenerative nodules measuring >3 mm in size. Liver shows more irregular pattern. Macronodular cirrhosis occurs due to hepatitis B, α_1-antitrypsin deficiency, and primary biliary cholangitis. Etiology, frequency, and histologic features in macronodular cirrhosis are given in Table 21.47.

Table 21.46 Etiology, frequency and light microscopic features in micronodular cirrhosis

Etiology	Frequency	Light Microscopy
Alcoholic cirrhosis	60–70%	Fatty change, neutrophilic infiltration and Mallory's bodies
Primary biliary cirrhosis	5%	Portal tract inflammation (early stage) and granulomas, medium-sized bile duct destruction, cholestasis in zone 1 in periportal region, Mallory's bodies in some cases
Large bile duct obstruction induced cirrhosis	<5%	Marked cholestasis and bile cell proliferation; and irregular outlines of cirrhotic nodules
Hemochromatosis induced cirrhosis	5%	Marked deposition of hemosiderin in hepatocytes, Kuppfer cells and epithelium of ducts
Intestinal bypass	Rare	Marked fatty change, and Mallory's bodies in some cases
Cystic fibrosis induced cirrhosis	Rare	Periodic acid–Schiff material present in proliferated ductules in portal tracts
Indian childhood cirrhosis	Rare	Mallory's bodies

Table 21.47 Etiology, frequency and histologic features in macronodular cirrhosis

Etiology	Frequency	Light Microscopy
Chronic viral hepatitis (HBV or HCV)	10–20%	Hepatocytes show ground glass appearance and viral antigens are demonstrated by immunohistochemistry technique
Cryptogenic cirrhosis	Common	Macronodular cirrhosis of unknown etiology
Wilson's disease	Rare	Excess deposition of copper in hepatocytes demonstrated by rubeanic acid, fatty change and sometimes Mallory's bodies
α_1-Antitrypsin deficiency	Rare	Periodic acid–Schiff stain positive and diastase resistant globules in hepatocytes demonstrated by immunohistochemistry technique
Drugs and toxins	Rare	Macronodular cirrhosis
Hereditary hemorrhagic telangiectasia	Rare	Large dilated blood vessels in fibrous septa of liver

Micronodular and Macronodular Cirrhosis

Micronodular and macronodular cirrhosis is characterized by presence of regenerative nodules ranging from <3 to >4 mm in diameter. Some portal tracts and central veins are spared. Mixed cirrhosis progresses into macronodular cirrhosis over time, indicative of incomplete expression of micronodular cirrhosis.

PATHOPHYSIOLOGY OF LIVER CIRRHOSIS

Liver is formed by hepatocytes (parenchymal cells), other non-parenchymal cells and hepatic sinusoids. The walls of hepatic sinusoids are lined by hepatic stellate cells (HSCs), liver sinusoidal endothelial cells (LSECs), and Kuffer's cells (KCs). Both hepatocytes (parenchymal cells) and cells lining hepatic sinusoids, cytokines, and microRNAs are involved in the initiation and progression of liver fibrosis and cirrhosis.

- Most important pathways leading to cirrhosis are chronic active hepatitis, steatohepatitis (fatty change with evidence of inflammation), portal fibrosis and fibrosis occurring in acinar zone 3.
- Chronic active hepatitis and alcohol liver disease are the most important causes of liver cirrhosis accounting >60% of cases. Pathologic changes that occur in cirrhosis, include irreversible hepatocellular injury, extensive fibrosis, and nodular tissue growth, resulting from hepatic parenchymal cell death; collapse of liver's supporting structure, distortion of the vascular bed, and nodular regeneration of remaining liver tissue. Toxins stimulate sinusoidal endothelial cells and Kupffer cells. Normal liver morphology and pathogenesis of cirrhosis are given in Fig. 21.42A and B.

Role of Activated Kupffer Cells

Activated Kuffer's cells present in the sinusoidal endothelium destroy hepatocytes by production of harmful chemical mediators, growth factors (PDGF, TNF-α, TGF-β, ET-1 and MCP-1. PDGF and TNF-α), which also stimulate the activation of hepatic stellate cells (HSCs). PDGF and MCP-1 released by activated Kupffer cells participate in chemotaxis of neutrophils and lymphocytes. Hepatocytes undergo dysfunction and apoptosis. Repeated cycles of apoptosis and regeneration of hepatocytes contribute to pathogenesis of liver cirrhosis.

Role of Liver Sinusoidal Endothelial Cells

Perisinusoidal endothelial cells synthesize endothelin 1 and platelet activating factor. Edothelin 1 (ET-1) stimulates quiescent extracellular matrix (ECM) synthesizing stellate cells (Ito cells). Liver sinusoidal endothelial cells (LSECs) constitute the sinusoidal endothelium. It is known that retinol deficiency can activate and transform hepatic stellate cells (HSCs) into myofibroblasts with an enhanced extracellular matrix (ECM) production, resulting in perisinusoidal fibrosis, and ultimately to liver cirrhosis. HSCs can synthesize cytokine IL-33 that activates HSCs and promote hepatic fibrosis. On the contrary, differentiated LSECs can promote reversion of activated LSECs to quiescent state thereby preventing progression to fibrosis through vascular endothelial growth factor (VEGF)-stimulated nitrous oxide production.

Role of Hepatic Stellate Cells

Hepatic stellate cells, also called Ito cells, reside in the space of Dissie in the normal liver and their main function is storage of vitamin A and other retinoids. Following multiple injurious insults and/or exposure to inflammatory cytokines such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and interleukin-1 (IL-1), HSCs undergo the transition from a quiescent state to activated state. Contraction of stellate cells causes decreased sinusoidal perfusion resulting in hypoxia. Activated HSCs play important role in initiation and progression of hepatic fibrosis, and synthesis of large amount of collagen and other ECM ultimately leading to hepatic fibrosis.

Role of Cytokines

At the molecular level, multiple cytokines are involved in regulation of signaling pathways that mediate activation of HSCs, fibrogenesis and development of cirrhosis.

- PDGF is the strong mitogen to HSCs. Transforming growth factor-α (TGF-α) is the strongest known inducer of fibrogenesis in liver by stimulating HSCs. TGF-β induces expression of the matrix-producing genes and inhibits degrading of ECM by downregulating expression of matrix metalloproteinases (MMPs) and promoting tissue inhibitor matrix proteinases (TIMP) resulting in excessive deposition of collagen fibers, promoting fibrogenesis and development of liver cirrhosis.
- Tumor necrosis factor-α (TNF-α) is mainly secreted by monocytes, macrophages, HSCs and Kupffer's cells. TNF-α plays an important role in the activation of HSCs and synthesis of ECM.
- Interferons (IFNs) downregulate fibrogenesis via triggering the apoptosis of HSCs. Similarly, interferon-γ (IFN-γ) downregulates extracellular matrix deposition by inhibiting activation of HSCs via TGF-β1/SMAD3 signaling pathways.
- Interleukins (ILs) are group of cytokines are secreted by leukocytes (monocytes/macrophages, CD4+ helper T cells, endothelial cells). ILs play important

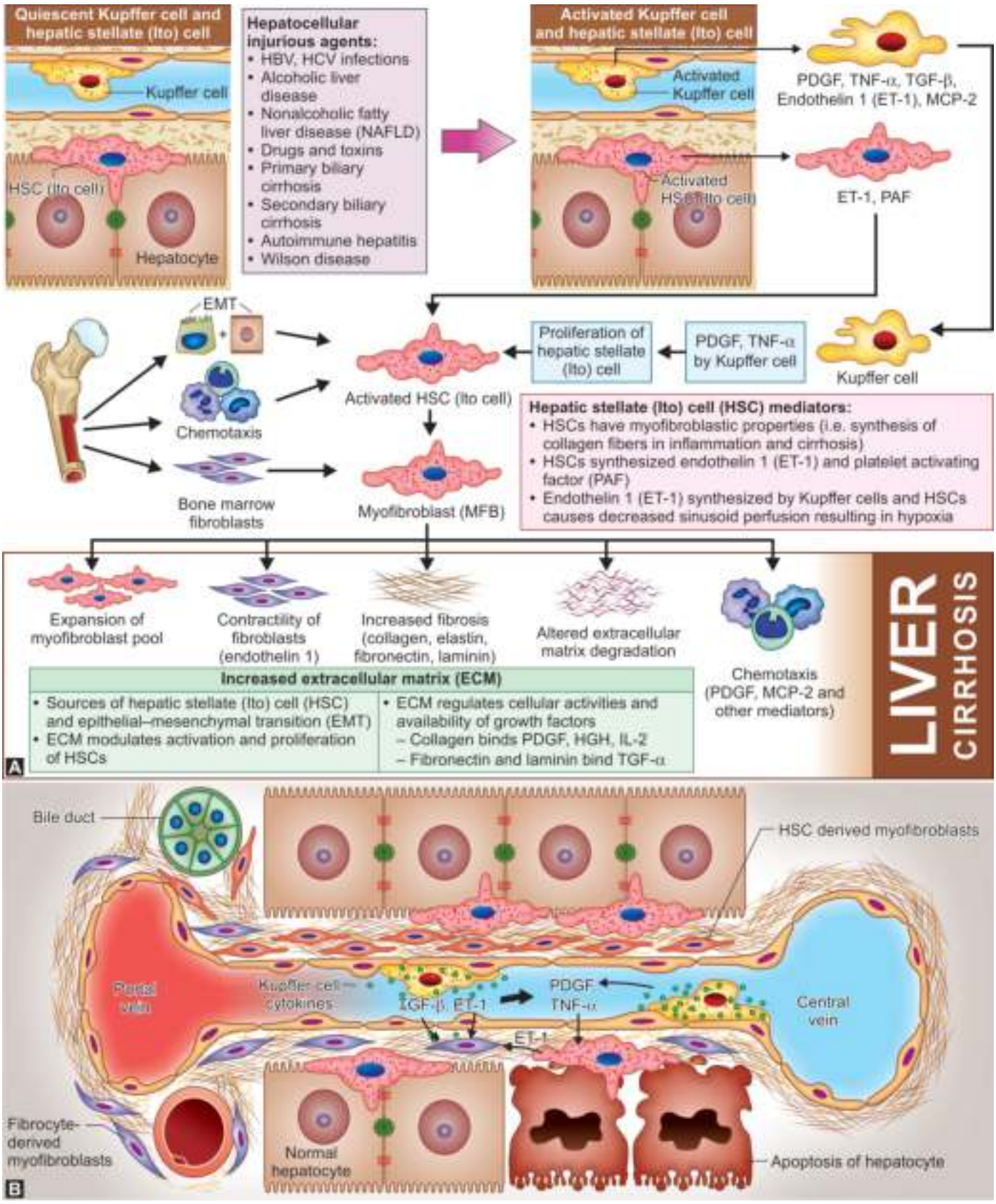


Fig. 21.42: (A) Normal liver morphology, (B) pathogenesis of cirrhosis. Kupffer cell activation and platelet activating factor synthesized by endothelial cells produce cytokines leading to influx of PMNs cells. Endothelial cells synthesize endothelins, which stimulate myofibroblast like Ito cells to synthesize collagen. Contraction of Ito stellate cells results in decreased sinusoidal perfusion and thus cause hypoxia.

role in inflammation, immune response, and liver fibrogenesis.

- **Pro-fibrogenic interleukins:** Kupffer cells and liver sinusoidal endothelial cells (LSECs) secrete interleukins in response to liver tissue damage. IL-1 stimulates hepatocyte stellate cells (HSCs) to secrete MMP-9, MMP-13 and TIMP-1 involved in hepatic fibrogenesis. IL-17 transforms HSCs to myofibroblasts, and upregulates TNF-α, TGF-β and collagen-1α involved in promoting hepatic fibrogenesis.
- **Antifibrogenic interleukins:** IL-10 downregulates the proinflammatory response and has modulatory effect on liver fibrogenesis. IL-22 promotes tissue repair, induces HSCs senescence, and restricts hepatic fibrinogenesis. Pretreatment of fibrotic liver with IL-6 improves hepatic microenvironment and primes it for mesenchymal stem cell transplantation resulting in improvement in hepatocellular injury after hepatic fibrosis.

Role of MicroRNAs

Recently, microRNAs as a regulator at post-transcription level, plays a pivotal role in hepatic fibrogenesis and development of cirrhosis. MicroRNAs can serve as biomarkers for HSCs activation and progression of hepatic fibrinogenesis, and may represent therapeutic targets for liver fibrosis and cirrhosis.

- **Pro-fibrogenic microRNAs:** MicroRNA-21 promotes liver fibrogenesis by downregulating TGF-β and HSCs activation. TGF-β induces expression of microRNAs (miR-18b) that can promote HSCs proliferation by regulating p27 and the cell cycle. Elevation of serum miR-18b level is suggestive of diagnostic biomarker for patients with liver cirrhosis. MicroRNAs (miR-214-5p) can increase the expression of fibrosis-related genes encoding MMP-2, MMP-9, TGF-β1, α-SMA, which activate HSCs leading to hepatic fibrinogenesis.
- **Antifibrogenic microRNAs:** MicroRNAs (miR-150, miR-194) can inhibit HSCs activation and extracellular matrix (ECM) deposition in the liver.

Surgical Pathology: Liver Cirrhosis

Gross Morphology

- **Early cirrhosis:** Liver may weigh >2 kg. External surface of liver may show diffuse nodularity. Cut surface shows yellow discoloration (Fig. 21.43).
- **Late cirrhosis:** Liver is shrunken in size weighing <1 kg. External surface of liver shows regenerative nodules (micronodules <3 mm or macronodules >3 mm or mixed pattern). Cut surface shows brown discoloration (Fig. 21.44A and B).

Light Microscopy

- Liver architecture is disrupted showing regenerating nodules of hepatocytes separated by fibrous bands of variable thickness resulting in disruption of architecture of entire liver.
- Fibrosis occurs as a result of synthesis of collagen by perisinusoidal hepatic stellate cells (Figs 21.45 and 21.46). Differences between cirrhosis and hepatic fibrosis are given in Table 21.48.

Pathology Pearls: Differential Diagnosis of Cirrhosis

- Cirrhosis should be differentiated from hepatocellular necrosis and nodular regenerating nodule:
  - Cirrhosis shows collagen deposition with regenerative nodules.
  - Hepatocellular necrosis shows a collapsed reticulin framework with lack of regenerative nodule.
  - Nodular regenerative hyperplasia lacks fibrous septa.
- Active cirrhosis should be differentiated from inactive cirrhosis:
  - Active cirrhosis shows presence of ongoing hepatocellular damage or interface hepatitis or steatohepatitis.
  - Inacative cirrhosis lacks liver cell necrosis, inflammation activity, and a sharp delineation of paucicellular septa and liver cell nodule.

CLINICAL FEATURES

Patient with a prior history of blood transfusion during surgical procedure presents with anorexia, fatigue, nausea, vomiting, weight loss, mild pain abdomen on right side, and varicose veins. Later, patient develops jaundice (yellow discoloration of sclera and skin), bruising (bleeding), ascites, splenomegaly, and edema in the lower extremities. Patient with cirrhosis can be

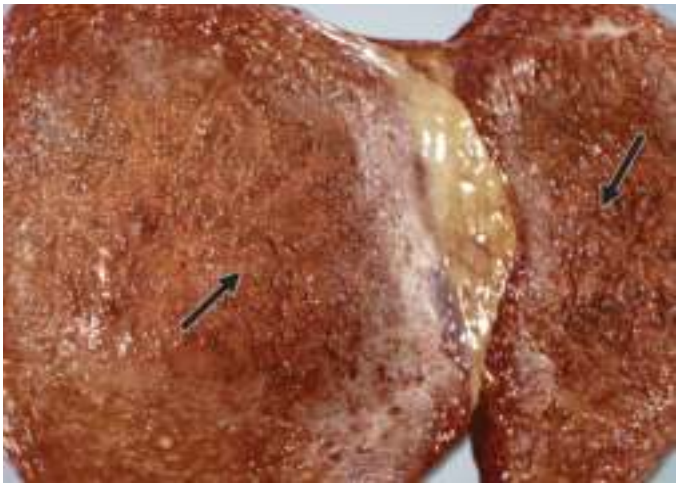


Fig. 21.43: Micronodular cirrhosis. This is an example of a micronodular cirrhosis. The regenerative nodules are quite small, averaging <3 mm in size. The most common cause for this is chronic alcoholism. The process of cirrhosis develops over many years (arrows).

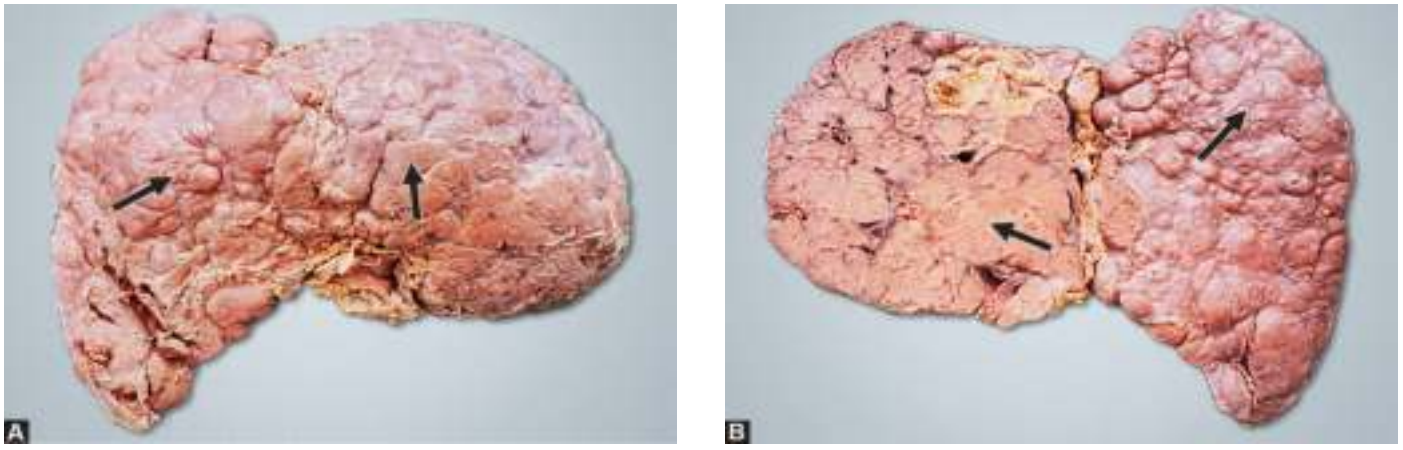


Fig. 21.44A and B: Macronodular cirrhosis. External as well as cut surface of liver shows large regenerative nodules >3 mm in diameter. HBV or HCV is the most common cause for macronodular cirrhosis. Cirrhosis may be linked to alcoholic liver disease, Wilson's disease and α_1-antitrypsin deficiency (arrows). (*Courtesy:* Dr. Sujata Kanetkar, Professor and Head, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

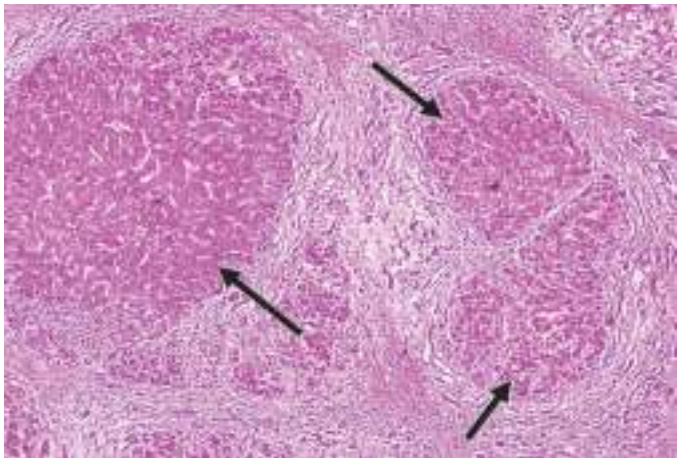


Fig. 21.45A: Liver cirrhosis. It shows parenchymal regenerative nodules surrounded by fibrous connective tissue that bridges between portal tracts. Within this collagenous tissue is scattered lymphocytes as well as a proliferation of bile ducts (arrows) (400X).

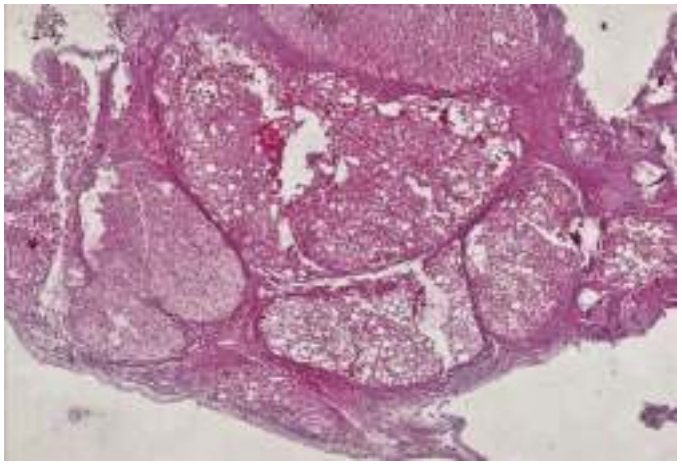


Fig. 21.45B: Liver cirrhosis. Histologic examination of nonbiliary cirrhotic liver reveals diffuse disruption in architecture of the entire liver (i.e. loss of normal central–portal relationship), bridging fibrous septa and rounded parenchymal nodules of regenerating hepatocytes (400X).

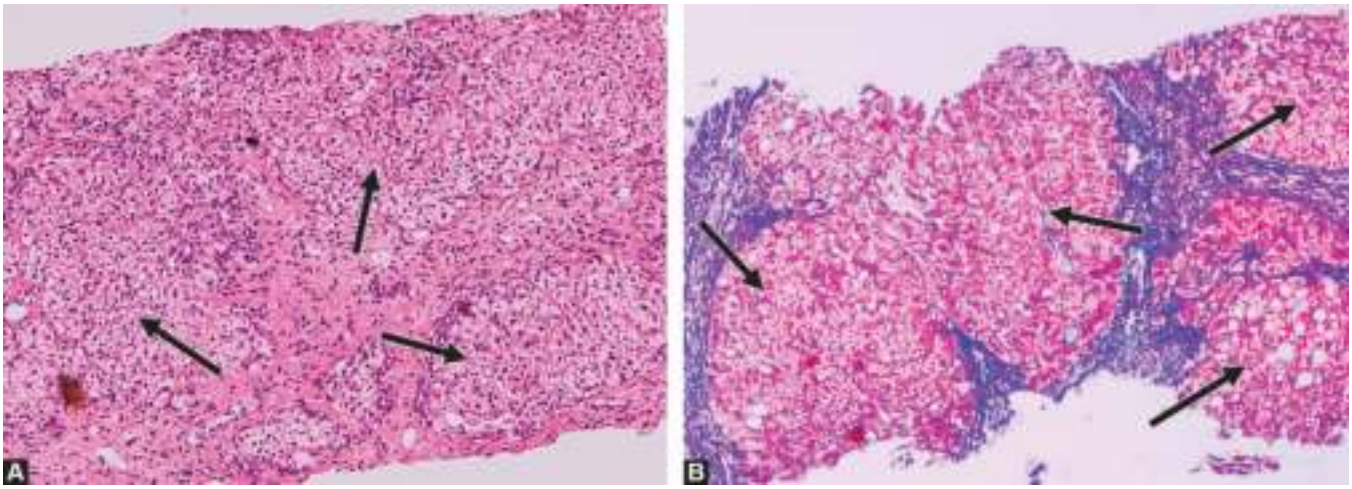


Fig. 21.46: (A) Liver cirrhosis. It shows regenerative nodules of hepatocytes surrounded by fibrous connective tissue that bridges between portal tracts. Within this collagenous tissue scattered lymphocytes as well as a proliferation of bile ducts (arrows), (B) regenerative nodules surrounded by dense connective tissue stained by Masson trichrome (arrows) (100X). (*Courtesy:* Prof. Zubaida Rasool, SKIMS, Srinagar, J & K.)

Table 21.48 Differences between cirrhosis and hepatic fibrosis

Parameters	Cirrhosis	Hepatic Fibrosis
Portal hypertension	Present	Present (marked)
Intrahepatic shunts contributing to portosystemic shunts	Present	Absent
Impaired hepatocyte functions	Present	Absent
Increased risk of development of hepatocellular carcinoma	Present	Absent

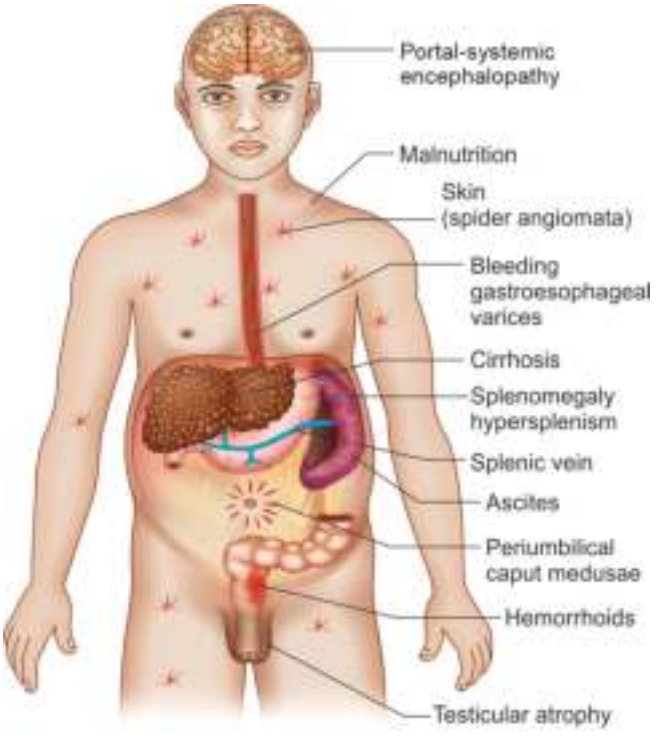


Fig. 21.47: Cirrhosis. It shows clinical manifestations.

asymptomatic or symptomatic, depending on whether cirrhosis is clinical compensated or decompensated. Clinical manifestations of liver cirrhosis are shown in Fig. 21.47.

Compensated Liver Cirrhosis

Patient with compensated cirrhosis is usually asymptomatic and detected incidentally on physical examination (hepatosplenomegaly), laboratory investigations (mild to moderate elevation of aminotransferases or gamma-glutamyl-glutamyl transpeptidase), and imaging.

Decompensated Liver Cirrhosis

Patient with decompensated cirrhosis presents with wide range of signs and symptoms arising from a combination of liver dysfunction and portal hypertension. Transition from compensated cirrhosis to decompensated cirrhosis is driven by the severity of portal hypertension with subsequent sequalae due to increased resistance to portal blood flow at the level of the sinusoids with development of venous collaterals (jaundice, esophageal varices, distended abdominal veins, i.e. caput medusae, splenomegaly, ascites, and edema in the lower extremities), hepatic encephalopathy (confusion, asterixis, i.e. flapping tremor on extension and dorsiflexion of hands, and '**fetor hepaticus**', i.e. sweet, musty smell due to high levels of ketones and dimethyl sulfide in the blood), spontaneous bacterial peritonitis, hepatocellular carcinoma, hyperdynamic circulation, and hepatorenal syndrome.

Physical Examination

Physical examination in patients with cirrhosis may reveal signs of chronic liver disease (jaundice, hypertrophic arthropathy, Dupuytren's contracture, and impaired estrogen metabolism related gynecomastia, hypogonadism, testicular atrophy, spider telangiectasias, palmer erythema), signs of portal hypertension (ascites, splenomegaly, caput medusae), and consequences of increased portal venous pressure (esophageal varices, distended abdominal veins (caput medusae), splenomegaly, and consequences of hypoalbuminemia (ascites, peripheral edema), a firm nodular liver edge just below the right costal margin, and soft protuberant abdomen with a shifting dullness on percussion, signs of hepatic encephalopathy (confusion, asterixis, i.e. flapping tremor on extension, and dorsiflexion of hands, and fetor hepaticus, i.e. sweet, musty smell due to high-levels of ketones and dimethyl sulfide in the blood), and scant chest/axillary hair. Liver cirrhosis may lead to hepatocellular failure and hepatocellular carcinoma in regenerating nodules.

- Liver cirrhosis can lead to hyperdynamic circulation, reduction in lean skeletal muscle, muscle cramps, and umbilical hernia. Spider nevi (central arteries surrounded by multiple small-sized vessels) are observed secondary to hyperesterogenemia.
- Peripheral blood smear examination reveals macrocytic anemia, and pancytopenia due to hypersplenism.

IMAGING TECHNIQUES

Abdominal ultrasonography with sonoelastography is commonly used to detect and characterize hepatic focal and diffuse fibrotic/cirrhotic lesions depending on the morphologic pattern.

- Abdominal ultrasonography reveals mild hepatosplenomegaly, nodular liver surface with heterogenous echotexture, loss of parenchymal uniformity, segmental atrophic right lobe, diffusely increased echogenicity of liver parenchyma, dilated portal vein

with slow venous blood flow, dilated splenic vein, and ascites consistent with cirrhosis.

- Sonoelastography is done to evaluate tissue stiffness and elasticity with high predictive value of 22.5 kilopascals indicative of cirrhosis (reference range 4.1–5.5).

LABORATORY DIAGNOSIS

A comprehensive work-up is performed to study electrocardiogram, B-type natriuretic peptide (BNP), complete blood count (CBC), serum alanine aminotransferases (ALT) and aspartate aminotransferases (AST); coagulation panel to evaluate the integrity of the patient's coagulation cascade [prothrombin time and international normalized ratio (PT–INR), and activated partial thromboplastin time (APTT)], and complete metabolic panel (serum albumin and total proteins—indicators of the synthetic activity of liver). Serum albumin and PT–INR are true indicators of synthetic hepatic function. Electrocardiogram findings are within normal limits. Appropriate laboratory investigations in patients with liver cirrhosis include hepatotropic virus serologies and liver biopsy.

Serum Hepatocellular Enzymes

Aminotransferases are usually elevated with AST greater that ALT; however, normal levels do not exclude cirrhosis. In chronic hepatitis except alcoholic hepatitis, the AST/ALT ratio is <1. As chronic viral hepatitis progresses to cirrhosis, there is reversal of this AST/ALT ratio. Alkaline phosphatase, gamma-glutamyl transferase (GGT) and 5′-nucleotide are elevated in cholestatic disorders.

Coagulation Profile Test

Prothrombin time and international normalized ratio (PT–INR) measures the activity of coagulation factors II, V, VIII and X involved in extrinsic and common pathways of coagulation system. Prothrombin time and international normalized ratio (PT–INR) is elevated in the settings of vitamin K deficiency, warfarin pharmacotherapy, liver disease, and disseminated intravascular coagulation. International normalized ratio (INR) is calculated standardization of the prothrombin time, used for assessment of extrinsic pathway and common pathway of coagulation system for monitoring the response to warfarin pharmacotherapy. Activated partial thromboplastin time (APTT) measures the activity of coagulation factors II, V, VIII, IX, X, XII involved in the intrinsic pathway of coagulation system. APTT is elevated in the settings of von Willebrand disease, antiphospholipid syndrome, in the presence of inhibitors, and disseminated intravascular coagulation.

Serum Proteins

Serum albumin is true indicator of synthetic hepatic function. Albumin synthesis is impaired in liver cirrhosis. Immunoglobulins, especially the gamma fraction, are usually elevated due to impaired clearance by the liver.

Complete Blood Count

Peripheral blood smear shows normocytic normochromic anemia, however macrocytic anemia can be seen in alcoholic liver cirrhosis. Leukopenia and thrombocytopenia are observed secondary to sequestration by splenomegaly as well as alcoholic suppression effect on the bone marrow.

Specific Tests in Newly Diagnosed Case of Cirrhosis

Serology and polymerase chain reaction techniques are used for viral hepatitis (HBV, HCV), autoimmune hepatitis (anti-nuclear antibodies, anti-smooth muscle antibodies) and primary biliary cirrhosis (anti-mitochondrial antibody).

- Serum ferritin and transferrin saturation is analyzed for hemochromatosis. Ceruloplasmin and urinary copper are analyzed for Wilson disease. A young patient with elevated liver enzymes, increased copper in the urine and Kayser-Fleischer rings in periphery of cornea on slit lamp eye examination has degeneration of the putamen in the brain.
- HCV most likely develop chronic viral hepatitis in patients requiring repeated blood transfusion over years for aplastic anemia. Serum α_1-antitrypsin level and protease inhibitor phenotype are analyzed for α_1-antitrypsin deficiency. Serum α-fetoprotein level is performed for hepatocellular carcinoma.

Liver Biopsy: Gold Standard for Cirrhosis Diagnosis

Liver biopsy is the gold standard for diagnosing liver cirrhosis as well as assessing the degree of inflammation (grade) and fibrosis (stage) of the disease.

- Liver biopsy in HBV is the indication to determine active hepatitis in patients, whose serological or biochemical tests do not paint a clear picture of disease status.
- Histologic examination of liver biopsy shows bridging fibrous septa surrounding parenchymal regenerative nodules, disruption of the architecture of the entire liver, steatosis, and portal inflammation.
- Histochemical trichrome stain is used to visualize connective tissues, particularly collagen, in tissue sections, which highlights as blue, circumferential bands of fibrosis surrounding regenerating parenchymal nodules (micronodular, macronodular, or mixed micronodular pattern of liver cirrhosis

elevating hepatic venous gradient). Reticulin stain enhances the fibrous septa dividing the hepatic regenerative nodules.

TREATMENT

Damage to the liver is permanent in liver cirrhosis. Further injury to the liver should be avoided to halt the progression of the liver disease. Chronic liver disease can be prevented by avoidance of alcohol, vaccination for hepatotropic viruses (HBV and HCV), good nutrition with a balanced diet, weight reduction, and early treatment of precipitating factors. Specific therapy most often targets the etiology such as antiviral mediations in viral hepatitis (HBV and HCV), corticosteroids and immunosuppressive agents in autoimmune disease, copper-chelating agents in Wilson disease, iron-chelating agents, and phlebotomy in hemochromatosis. Alcohol abstinence is beneficial in alcoholic cirrhosis. Weight reduction of at least 7% is beneficial in nonalcoholic steatohepatitis (NASH).

PORTAL HYPERTENSION

Portal hypertension is characterized by development of venous collaterals with varices in the submucosal veins of the esophagus, hemorrhoidal plexus, and other sites.

- Patient with portal hypertension has fatal outcome due to development of hepatic encephalopathy, massive bleeding in GIT, recurrent infection, hepatorenal syndrome and hepatocellular carcinoma.
- Normal pressure in portal vein is 7 mm Hg. As in other vascular bed, pressure in portal vein results from the interaction between the amount of blood flowing through the system and resistance to the blood flow.
- Portal hypertension occurs due to increased resistance to blood flow anywhere in the portal system or increased blood flow through the systemic circulation or combination of both mechanisms.

  In majority of cases, portal hypertension occurs due to resistance to blood flow in prehepatic, hepatic or posthepatic vascular tree.
- Portal hypertension causes significant diversion of blood from the portal circulation into the systemic circulation.

ETIOLOGIC CLASSIFICATION

At a functional level, portal hypertension may be divided into three main groups based on site of portal venous obstruction (pre-sinusoidal, sinusoidal and post-sinusoidal) as shown in Fig. 21.48 and Table 21.49.

- In pre-sinusoidal portal hypertension, hepatic venous pressure is normal even though increased

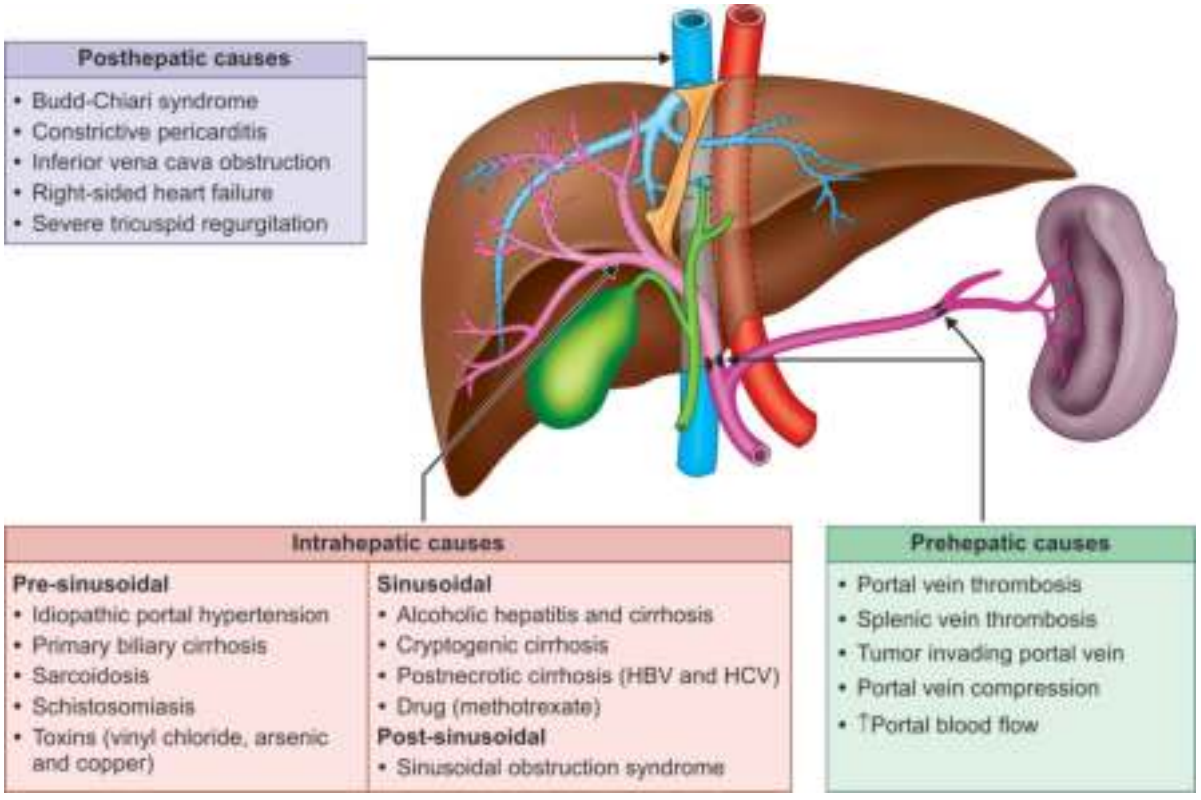


Fig. 21.48: Portal hypertension. It shows prehepatic, intrahepatic and posthepatic causes.

Table 21.49 Etiological classification of portal hypertension

Site of Lesions	Causes
Post-sinusoidal etiology	
Heart	Constrictive pericarditis and congestive heart failure
Inferior vena cava	Fibrous tissue webs, thrombosis and malignant tumor invasion
Large extrahepatic veins	Thrombosis (Budd-Chiari syndrome), tumor invasion and fibrous webs
Small intrahepatic veins	Veno-occlusive disorder, alcoholism and central hyaline sclerosis
Sinusoidal etiology	
Intrahepatic lesions	Cirrhosis (most common), acute alcoholic hepatitis, viral hepatitis (HBV and HCV), cytotoxic drug (methotrexate), vitamin A intoxication
Pre-sinusoidal etiology	
Portal tract lesions	Schistosomiasis, early primary biliary cirrhosis, congenital hepatic fibrosis, chronic active hepatitis, toxins (vinyl chloride, arsenic, copper), sarcoidosis, tuberculosis, and idiopathic
Portal and splenic veins	Thrombosis, compression and tumor invasion
Increased portal blood flow	Idiopathic tropical splenomegaly and arteriovenous fistula

pressure in portal vein. Liver functions are normal. Bleeding episode as a rule is not followed by hepatocellular failure.

- In sinusoidal as well as post-sinusoidal portal hypertension, the resistance to blood flow extends from the central hepatic veins. Hence, pressure in both hepatic and portal veins is increased. Patient with sinusoidal portal hypertension in cirrhosis frequently develops hepatocellular failure following a variceal bleed.

Portal Hypertension with Portal Vein Obstruction

Thrombosis in portal vein is the principal pathologic cause of extrahepatic portal vein obstruction.

- **In neonates**, infection may spread along umbilical vein to reach the left portal vein resulting in its obstruction.
- **In children**, intra-abdominal sepsis such as appendicitis may be responsible for portal vein thrombosis. Probable predisposing factors include dehydration associated with gastroenteritis and infection.
- **In adults**, portal vein thrombosis may occur in ulcerative colitis, Crohn's disease, sclerosing cholangitis, postoperative period, hypercoagulable state in myeloproliferative disorder and tumor invasion in portal vein especially hepatocellular carcinoma.

Portal Hypertension without Portal Vein Obstruction

Pre-sinusoidal portal hypertension is characterized by splenomegaly, functional evidence of hypersplenism without portal vein obstruction in non-cirrhotic liver. Portal blood pressure rises due to increased hepatic resistance to portal blood inflow, which probably occurs as a result of injury to the endothelial lining of intrahepatic portal vein radicles and sinusoids. This entity is common in middle-aged Japanese women.

CLINICAL FEATURES

In cirrhosis, liver is unable to receive blood from the gastrointestinal tract and accessory organs. As the pressure in the portal vein rises; blood flows back into the spleen and flows through collateral channels to the venous system, bypassing the liver. Liver fails to synthesize essential vital blood components, filter substances or neutralize toxic substances.

- Patient with portal hypertension presents with jaundice, hypoalbuminemia, hyperammonemia (hepatic encephalopathy), fetor hepaticus, palmar erythema, spider angiomas, caput medusae, gynecomastia, respiratory renal failure and bleeding diathesis. Death occurs due to hepatic coma, massive GIT bleeding, hepatorenal syndrome and hepatocellular carcinoma (Table 21.50).
- Clinical manifestations of portal hypertension are portosystemic shunts, massive splenomegaly and ascites. Later, patient develops various complications associated with fatal outcome.

Portosystemic Shunts

Pressure in the venous system causes distension and varicosity of the veins in esophagus and around umbilicus. Varicose veins can hemorrhage during vomiting, resulting in bloody vomit, internal hemorrhage, and shock associated with fatal outcome.

Table 21.50 Clinical manifestations in cirrhosis

Clinical Sequelae	Clinical Findings
Portal hypertension	Esophageal varices, splenomegaly, caput medusae, ascites, ankle edema, hypersplenism (anemia, leukopenia and thrombocytopenia) anemia, and hemorrhagic tendencies
Hyperestrinism	Spider nevi, pectoral alopecia, gynecomastia, altered hair distribution, palmar erythema, testicular atrophy
Hepatic encephalopathy	Jaundice, hemorrhagic tendencies due to decreased synthesis of coagulation factors, edema due to decreased synthesis of albumin by liver. Asterixis is a flapping tremor associated with hepatic encephalopathy

Esophageal Varices

In many patients, the first sign of portal hypertension is bleeding esophageal varices.

- **Location of esophageal varices:** Esophageal veins are dilated veins immediately beneath the mucosa in the lower-third of esophagus especially in cirrhosis. Esophageal veins are linked to the portal system through gastroesophageal anastomoses. Left gastric vein is also dilated in these patients. Esophageal varices are associated with atrophy of muscularis mucosae.
- **Rupture of esophageal varices:** Esophageal varices >5 mm in diameter are prone to rupture and life-threatening hematemesis; requiring emergency care to control hemorrhage and prevent hypovolemic shock.
- **Endoscopy:** Endoscopy is the primary diagnostic tool for confirming esophageal varices. These are graded according to severity. Grade 1 esophageal varices can be depressed by pressure exerted by endoscopy. Grade 2 esophageal varices cannot be depressed by endoscopy. Grade 3 esophageal varices are confluent and extend circumferentially around esophageal lumen.
- **Sclerotherapy:** Esophageal varices are treated by sclerotherapy.
- **Imaging techniques:** Imaging techniques demonstrate numerous prominent blue venous channels beneath the mucosa of the everted esophagus, particularly above the gastroesophageal junction.

Caput Medusae Around Umbilicus

The umbilical vein remnants of fetal circulation present in falciparum ligament. It can become anastomotic link between portal vein and epigastric veins of anterior abdominal wall around umbilicus. Caput medusae results from dilation of the periumbilical venous collaterals due to portal hypertension and activation of portocaval anastomoses.

Hemorrhoids in Rectal Submucosa

Superior and middle hemorrhoidal veins form part of the portal venous drainage, whereas the inferior hemorrhoidal vein drains into the inferior vena cava. Anastomoses formed between these two systems lead to development of rectal varices (hemorrhoids).

- Internal hemorrhoids are formed due to dilatation of superior hemorrhoidal veins, which may protrude from the anus.
- External hemorrhoids are formed due to dilatation of inferior hemorrhoidal veins.

Shunting of Blood in Left Renal Vein

Shunting of blood to left renal vein occurs derived directly from splenic vein or pancreatic or gastric veins.

Massive Splenomegaly

Morphologic consequences of portal hypertension are esophageal varices and massive splenomegaly.

Surgical Pathology: Massive Splenomegaly in Portal Hypertension

Gross Morphology

- The spleen is enlarged, as it is unable to drain through portal vein. Due to excess of blood in spleen, the risk for rupture and internal hemorrhage is very high.
- Due to pooling of platelets in enlarged spleen, patient develops thrombocytopenia (Fig. 21.49).

Light Microscopy

- Sinusoids of spleen are dilated and lined by prominent endothelial cells.
- Macrophages are prominent and containing hemosiderin granules.
- Spleen may show small hemorrhages.

Ascites

Ascites is defined as the accumulation of free fluid in the peritoneal cavity. Clinical examination does not detect ascites if fluid is <500 ml.

- In clinical practice, patient with decompensated cirrhosis may accumulate excessive fluid in the peritoneal cavity leading to gross distension of the abdominal wall.
- Protein content of fluid reveals low protein content about 1–2 g/dl indicating presence of transudate.



Fig. 21.49: Massive splenomegaly in portal hypertension. The spleen is enlarged from the normal 300 g or less to between 500 and 1000 g. Another finding here is the irregular pale tan plaques of collagen over the purple capsule known as sugar icing or hyaline perisplenitis which follows the splenomegaly and/or multiple episodes of peritonitis that are a common accompaniment to cirrhosis of liver.

- Higher concentration of protein in the ascitic fluid reflects either superadded infection or cirrhosis progressing to hepatocellular carcinoma. Pathogenesis of ascites and generalized edema in portal hypertension is shown in Fig. 21.50.

Pathogenesis

Patient develops ascites due to sinusoidal hypertension, percolation of hepatic lymph, intestinal capillary fluid leakage and renal retention of Na^+ and water. There is seepage of plasma out of veins and lymphatic channels into the peritoneal space resulting in ascites and abdominal distension.

Normal flora of gut may seep out to produce infection in ascitic fluid, resulting in life-threatening spontaneous bacterial peritonitis. Patient with cirrhosis develops peripheral edema, ascites and hydrothorax by the following mechanisms:

- Increased pressure at the venous end of splanchnic capillaries leads to transudation of fluid from vascular to the extravascular compartment, which occurs in the presence of sinusoidal portal hypertension as a result of deposition of collagen fibers in the space of Disse.
- Lymphatic channels are dilated at the hilum of the liver. Flow of lymph in thoracic duct is also increased. It is likely that escape of lymph from the liver surface occurs only when the capacity of the thoracic duct is exceeded.
- In the setting of cirrhosis, decreased intravascular oncotic pressure due to hypoalbuminemia is also an important factor in the pathogenesis of ascites. Overall, imbalances in Starling forces lead to transudation of fluid into the abdominal cavity.
- Concentration of aldosterone and angiotensin II is increased due to decreased degradation of these metabolites in cirrhotic patients. Increased aldosterone level acts on distal convoluted tubules and enhances excessive absorption of sodium and water. It is worth mentioning that angiotensin II stimulates adrenal cortex to synthesize aldosterone.

COMPLICATIONS

Complications of portal hypertension include hepatic encephalopathy, altered metabolic activity, disruption of hormonal inactivation, bleeding and bruising; skin lesions and hematologic abnormalities (Figs 21.51 and 21.52). Clinical consequences of cirrhosis are shown in Table 21.51.

Hepatic Encephalopathy

Patient with portal hypertension developing neuropsychiatric disturbances is known as hepatic encephalopathy. It may occur in a background of chronic liver disease such as cirrhosis in association with massive hepatocellular necrosis of liver leading to acute hepatocellular failure. The progressive loss of liver function can

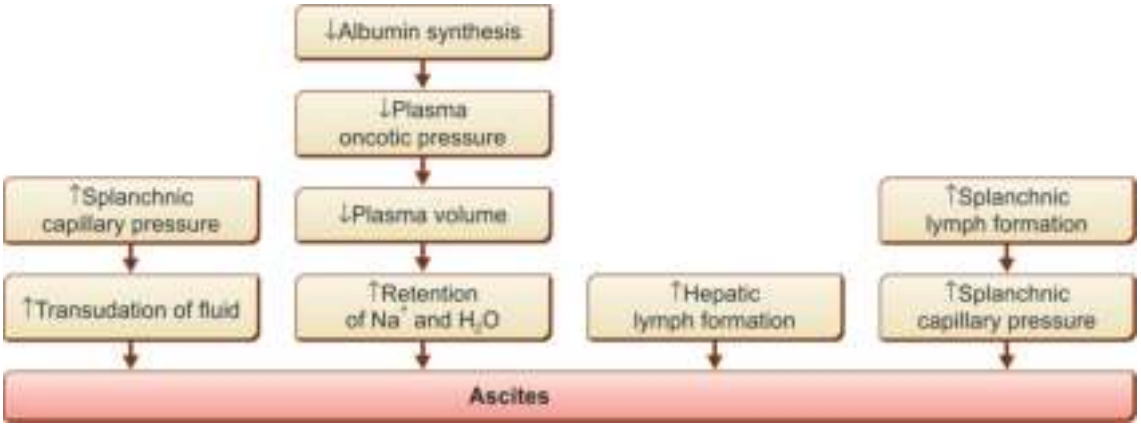


Fig. 21.50: Pathogenesis of ascites and generalized edema in portal hypertension.

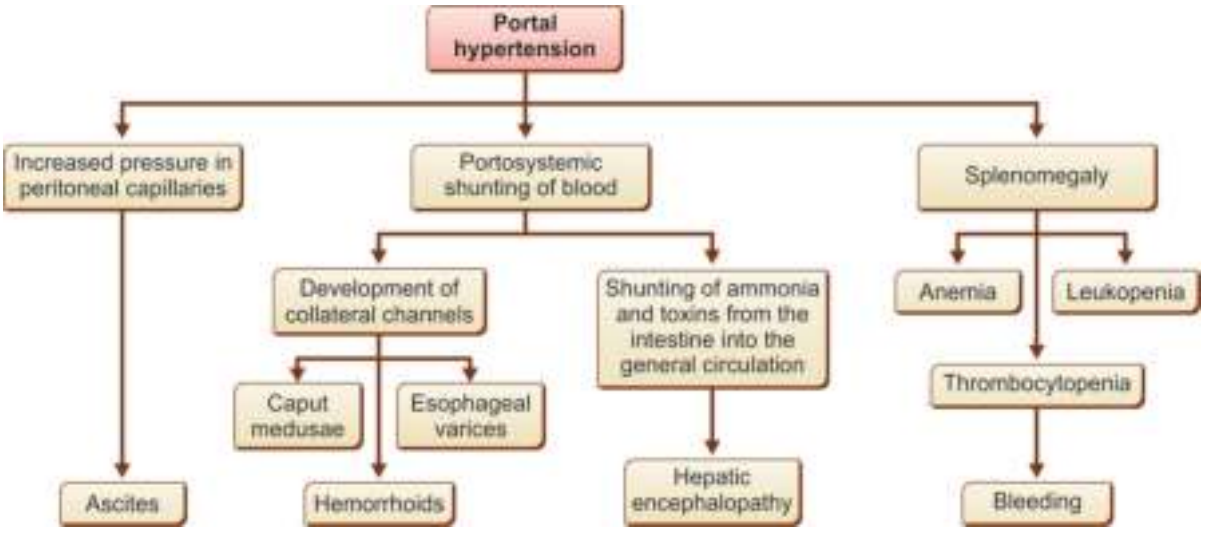


Fig. 21.51: Mechanism of disturbed liver functions related to portal hypertension.

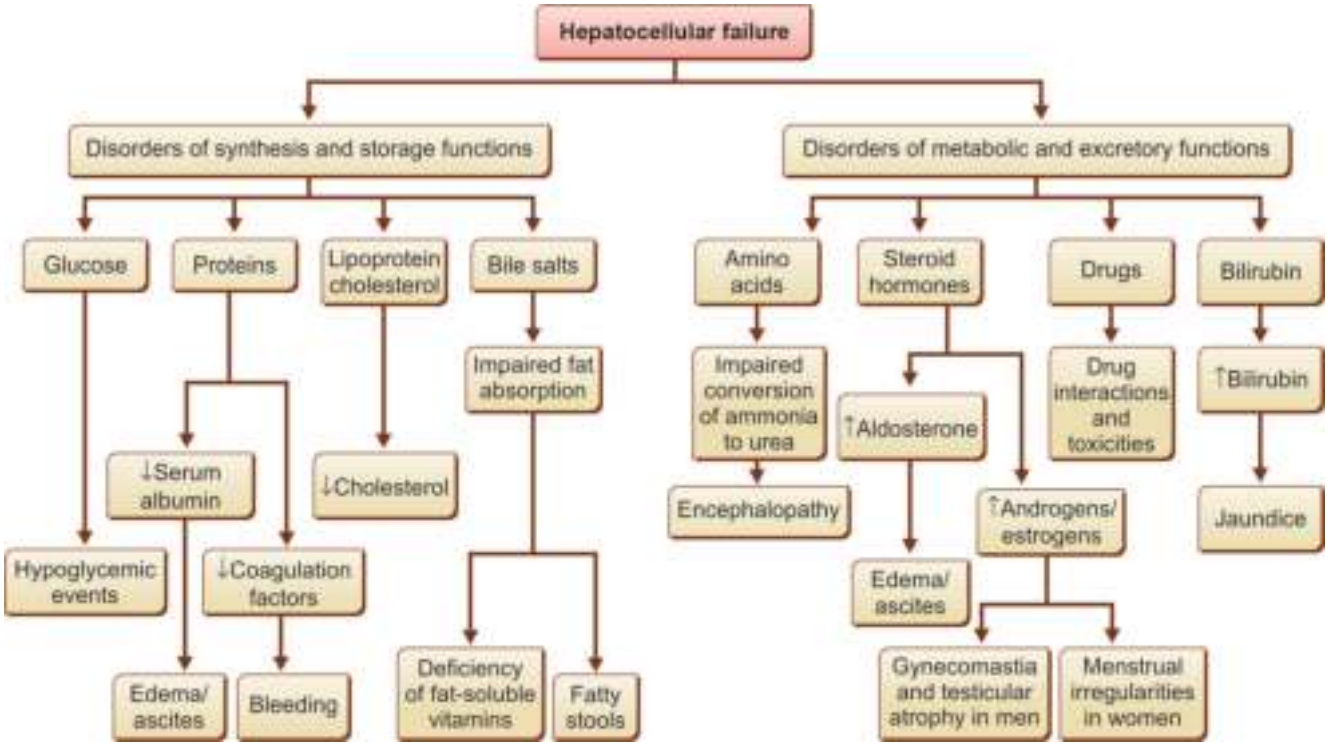


Fig. 21.52: Alterations in liver function and clinical manifestations of hepatocellular failure.

lead to liver failure. A person with end-stage hepatocellular failure needs liver transplantation for survival.

Clinical Course of Hepatic Encephalopathy

- **Acute hepatocellular failure:** Patient with acute hepatocellular failure due to fulminant hepatitis or drug-induced hepatocellular injury develops rapid hepatic encephalopathy associated with poor outcome.
- **Chronic liver disease:** Clinical manifestation is different in patient with chronic liver disease. During clinical course, features of gradual hepatic encephalopathy occur.

Pathogenesis of Hepatic Encephalopathy

Central to the development of hepatic encephalopathy is shunting of portal blood to systemic circulation.

Table 21.51 Clinical consequences of cirrhosis

Parameters of Cirrhosis	Clinical Consequences
Portal hypertension	▪ Ascites ▪ Portosystemic shunts formation • Esophageal varices • Caput medusae around umbilicus • Hemorrhoids ▪ Massive splenomegaly
Hepatocellular dysfunction	▪ Hypoalbuminia ▪ Coagulation defects ▪ Hepatic encephalopathy • Altered metabolic activity • ↑Ammonia in blood • Fetor hepaticus induced by mercaptans • ↑γ-Aminobutyric acid (GABA) in blood • ↑Glutamine in CSF ▪ Hepatorenal syndrome ▪ Hepatopulmonary syndrome ▪ Jaundice ▪ Palmar erythema ▪ Spider nevi
Increased incidence of malignancy	▪ Hepatocellular carcinoma

- **Functional basis:** Massive hepatocellular necrosis resulting from fulminant or toxic damage fails to metabolize the constituents of the portal blood.
- **Anatomical basis:** In patient with cirrhosis, shunting of portal blood to systemic circulation is purely based on disrupted anatomical architecture of liver.

Clinical Features in Hepatic Encephalopathy

Patient with hepatic encephalopathy presents with somnolence, confusion, neck rigidity, flapping tremors of hands (asterixis), hallucinations, and coma with fatal outcome.

Flapping tremors are characteristic of the hepatic encephalopathy but not pathognomonic of portal hypertension, because these may also be seen in patient with uremia, respiratory failure, cardiac failure and hypoglycemia (Fig. 21.53).

Surgical Pathology: Brain Histology in Hepatic Encephalopathy

Gross Morphology

The brain appears normal. Cerebral edema is seen in patient with fulminant hepatitis in 50% of cases, who have been in coma for some days.

Light Microscopy

- **Early histologic changes in brain:** In hepatoencephalopathy, early histologic changes in brain include increase in size and number of protoplasmic astrocytes in the deep cortex, basal ganglion and dentate nucleus in cerebellum. Astrocytes show pale cytoplasm and lobulated nucleus. Such altered astrocytes are also known as Alzheimer type 2 cells.
- **Late histologic changes in brain:** In patients with history of recurrent episodes of hepatic encephalopathy over time, the cerebral cortex is thinned out due to irreversible loss of neurons and fibers. Demyelination is an occasional finding demonstrated in the spinal tracts producing spastic paraplegia.

Biochemical Abnormalities in Hepatic Encephalopathy

- **Altered metabolic activity in hepatic encephalopathy:** Advanced cases of cirrhosis fail to detoxify toxic substances. Further blood–brain barrier in central nervous system becomes less effective in these patients.
  - Altered metabolic activity in hepatic encephalopathy include increased concentration of ammonia, mercaptans, octopamine and γ-aminobutyric acid (GABA).
  - Toxic substances such as ammonia and other enteric degradation products shunting from liver to the systemic circulation deliver neurotoxic substances and cause brain damage (Fig. 21.54). Factors triggering hepatic encephalopathy are given in Table 21.52.
- **Increased blood ammonia concentration:** It is not a reliable indicator of hepatic encephalopathy, because some patients with unequivocal coma may show normal blood ammonia concentration.
- **Mercaptans:** Derivatives of aromatic amino acids such as methionine, phenylalanine, tyrosine and tryptophan are known as mercaptans, which are responsible for the curious odor of breath fetor hepaticus in patient with acute hepatocellular failure.
- **Octopamine false neurotransmitter:** It is derived from colon as a result of bacterial infection.
- **Gamma aminobutyric acid (GABA) neurotransmitter:** Patient with hepatic encephalopathy exhibits increased binding of benzodiazepines and increased blood concentration of GABA.
- **Glutamine concentration in CSF:** Glutamine concentration in CSF is most often increased in hepatic encephalopathy. Its measurement is a reliable diagnostic tool. Glutamine in cerebrospinal fluid is derived from aromatic amino acids such as methionine, phenylalamine, tyrosine and tryptophan.

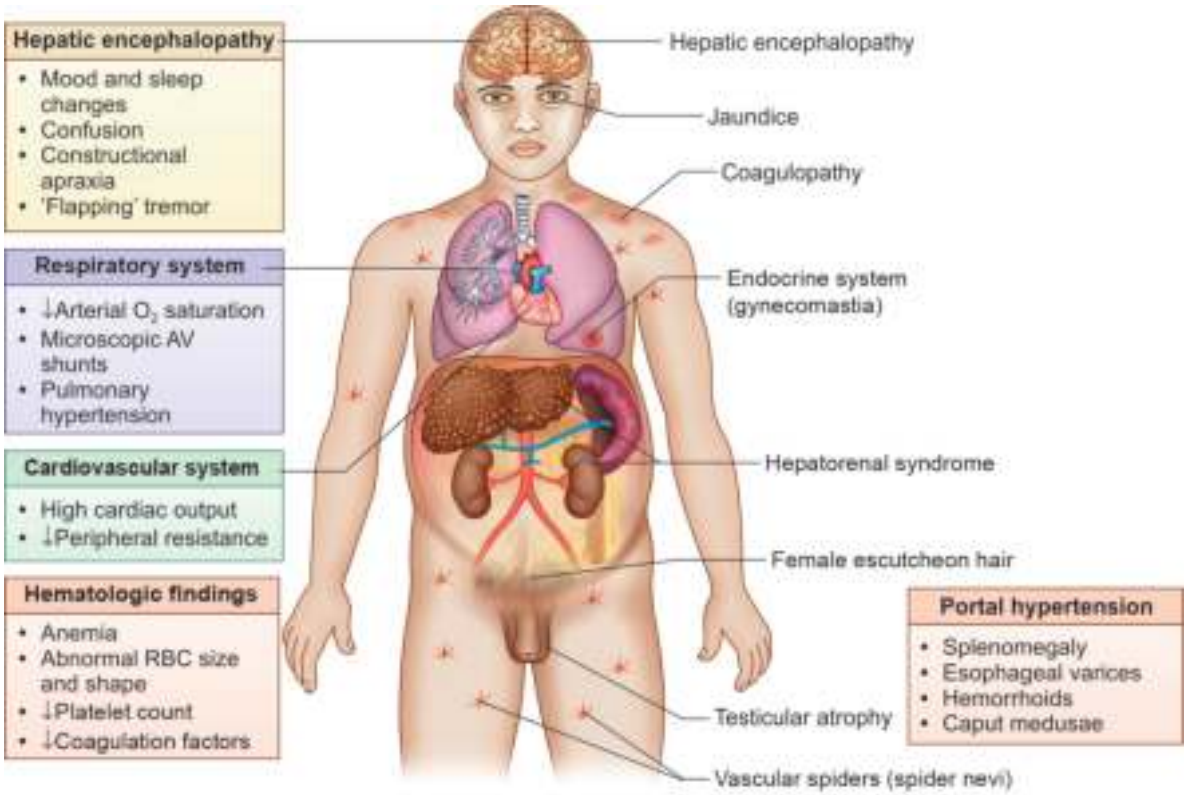


Fig. 21.53: Clinical manifestations and complications of hepatic encephalopathy in a patient of cirrhosis with portal hypertension.

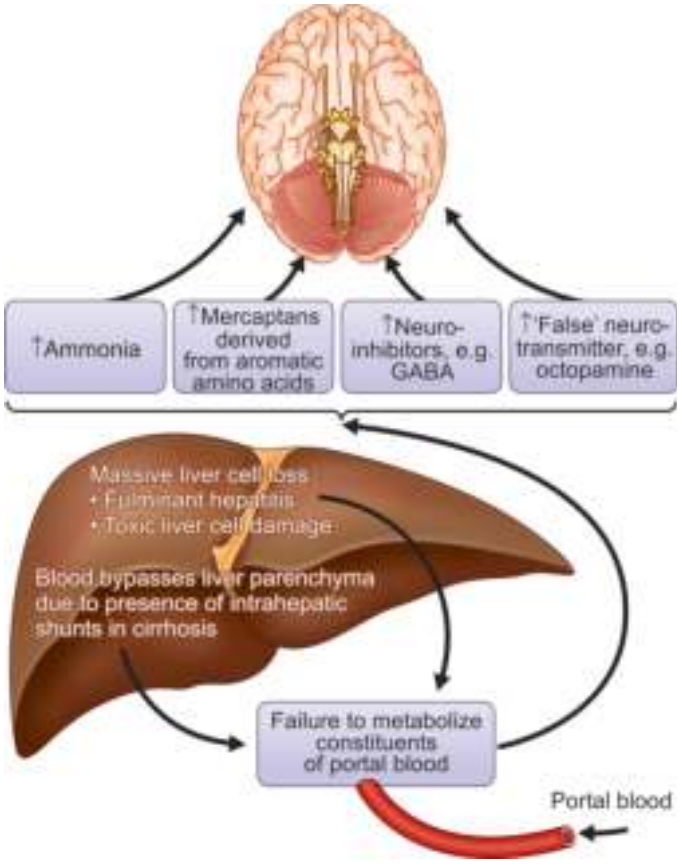


Fig. 21.54: Pathogenesis of hepatic encephalopathy.

Laboratory Findings in Hepatic Encephalopathy

Laboratory findings in hepatoencephalopathy in advanced cirrhosis are elevated conjugated and unconjugated bilirubin, decreased serum albumin, prolonged prothrombin time–INR, anemia, thrombocytopenia, elevated levels of blood creatinine and blood urea nitrogen, and hypoglycemia. Commonly observed laboratory findings in hepatoencephalopathy in advanced cirrhosis are given in Table 21.53.

Hepatorenal Syndrome

Advanced cirrhosis can impair blood flow to the kidneys resulting in hepatorenal syndrome. Renal involvement leads to oliguria, azotemia, and increased levels of serum creatinine.

- Renal failure occurs due to vasoconstriction and hypoperfusion of the kidneys mediated by various hormones and vasoactive substances, some of which may not be cleared by the cirrhotic liver.
- Hepatorenal syndrome is an emergency that requires a liver transplant.

Disruption of Hormones Inactivation

- **Hyperestrinism in men:** Liver normally inactivates estrogen in men. Male patients with cirrhosis no longer inactivate estrogen. Patient presents with gynecomastia, loss of chest and pubic hair; impotence, testicular atrophy and palmar erythema, spider nevi

Table 21.52 Factors triggering hepatic encephalopathy in advanced cirrhosis

Factors	Examples
Infections	Bacterial peritonitis, chest infections, urinary tract infections
Hemorrhage	Esophageal varices, peptic ulcer, Mallory-Weiss syndrome (vomiting induced bleeding)
Electrolytes imbalance	Diuretics
Drugs and toxic substances	Morphea, benzodiazepines, barbiturates, and alcohol

Table 21.53 Commonly observed laboratory findings in hepatoencephalopathy in advanced cirrhosis

Characteristics	Comment(s)
Elevated conjugated and unconjugated bilirubin	Defects in conjugation and excretion in bilirubin
Hypoalbuminemia	Decreased synthesis of albumin
Elevated ALT (alanine transferase) and AST (aspartate transaminase)	Elevation of ALT and AST to 100-fold with acute hepatocellular failure, rapid decline to normal can indicate permanent loss of hepatocytes. ALT and AST can be normal in chronic liver disease
Hyperammonemia	Impaired urea cycle
Hypoglycemia	Impaired gluconeogenesis in the fasting state after glycogenolysis has exhausted liver glycogen stores
Prolonged prothrombin time–INR	Decreased synthesis of coagulation factors, malabsorption of vitamin K, and decreased clearance fibrin-split products
Thrombocytopenia	As a result of disseminated intravascular coagulation or thrombopoietin deficiency
Anemia	Bone marrow suppression leads to chronic anemia, blood loss from esophageal varices
Elevated creatinine and blood urea nitrogen	Decreased urine output, the hepatorenal syndrome may be present; elevated BUN and a normal creatinine can indicate a gastrointestinal bleeding

(capillary telangiectasis) of the face, upper arms, and chest. These findings are attributed to reduced hepatic catabolism of estrogens (i.e. hyperestrogenism).

- **Hyperestrinism in women:** Women patients present with cessation of menstrual periods, infertility and growth of body hair. Both men and women with cirrhosis can expect a decreased sex drive.

Bleeding and Bruising

Patient with chronic liver disease (cirrhosis) presents with bleeding and bruise for prolonged periods. Affected person may show several functional bleeding defects. Bleeding and bruise occur by the following mechanisms.

- **Decreased synthesis of coagulant and anticoagulant proteins:** Liver synthesizes inadequate vitamin K-dependent coagulation factors and substrates (II, VII, IX, and X); natural occurring anticoagulants such as protein C, protein S and antithrombin III; and antifibrinolytic protein (plasminogen).
- **Synthesis of abnormal forms of coagulant proteins:** Liver synthesizes abnormal forms of vitamin K-dependent clotting factors, fibrinogen, and factor VIII except von Willebrand factor.
- **Decreased clearance of coagulant proteins:** There is impaired clearance of activated coagulation factors, thrombin–antithrombin III complex and plasminogen activators synthesized by vascular endothelium.

Skin Lesions

- **Jaundice:** Bilirubin, a product of dead red blood cells is produced in the spleen and then recycled in the liver as a component of bile. Cirrhosis interferes with this process; conjugated and unconjugated bilirubin is accumulated in the bloodstream. Bilirubin has affinity for sclera giving yellow discoloration.
- **Spider nevi:** Patient with chronic liver disease (cirrhosis) may develop spider nevi. These lesions consist of a centrally dilated arteriole from which numerous small vessels radiate. Pressure exerted on the central arteriole causes blanching of the lesions. Number of spider nevi increases in patient with worsening chronic liver disease and the converse is true if liver pathology improves.
- **Palmar erythema:** Patient with chronic liver disease (cirrhosis) most often develops warm red palms with eminent thenar and hypothenar regions.

Hematologic Abnormalities

Patient with chronic liver disease (cirrhosis) may show hematologic abnormalities. Patient develops anemia as a result of hypersplenism induced hemolysis, increased plasma volume (sodium and water absorption), recurrent bleeding associated with esophageal varices and bone marrow depression.

- **RBC abnormalities:** Patient may develop macrocytic anemia due to disturbance of vitamin B_{12} and folic acid metabolism. Microcytic anemia occurs as a result of recurrent bleeding. Presence of target cells is associated with increased amount of cholesterol and phospholipid in the cell membrane. Echinocytes are associated with binding of an abnormal high density lipoprotein to the red blood cell surface. Acanthocytes are bizarre cells with many projections on their surfaces. Mechanism of presence of acanthocytes is not known.
- **WBC abnormalities:** Leukocytosis is associated with fulminant hepatitis, hepatic malignancy, ascending cholangitis and alcoholic liver disease. Leukopenia is associated with alcoholic cirrhosis as a result of hypersplenism and toxic effects of alcohol on bone marrow.
- **Platelet abnormalities:** Thrombocytopenia occurs due to increased pooling of platelets in the spleen, shortening of platelet's life span and inability of the bone marrow to compensate fully for platelet abnormalities. There may be abnormality of aggregation defects of platelets.

METABOLIC LIVER DISORDERS

ALCOHOLIC LIVER DISEASE

Alcohol toxicity is the most common cause of morbidity and mortality worldwide. Alcohol is the most common cause of cirrhosis, but disorders related to other systems also occur such as pancreatitis, cardiomyopathy, fetal alcohol syndrome, GIT bleeding, Wernicke-Korsakoff psychosis, polyneuropathy and spontaneous abortion.

- Daily intake of 80–160 g alcohol increases the risk for alcoholic liver disease.
- Chronic alcoholics develop fatty liver (60%), steatohepatitis (20–30%), and micronodular cirrhosis over a span of 10–15 years (10–15%).
- Only 10% of chronic alcoholic cases of micronodular cirrhosis may develop hepatocellular carcinoma. Alcoholic liver disease is shown in Fig. 21.55A to C. Alcohol oxidative pathways and their role in pathogenesis of alcoholic liver disease are given in Table 21.54.

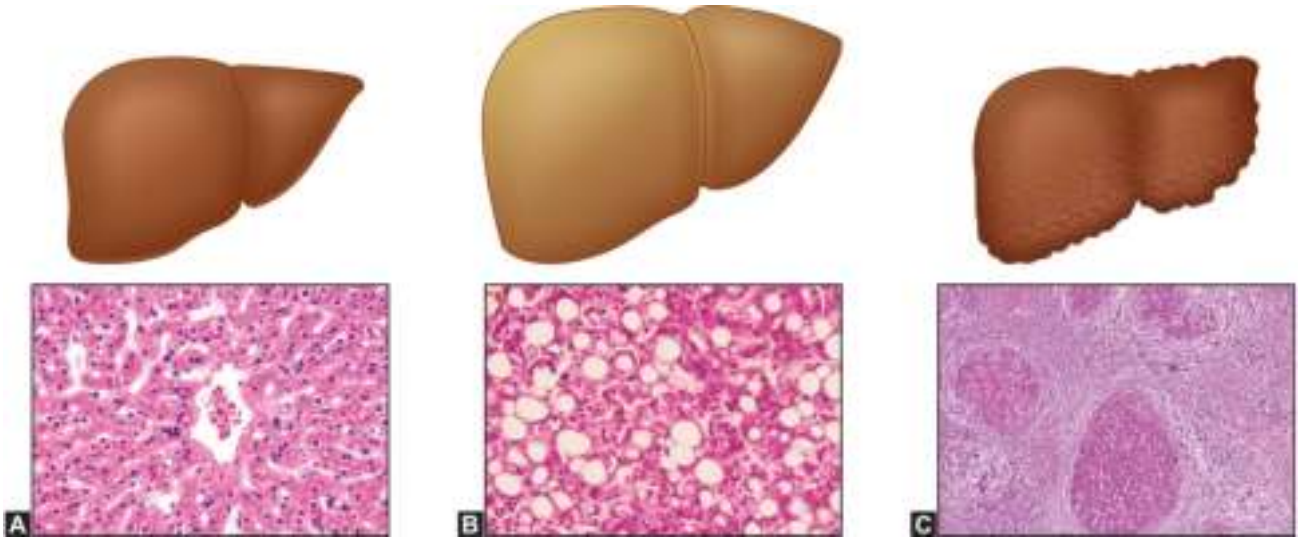


Fig. 21.55: Alcoholic liver disease (ALD). Approximately 60% of chronic alcoholics develop fatty liver. Around 40% develop steatohepatitis, showing inflammation and early fibrosis on histologic examination. Approximately 10–15% may develop micronodular cirrhosis. Only 10% of persons with cirrhosis develop hepatocellular carcinoma. (A) Normal liver, (B) fatty liver, (C) cirrhosis of liver.

Table 21.54 Alcohol oxidative pathways and their role in pathogenesis of alcoholic liver disease

Alcohol Oxidative Pathways	Adverse Effects on Liver
Direct toxic effects of alcohol	
Lipid peroxidation	Synthesis of fatty acid ethyl esters, phosphatidyl-ethanol resulting in alteration in membrane fluid
Alcohol toxic effect	▪ Decreased synthesis of vitamin A, vitamin E, mitochondrial GSH, SAMe ▪ Induces CYP2E1, and hydroxyethyl radicals ▪ Iron deposition
Endotoxemia	Kupffer cell dysfunction
Perivenular hypoxia	Sinusoidal dysfunction
Toll-like receptors, heat shock proteins	Inflammatory cytokines
Acetaldehyde metabolic product	
Acetaldehyde toxic effect	▪ DNA oxidation ▪ Protein oxidation ▪ Lipid peroxidation ▪ Oxidative stress
Acetaldehyde adducts	▪ Enzyme inhibition, malondialdehyde adducts ▪ Adverse immune response ▪ Impaired cytoskeleton function ▪ Mitochondrial injury, decreased ATP, decreased GSH and cysteine, increased collagen synthesis, activation of NF-κB and AP-1 leading to dysfunction of hepatocyte, Kupffer cells, and stellate cell.

AP-1; activator protein 1, ATP; adenosine triphosphate, CYP2E1; cytochrome p-450 system, GSH; glutathione; SAMe; S-adenosyl methionine, NF-κB; nuclear factor κB.

Pathology Pearls: Pathogenesis of Alcoholic Liver Disease

Ethanol absorbed by gastrointestinal tract is metabolized in liver by three pathways: alcohol dehydrogenase (most important), microsomal ethanol oxidizing P450 system and catalase. Alcohol mobilizes fatty acids from peripheral adipose tissue.

Ethanol alters mitochondrial and smooth endoplasmic reticulum thus inhibits fatty acid oxidation to ketone bodies and CO_2. Alcohol also decreases synthesis of lipoproteins resulting in accumulation of fat in liver cells.

- **Mobilization and biosynthesis of fatty acids:** Ethanol participates in mobilization of fatty acids to liver from peripheral stored fat. Alcohol dehydrogenase generates excessive NADH resulting in biosynthesis of lipid.
- **Mitochondrial dysfunction:** Normally, mitochondria participates in metabolism of ethanol to acetaldehyde. Ethanol disrupts normal functions of mitochondria and microtubules resulting in decreased fatty acid metabolism.

  Fatty acids accumulate in smooth endoplasmic reticulum of hepatocytes. There is decreased synthesis of lipoproteins, hence, liver is unable to release fatty acids to peripheral tissues.
- **Lipid peroxidation by acetaldehyde:** Acetaldehyde, metabolic product of ethanol induces lipid peroxidation with formation of malondialdehyde-acetaldehyde adduct. Autoantibody formed against this adduct results in hepatocellular injury.
- **Role of cytokines in cirrhotic nodules formation:** Ethanol stimulates Kupffer cells to synthesize cytokines such as TNF-α, IL-1, IL-6 and TNF-β, responsible for inflammation in liver. These cytokines stimulate stellate cells to synthesize malondialdehyde-acetaldehyde adduct responsible for cirrhotic nodules formation by laying down of collagen fibers.
- **Oxygen-derived free radicals induced hepatocellular injury:** Microsomal ethanol oxidizing system (P450) participates in oxidation of ethanol and generation of oxygen-derived free radicals leading to hepatocellular injury. Clinical consequences of chronic alcohol consumption are given in Table 21.55.

FATTY CHANGE (STEATOSIS) IN LIVER

Fatty change in liver is most frequent morphologic abnormality caused by alcohol intake and characterized by the accumulation of triglycerides within parenchymal cells. Fatty change in liver results from an imbalance between the uptake, utilization, and mobilization of fat from liver cells.

The amount of fat deposited varies with the amount of alcohol consumed, as well as the patient's hormonal status, diet, and other factors. Accumulation of triglyceride by itself is not ordinarily damaging liver, and the condition is fully reversible upon discontinuation of alcohol abuse.

Etiology

Alcohol abuse and diabetes mellitus associated with obesity are the most common causes of fatty change in liver. Alcoholic fatty liver may be reversible with complete abstinence from alcohol. Nonalcoholic fatty liver disease (NAFLD) is a related condition unrelated to alcohol consumption. Normal liver metabolism of free fatty acids (FFAs) is shown in Fig. 21.56.

- Alcohol intake alters mitochondrial and smooth endoplasmic reticulum function and thus inhibits

Table 21.55 Clinical consequences of chronic alcohol consumption

Organs	Disorders
Liver	▪ Steatosis ▪ Steatohepatitis ▪ Cirrhosis ▪ Hepatocellular carcinoma
Gastrointestinal tract	Gastritis
Pancreas	Pancreas
Central nervous system	Wernicke-Korsakoff pshycosis
Pregnancy	Fetal alcohol syndrome
Heart	Congestive/dilated cardiomyopathy due to thiamine deficiency
Other manifestations	▪ Malnutrition ▪ Vitamins deficiency ▪ Anemia

Pathogenesis of hepatic steatosis

- **Dietary factors and obesity:** Excessive consumption of saturated fatty acids leads to hepatic steatosis.
- **Chronic alcohol consumption:** It causes hepatic steatosis progressing to cirrhosis due to various mechanisms: (a) ethanol is oxidized, first to acetaldehyde and then to acetate and finally to enter citric acid cycle virtually in all tissues. This process is irreversible and unregulated, which decreases both cytoplasmic and intramitochondrial NAD, which causes imbalance of NADP: NADPH ratio and thus inhibits free fatty acids oxidation to make energy in tissues, (b) increased mobilization of free fatty acids from peripheral tissue to liver, (c) altering functions of mitochondria and smooth endoplasmic reticulum, inhibit oxidation of free fatty acids, and (d) inhibiting lipoprotein synthesis.
- **Insulin-resistant type 2 diabetes mellitus:** It causes hepatic steatosis progressing to cirrhosis and hepatocellular carcinoma due to various mechanisms: (a) increased mobilization of free fatty acids to liver, (b) imbalance of adipocytokines (i.e. decreased adiponectin and/or increased proinflammatory cytokines), (c) both free fatty acids and adipocytokines activate inflammatory pathways that include protein kinase C, transcription factor NF-κB and c-Jun N-terminal kinases consist of 10 isoforms derived from three genes: JNK1 (4 isoforms), JNK2 (4 isoforms) and JNK3 (2 isoforms) and can accelerate the progression of hepatic steatosis to nonalcoholic steatohepatitis and cirrhosis.

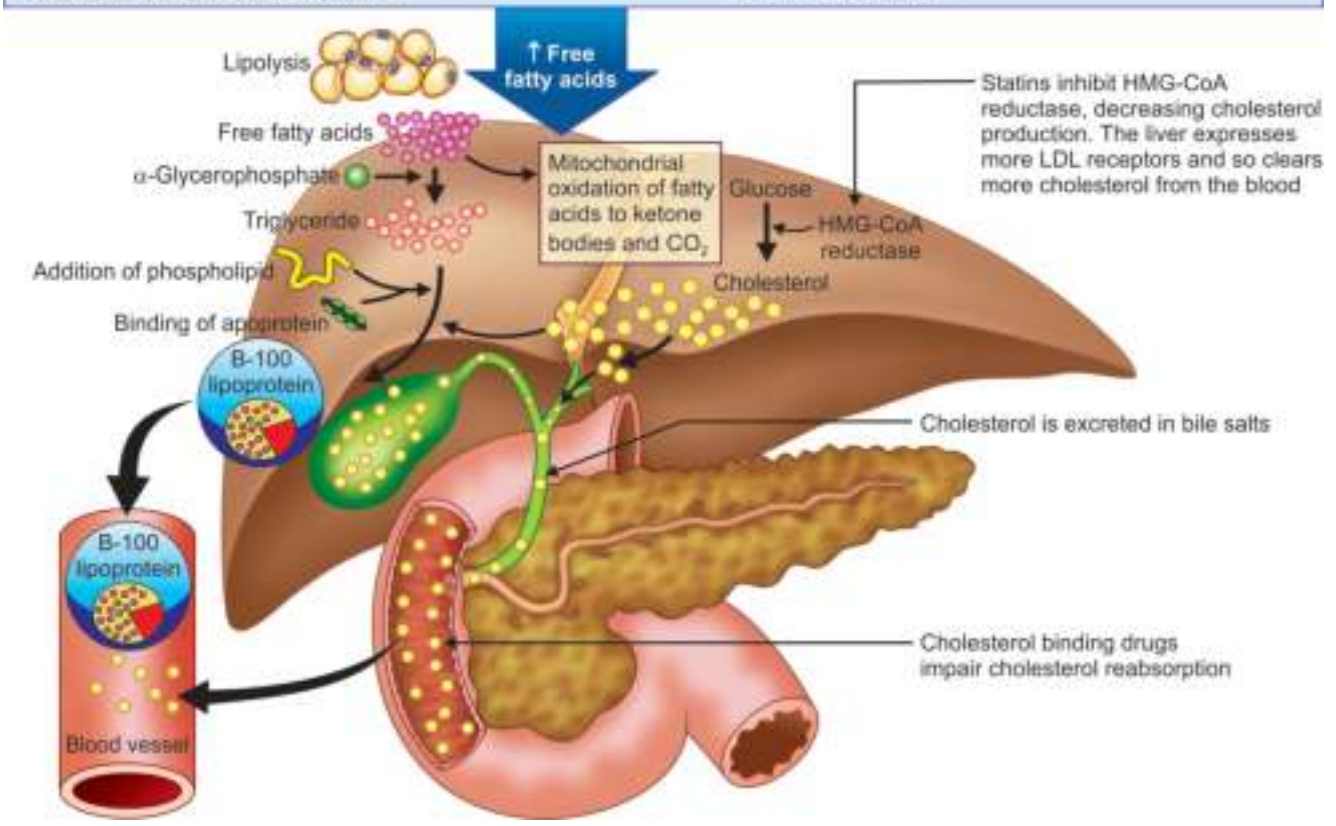


Fig. 21.56: Normal liver metabolism of free fatty acids (FFA). The cholesterol pathway is also shown. Cholesterol is also brought to the liver by high-density lipoproteins (HDL). The point at which alcohol interacts are shown. The effects of drug statin treatment are shown.

Table 21.56 Patterns and etiology of fatty change in liver (hepatic steatosis)

Hepatic Steatosis Pattern	Disorder	
Macrovesicular hepatic steatosis (large droplets)	▪ Alcoholic steatosis/steatohepatitis ▪ Nonalcoholic steatosis/steatohepatitis ▪ Bypass surgery for morbid obesity ▪ Drug- and toxin-induced (e.g. methotrexate, corticosteroids, amiodarone, mushrooms) ▪ Pancreatic insufficiency ▪ Inflammatory bowel disease (Crohn's disease and ulcerative colitis)	▪ Kwashiorkor (late stage) ▪ Metabolic disorders (e.g. abetalipoproteinemia, galactosemia, glycogen liver disease Ia, II,VI) ▪ Septicemia ▪ Viral hepatitis, chronic hepatitis, secondary to hepatitis C virus ▪ Wilson disease
Microvesicular hepatic steatosis (small droplets)	▪ Acute fatty liver of pregnancy* ▪ Alcoholic foamy degeneration ▪ Drug- and toxin-induced (e.g. tetracycline, cocaine, valproic acid, phosphorus) ▪ Kwashiorkor (early disease)	▪ Viral hepatitis, chronic hepatitis, secondary to HDV ▪ Metabolic disorders (e.g. Wolman disease, Alpers disease, cholesterol ester storage) ▪ Reye syndrome

**Acute fatty liver of pregnancy in the third trimester can develop hepatocellular failure with fatal outcome.*

fatty acid oxidation resulting in accumulation of lipids in hepatocytes.
- Protein malnutrition causes decreased synthesis of lipoproteins. Anoxia inhibits fatty acid oxidation.
- Starvation leads to mobilization of fatty acids. Diabetes mellitus and obesity may also cause fatty change.

Patterns of Fatty Change in Liver

Patterns and etiology of fatty change in liver (hepatic steatosis) are given in Table 21.56.

- **Microvesicular steatosis:** Minute multiple droplets of fats are present in hepatocytes not displacing nuclei to periphery.
- **Macrovesicular steatosis:** Single large lipid droplet is present in hepatocytes displacing nuclei to periphery.

Pathophysiology of Fatty Change in Liver

- **Physiologic state:** Free fatty acids from adipose tissue or ingested are normally transported into hepatocytes, where these are esterified to triglycerides, converted into cholesterol or phospholipids, or oxidized to ketone bodies. Some of the fatty acids are synthesized from acetate within hepatocytes as well. Egress of the triglyceride requires the formation of complexes with apoproteins to form lipoproteins, which are able to enter the circulation.
- **Pathologic state:** Defects in any of the steps of uptake, catabolism, or secretion can lead to accumulation of triglycerides in hepatocytes. Hepatotoxins (e.g. alcohol) alter mitochondrial and smooth endoplasmic reticulum function and thus inhibit fatty acid oxidation. Carbon tetrachloride and protein malnutrition decrease the synthesis of apoproteins. Anoxia inhibits fatty acid oxidation. Starvation increases fatty acid mobilization to liver from peripheral stores.

Clinical Significance

Mild fatty change in liver has no effect on cellular functions. Severe fatty change in liver may transiently impair cellular functions. But in carbon tetrachloride poisoning, fatty change in liver is irreversible.

Clinical Features

Patient with fatty change in liver shows hepatomegaly and mild elevation of serum bilirubin and alkaline phosphatase.

Surgical Pathology: Fatty Change in Liver

Gross Morphology

- Fatty change in liver starts at centrilobular area and progresses to involve whole lobule.
- Mild fatty change in liver may not affect the gross appearance.
- Severe fatty change in liver results in enlargement of liver, which becomes progressively yellow on cut surface until it may weigh 3–6 kg. This uniform change is consistent with fatty metamorphosis (fatty change). Gross morphology of fatty liver is shown in Fig. 21.57.

Light Microscopy

- **Fatty change in liver:** Initially, hepatocytes show small fat vacuoles around nucleus (mild fatty change or microvesicular) in hematoxylin and eosin stained sections. Progressive accumulation of fat vacuoles in the cytoplasm coalesces to create spaces that displace the nucleus toward periphery indicating severe fatty change.
- **Fatty cysts in liver:** Occasionally contiguous cells rupture, and the enclosed fat globules unite to produce so-called fatty cysts.
- **Distribution of lipid in liver:** Distribution of lipid vacuoles may be diffuse, focal or zonal, midzonal or peripheral region of liver depending on severity and etiology (Fig. 21.58).



Fig. 21.57: Fatty change in liver. This is a larger liver with more pronounced fatty change in liver. Such fatty change is most often nutritional in etiology when diet is poor in protein and/or when fatty acid metabolism is deranged and/or when liver cell function is impaired.

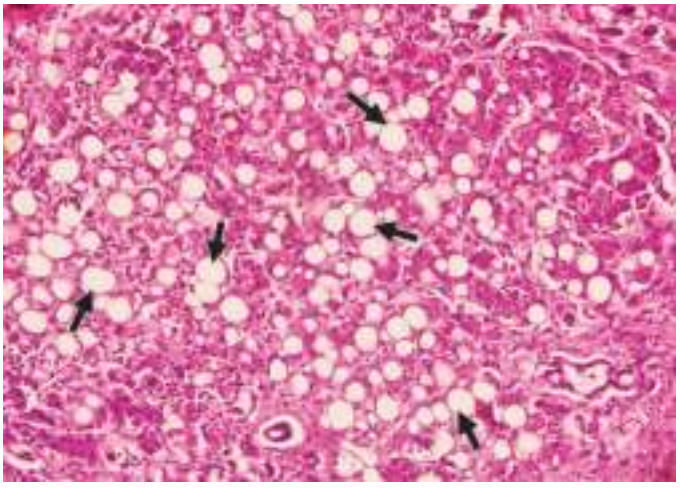


Fig. 21.58: Fatty change in liver. The lipid accumulates in the hepatocytes as vacuoles. These lipid vacuoles have a clear appearance with H&E staining. Alcohol, the most common cause, is a hepatotoxin that interferes with mitochondrial and microsomal function in hepatocytes, leading to an accumulation of lipid. In developing countries, fatty change in liver is seen in kwashiorkor, diabetes mellitus, obesity, and severe gastrointestinal tract malabsorption (arrows) (400X).

Fat Demonstration in Fatty Change in Liver

In paraffin-embedded sections, fat is dissolved during processing of tissue. Fat can be demonstrated by frozen section technique with the fat stains such as Sudan III, Sudan IV, Sudan black, Oil red O and osmic acid.

ACUTE ALCOHOLIC STEATOHEPATITIS

Acute alcoholic steatohepatitis is characterized by hepatic steatosis and hydropic swelling of hepatocytes, focal hepatocellular necrosis, infiltration by neutrophils, and cytoplasmic hyaline inclusions within the hepatocytes (Mallory bodies), which represent precipitated intermediate filament proteins (cytokeratin 8 and 18). Development of irreversible perivenular and perisinusoidal fibrosis in acute alcoholic steatohepatitis may progress to cirrhosis.

Clinical Features

Patient with acute alcoholic steatohepatitis typically presents with malaise and anorexia, fever, loss of weight, pain in right upper quadrant due to hepatomegaly, and jaundice. Serum bilirubin and alkaline phosphatase levels are raised with peripheral neutrophilic leukocytosis.

Surgical Pathology: Acute Alcoholic Steatohepatitis

Light Microscopy

Liver biopsy examined under light microscope shows hepatic steatosis and hydropic swelling of hepatocytes, focal hepatocellular necrosis, infiltration by neutrophils, and cytoplasmic Mallory's hyaline inclusions within the hepatocytes (Fig. 21.59).

- **Mallory-Denk hyaline bodies:** These are demonstrated in hepatocytes.
- **Necroinflammatory change:** Sclerosing hyaline necrosis, lobular focal necrosis and neutrophilic infiltrate may be demonstrated. Hepatocyte ballooning may also be seen.

Electron Microscopy

Giant mitochondria (megamitochondria) are ultrastructural feature of alcoholic liver disease.

Pathology Pearls: Mallory-Denk Hyaline Inclusions

- In histopathology, Mallory-Denk hyaline inclusions are demonstrated in the cytoplasm of hepatocytes.
- These are damaged intermediate filaments within hepatocytes.
- Mallory's hyaline inclusions are also known as **Mallory-Denk hyaline**. These are present in primary biliary cirrhosis but absent in secondary biliary cirrhosis. Liver disorders associated with Mallory-Denk bodies are given in Table 21.57.

ALCOHOLIC CIRRHOSIS

Development of irreversible perivenular and perisinusoidal fibrosis in acute alcoholic steatohepatitis may progress to cirrhosis. Chronic alcoholism is the most frequent cause of cirrhosis.

Initially, patient develops micronodular cirrhosis and later progresses to typical hobnail liver with large, irregular parenchymal regenerative nodules (macronodular cirrhosis). Laennec cirrhosis shows replacement of entire liver lobule by tough pale fibrous scar tissue.

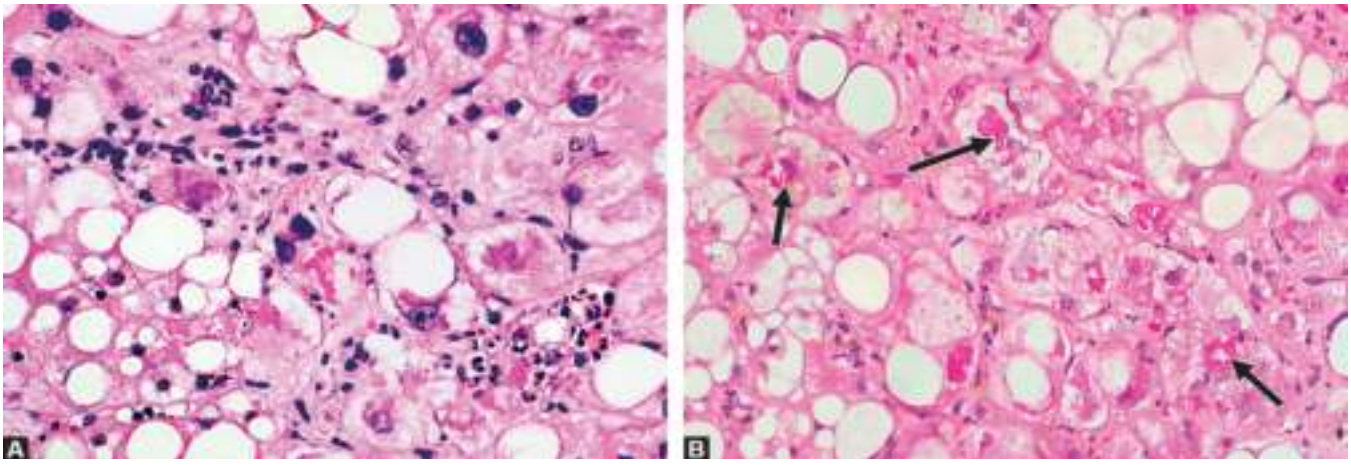


Fig. 21.59: Acute alcoholic steatohepatitis. (A) Hepatocytes show an accumulation of fat and Mallory's hyaline. It is infiltrated by neutrophils, (B) Mallory's hyaline. At high magnification can be seen globular red hyaline material. This is Mallory's hyaline, also known as alcoholic hyaline because it is most often seen in conjunction with chronic alcoholism. The globules are aggregates of intermediate filaments in the cytoplasm resulting from hepatocyte injury (arrows) (400X).

Table 21.57 Liver disorders associated with Mallory-Denk bodies

Category	Disorder
Excessive alcohol consumption	Alcoholic liver disease
Biliary tract disease	▪ Biliary atresia ▪ Extrahepatic bile duct obstruction ▪ Primary biliary cirrhosis ▪ Primary sclerosing cholangitis
Inherited disorders	▪ α_1-Antitrypsin deficiency ▪ Abetalipoproteinemia ▪ Glycogen storage disease 1α ▪ Wilson disease
Drug-induced disorders	▪ Ethanol ▪ Amiodarone
Cirrhosis of unknown etiology	▪ Indian childhood cirrhosis ▪ Nonalcoholic steatohepatitis
Hepatocellular tumors	▪ Hepatic adenoma ▪ Hepatocellular carcinoma
Other cause	Focal nodular hyperplasia

Surgical Pathology: Alcoholic Cirrhosis

Gross Morphology

- The liver may be enlarged or small and shrunken. The pattern is most often micronodular.
- In late stages, the regenerating nodules tend to become larger and irregular; this pattern results in a scarred, shrunken liver termed the hobnail liver (Fig. 21.60).

Light Microscopy

- Hepatic architecture is obscured by fibrous bands surrounding regenerating nodules of distorted liver cell plates.
- The fibrous bands show proliferating bile ducts, lymphocytes and plasma cells (Fig. 21.61).



Fig. 21.60: Alcoholic cirrhosis. Cut surface of liver shows parenchymal regenerative nodules of variable size.

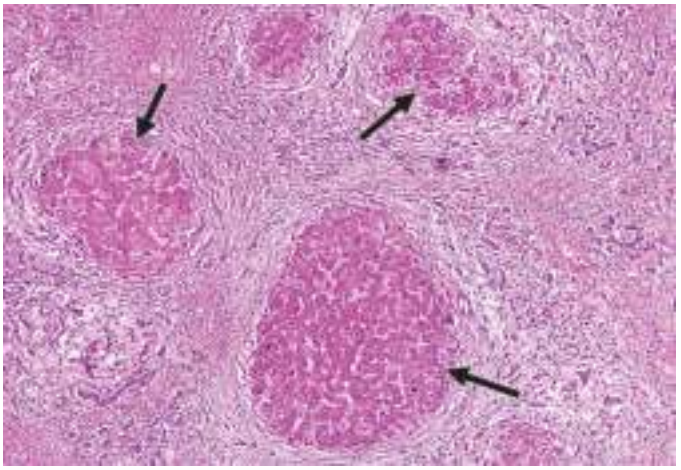


Fig. 21.61: Alcoholic liver cirrhosis. It shows parenchymal regenerative nodules surrounded by fibrous connective tissue that bridges between portal tracts. Within this collagenous tissue is scattered lymphocytes as well as a proliferation of bile ducts (arrows) (100X).

Table 21.58 Differences between alcoholic and post-necrotic cirrhosis

Parameters	Alcoholic Cirrhosis	Post-necrotic Cirrhosis
Nodules in liver		
Size of nodules in liver	Initially micronodular cirrhosis later mixed micronodules and macronodules giving hobnail appearance	Macronodules
Gross morphology		
External surface	Initially, liver yellow-tan in color, later brown colored	Liver small, shrunken and distorted. It shows irregular coarse scars and nodules
Cut surface	Cut surface showing multiple nodules of variable sizes	Cut surface revealing macronodules of variable sizes
Light microscopy		
Fibrous septa	Initially delicate and later thick fibrous septa	Generally thick fibrous septa
Inflammatory cells	Sparse infiltration of fibrous septa by macrophages, lymphocytes and plasma cells	Heavy infiltration of fibrous septa by macrophages, lymphocytes and plasma cells resulting in formation of lymphoid follicles
Mallory body	May be present but rare	Absent
Proliferation of bile ducts	Minimal proliferation	Extensive proliferation
Fatty change in liver	May show fatty change in liver	Absent

Consequences of Cirrhosis

Consequences of intrahepatic scarring with increased portal venous pressure lead to esophageal varices often resulting in upper gastrointestinal tract hemorrhage, rectal hemorrhoids, periumbilical venous collaterals (caput medusae) and splenomegaly.

Differences between alcoholic and post-necrotic cirrhosis are given in Table 21.58.

Clinical Features

Patient with alcoholic cirrhosis presents with jaundice, distended abdomen, esophageal varices, caput medusae, massive splenomegaly with elevated serum transaminase (AST and ALT) levels and serum alkaline phosphatase. Serum protein levels are decreased. Clinical manifestations associated with hepatocellular damage and hepatocellular failure are described as under.

- **Jaundice:** It is most often mixed conjugated and unconjugated.
- **Hypoalbuminemia:** It is caused by decreased albumin synthesis in damaged hepatocytes.
- **Decreased synthesis of coagulation factors:** Liver synthesizes less amount of coagulation factors with the exception of von Willebrand factor.
- **Hyperestrinism:** Patient presents with palmar erythema; spider nevi (capillary telangiectasia) of the face, upper arms, and chest; loss of body and pubic hair; testicular atrophy; and gynecomastia.
- **Peripheral edema, ascites or hydrothorax:** These manifestations occur by various mechanisms. Liver fails to synthesize albumin leading to decreased plasma oncotic pressure. Increased portal venous pressure leads to increased production of hepatic lymph, retention of sodium and water as a result of decreased hepatic degradation of aldosterone, activation of the renin-angiotensin system, or both.
- **Hepatic encephalopathy (portal-systemic encephalopathy):** It is facilitated by shunting from the portal to the systemic circulation, with delivery of neurotoxic substances, such as ammonia and other enteric degradation products, directly into the systemic circulation. Neurological manifestations varying from slight confusion to deep coma along with asterixis (flapping tremor of hands) are characteristic features.

NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) is so named because it closely resembles alcoholic fatty liver disease. This condition represents a spectrum of hepatocellular injuries that initially display steatosis, with or without hepatitis. NAFLD may progress to bridging fibrosis and cirrhosis of liver. It is consistently associated with metabolic syndromes characterized by central obesity, type 2 diabetes mellitus, dyslipidemia, and microalbuminuria.

- Nonalcoholic fatty liver disease occurs in the settings of insulin resistant diabetes mellitus, lack of exercise

and genetic/epigenetic alteration mechanisms. Hepatocellular injury is caused by oxidants and inflammatory cells induced by chemical mediators such as TNF-α and IL-1. Activation of stellate cells by TNF-α, TNF-β and NK cell results in fibrosis in liver.

Surgical Pathology: Nonalcoholic Fatty Liver Disease

Light Microscopy

- Steatosis is defined as the involvement of >5% of hepatocytes.
- Essential features of nonalcoholic fatty liver disease (NAFLD) include macrovesicular hepatocytes in the zone 3, mild mixed acinar inflammation and hepatocellular injury in the form of hepatocellular degeneration in zone 3 and/or pericellular fibrosis.
- In comparison to acute alcoholic steatohepatitis, Mallory-Denk hyaline bodies are less prominent, but mild mixed acinar inflammation is more prominent. Nonalcoholic fatty liver disease may lead to cirrhosis over years (Fig. 21.62A and B). Comparison between acute alcoholic steatohepatitis and nonalcoholic fatty liver disease is given in Table 21.59.
- Other histologic features may be present, but not essential for diagnosis include Mallory-Denk hyaline bodies in zone 3, mild iron deposition in hepatocytes or sinusoidal cells and megamitochondria; and glycogenated nuclei in zone 1, lipogranulomas in the lobular parenchyma or portal tracts and occasional eosinophilic bodies.

- Grading and staging of nonalcoholic fatty liver disease (NAFLD) is given in Tables 21.60 and 21.61.

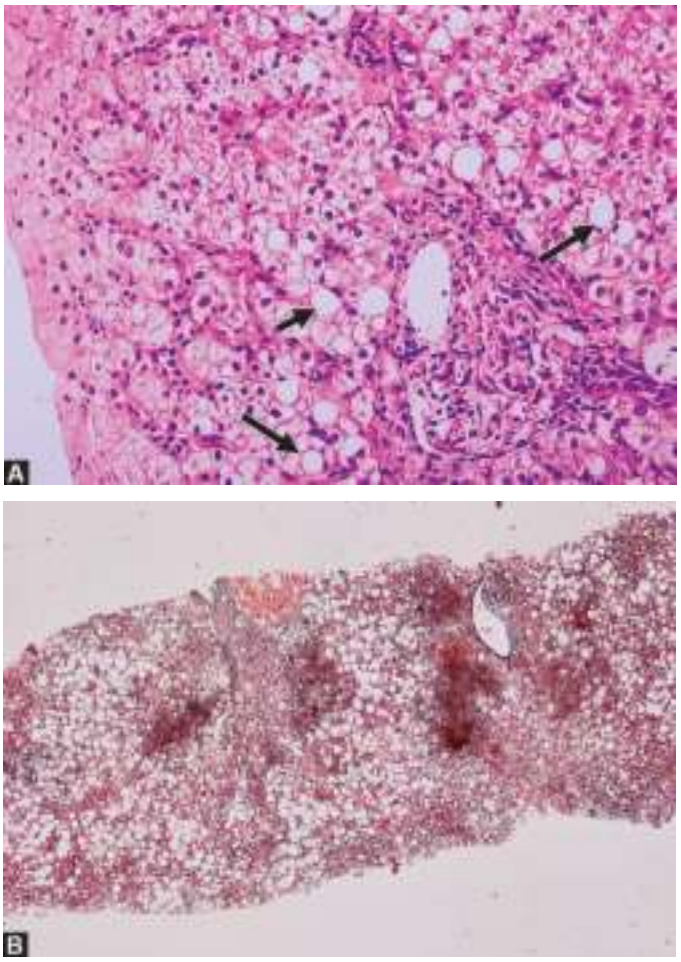


Fig. 21.62A and B: Nonalcoholic fatty liver disease (NAFLD). (A) Histologic examination of liver reveals steatosis involving more than 5% of hepatocytes in zone 3, lobular inflammation (polymorphonuclear cells, lymphocytes, plasma cells), and macrophages, hepatocellular ballooning in zone 3 and perisinusoidal fibrosis in zone 3. Small lipogranulomas or acidophil PAS stained Kupffer cells can be demonstrated in the lobular parenchyma (arrows) (400X). (B) Reticulin staining is done to demonstrate fibrosis in nonalcoholic fatty liver disease. The characteristic pattern of fibrosis in nonalcoholic steatohepatitis is sinusoidal/pericellular (chicken wire) fibrosis, which usually begins in zone 3 and demonstrated by reticulin staining technique.

HEMOCHROMATOSIS

Hemochromatosis is characterized by excessive deposition of iron deposition in the parenchymal cells of liver, pancreas, heart, skin, joints and other organs resulting in organ damage. Disease manifests when body storage iron exceeds 20 g.

- Clinical manifestations occur due to excessive hemosiderin deposition in various organs. Iron in tissues

Table 21.59 Comparison between acute alcoholic steatohepatitis and nonalcoholic steatohepatitis

Parameters	Acute Alcoholic Steatohepatitis	Nonalcoholic Steatohepatitis
History	Alcohol intake	▪ Diabetes mellitus ▪ Hyperlipidemia ▪ Metabolic diseases ▪ Bypass surgery
AST/ALT ratio	AST/ALT >2	AST/ALT <1
Steatosis features	▪ Macrovesicular steatosis ▪ Central venular distribution of steatosis ▪ Fibrosis around central venules	▪ Microvesicular steatosis ▪ Diffuse distribution of steatosis ▪ Portal fibrosis
Mallory-Denk hyaline bodies	Frequent	Less frequent

Table 21.60 Component scores for grading nonalcoholic fatty liver disease (NAFLD)

Stage	Steatosis	Lobular Inflammation	Hepatocellular Ballooning
Stage 0	<5%	None	None
Stage 1	5–33%	<2/20 × per field	Mild (few)
Stage 2	34–66%	2–4/20 × per field	Moderate to marked
Stage 3	>66–100%	>4/20 × per field	Moderate to marked

NAFLD activity score: NAS 0–8 (steatosis + lobular inflammation + ballooning)

Table 21.61 Fibrosis scoring: Based on Masson trichrome stain for nonalcoholic fatty liver disease (NAFLD)

Stage	Masson Trichrome
Stage 0	None
Stage 1a	Delicate zone 3 perisinusoidal fibrosis and requires trichrome stain
Stage 1b	Dense zone 3 perisinusoidal fibrosis, visible on hematoxylin and eosin
Stage 1c	Portal/periportal fibrosis
Stage 2	Zone 3 perisinusoidal and portal fibrosis
Stage 3	Bridging fibrosis
Stage 4	Cirrhosis

is demonstrated by Perls Prussian blue staining technique.

- Patient may present with triad of hepatomegaly, diabetes mellitus and skin pigmentation is known as bronze diabetes.
- Patients are at great risk of developing congestive heart failure secondary to iron deposition in the heart; cirrhosis and hepatocellular carcinoma.
- Serum iron, transferrin saturation and serum ferritin levels are increased. Hemochromatosis is treated by repeated phlebotomy and deferoxamine. Distribution of normal iron in adults is given in Table 21.62. Causes of primary and secondary hemochromatosis are given in Table 21.63.

Table 21.62 Distribution of normal iron in adults

Iron	Examples of Iron in Body	Values of Total Body Iron (3.0–5.0 g)
Functional iron	▪ Hemoglobin ▪ Essential/functional/nonavailable iron	▪ 1.5–3.0 g ▪ Myoglobin: 0.3 g ▪ Enzymes of cellular respiration: 0.05 g
Storage/available tissue iron	▪ Ferritin ▪ Hemosiderin	1.2–2.0 g
Plasma iron	Transferrin bound iron for transport	3–4 mg

Genetic hemochromatosis refers to >50 g of total body iron. Normal liver contains 0.5 g in 98% of hepatocytes.

Table 21.63 Causes of primary and secondary hemochromatosis

Causes	Disorders	
Primary hemochromatosis		
Genetic hemochromatosis	Mutation of HFE gene on chromosome 6	
Secondary hemochromatosis		
Parenteral iron overload due to frequent blood transfusions and iron dextran injections	▪ Aplastic anemia ▪ Leukemias ▪ Sickle cell anemia	▪ Myelodysplastic syndrome ▪ Long-term hemodialysis
Ineffective erythropoiesis with increased erythroid activity	▪ β-Thalassemia ▪ Sideroblastic anemia	▪ Pyruvate kinase deficiency
Increased oral intake of iron	Bantu siderosis also called African iron overload	
Chronic liver disease	▪ Chronic alcohol disease	▪ Porphyria cutanea tarda
Congenital atransferrinemia	Hereditary disorder	
Neonatal hemochromatosis	Abnormal accumulation of iron in the tissues	

- Primary hemochromatosis is caused by a mutation in the type 1 HH, an autosomal recessive disorder (HFE gene), type 2 HH is more severe, an autosomal recessive disorder (hemojuvelin and hepcidin), type 3 HH, an autosomal recessive disorder (transferrin receptor type 2) and type 4 HH, an autosomal dominant disorder (ferroportin 1). Hepcidin is the main regulator of iron absorption. It is also known as liver expressed antimicrobial peptide (LEAP) encoded by HAMP gene located on the short arm of chromosome 6p21.3 in close linkage with HLA locus.
- Secondary hemochromatosis is associated with chronic blood transfusion therapy, ineffective erythropoiesis, or increased oral intake of iron.

PRIMARY/HEREDITARY HEMOCHROMATOSIS

Primary/hereditary hemochromatosis (HH) is caused by a mutation in the type 1 HH is an autosomal recessive disorder (HFE gene), type 2 HH more severe, an autosomal recessive disorder (hemojuvelin and hepcidin), type 3 HH, an autosomal recessive disorder (transferrin receptor type 2) and type 4 HH, an autosomal dominant disorder (ferroportin 1). Unrestricted intestinal iron absorption and mobilization of iron from storage sites result in excessive deposition of iron in parenchymal organs (liver, pancreas, myocardium, endocrine glands, joints, testes and skin) and cause organs damage. Iron stimulates hepatic stellate cells, which promote fibrosis in liver.

Molecular Pathogenesis: Hereditary Hemochromatosis

Four types of primary/hereditary hemochromatosis are given in Table 21.64.

Hereditary Hemochromatosis Type 1

- Hepcidin is the main regulator of iron absorption. It is also known as LEAP (liver expressed antimicrobial peptide) encoded by HAMP gene located on the short arm of chromosome 6p21.3 in close linkage with HLA locus.
- HH type 1 is an autosomal recessive disorder due to hepcidin mutation. Hepcidin mutation leads to low hepcidin level and consequent excess iron absorption and increased mobilization of iron from macrophages.
- HH type 1 is the most common type of hemochromatosis. Two most commonly described mutations are C282Y (substitution of cytosine to tyrosine to aspartate an amino acid at position 282) and H63D (substitution of histidine to aspartate an amino acid at position 63).
- Excessive iron deposition in hepatocytes is more prominent in the periportal region. Patients can be autosomal recessive C282Y, homozygous C282Y and double heterozygous.
- Patient presents in third and fourth decades due to excessive deposition of iron in the liver, pancreas, heart, skin and joints.
- Evidence of excessive iron overload can be assessed by serum ferritin, MRI and liver biopsy.
- Serum ferritin is a highly sensitive index of elevated iron.
- Normal serum ferritin excludes iron overload. Serum ferritin more than 1000 ng/L, elevated ALT, hepatomegaly are risk factors of fibrosis resulting in cirrhosis and hepatocellular failure. Liver biopsy can be considered in these patients.

Hereditary Hemochromatosis Type 2 (Juvenile Type 2 HH)

- HH type 2 occurs in two forms: autosomal recessive mutations involving hemojuvelin located on chromosome 1 (2A) or hepcidin located on chromosome 19 (2B). Hemojuvelin (HJV) mutation leads to low hepcidin level resulting in increased iron mobilization from intestine and macrophages.
- Most cases equally affecting males and females occur due to hemojuvelin gene mutation.
- Excessive iron deposition occurs in the hepatocytes associated with cardiomyopathy and hypogonadism. Patient presents in first three decades with more severe disease compared to HFE associated type 1 (HH).

Hereditary Hemochromatosis Type 3

- HH type 3 is an autosomal recessive disorder. It occurs due to mutation involving transferrin receptor type 2 gene located on chromosome 7.
- It tends to similar to type 1 HFE-HH. Excessive iron deposition occurs in hepatocytes.
- Patient presents in third and fourth decades due to excessive deposition of iron in the liver, pancreas, heart, skin and joints.

Hereditary Hemochromatosis (Ferroportin Disease) Type 4

- HH type 4 is an autosomal disorder caused by mutation involving ferroportin 1 located on chromosome 2.
- Patient presents in fourth decade; severity varies with type of mutation.

Organs Involved and Resulting Complications

Many organs are involved in primary hemochromatosis (Fig. 21.63).

- **Liver:** It shows features of micronodular cirrhosis. Hepatocytes as well as Kupffer cells show excessive deposition of brown granules of hemosiderin. Liver iron concentration is >400 mg/100 g of wet liver in primary hemochromatosis. Normal wet liver contains iron concentration <30 mg/100 g of wet liver Perls Prussian blue iron stain demonstrates the blue granules of hemosiderin in hepatocytes and Kupffer cells (mononuclear phagocyte system). Most common causes of death are **cirrhosis** and **hepatocellular carcinoma**.
- **Pancreas:** Excessive deposition of iron in islets of Langerhans of pancreas causes diffuse interstitial fibrosis resulting in diabetes mellitus.
- **Myocardium:** Excessive deposition of iron in myocardium may cause restrictive myocarditis, cardiac arrhythmias and congestive heart failure.

Table 21.64 Types of primary/hereditary hemochromatosis (HH)

Type	Genetics	Liver Biopsy	Clinical Features
Type 1 (HFE-associated HH)	Autosomal recessive disorder involving HFE gene located on chromosome 6 (C282Y, homozygous C282Y and double heterozygous)	Excessive iron deposition in hepatocytes, more prominent in the periportal region	Patient presents in third and fourth decades due to excessive deposition of iron in the liver, pancreas, heart, skin and joints
Type 2 (juvenile HH)	Autosomal recessive disorder with mutations involving hemojuvelin on chromosome 1 (2A) or hepcidin on chromosome 19 (2B)	Excessive iron deposition in hepatocytes	Patient presents in first three decades with more severe disease compared to HFE associated HH
Type 3 (HH)	Autosomal recessive disorder with mutation involving transferrin receptor type 2 gene located on chromosome 7	Excessive iron deposition in hepatocytes	Patient presents in third and fourth decades similar to HFE-associated HH but intermediate between type 1 and type 2 due to excessive deposition of iron in the liver, pancreas, heart, skin and joints
Type 4 (HH)/ ferroportin disease	Autosomal dominant with mutation involving ferroportin 1 located on chromosome 2	First subtype excessive iron deposition in hepatocytes, second subtype excessive iron deposition in Kupffer cells	Patient presents in fourth decade; severity varies with type of mutation

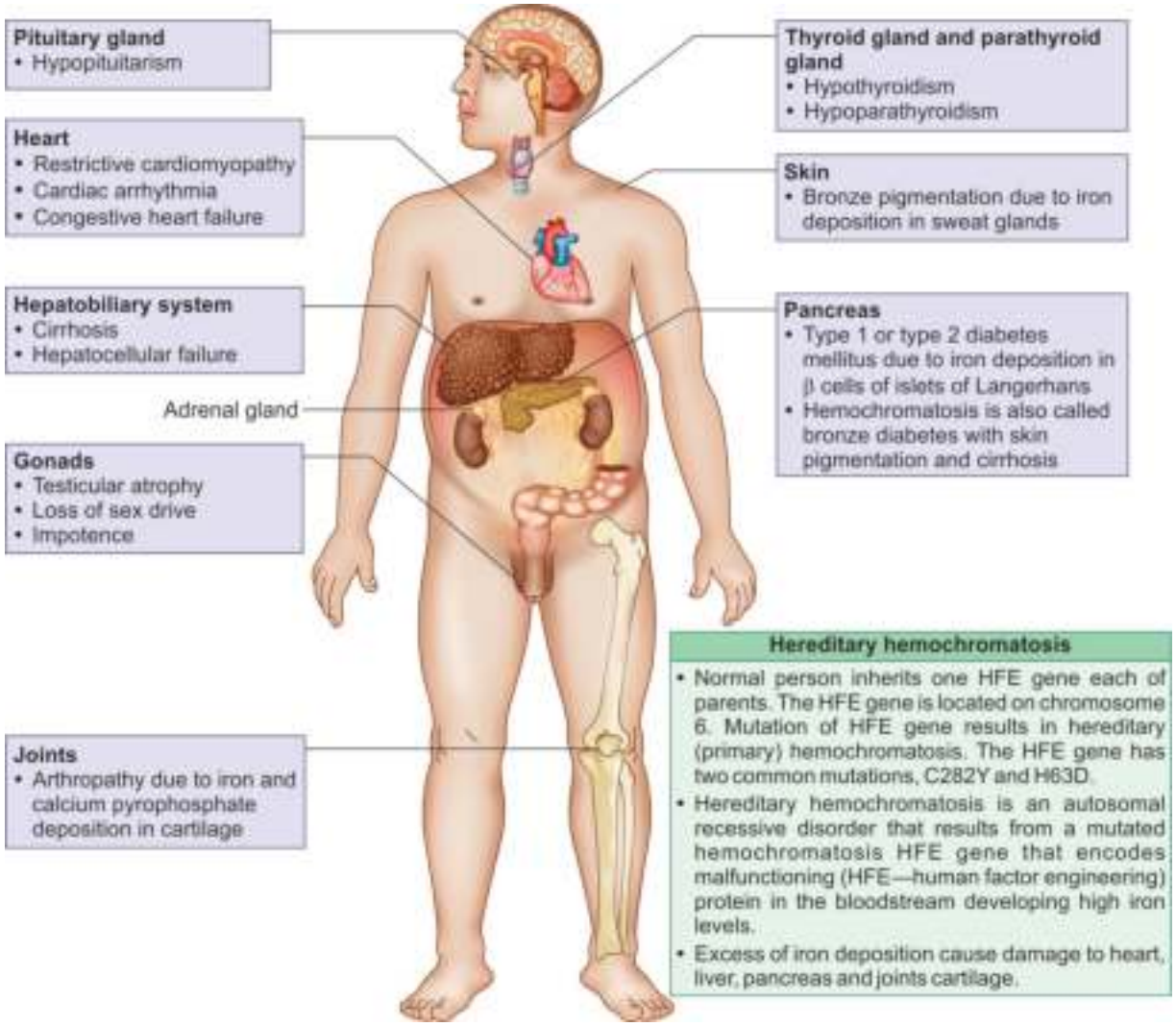


Fig. 21.63: Primary hemochromatosis. It shows involvement of multiple organs due to deposition of iron.

- **Endocrine glands:** Excessive deposition of iron in thyroid, parathyroid, pituitary and adrenal glands may lead to endocrinal gland dysfunction.
- **Joints:** Excessive iron is deposited in synovial linings of joints in 30 to 50% of cases. Cartilage damage leads to disabling polyarthritis.
- **Skin pigmentation:** Skin pigmentation on face, neck and extensor surface of forearms occurs due to deposition of melanin and hemosiderin in 70 to 90% of patients. It is component of bronze diabetes mellitus.
- **Testes:** Small atrophic testes (hypogonadism) in primary hemochromatosis occur due to impairment of hypothalamopituitary function due to iron deposition and not due to excessive deposition of iron in testes.

Clinical Features

Patient with primary hemochromatosis presents with hepatomegaly, abdominal pain, skin pigmentation, diabetes mellitus or deranged glucose metabolism, cardiac dysfunction, amenorrhea in females, decreased libido and impotence in males. Cause of death is due to development of hepatocellular carcinoma, cardiac dysfunction and hepatocellular failure. Chelating agent **desferrioxamine** is administered in these patients with severe anemia and hypoproteinemia.

Surgical Pathology: Primary Hemochromatosis in Liver

Light Microscopy

Hepatocytes as well as Kupffer cells show excessive deposition of brown granules of hemosiderin (Fig. 21.64).

Screening Tests

The recommended screening test for primary hemochromatosis is increased serum transferrin saturation. In selected cases, molecular testing is done to demonstrate mutations in the HFE gene in tissues. Early detection and occasional phlebotomy can prevent multiorgan failure attributed to iron accumulation in tissues.

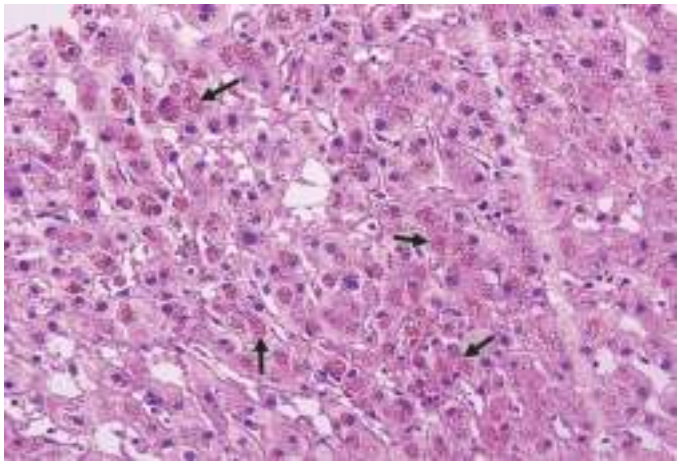


Fig. 21.64: Primary hemochromatosis involving liver. Hepatocytes as well as Kupffer cells show excessive deposition of brown granules of hemosiderin (arrows) (400X).

Laboratory Diagnosis

- There is marked increase in serum iron and modest reduction in total iron-binding capacity (TIBC, transferrin). This combination results in increased transferrin iron saturation (100%).
- Serum ferritin levels are increased >500 ng/ml (normal value is <150 ng/ml) in these patients.
- Blood biochemistry such as blood sugar and urine analysis should be done in patients with bronze diabetes. Iron is demonstrated by Perls Prussian blue reaction in the tissues. Scheuer system of grading of liver iron is given in Table 21.65.

Pathology Pearls: Liver Biopsy for Iron Analysis in Primary Hemochromatosis

- Liver biopsy specimen should be sent in two cores. One core fixed in 10% formalin is submitted for histopathologic examination. Other core wrapped in lens paper submitted for measurement of drug weight iron on molecular testing.
- Hepatic iron index is calculated by hepatic iron in micromole/gram dry weight and age. Hepatic iron <1 is normal.
- Hepatic iron >2 g is diagnostic of primary hemochromatosis.

Prognosis

Common causes of death from primary hemochromatosis are liver cirrhosis, cardiac failure and hepatocellular carcinoma.

SECONDARY HEMOCHROMATOSIS

Secondary hemochromatosis is most often associated with a combination of ineffective erythropoiesis and multiple blood transfusions in β-thalassemia major, high intake of iron and '**Bantus siderosis**' due to consumption of traditional beer home-brewed in steel drums.

Excessive iron is deposited largely in mononuclear phagocytic system, which in some instances, may spill over into parenchymal cells, producing a hybrid condition.

Table 21.65 Scheuer system of grading of liver iron

Iron Grade	Interpretation of Results
Grade 0	Hemosiderin granules absent or barely discernible at 400X
Grade 1	Hemosiderin granules barely discernible at 250X, easily demonstrated
Grade 2	Discrete hemosiderin granules demonstrated at 100X
Grade 3	Discrete hemosiderin granules demonstrated at 25X
Grade 4	Masses of hemosiderin granules demonstrated at 10X

WILSON DISEASE

Wilson disease is an autosomal recessive disorder of copper metabolism in which excess copper can be deposited in the liver and brain due to impaired transport of copper across lysosomal membrane or bile canaliculi. Ceruloplasmin levels are decreased in Wilson disease secondary to copper deposition in liver, basal ganglion and corneal limbus.

- **Normal copper metabolism:** Copper absorbed from proximal small intestine is bound to apoceruloplasmin in liver to form ceruloplasmin, that is secreted into the blood, which is then taken up by liver and broken down in lysosomes. Released copper is then excreted through the bile.
- **Pathogenesis:** In Wilson disease, mutation of ATP7B gene located on chromosome 13 leads to deficiency of a copper transporting ATPase resulting in decrease in copper transport into bile and impairing its incorporation into ceruloplasmin. Accumulated copper causes toxic injury to organs by oxygen-derived free radical mediated injury and displacing of metals from metalloenzymes (Table 21.66).

Table 21.66 Organs involved in Wilson disease and consequences

Organs Involved	Wilson Disease
Liver	▪ Steatosis ▪ Acute/chronic hepatitis ▪ Micronodular/macronodular cirrhosis
Eye	Kayser-Fleischer rings due to brown deposits of copper in Descemet membrane in the limbus of cornea demonstrated by slit-lamp eye examination
Kidney tubules	▪ Aminoaciduria ▪ Glycosuria
Brain	Basal ganglia especially putamen involvement impairing extrapyramidal functions, e.g. psychosis and tremors

- **Pathology:** Accumulation of copper in various organs results in liver (e.g. chronic hepatitis to micronodular or macronodular cirrhosis), brain (e.g. basal ganglia especially the putamen of the lenticular nucleus impairing extrapyramidal motor functions), kidney tubular cells (e.g. aminoaciduria and glycosuria), and eye (e.g. Descemet membrane known as '**Kayser-Fleischer ring**').
- **Clinical features:** Patient presents with neuropsychiatric symptoms and movement disorders, rigid dystonia as a result of basal ganglia involvement. Patient may also present with symptoms of acute or chronic liver disease and ocular Kayser-Fleischer ring.

Surgical Pathology: Cirrhosis in Wilson Disease

Light Microscopy

- Liver biopsy shows chronic hepatitis and fatty change.
- Liver cell nuclei are vacuolated.
- Mallory bodies in periportal hepatocytes and piecemeal necrosis are present in the cytoplasm.
- Stainable copper is present in liver cells.
- Cirrhosis occurs following chronic hepatitis.

Histochemistry

Excessive copper in tissues can be demonstrated by **rhodamine stain** or reddish orange orcein for copper and orcein stain for copper associated protein.

Biochemical Investigations

Decreased serum ceruloplasmin, increased hepatic copper level (>250 μg/1 g dry weight of liver) and increased urinary copper are consistently seen in Table 21.67).

α_1-ANTITRYPSIN DEFICIENCY

The α_1-antitrypsin deficiency is the most common autosomal recessive disorder of liver disease in infants and children in which liver transplantation is indicated.

Table 21.67 Laboratory diagnosis of Wilson's disease

Test	Results
Ocular findings	Kayser-Fleischer rings
Hepatic copper content (confirmatory test)	250 g copper per 10 g of dried liver tissue
Serum ceruloplasmin level (most specific screening)	Decreased
Urinary excretion of copper	Increased
Rhodamine stain	Used to stain copper
Reddish orange orcein stain	Used to stain copper associated protein

Table 21.68 Liver diseases associated with α_1-antitrypsin deficiency in age groups

Liver Disorder	Clinical Features	Light Microscopy
Neonatal hepatitis	Cholestasis jaundice	▪ Cholestasis (marked) ▪ Liver cell necrosis ▪ Lymphocytes, plasma cells and macrophages infiltration ▪ Globules of α_1-antitrypsin demonstrated after attaining 6 months of age ▪ Recovery is common
Childhood cirrhosis	Persistence of jaundice >6 months	Cirrhosis
Adult macronodular cirrhosis (60–70 years)	Only liver involved and lungs spared	▪ Cirrhosis (globules of α_1-antitrypsin demonstrated) ▪ Hepatocellular carcinoma

Liver may be involved with or without lung disease in the form of emphysema. There is increased risk of development of hepatocellular carcinoma.

- The disorder may affect infants, children and adults. Liver diseases associated with α_1-antitrypsin deficiency in these age groups are given in Table 21.68.
- Normally α_1-antitrypsin (AAT) is a protein encoded by SERPINA-1 gene on the proteinase inhibitor locus (Pi) on chromosome 14. It is synthesized by liver in normal genotype persons (PiMM phenotype). This protein prevents elastases to degrade normal host tissue. It accounts for 90% of antiproteinase activity in the blood.
- The α_1-antitrypsin (AAT) deficiency is an autosomal dominant disorder equally affecting men and women. Most patients with clinically-diagnosed emphysema <40 years of age have α_1-antitrypsin (PiZZ phenotype) deficiency.

Pathology Pearls: Clinical Consequences in α_1-Antitrypsin Deficiency

- Homozygous PiZZ genotype patients deficient in α_1-antitrypsin (AAT) (<11 μm/L) are prone to develop panacinar emphysema, cirrhosis, cutaneous panniculitis, neonatal hepatitis, bronchiectasis and Wegener's granulomatosis. Serum electrophoresis reveals absence of α_1-globulin peak.
- Tobacco smoking appears to be the most important risk factor for the development of panacinar emphysema among AAT deficient persons. Among smokers, even mild to moderate reduction in AAT levels may be associated with panacinar emphysema resulting in more rapid decline in lung function.
- Destruction of lung parenchyma and consequent emphysema results from an imbalance between the major natural antiprotease, AAT and inflammatory proteases (especially leukocyte elastase). Intravenous purified pooled human plasma is recommended to enhance ATT levels in these patients.

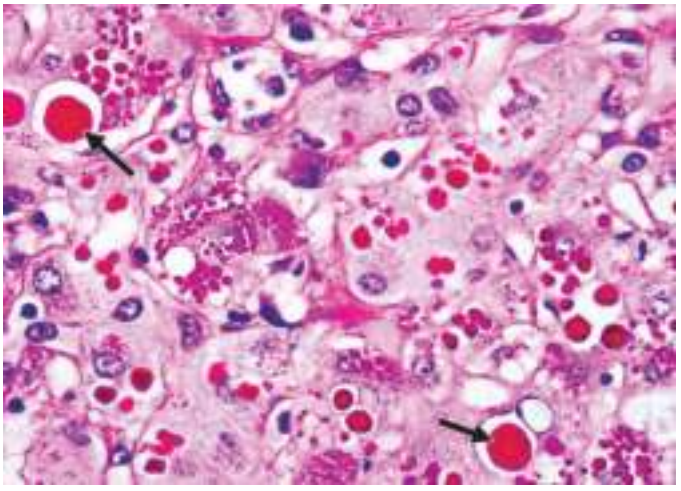


Fig. 21.65: The α_1-antitrypsin (AAT) deficiency. The periportal red hyaline globules seen here with periodic acid–Schiff (PAS) stain are characteristic for α_1-antitrypsin deficiency (a person with homozygous PiZZ genotype). The globules are intrahepatic collections of α_1-antitrypsin that is not being excreted from the hepatocytes. This may eventually lead to a macronodular cirrhosis. These patients are also prone to develop panacinar emphysema of lungs (arrows) (400X).

Surgical Pathology: Liver in α_1-Antitrypsin Deficiency

Light Microscopy

- Histologic examination of liver biopsy shows round-to-oval cytoplasmic globular inclusions of misfolded α_1-antitrypsin PAS positive diastase resistant proteins in hepatocytes.
- These globules stain red with PAS after removing glycogen with diastase (Fig. 21.65).

GLYCOGEN STORAGE DISEASE TYPE 4

Andersen disease, also known as glycogen storage disease type 4, is an autosomal recessive disorder caused by deficiency of a glycogen-branching enzyme. This enzyme deficiency results in the accumulation of abnormal glycogen (amylopectin) in the liver, muscle, and other tissues.

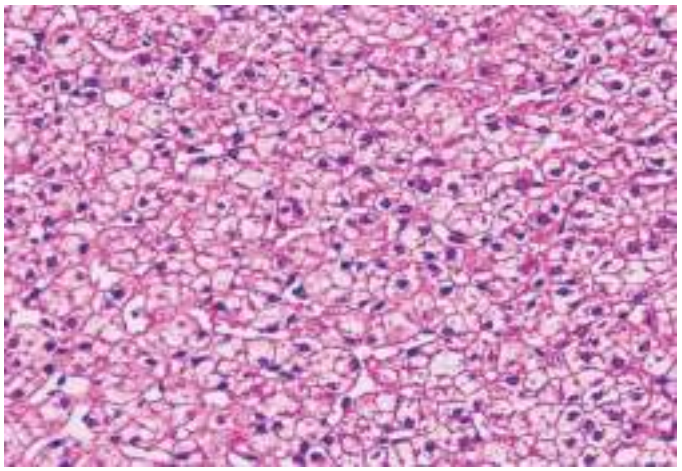


Fig. 21.66: Glyocogen storage disease in liver. It shows deposition of glycogen in hepatocytes giving mosaic appearance (400X).

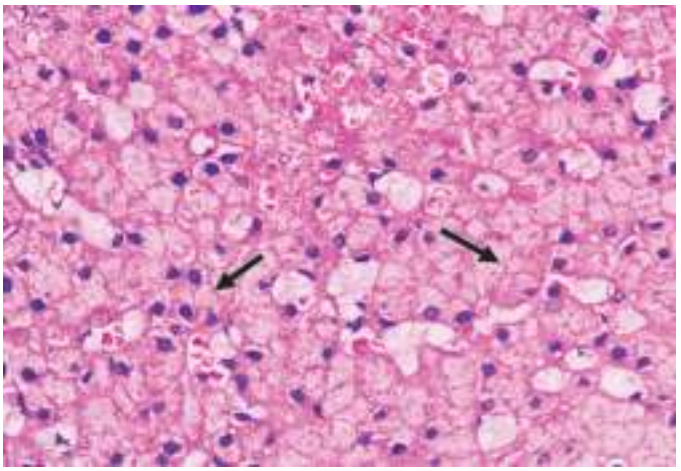


Fig. 21.67: Niemann-Pick disease. Liver Kupffer cells show foamy histiocytes containing sphingomyelin (arrows) (400X).

- In most affected persons, the disorder becomes evident in the first month of life. Patient presents with failure to thrive and hepatomegaly. Disease progresses to hepatic fibrosis leading to cirrhosis and hepatocellular failure.
- Histologic examination of liver biopsy shows pale distended hepatocytes giving mosaic appearance.
- PAS diastase histochemical staining and electron microscopy may help in confirming diagnosis (Fig. 21.66).

NIEMANN-PICK DISEASE

Niemann-Pick disease is a hereditary lysosomal storage disease due to deficiency of sphingomyelinase resulting in accumulation of sphingomyelin in phagocytes (types A and B Niemann-Pick disease). Type C Niemann-Pick disease is caused by defect in a gene involved in cholesterol transport with cholesterol accumulation within phagocytes.

- Diagnostic hallmark of Niemann-Pick disease is presence of foamy histiocytes containing sphingomyelin in liver, spleen, lymph nodes, and skin.
- Child presents with hepatosplenomegaly, anemia, fever and neurologic manifestations. Approximately 50% of the patients develop cherry red spot in the macula similar to that of Tay-Sachs disease. Death occurs by 3 years of age.

Surgical Pathology: Liver in Niemann-Pick Disease

Light Microscopy

Histologic examination of liver biopsy shows foamy histiocytes containing sphingomyelin in Kupffer cells (Fig. 21.67).

GAUCHER'S DISEASE

Gaucher's disease is an autosomal recessive disorder most often seen in persons of European Ashkenazi Jewish lineage. Disease is characterized by accumulation of glucosylceramide (membrane glycosphingolipids) derived from the catabolism of senescent leukocytes, primarily in the lysosomes of macrophages.

The membranes of macrophages are rich in the cerebrosides. Their degradation is blocked by the deficiency of glucocerebrosidase (lysosomal acid β-glucosidase), an intermediate metabolite such as glucosylceramide accumulates.

- **Adult Gaucher's disease (type 1):** It accounts for 80% of cases. Patient presents with hepatosplenomegaly, bone pain and fracture due to erosion of femoral head and other long bones.
- **Infantile Gaucher's disease (type 2):** Infant develops severe CNS involvement associated with fatal outcome before 1 year of age.
- **Juvenile Gaucher's disease (type 3):** Its onset usually occurs in early childhood. There is involvement of brain and visceras. It is less severe than Gaucher's disease (type 2).

Surgical Pathology: Liver in Gaucher's Disease

Light Microscopy

- Histologic examination of liver biopsy shows Gaucher's cells with a distinctive cigarette paper-like cytoplasmic appearance and eccentric nuclei, which are lipid-laden macrophages present along liver sinusoids (Kupffer cells).
- Diagnosis is confirmed by presence of Gaucher's cells, and assay of specific enzymes (chitotriosidase and angiotensin-converting enzyme markers of macrophage proliferation) (Fig. 21.68).

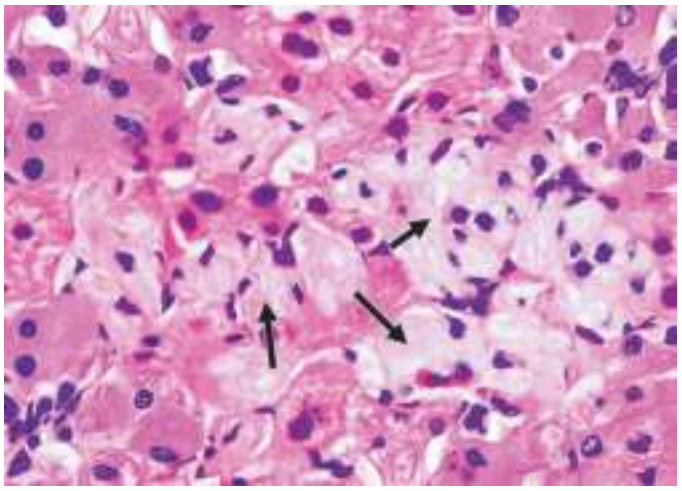


Fig. 21.68: Gaucher's disease. It shows cell containing cigarette paper-like cytoplasmic appearance and eccentric nuclei, which are lipid-laden macrophages (arrows) (400X).

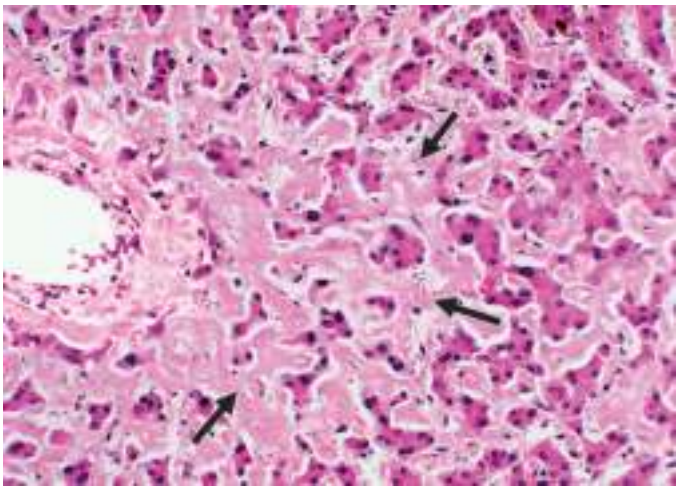


Fig. 21.69A: Amyloid deposit of liver. It shows deposition of amyloid material in the space of Disse between the hepatocytes and vascular sinusoids leading to compression of hepatic cords (arrows) (400X).

AMYLOIDOSIS OF LIVER

Amyloidosis is a well-known complication of chronic inflammatory disorders, i.e. tuberculosis, bronchiectasis, rheumatoid arthritis, or osteomyelitis. It stimulates the production of amyloid from the serum amyloid A (SAA) protein, an acute phase reactant secreted by the liver. The kidneys in 80% of cases, liver, spleen, and adrenals are the most common organs involved.

Surgical Pathology: Amyloidosis of Liver
Gross Morphology
Amyloid liver is usually grossly enlarged (as much as 900 g), pale, and smooth surface, firm in consistency. When sectioned, it shows sharp rigid edges.
Light Microscopy
▪ Amyloid material is initially deposited in the space of Disse between the hepatocytes and vascular sinusoids. As more amyloid accumulates, it compresses the hepatic cords and sinusoids. ▪ The hepatic cords undergo nutritional and pressure atrophy and become displaced or replaced by bands and nodules of amyloid (Fig. 21.69A and B).

Amyloid Stains

Amyloid material is demonstrated by Congo red staining under light microscopy. Pretreatment of tissue with potassium permanganate and stained by Congo red demonstrates amyloid. AL amyloid only but not AA amyloid. Various stains used to demonstrate amyloid material are given in Table 21.69.

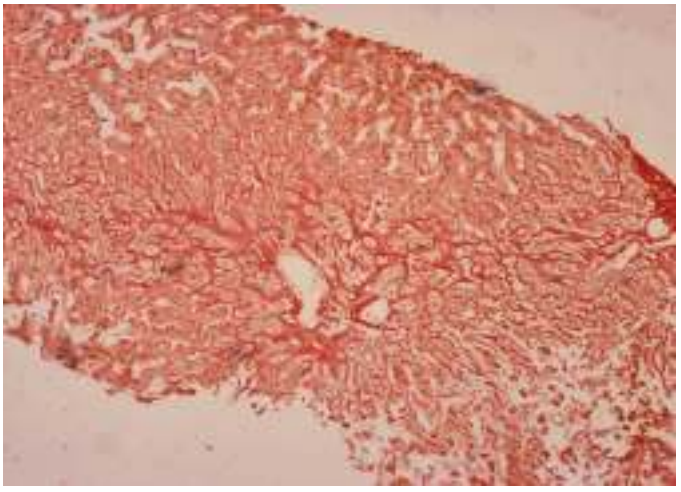


Fig. 21.69B: Congo red stain demonstrates amyloid in liver.

INDIAN CHILDHOOD CIRRHOSIS

Indian childhood cirrhosis most often affects children between 6 months to 5 years in Indian population. Exact etiology is unknown. It is most often seen in Aggarwal, Brahmin, Kayastha and Sikhs. Serum copper and copper binding protein levels are increased in these patients. Child presents with hepatosplenomegaly and jaundice. Child develops portal hypertension with fatal outcome.

Surgical Pathology: Indian Childhood Cirrhosis
Light Microscopy
Liver biopsy shows micronodular cirrhosis, Mallory's hyaline body, and diffuse fibrosis around individual hepatocytes (known as creeping fibrosis), chronic inflammatory cells, bile stasis and ductular proliferation.

Table 21.69 Stains used to demonstrate amyloid material

Amyloid Stain	Color Imparted
Congo red with light microscopy	Pink color
Congo red with polarizing microscopy	Apple green birefringence (color of a Granny Smith apple)
Methyl violet (metachromatic stain)	Bright pink color
Crystal violet (metachromatic stain)	Pink color
Fluorescent stains: Thioflavin T, thioflavin S	Greenish color (immunofluorescence study)
Van Gieson stain	Khaki color
PAS stain	Magenta color

ACUTE FATTY LIVER OF PREGNANCY

Acute fatty liver of pregnancy (AFLP) is a rare life-threatening complication of pregnancy associated with fatal outcome. It usually occurs in late third trimester. Acute hepatocellular failure is associated with microvesicular fatty liver.

- Patient develops severe liver dysfunction with hypoalbuminemia, hypofibrinogenemia, prolonged clotting times and hypocholesterolemia.
- The most critical component of caring for a woman with AFLP is the delivery of her fetus. While the natural history of the disease is improvement within 24–48 hours of delivery.

REYE SYNDROME

Reye syndrome is a potentially fatal pediatric disorder defined as acute non-inflammatory encephalopathy with fatty microvesicular hepatocellular failure. Child presents with vomiting and confusion, rapid progression to coma and fatal outcome. Reye syndrome is associated with aspirin administration in children associated with fatal outcome.

BILE DUCT DISORDERS

BILE DUCT DISORDERS: OVERVIEW

Biliary cirrhosis occurs as primary or secondary disorder. Primary biliary cirrhosis (PBC) is an autoimmune chronic progressive disorder and more frequent than secondary biliary cirrhosis due to extrahepatic biliary obstruction. Ductular proliferation is the single most important histologic feature on liver biopsy for establishing diagnosis of extrahepatic biliary atresia in a neonate. Causes of biliary tract disease are given in Table 21.70.

Table 21.70 Causes of biliary tract disease

Failure of Formation of the Bile Ducts
Biliary atresia
Obstruction or Obliteration of the Bile Ducts
▪ Cholelithiasis ▪ Cholangitis ▪ Primary biliary cirrhosis ▪ Primary sclerosing cholangitis ▪ Post-surgical strictures ▪ Parasitic infection
Compression of the Bile Ducts Outside the Liver
▪ Pancreatic cancer ▪ Pancreatitis ▪ Hepatic cancers

In biliary tract disease, the relative elevation in ALP exceeds the relative elevation in ALT and AST.

PRIMARY BILIARY CIRRHOSIS

Primary biliary cirrhosis, a nonsuppurative destructive cholangitis, occurs due to chronic destruction of intrahepatic bile ducts in the portal tracts. It evolves through ductal lesions, scarring, and eventually cirrhosis.

- The hallmark of PBC is the presence of antimitochondrial antibodies in serum. These autoantibodies recognize epitopes associated with the mitochondrial pyruvate dehydrogenase complex.
- Primary biliary cirrhosis occurs more often in women than men (10:1 female predominance) between 40 and 50 years of age.

Predisposing Factors

Majority of patients are associated with autoimmune disorder such as thyroiditis, rheumatoid arthritis, scleroderma, Sjögren syndrome, or systemic lupus erythematosus.

Pathogenesis

Intrahepatic bile duct destruction is caused by circulating antimitochondrial antibodies in >95% of patients. These antimitochondrial antibodies play no role in the pathogenesis of the disease. The complement system is chronically activated.

Clinical Features

Patient presents with itching of skin, jaundice, hepatosplenomegaly, passage of clay-colored stool and dark-colored urine. Serum cholesterol level is raised secondary to severe obstructive jaundice. Hypercholesterolemia leads to cutaneous xanthomas formation.

Surgical Pathology: Primary Biliary Cirrhosis

Light Microscopy

- Histologic examination of liver biopsy shows nonsuppurative inflammation of interlobular ducts (florid duct lesions). The cells surrounding bile duct destruction site include predominant suppressor/cytotoxic CD8+ T cells, macrophages, B cells and plasma cells in some portal tracts.
- Bile duct destruction is mediated by these suppressor/cytotoxic CD8+ T cells.
- There may be presence of epithelioid cell granulomas. Proliferation of bile ductules within portal tracts is common and may be florid.
- It is worth mentioning that macrophages and B cells cause periductal inflammation but do not mediate epithelial cytotoxicity (Fig. 21.70).

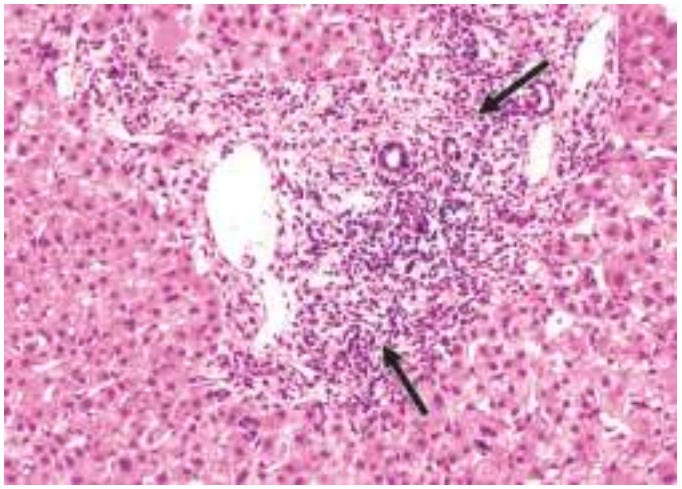


Fig. 21.70: Primary biliary cirrhosis. This is a case of primary biliary cirrhosis, a rare autoimmune disease (mostly of middle-aged women) that is characterized by destruction of bile ductules within the triads of the liver. Antimitochondrial antibody can be detected in serum. Seen here in a portal tract is an intense chronic inflammatory infiltrate with loss of bile ductules. Micronodular cirrhosis ensues (arrows) (400X).

Table 21.71 Grading and staging of primary biliary cirrhosis

Stages	Scheuer System	Ludwig System
Stage 1	▪ Florid duct lesion ▪ Bile duct damage ▪ Portal inflammation	*Portal stage:* Portal inflammation
Stage 2	▪ Ductular proliferation (reaction) ▪ Expanded portal tracts ▪ Proliferated ductules ▪ Piecemeal necrosis	*Periportal stage:* Periportal inflammation ± and piecemeal necrosis
Stage 3	▪ Scarring ▪ Bridging fibrosis ▪ Loss of ducts	*Septal stage:* Fibrous septa and bridging necrosis
Stage 4	Nodular cirrhosis	Cirrhosis

Laboratory Findings

Serum conjugated bilirubin, alkaline phosphatase and cholesterol levels are increased.

Diagnostic Tool

Serum antimitochondrial antibodies (IgM especially M2) are analyzed in the diagnosis of primary biliary cirrhosis.

Prognosis

Patient with primary biliary cirrhosis may develop micronodular cirrhosis. Hepatocellular carcinoma does not develop. Most common causes of death include liver failure and variceal bleeding.

Grading and Staging

Grading and staging of primary biliary cirrhosis is given in Table 21.71.

SECONDARY BILIARY CIRRHOSIS

Secondary biliary cirrhosis is caused by extrahepatic biliary obstruction due to passage of stone in common bile duct or carcinoma head of pancreas.

- Persistent extrahepatic obstruction increases the pressure within intrahepatic bile ducts and cholangioles resulting in ductal injury.
- Lodgment of stones in the common bile duct leads to obstructive jaundice, cholangitis, and acute pancreatitis. Men and women are equally affected.

Clinical Features

Patient presents with pruritus, jaundice, malaise, diarrhea, steatorrhea, weight loss, dark urine, clay-colored stool and hepatosplenomegaly.

Laboratory Findings

Serum conjugated bilirubin, cholesterol and alkaline phosphatase levels are raised. Urine shows bile salts and bile pigments.

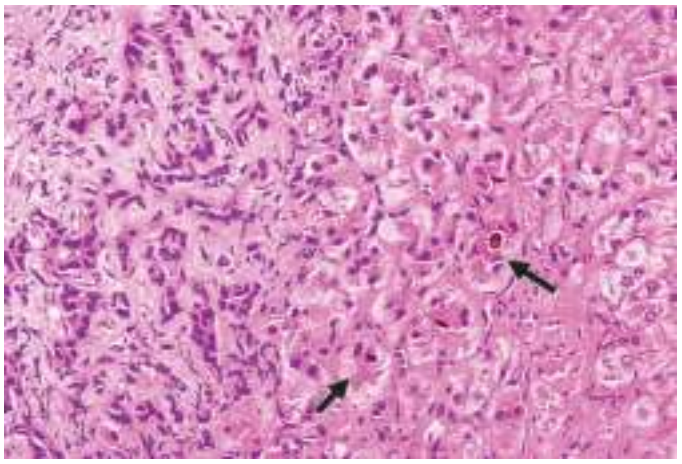


Fig. 21.71: Extrahepatic biliary obstruction. It shows bile stasis forming bile lakes as a result of accumulation of bile within hepatic parenchyma. Mononuclear inflammation is seen in ducts and around ducts (arrows) (400X).

Surgical Pathology: Secondary Biliary Cirrhosis

Light Microscopy

- Histologic examination of liver biopsy shows bile stasis and bile lakes, accumulations of bile within hepatic parenchyma.
- Inflammation is seen in ducts and periductal region. Healing occurs by formation of fibrous tissue (Fig. 21.71).

Complications

Complications of secondary biliary cirrhosis include ascending cholangitis and bacterial inflammation of the intrahepatic bile ducts. Differences between intrahepatic and extrahepatic biliary obstruction are given in Table 21.72.

PRIMARY SCLEROSING CHOLANGITIS

Primary sclerosing cholangitis (PSC) is rare pathologic progressive fibro-obliterative disorder of unknown etiology. It primarily affects young men between 30 and 50 years of age group.

Men are more affected than women in 2:1 ratio. Approximately 70% of patients with PSC have long-standing ulcerative colitis indicating autoimmune disorder. It is associated with HLAB-8, DR-3 and DQ-2.

Pathology

Primary sclerosing cholangitis is characterized by inflammation, onion skin fibrosis and obliteration of intrahepatic and extrahepatic bile ducts, with dilation of preserved segments, which eventually develops into biliary cirrhosis. Cholangiocarcinoma is a late complication in these patients.

Imaging Techniques

Definite diagnosis of primary sclerosing cholangitis rests on imaging by MRCP and ERCP. Radiograph shows beaded biliary tree, representing sporadic strictures.

Clinical Features

Patient presents with pruritus, jaundice, malaise, hepatosplenomegaly, passage of clay-colored stool and dark-colored urine. Complications of primary sclerosing cholangitis are secondary biliary cirrhosis, chronic pancreatitis, hepatocellular carcinoma and cholangiocarcinoma in 10% of cases.

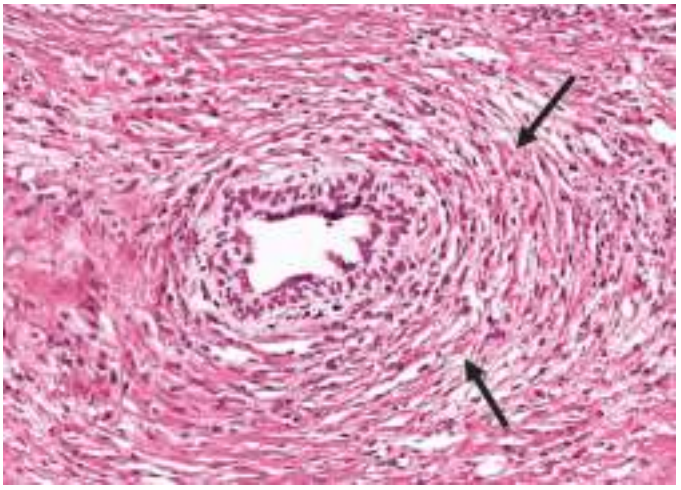


Fig. 21.72: Primary sclerosing cholangitis. Microscopically, this bile duct in a case of sclerosing cholangitis is surrounded by marked collagenous connective tissue deposition (arrows) (100X).

Table 21.72 Differences between intrahepatic and extrahepatic biliary obstruction

Characteristics	Intrahepatic Biliary Obstruction	Extrahepatic Biliary Obstruction
Bile pigment	Bile pigment is present in hepatocytes and Kupffer cells	Bile plugs present in bile ducts and ductules result in rupture of bile canaliculi
Cholestasis severity	Mild degree	Bile lakes present
PMN cells infiltration around bile ducts and canaliculi	Absent	Present
Feathery degeneration of hepatocytes	Marked	Not much marked

Laboratory Findings

Serum conjugated bilirubin, cholesterol, and alkaline phosphatase, IgM levels are raised.

Surgical Pathology: Primary Sclerosing Cholangitis

Light Microscopy

- Extrahepatic duct biopsy in primary sclerosing cholangitis is performed to rule out malignancy. Histologic examination reveals inflammation, onion skin fibrosis and obliteration of bile ducts.
- Liver biopsy exaination shows concentric fibrosis around ducts in onion skin appearance, segmental stenosis of extrahepatic and intrahepatic bile ducts.
- Chronic cholestasis is typical finding (Fig. 21.72).
- Distinction between primary biliary cirrhosis and primary sclerosing cholangitis is given in Table 21.73.

Grading and Staging

Grading and staging of primary sclerosing cholangitis is given in Table 21.74.

Table 21.73 Distinction between primary biliary cirrhosis and primary sclerosing cholangitis

Feature	Primary Biliary Cirrhosis	Primary Sclerosing Cholangitis
Epidemiology	Female predominance, age 40–50 years	Male predominance, age 30–50 years
Associated antibody	Anti-mitochondrial antibody (IgM)	p-ANCA
Histologic features	Chronic destruction and scarring of intrahepatic bile ducts	Concentric fibrosis around ducts within portal tracts resulting in chronic cholestasis
Clinical features	Jaundice, pruritus, hepatomegaly, clay-colored stool	Jaundice, pruritus, fever and chills; and 70% of cases have ulcerative colitis
Complication	Micronodular cirrhosis	Secondary biliary cirrhosis, chronic pancreatitis, hepatocellular carcinoma, cholangiocarcinoma (10%)

Table 21.74 Grading and staging of primary sclerosing cholangitis

Stages	Scheuer System	Ludwig System
Stage 1	Portal stage	Portal inflammation or bile duct abnormalities
Stage 2	Periportal stage	Periportal fibrosis or enlargement of portal tract
Stage 3	Septal stage	Fibrous septal or bridging necrosis
Stage 4	Cirrhotic stage	Cirrhosis

VASCULAR DISORDERS OF LIVER

Vascular disorders of liver occur due to impairment of hepatic venous drainage, arterial underperfusion and miscellaneous intrahepatic vascular conditions.

IMPAIRMENT OF HEPATIC VENOUS DRAINAGE (CHRONIC VENOUS CONGESTION OF LIVER)

In long-standing right-sided heart failure, increased venous pressure leads to chronic venous congestion of liver due to impairment of hepatic veins drainage into inferior vena cava. Pitting edema is seen in lower extremities.

Chronic venous congestion of the liver occurs due to right-sided heart failure, chronic obstructive pulmonary disease (COPD) and thrombosis in hepatic vein or inferior vena cava.

Surgical Pathology: Chronic Venous Congestion of Liver

Gross Morphology

- Liver is enlarged, tense, and cyanotic with rounded edges.
- Cut surface of the liver can assume a variegated red and yellow appearance referred to as nutmeg liver, with dark red congested centrilobular areas alternating with pale unaffected peripheral portions of the lobules.
- Cut surface resembles a cross section of a nutmeg, and so-called as nutmeg liver.

Light Microscopy

Macrophages contain iron pigment. Bile stasis within canaliculi may also be seen. Acinar pattern of liver becomes exaggerated due to these changes. Post-congestion fibrosis may produce finely nodular liver known as cardiac cirrhosis (Fig. 21.73).

- **Dark areas of the nutmeg appearance:** These are represented by the congested and dilated venous radicles and sinusoids of zone 3 region. These changes cause pressure atrophy of the centrilobular liver cords. In severe cases, the hepatocytes show necrosis.
- **Pale hypoxic areas of the liver:** These are represented by the hypoxic fatty change in hepatocytes. Hepatocytes in mid-zone show fatty change. Hepatocytes are normal in the peripheral zones. Left heart failure leads to decreased perfusion resulting in centrilobular ischemic necrosis of hepatocytes.
- **Centrilobular hemorrhagic necrosis:** Combination of decreased perfusion and congestion causes centrilobular hemorrhagic necrosis. Liver shows variegated mottled appearance. There is sharp demarcation of viable periportal necrotic centrilobular hepatocytes.
- **Cardiac cirrhosis of liver:** Long-standing chronic passive congestion of liver leads to bridging fibrosis in extreme cases resulting in cardiac cirrhosis of liver. With the progression of fibrosis around portal region with bridging between adjacent hepatic portal region (Figs 21.74 and 21.75).

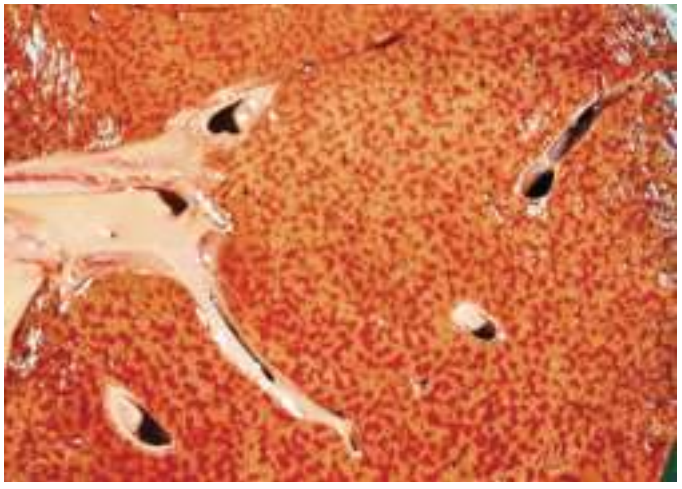


Fig. 21.73: Chronic venous congestion of liver. Here is an example of a nutmeg liver seen with chronic passive congestion of the liver. Note the dark red congested regions that represent accumulation of RBCs in centrilobular regions.

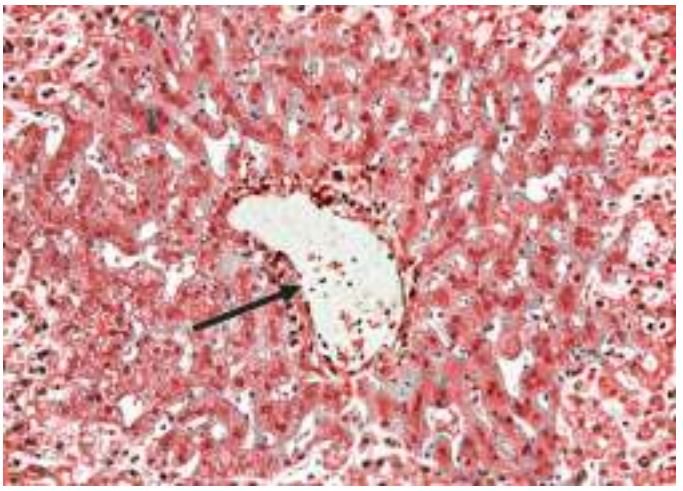


Fig. 21.74: Chronic venous congestion of liver. Microscopically, the nutmeg pattern results from congestion around the central veins, as seen here. This is usually due to a right-sided heart failure (arrow).

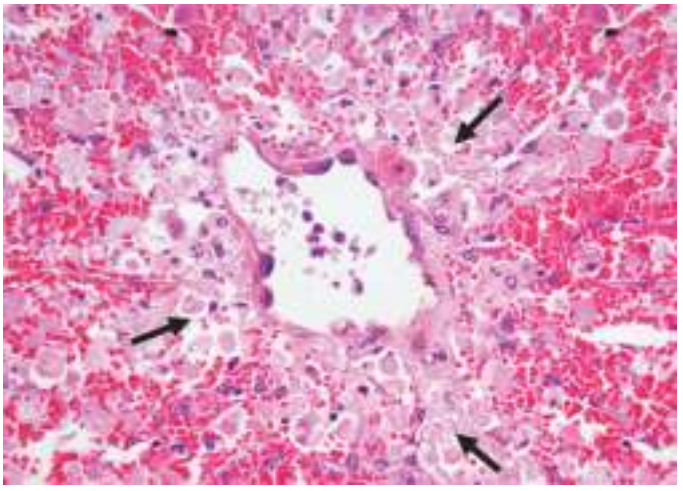


Fig. 21.75: Cardiac cirrhosis. Chronic venous congestion of liver continues for a long time, a condition is called cardiac cirrhosis may develop in which there fibrosis is bridging between central zonal regions, as shown below, so that the portal tracts appear to be in the center of the reorganized lobule. This process is best termed cardiac sclerosis because, unlike a true cirrhosis, there is minimal nodular regeneration (arrows) (400X).

BUDD-CHIARI SYNDROME

Budd-Chiari syndrome occurs due to thrombotic occlusion in major hepatic veins at their origin or inferior vena cava distal to its junction with the hepatic veins and is characterized by classic triad: hepato-megaly, abdominal pain and ascites. It most often occurs due to thrombosis or invasion of the hepatic veins. Etiology of large hepatic vein thrombosis is given in Table 21.75.

- Classic clinical manifestations in a case of Budd-Chiari syndrome are marked hepatomegaly in caudate lobe of liver demonstrated by imaging techniques. Lumbar veins in posterior region show visible dilatation. Patient improves after mesocaval shunt.
- Patient presents with abdominal pain, mild jaundice, ascites, and eventual acute hepatocellular failure and death. Clinical features and their frequency in Budd-Chiari syndrome are given in Table 21.76.

Surgical Pathology: Budd-Chiari Syndrome
Gross Morphology
Thrombus in the hepatic veins causes obstruction to blood flow to liver leading to venous congestion of the liver (Fig. 21.76).

Light Microscopy

- Histologic examination of liver biopsy shows severe centrilobular necrosis and hemorrhage.
- The sinusoids of the central zone are dilated and packed with erythrocytes showing chronic passive congestion as a result of localized obstruction to venous drainage.
- Rim of hepatocytes in zone 1 shows survival.

SINUSOIDAL OBSTRUCTION SYNDROME (VENO-OCCLUSIVE DISORDER)

Veno-occlusive disorder, also known as sinusoidal obstruction syndrome, is characterized by non-thrombotic luminal narrowing affecting hepatic vein radicles and sublobular veins. Narrowing of these blood vessels occurs due to growth of a new layer of subendothelial connective tissue. Patient presents with abdominal pain, ascites and hepatosplenomegaly similar to Budd-Chiari syndrome. Predisposing factors for veno-occlusive disorder are described as under:

- Poisoning with pyrrolizidine alkaloids occurs either due to contamination of flour or ingestion of products derived from alkaloid-containing plants such as *Crotalaria* and *Senecio* species.
- Irradiation of liver
- Treatment with anticancer drugs
- Bone marrow transplantation within 4 weeks in 20% of cases due to high doses of chemotherapeutic agents (cyclophosphamide) and total body irradiation
- Graft-versus-host reaction

Table 21.75 Etiology of large hepatic vein thrombosis

Primary myeloproliferative disorders (polycythemia vera)
Paroxysmal nocturnal hemoglobinuria
Acute promyelocytic leukemia
Protein C deficiency
Protein S deficiency
Antithrombin III deficiency
Antiphospholipid autoantibodies (lupus anticoagulant)
Hepatocellular carcinoma
Behçet's disease
Allergic vasculitis
Oral contraceptives
Pregnancy and postpartum state
Certain solid tumors
Idiopathic (10% cases)

Table 21.76 Budd-Chiari syndrome, clinical features and their frequency

Clinical Manifestations	Frequency
Ascites	96%
Hepatomegaly	90%
Abdominal pain	80%
Splenomegaly	64%
Edema	46%
Jaundice	44%
Fever	40%
Hepatic encephalopathy	22%
Gastrointestinal bleeding	14%

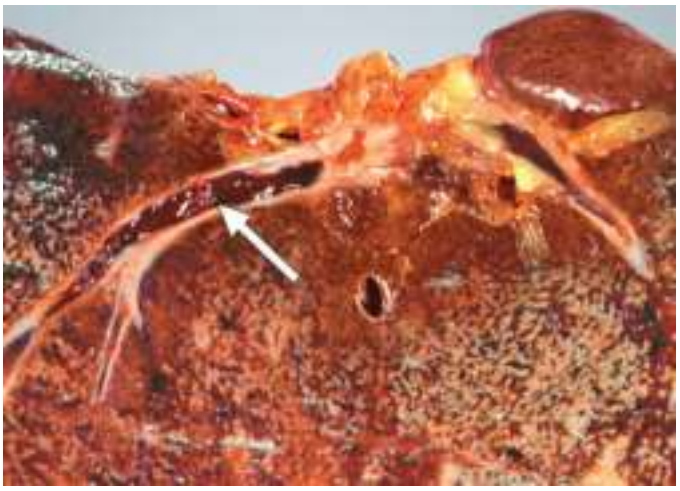


Fig. 21.76: Budd-Chiari syndrome. The liver has been sliced coronally, with the caudate lobe in the middle. Thrombus can be seen in the hepatic veins. This caused obstruction, which resulted in venous congestion of the liver, most marked in the caudate lobe (arrow).

NONCIRRHOTIC PORTAL HYPERTENSION

Noncirrhotic portal hypertension is also known as idiopathic portal hypertension, which usually occurs as a result of presinusoidal causes. It may also occur due to hepatic causes such as sarcoidosis, schistosomiasis or congenital hepatic fibrosis. Clinically liver functions are maintained. Noncirrhotic portal hypertension can occur in the settings of following:

- Nodular regenerative hyperplasia of liver occurs due to aberrant blood flow hepatic lobules. Liver parenchyma is diffusely nodular without fibrosis. Reticulin stain highlights regenerative hepatocytes outlined by atrophic cords.
- Hepatoportal sclerosis due to intrahepatic portal vein injury occurs due to intrahepatic portal vein injury and scarring. Portal vein remains initially patent, that later on shows marked thickening with luminal narrowing.

- Hemorrhagic (red) infarcts in liver are rare due to its dual blood supply, e.g. portal vein and hepatic arteries. Liver receives blood from hepatic arteries and portal vein. Interruption of blood supply to liver due to blockage in hepatic artery or portal vein results in hemorrhagic (red) infarct in liver. Hemorrhagic (red) infarct region receives constant blood supply from normal spared vessel of liver, allows RBCs to diffuse through necrotic tissue.
- Hemorrhagic (red) infarct in liver occurs due to embolism, sepsis, polyarteritis nodosa, disseminated intravascular coagulation and Budd-Chiari syndrome (portal vein thrombosis).

MISCELLANEOUS INTRAHEPATIC VASCULAR CONDITIONS

PELIOSIS HEPATITIS

Peliosis hepatitis is reversible sinusoidal dilatation of liver and of unknown etiology and characterized by blood-filled cystic spaces partly lined by endothelial cells.

- Peliosis hepatitis is associated with tuberculosis, cancers, AIDS, post-transplantation immunodeficiency, therapeutic use of anabolic hormones, oral contraceptives and danazol.
- Peliosis hepatitis has also been observed in patients to control renal transplant rejection with azathioprine.
- Patient presents with hepatomegaly and jaundice.

Surgical Pathology: Peliosis Hepatitis
Light Microscopy
Histologic examination of liver shows numerous blood-filled spaces partly lined by endothelial cells.

RENDU-OSLER-WEBER DISEASE

Rendu-Osler-Weber disease is an autosomal dominant hemorrhagic telangiectasia characterized by the presence of numerous blood-filled spaces affecting many tissues.

Liver lesions in hereditary hemorrhagic telangiectasia type 2 occur due to mutation of ALK gene located on chromosome 12. Patient presents with noise bleed.

Surgical Pathology: Rendu-Osler-Weber Disease
Gross Morphology
Liver contains subcapsular enlarged blood vessels.
Light Microscopy
Hepatic lesions are composed of thick-walled veins, scattered dilated sinusoids and abnormal-sized portal tracts.

NEOPLASTIC DISORDERS OF LIVER

Current 2019 WHO classification of primary hepatic neoplasms and non-neoplastic lesions are given in Table 21.77. Most common type of metastases from various organs in noncirrhotic liver. Metastases most often present as multiple nodules in contrast to the single nodules of primary tumors. Metastases to a cirrhotic liver are very uncommon.

Immunohistochemistry technique plays an important role in differential diagnosis of primary liver tumors and tumor-like lesions; and metastases in

Table 21.77 Current 2019 WHO classification of primary hepatic neoplasms and non-neoplastic lesions

Cell Origin	Benign Tumors	Malignant Tumors
Epithelial origin tumors		
Hepatocellular origin	▪ Hepatocellular adenoma ▪ Focal nodular hyperplasia ▪ Nodular regenerative hyperplasia ▪ Macrogenerative nodule ▪ Dysplastic nodule ▪ Compensatory lobar hyperplasia ▪ Accessory lobe	▪ Hepatocellular carcinoma ▪ Fibrolamellar hepatocellular carcinoma ▪ Combined hepatocellular carcinoma and cholangiocarcinoma ▪ Hepatoblastoma epithelial type
Biliary epithelial origin	▪ Bile duct hamartoma (von Meyenburg complex) ▪ Bile duct adenoma	▪ Cholangiocarcinoma ▪ Intraductal papillary biliary neoplasia

Contd...

Table 21.77 Current 2019 WHO classification of primary hepatic neoplasms and non-neoplastic lesions (*Contd...*)

Cell Origin	Benign Tumors	Malignant Tumors
	▪ Biliary cysts (non-neoplastic) • Solitary cyst • Polycystic disease • Caroli disease • Multiple hilar cysts ▪ Biliary cystadenoma • Mucinous biliary cystadenoma (+/– mesenchymal stroma) • Serous biliary cystadenoma	▪ Adenosquamous carcinoma ▪ Mucoepidermoid carcinoma ▪ Squamous cell carcinoma ▪ Combined cholangiocarcinoma and neuroendocrine tumor ▪ Biliary cystadenocarcinoma
Mesenchymal origin tumors		
Vascular tumors	▪ Hemangioma ▪ Infantile hemangioma ▪ Hemangiomatosis ▪ Lymphangioma ▪ Hereditary hemorrhagic telangiectasia ▪ Peliosis hepatic	▪ Angiosarcoma ▪ Epithelioid hemangioendothelioma ▪ Kaposi sarcoma
Adipocytic tumors	▪ Angiolipoma (and related tumors) ▪ Pseudolipoma ▪ Focal hepatocellular fatty change	Liposarcoma
Other mesenchymal tumors	▪ Solitary fibrous tumor ▪ Inflammatory myofibroblastic tumor (pseudotumor) ▪ Leiomyoma	▪ Embryonal sarcoma (undifferentiated) ▪ Rhabdomyosarcoma ▪ Fibrosarcoma ▪ Undifferentiated pleomorphic sarcoma (malignant fibrous histiocytoma) ▪ Leiomyosarcoma ▪ Osteosarcoma
Mixed tumors with epithelial and mesenchymal components		
Mixed tumors with epithelial and mesenchymal components	▪ Necrotic/fibrous nodule ▪ Heterotopia (adrenal, pancreas, spleen) ▪ Endometrial cyst (endometrioma) ▪ Benign nerve sheath tumors ▪ Sarcoid pseudotumor (sarcoidoma) ▪ Abscesses ▪ Ciliated foregut cyst ▪ Alimentary duplication cyst ▪ Pseudocysts (pancreatic, traumatic)	▪ Malignant schwannoma ▪ Germ cell tumors (teratoma, yolk sac tumor) ▪ Malignant rhabdoid tumor ▪ Primary lymphoma ▪ Primary neuroendocrine tumor ▪ Pheochromocytoma ▪ Solid and cystic tumor
Other tumors and tumor-like masses		
Other tumors and tumor-like masses	▪ Necrotic/fibrous nodule ▪ Heterotopia (adrenal, pancreas, spleen) ▪ Endometrial cyst (endometrioma) ▪ Benign nerve sheath tumors ▪ Sarcoid pseudotumor (sarcoidoma)* ▪ Abscesses ▪ Parasitic cysts ▪ Ciliated foregut cyst ▪ Alimentary duplication cyst ▪ Pseudocysts (pancreatic, traumatic)	▪ Malignant schwannoma ▪ Germ cell tumors (teratoma, yolk sac tumor) ▪ Malignant rhabdoid tumor ▪ Primary lymphoma ▪ Primary neuroendocrine tumor ▪ Pheochromocytoma ▪ Solid and cystic tumor

***** *Sarcoid pseudotumor (sarcoidoma) is borderline tumor, however placed in benign category.*
Liver metastases occur from epithelial origin carcinomas (breast, lung, stomach, colon, rectum, pancreas, prostate, endometrium, thyroid and kidney) and mesenchymal origin sarcomas (angiosarcomas. rhabdomyosarcoma) and round cell tumors (Wilm's tumors, Ewing sarcoma, neuroblastoma and bronchopulmonary neuroendocrine tumors).

liver arising from epithelial and mesenchymal tumors. Panel of immunohistochemical markers applied in conventional and fibrolamellar variants of hepatocellular carcinoma, hepatoblastoma and cholangiocarcinoma is given in Table 21.78.

PRIMARY BENIGN TUMORS

HEPATOCELLULAR ADENOMA

Hepatocellular adenoma is a rare tumor in young women taking oral contraceptives. It usually occurs as a solitary, sharply demarcated encapsulated mass up to 40 cm in diameter and 3 kg in weight which is located anywhere in liver beneath capsule. Subtypes of hepatocellular adenoma are given in Table 21.79.

Complications

Subcapsular hepatocellular adenoma has a tendency to rupture resulting in severe bleeding in peritoneal cavity especially during pregnancy which induces hypovolemic shock that requires emergency treatment.

Pathogenesis

Hepatocellular adenoma has three variants. Each has risk of development of malignant transformation.

Table 21.78 Panel of immunohistochemical markers applied in conventional and fibrolamellar variants of hepatocellular carcinoma, hepatoblastoma and cholangiocarcinoma

Immunohistochemical Markers	Expression
Hepatocellular origin	
Conventional hepatocellular carcinoma	
Hepatocyte paraffin 1 (Hep Par 1)	Positive (95%)
p-CEA	Positive (80%), highly specific canalicular marker
AFP (α-fetoproteins)	Positive (30–60%)
CD10	Positive (canalicular)
Villin	Positive (canalicular)
CK20	Positive (up to 20%)
CK AE1/AE3 (pan-CK)	Positive
CAM 15.2 (LMWCK) pan-CK	Positive (5–20%)
CK903 (HMWCK)	Positive
Glypican 3	Positive (promising new marker of hepatocellular differentiation)
Arginase 1	Positive (new marker of hepatocellular differentiation)
α_1-Antitrypsin	Positive (up to 50%)
TTF-1 (thyroid transcription factor 1)	Positive (cytoplasmic staining)
Cytokeratin 7 (CK7)	Negative
EMA (epithelial membrane antigen)	Negative
MOC-31	Negative
Mucicarmine	Negative
Fibrolamellar hepatocellular carcinoma	
Neurotensin	Positive
AFP (α-fetoproteins)	Negative
Hepatoblastoma	
AFP (α-fetoproteins)	Positive
Hep Par 1 (hepatocyte paraffin 1)	Positive
DLK (delta-like protein)	Positive
hCG (human chorionic gonadotropin)	Positive
Cytokeratins (CKs)	Positive
CEA (carcinoembryonic antigen)	Positive
Glypican 3	Positive
EMA (epithelial membrane antigen)	Positive
Vimentin	Positive
Hepatic small ducts or large extrahepatic bile ducts	
Cholangiocarcinoma	
CK19 (cytokeratin 19)	Positive (70–100%)
m-CEA (carcinoembryonic antigen monoclonal)	Positive
p-CEA	Positive (cytoplasmic staining)
CK7 (cytokeratin 7)	Positive
CK20 (cytokeratin 20)	Positive
CK AE1/AE3 (pan-CK)	Positive
CAM 15.2 (LMWCK) pan-CK	Positive
CK903 (HMWCK)	Positive
Hep Par 1 (hepatocyte paraffin 1)	Negative
AFP (α-fetoproteins)	Negative/positive
CD10	Negative
Villin	Negative
Glypican 3	Negative
Arginase 1	Negative

Contd...

Table 21.79 Subtypes of hepatocellular adenoma

Subtype	Molecular Genetics	Histologic Features
HNF1α (hepatic nuclear factor 1α) inactivated subtype	Biallelic inactivation of HNF1α	Liver demonstrates marked fatty change
β-Catenin activated subtype	CTNNB1 mutation	Hepatocytes demonstrate nuclear atypia
Inflammatory subtype	In-frame deletions of gpl 190 (60%) and JAK/STAT pathway activation	Sinusoidal dilatation; patchy inflammation; and variable number of ductules
Unclassified subtype	No specific genetic abnormality	No specific histologic features

- **HNF-α inactivated mutation-linked hepatocellular adenoma:** HNF-α encodes for transcriptional factor. Approximately 80% of these tumors show somatic mutations of HNF-α. Heterozygous germline mutations of HNF-α are responsible for autosomal dominant maturity-onset diabetes of the young type 3 (MODY-3).
- **β-Catenin activated mutation-linked hepatocellular adenoma:** Mutation of β-catenin is associated with hepatocellular adenoma undergoing malignant transformation.
- **Inflammatory hepatocellular adenoma:** It is associated with small risk of development of malignant transformation. It is associated with gp130 a co-receptor for IL-6, that leads to consecutive JAK-STAT signaling and overexpression of acute phase reactant.

Surgical Pathology: Hepatocellular Adenoma

Gross Morphology

- Hepatocellular adenoma is well circumscribed tumor. The cut surface demonstrates greenish bile staining, indicating that it is of hepatocyte origin.
- Occasionally, tumor has pseudopods projecting into liver substance mimicking hepatocellular carcinoma (Fig. 21.77).

Light Microscopy

- Tumor is composed of uniform population of hepatocytes with a normal nucleus to cytoplasm ratio.
- Tumor cells are arranged in one- to three-cell-thick plates with intact reticulin framework similar to that in normal liver. The limiting plates are usually more irregular and nonlinear than in the normal liver. Portal tracts are absent.
- The presence of arterioles without accompanying bile ducts and surrounded by scant connective tissue is a characteristic feature of hepatocellular adenoma with naked arterioles (Fig. 21.78).

HEPATIC HEMANGIOMA

Hepatic hemangioma is the most common benign tumor of liver. Occasionally, spontaneous bleeding and rupture and thrombocytopenia due to sequestration of platelets.



Fig. 21.77: Hepatocellular adenoma. The cut surface of the hepatic adenoma demonstrates greenish bile staining, indicating that it is of hepatocyte origin (no other neoplasm could accumulate bile pigment). Such neoplasms are rare. One setting for its occurrence is young women taking oral contraceptives (arrow).

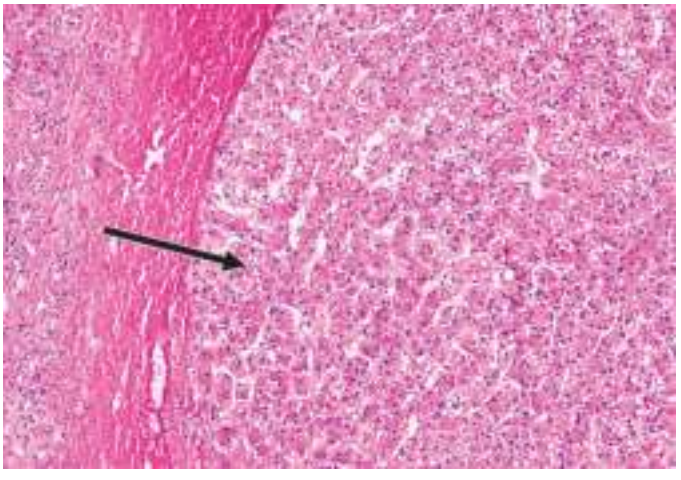


Fig. 21.78: Hepatocellular adenoma. Normal liver tissue with a portal tract is seen on the left. The hepatic adenoma is on the right and is composed of tumor cells that closely resemble normal hepatocytes, but the neoplastic liver tissue is disorganized hepatocyte cords and does not contain normal lobular architecture (arrow).

Surgical Pathology: Hepatic Hemangioma
Gross Morphology
Tumor is most often <1 cm located just beneath liver capsule. Cut surface is brown (Fig. 21.79).
Light Microscopy
Hepatic hemangioma is composed of vascular channels lined by endothelial cells (Fig. 21.80).

HEPATIC LYMPHANGIOMA

Hepatic lymphangioma occurs in infants and children. Liver involvement occurs as a part of multicentric process affecting other organs.



Fig. 21.79: Hepatic hemangioma. This is benign hemangioma of the liver just beneath the capsule. Perhaps one person in 50 has such a neoplasm, which is typically just an incidental finding, since most are 1 cm or less. They can sometimes be multiple (arrow).

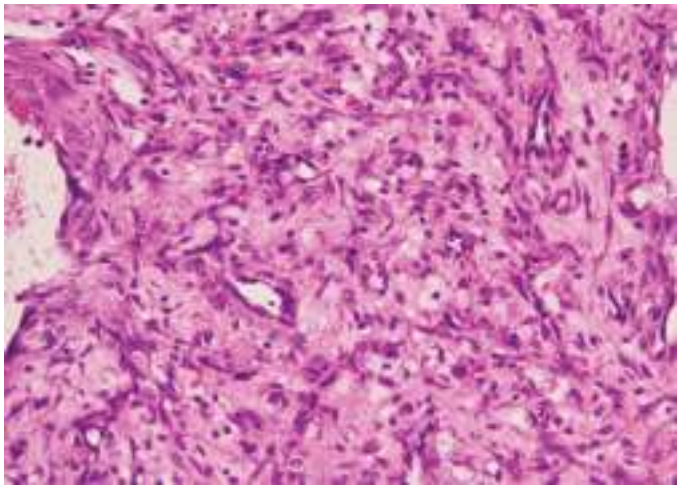


Fig. 21.80: Hepatic hemangioma. It shows vascular channels lined by endothelial cells (100X).

MULTIPLE BILIARY HAMARTOMAS (VON MEYENBURG COMPLEX)

Multiple biliary hamartomas are asymptomatic lesions also known as von Meyenburg complex, which show irregularly dilated bile ducts. This disorder is characterized by multiple bile duct hamartomas and biliary microhamartomas <5 mm.

It is important to distinguish multiple biliary hamartomas from multiple hepatic metastases. Women are more affected than men.

Predisposing Factors

Multiple biliary hamartomas are attributed to ischemia, inflammation or genetic abnormalities. These are known to be associated with polycystic liver disease and autosomal polycystic disease of kidney. Several reports suggest that these patients may develop hepatocellular carcinoma and cholangiocarcinoma.

Surgical Pathology: Multiple Biliary Hamartomas
Gross Morphology
▪ Liver surface shows creamy nodules in multiple biliary hamartomas mimicking secondary deposits from colon carcinoma. ▪ Histologic examination of the nodule shows proliferation of bile ducts (Fig. 21.81).
Light Microscopy
Multiple biliary hamartomas are composed of small disorganized clusters of dilated cystic bile ducts lined by columnar cells enclosed within fibrocollagenous stroma (Fig. 21.82).



Fig. 21.81: Multiple bile duct hamartomas. Liver surface shows creamy nodules consisting of proliferation of bile ducts. During surgery for bowel cancer, surgeon may consider metastatic deposits in liver. Histologic examination of one or two nodules confirms diagnosis that these are not secondary deposits (arrows).

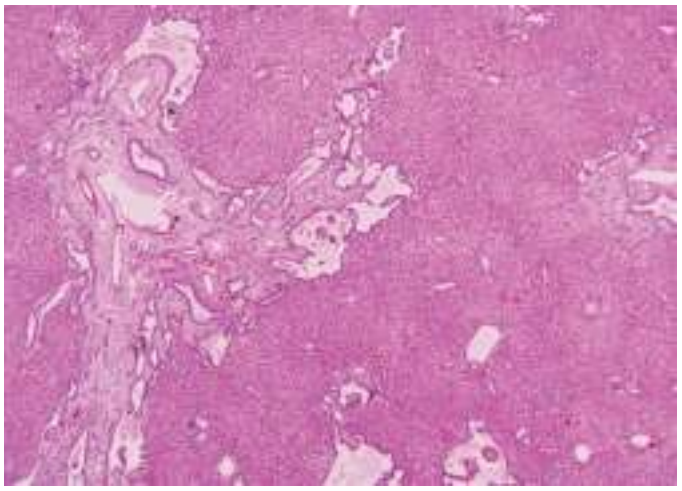


Fig. 21.82: Multiple bile duct hamartomas are also known as von Meyenburg complex. Hamartoma is composed of small disorganized clusters of dilated cystic bile ducts lined by columnar cells enclosed within fibrocollagenous stroma (100X).

HEPATOCELLULAR NODULES

Liver cirrhosis is characterized by irreversible remodeling of the hepatic architecture with advanced bridging fibrosis and a spectrum of hepatocellular nodules observed under light microscopy.

- The hepatocellular nodules are classified into various categories: (a) regenerative lesions (e.g. regenerative nodules, lobar hyperplasia, segmental hyperplasia, focal nodular hyperplasia), and (b) dysplastic or neoplastic lesions (e.g. dysplastic foci, dysplastic nodules, hepatocellular carcinoma).
- Cirrhosis-associated hepatocellular nodules are formed due to proliferation of hepatocytes and their supporting stroma in response to hepatocellular injury. Majority are benign regenerative nodules; however, regenerative nodules may progress along a well-described carcinogenic pathway to become dysplastic nodules or hepatocellular carcinomas.
- The differentiation of these hepatocellular lesions is important because regenerative nodules are benign in nature. On the other hand, dysplastic nodules are premalignant and neoplastic nodules are malignant in nature, respectively. However, their accurate characterization may be difficult even at histopathologic examination.
- Differential diagnosis of cirrhosis associated hepatocellular nodules may be facilitated by comparing the clinical, gross morphologic and histologic findings with radiologic imaging features; in particular, size of nodules, vascularity, hepatocellular function, Kupffer cell densities assessed at magnetic resonance imaging (MRI) using various contrast media, which provides better soft-tissue contrast and a more nuanced depiction of different tissue properties. Therefore, MRI is for optimal diagnosis and management of cirrhotic disease.

HEPATOCELLULAR NODULES: CLASSIFICATION

There are four classes of hepatocellular nodular lesions that characteristically are found in the cirrhotic liver: regenerative nodules, dysplastic foci, dysplastic nodules, and hepatocellular carcinomas. These lesions are referred to collectively as cirrhosis associated hepatocellular nodules.

Regenerative Nodules in Liver

Regenerative nodules typically occur in the cirrhotic liver in response to injury and altered circulation and/or other stimuli, hence referred as cirrhotic nodules. In this setting, regenerative nodules are surrounded by fibrous septa. Less characteristically, regenerative nodules may also arise in an otherwise normal liver; in this setting, the regenerative nodules are surrounded by normal liver parenchyma instead of fibrotic septa.

- **Large regenerative nodules in liver:** Large regenerative nodules measuring >1.00 to <1.00 cm in the setting of cirrhosis. On histologic examination, regenerative nodules in the liver resemble cirrhotic nodules with an intact reticulin framework similar to that of normal liver. Liver cell limiting plates are one to two cells thick. Portal tracts are usually present within the nodule, and bile ductular reaction may be prominent. Large regenerative nodules have been associated with an increased incidence of hepatocellular carcinoma.
- **Diffuse hepatocellular hyperplasia:** Diffuse hepatocellular hyperplasia occurs without fibrosis. It is associated with rheumatoid arthritis (most common), myeloproliferative disorders, hyperviscosity syndromes, transplantation of liver and kidney, HIV infection, vasculitis and drugs (anabolic drugs, cytotoxic drugs).
- **Focal nodular hyperplasia in liver:** Focal nodular hyperplasia in liver is a benign non-neoplastic lesion often diagnosed as an incidental finding during radiological investigations done for other causes. It is commonly seen in young women in 3rd to 4th decades. It is associated with use of anabolic steroid or oral contraceptives. Liver shows central stellate fibrous scar with arterial blood vessels. Liver functions are normal. AFP levels are not raised. It rarely grows or bleeds. It does not undergo malignant transformation. Patient presents with upper abdominal mass. Risk of hemorrhage and rupture is less as compared to hepatocellular adenoma. It is treated by local resection.

Surgical Pathology: Focal Nodular Hyperplasia in Liver

Gross Morphology

- Focal nodular hyperplasia in liver is most often a solitary well circumscribed lesion measuring <5 mm in diameter. It is usually located near the capsule of liver in 80% of cases.
- Multifocal lesions can also be seen in 20% of cases, which may be focal or diffuse. Focal lesion appears nodular, well demarcated, unencapsulated with lighter color when compared to surrounding liver. Focal nodular hyperplasia in liver has a characteristic central scar on gross examination and is shown in Fig. 21.83A.

Light Microscopy

- Focal nodular hyperplasia has normal-appearing hepatocytes arranged in nodules and separated by fibrous tissue.
- The limiting plate layer is of 2–3 cells in thickness and the reticulin framework intact similar to normal liver.
- Rarely foci of mild nuclear atypia and conspicuous nucleoli can be seen. Histology of focal nodular hyperplasia is shown in Fig. 21.83B.
- Comparison of benign hepatocellular nodules (adenoma, focal nodular hyperplasia, regenerative nodule in cirrhosis and conventional hepatocellular carcinoma is given in Table 21.80.

Immunohistochemistry Technique

Hepatocyte markers such as Hep Par 1, CAM 5.2, and polyclonal CEA are positive. Glutamine synthetase has a characteristic map-like pattern in FNH. AFP is negative.

Markers	Expression
▪ Hep Par 1	▪ Positive
▪ CAM 5.2	▪ Positive
▪ Carcinoembryonic antigen (CEA) polyclonal	▪ Positive
▪ Glutamine synthetase	▪ Positive
▪ α-Fetoprotein (AFP)	▪ Negative

Dysplastic Nodules in Liver

Cirrhosis-associated dysplastic and neoplastic nodules are composed of hepatocytes that exhibit histologic features of abnormal growth caused by a presumed or proved genetic alteration.

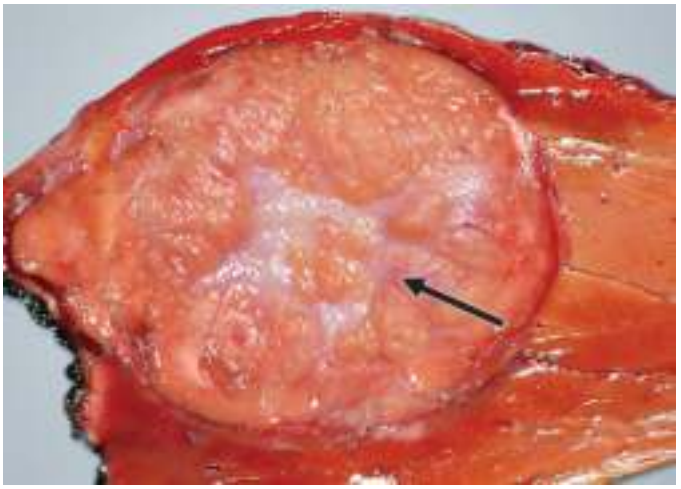


Fig. 21.83A: Gross morphology of focal nodular hyperplasia in liver. This well circumscribed mass in the liver is a benign lesion. It is regarded as being a hamartoma and is distinguished from a true adenoma by the presence of the central stellate scar, which contains bile ductules. This is a large example of FNH. The abnormal areas are usually multiple and smaller than this one (arrow).

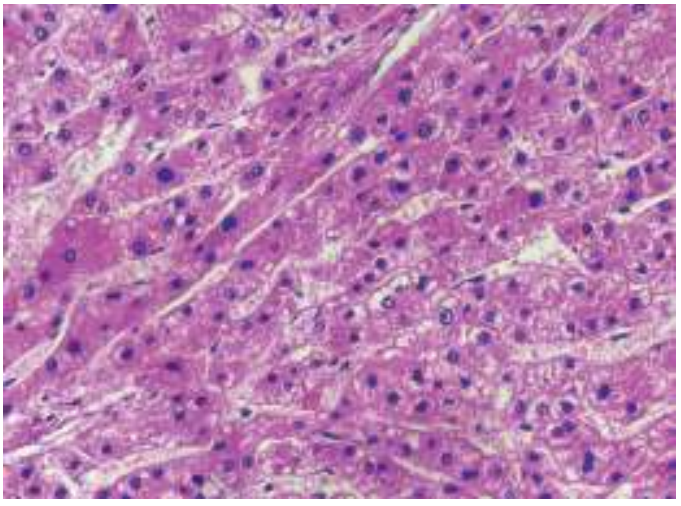


Fig. 21.83B: Histology of focal nodular hyperplasia in liver. Focal nodular hyperplasia has normal-appearing hepatocytes arranged in nodules and separated by fibrous tissue. The limiting plate layer is of 2–3 cells in thickness and the reticulin framework intact similar to normal liver.

Table 21.80 Comparison of benign hepatocellular nodules (adenoma, focal nodular hyperplasia, regenerative nodule in cirrhosis) and conventional hepatocellular carcinoma

Histochemical Stain and Immunohistochemical Marker	Benign Hepatocellular Nodules (Adenoma, Focal Nodular Hyperplasia, Regenerative Nodule in Cirrhosis)	Conventional Hepatocellular Carcinoma
Reticulin stain	Hepatic platelets are of normal thickness (1–2 cells in thickness)	Hepatic platelets are thickened (>3 cells in thickness)
Glypican 3	Negative	Positive
CD34	Negative	Positive (sinusoidal capillarization)
Factor VIII	Negative	Positive (sinusoidal capillarization)

- Dysplastic nodules do not satisfy the histologic criteria for malignancy or invasion, which may be either dysplastic foci (<1 mm in diameter) or dysplastic nodules (≥1 mm in diameter).
- A dysplastic focus is a cluster of dysplastic hepatocytes and recognized microscopically measuring <1 mm in diameter. Dysplastic nodule is a large lesion measuring ≥1 mm in diameter, that is grossly or radiologically apparent.
- Small cell dysplastic nodule is characterized by smaller than the normal hepatocytes, higher than normal nucleus to cytoplasm ratio, and hyperchromatic nuclei. Small expansive foci of small cell dysplastic nodules are closely associated with hepatocellular carcinoma than large cell dysplastic nodules.
- Large cell dysplastic nodule is characterized by nuclear enlargement, hyperchromasia, prominent nucleoli, but with abundant cytoplasm and hence, a normal nucleus to cytoplasm ratio. It may represent a regenerative phenomenon or a response to prolonged cholestasis. Large cell dysplastic nodules usually arise in cirrhosis and may be classified as low-grade or high-grade according to the degree of dysplasia. It is difficult to differentiate dysplastic nodules from other cirrhosis-associated hepatocellular nodules on histologic examination, hence molecular genetic techniques may be essential in this context.

Pathology Pearls: Dysplastic Nodules in Liver

- Dysplastic focus measures <1 mm
- Dysplastic nodules ≥1 mm in diameter
- Early hepatocellular carcinoma's cut of size is 2 cm

Neoplastic Nodules in Liver

Dysplastic nodules and neoplastic nodules in liver may transform into hepatocellular carcinoma slowly overtime. Therefore, understanding histologic and magnetic resonance imaging features of dysplastic nodules is essential for diagnosis and management of the patients.

- Hepatocellular carcinomas are malignant neoplasms composed of dedifferentiated hepatocytes. Neoplastic nodules are categorized either small (<2 cm in diameter) or large (≥2 cm in diameter). Based on gross morphologic features, hepatocellular carcinoma is classified into three major types: nodular, massive and diffuse. Hepatocellular carcinomas are scored by using World Health Organization grading system.
- Nodular hepatocellular carcinomas are small lesions with distinct margins. Massive hepatocellular carcinomas are larger than nodular types and characterized by multiple infiltrating tumors.
- Diffuse hepatocellular carcinomas are characterized by multiple infiltrating tumors.

Pathology Pearls: Hepatocarcinogenesis in Cirrhotic Liver

Cirrhosis is the most common predisposing factor for hepatocellular carcinoma in 80–90% of patients. Other predisposing factors for hepatocellular carcinoma in cirrhotic nodules include nonalcoholic steatohepatitis, hemochromatosis, Wilson disease, α_1-antitrypsin deficiency and aflatoxin B from contaminated food.

- Recent studies have elucidated the molecular mechanisms and morphologic features of hepatocellular carcinoma in cirrhosis-associated hepatocellular nodules.
- Oxidative stress induced by chronic injury, inflammation, and continuous liver cellular regeneration predispose hepatocytes to mutation in the TP53 tumor suppressor gene that results in abnormal cellular proliferation. The dysplastic foci initially congregate in dysplastic foci or dysplastic nodules then followed by further cumulative mutations.
- Cellular architecture becomes disorganized that results in increased nodule size and vascularity, nodule cellular density; and change in hepatocellular function and Kupffer cells. This pathway involves processes, such as capillarization and neoangiogenesis that result in gradual change in blood supply from portal to arterial pattern, and thus uncontrolled cellular growth and overtime to produce hepatocellular carcinoma via a dysplasia-carcinoma sequence.
- An oncoprotein produced by hepatitis B virus can directly bind to and inactivate T53 tumor suppressor gene thereby inducing hepatocellular carcinoma without cirrhosis. Cirrhosis is more prevalent in hepatitis C virus infection.

PRIMARY MALIGNANT TUMORS

CONVENTIONAL HEPATOCELLULAR CARCINOMA

Conventional hepatocellular carcinoma (HCC) is the most common primary malignant tumor with hepatic differentiation. It constitutes 85% of hepatic malignancies, which usually affects adults of more than 50 years of age without cirrhosis. However, it may affect children in 30% of cases. HCC is marked by increased serum concentration of α-fetoprotein. There is propensity for invasion of vascular channels with hematogenous dissemination. HCC may obstruct the biliary ducts and results in increased serum alkaline phosphatase. HCC is prone to hemorrhage and necrosis resulting in hemoperitoneum. Adenomatous hyperplasia and liver dysplasia are thought to be precursor of hepatocellular carcinoma.

Epidemiology

Conventional hepatocellular carcinoma is the fifth most common malignancy worldwide. Highest rates

of conventional HCC is observed in Korea, Taiwan, Southeast China, Japan and sub-Sahara Africa. Rate of incidence varies due to difference in the risk factors such as infection with HBV, HCV; aflatoxin β_1 exposure and cirrhosis. Other less common risk factors are thorotrast exposure, androgenic steroids and tyrosinemia.

Risk Factors

- **Aflatoxin β_1:** It is produced by *Aspergillus flavus* (aflatoxin β_1) and *Aspergillus parasiticus*, which contaminate grain particularly peanuts stored in warm and humid conditions in tropical and subtropical regions. Aflatoxin β_1 is a potent carcinogen can bind covalently to DNA of hepatocytes resulting in formation of mutagenic adducts with guanosine, which are thought to cause specific mutation in the TP53 tumor suppressor gene and resulting in hepatocellular carcinoma. Normally, enzymes in hepatocytes can detoxify aflatoxin β_1. People in Africa and China have mutations in hepatic enzymes, that normally detoxify aflatoxin β_1.
- **Cirrhosis:** Major risk factors for hepatocellular carcinoma include alcoholism, α_1-antitrypsin deficiency, HCV, nonalcoholic fatty liver disease (NAFLD), hereditary tyrosinemia, primary biliary cirrhosis and primary hemochromatosis. Hepatocellular carcinoma occurs without chronic liver disease in elderly persons or associated with hepatocellular adenoma with β-catenin gene mutations.
- **Hepatitis B virus:** HBV DNA is integrated into the host genome and inducing genomic instability. HBV contains four open reading frames. HBV-X protein disrupts normal growth control by transcriptional activation of insulin-like growth factor II (IGF-II) and receptors for insulin-like growth factor I. HBV-X protein binds to TP53 tumor suppressor gene resulting in its inactivation. HBV-X protein also disrupts β-catenin gene.
- **Hepatitis C:** Conventional hepatocellular carcinoma is rare in the absence of cirrhosis, because HCV lacks direct carcinogenic role. HCC first induces post-necrotic cirrhosis, that results in development of conventional hepatocellular carcinoma.
- **Drugs:** Therapeutic administration of thorotrast radiocontrast dye, androgens, anabolic steroids and oral contraceptives increases risk for hepatocellular carcinoma.
- **Chemical agents:** Azo dyes derivatives of aromatic amines such as dimethylaminobenzene is used in imparting yellow color to butter increase risk for hepatocellular carcinoma (Tables 21.81 and 21.82).
- **Pathogens:** Pathogens inducing hepatocellular carcinoma include viruses (HBV, HCV), fungus (*Aspergillus flavus*) and parasites (*Clonorchis sinensis*) increase risk for hepatocellular carcinoma.

Clinical Features

Clinical symptoms of conventional hepatocellular carcinoma may be masked in the background of chronic viral hepatitis or cirrhosis.

- Patient presents with abdominal fullness, upper abdominal pain, weight loss and generalized weakness.

Table 21.81 Pathogens induced liver cell cancers

Organisms	Pathogens	Associated Tumors
Viruses	▪ HCV (RNA) ▪ HBV (DNA)	Hepatocellular carcinoma
Fungus	*Aspergillus flavus* (aflatoxin β_1 product)	Hepatocellular carcinoma
Parasites	▪ *Clonorchis sinensis* (liver fluke) ▪ *Opisthorchis viverrini* ▪ *Fasciola hepatica*	▪ Hepatocellular carcinoma ▪ Cholangiocarcinoma (bile duct carcinoma) ▪ Pancreatic carcinoma ▪ Cholangiocarcinoma (bile duct carcinoma)
Steatohepatitis	▪ Alcoholic etiology ▪ Nonalcoholic etiology	Hepatocellular carcinoma
Metabolic disorders	▪ Hemochromatosis ▪ α_1-Antitrypsin deficiency ▪ Tyrosinemia ▪ Porphyria cutanea tarda	Hepatocellular carcinoma
Drugs	▪ Thorostat ▪ Androgens ▪ Anabolic steroids ▪ Oral contraceptives	Hepatocellular carcinoma

Table 21.82 Indirect acting chemical carcinogens induced liver cancers

Chemical Carcinogen	Examples	Associated Cancers
Azo dyes derivatives of aromatic amines	Dimethylaminoazobenzene, known as butter yellow imparts an appetizing yellow color to margarine	Hepatocellular carcinoma
Natural carcinogens	Aflatoxin β_1 synthesized by fungus *Aspergillus flavus* in growing stored grains, nuts and peanut	Hepatocellular carcinoma
Organohalogen compounds	Polyvinyl chloride, carbon tetrachloride, chloroform, hexachlorobenzene, trichloroethylene	Liver angiosarcoma
Occupational carcinogens	Arsenic used in alloys, medication, preparation of fungicides and herbicides	Liver angiosarcoma, skin squamous cell carcinoma and basal cell carcinoma, lung carcinoma

- Other clinical features are due to hypoglycemia, polycythemia, increased serum calcium level, hypercholesterolemia and porphyria.
- Patient reaches into terminal stage characterized by cachexia, gastrointestinal or esophageal variceal bleeding, and hepatocellular failure with hepatic coma or rupture of tumor with fatal hemorrhage.
- On clinical examination, liver is enlarged with irregular nodularity surface.

Surgical Pathology: Conventional Hepatocellular Carcinoma

Gross Morphology

- Conventional hepatocellular carcinoma (HCC) can be unifocal or multifocal or diffusely infiltrative soft tumor, paler than surrounding normal liver. Color of the HCC may be green due to presence of bile. Extensive intrahepatic metastases are common. Tumor may be partially or completely encapsulated.
- Snake-like masses of tumor may involve the portal vein (35–80%), hepatic vein (20%) or inferior vena cava (similar to renal cell carcinoma).
- Cut surface of tumor has greenish yellow hue with necrosis and hemorrhage.
- Occasional tumor may be pedunculated arising from posterior and inferior surfaces of right lobe or accessory lobe in <1% of cases.
- Tumor <2 cm in diameter is referred to as small hepatocellular carcinoma lacking gross vascular invasion necrosis or hemorrhage. Gross morphology of conventional hepatocellular carcinoma is shown in Fig. 21.84.

Light Microscopy

Histology of conventional hepatocellular carcinoma is shown in Fig. 21.85. Histologic grading of conventional hepatocellular carcinoma (HCC) is given in Table 21.83.

Well-differentiated conventional hepatocellular carcinoma:

- Tumor is composed of large polyhedral cells with hyperchromatic round nuclei, thick nuclear membrane, prominent nuclei, intracytoplasmic globular hyaline inclusions and atypical mitoses.
- Tumor cells are arranged in trabecular (sinusoidal, plate-like) pattern with ≥3 cells much wider than normal liver plate thickness of 2 cells thick.
- Tumor cells may also be arranged in solid (compact), tubular, pseudoglandular or sarcomatoid patterns.
- Tumor cells are surrounded by layer of flattened endothelial cells. Presence of sinusoidal vessels surrounding tumor cells is an important diagnostic factor.
- The reticulin framework is generally reduced or absent.
- Common features in conventional HCC are portal vein thrombosis, vascular invasion and mitotic figure.
- Variable amount of fat, bile within bile canaliculi and Mallory-Denk hyaline bodies may be demonstrated.

Moderately-differentiated conventional hepatocellular carcinoma:

- Tumor is composed of large polyhedral cells with hyperchromatic round nuclei, thick nuclear membrane, prominent nuclei, more eosinophilic cytoplasm and atypical mitoses.
- Tumor cells are arranged in trabecular pattern with ≥3 cells much wider than normal liver plate thickness of 2 cells thick.

Poorly-differentiated conventional hepatocellular carcinoma:

- Tumor shows marked pleomorphism, scant cytoplasm, bizarre mitotic figures, tumor giant cells, prominent nuclei with nucleoli.
- Confluent growth or compression of adjacent trabeculae results in compact (solid) growth pattern with conspicuous or obliterated sinusoids.
- Cytoplasmic inclusions such as Mallory-Denk bodies, globular eosinophilic bodies composed of proteins including albumin, fibrinogen, α_1-antitrypsin, or ferritin, may be present.
- Pale bodies in large cytoplasmic inclusions containing fibrinogen are seen in fibrolamellar hepatocellular carcinoma.

Other Histologic Variants of Hepatocellular Carcinoma

Pleomorphic (giant cell) HCC

- Tumor cells demonstrate marked pleomorphic bizarre tumor giant cells with marked loss of cell cohesion.
- Distinct trabecular pattern is difficult to demonstrate in this HCC variant.

Clear cell HCC

- Tumor is composed of clear cytoplasm resulting from accumulation of cytoplasmic glycogen and/or fat that is lost in the paraffin embedding process.
- It is difficult to differentiate from metastatic renal, adrenal, or ovarian carcinoma.
- Immunohistochemical analysis is essential to establish diagnosis by highlighting bile canliculi (polyclonal CEA, pCEA) or hepatocellular differentiation (Hep Par 1 or glypican 3).
- Elevated serum AFP level, presence of chronic liver disease, and the absence of an extrahepatic mass indicate a hepatic primary neoplasm.

Sarcomatoid (spindle cell, pseudosarcomatous) HCC

- Tumor is composed of spindle-shaped cells. Sometimes, the tumor has been reported as carcinosarcoma when foci of more differentiated sarcoma such as osteosarcoma, chondrosarcoma, leiomyosarcoma and rhabdomyosarcoma are demonstrated.
- Epithelial component shows positivity for CAM 5.2 in 60% of cases.
- Spindle cell component demonstrates positivity for vimentin, smooth muscle actin and desmin. S-100 positivity is demonstrated in small number of cases. Serum AFP level is increased in 50% of cases.

Combined HCC and cholangiocarcinoma

- Combined HCC and cholangiocarcinoma constitutes <1% of primary hepatocellular carcinomas.

Diffuse cirrhosis-like HCC

- Diffuse and extensive liver involvement by small cirrhosis-like nodules evade clinical and radiographic detection of hepatocellular carcinoma.

Steatohepatitis HCC

- Tumor has been recently described associated with non-alcoholic fatty liver.
- Tumor has firmer consistency due to fibrosis.
- Histologic examination of tumor shows steatosis, hepatic ballooning degeneration, inflammation, and pericellular fibrosis with neoplastic element.

Immunohistochemistry Technique

Conventional hepatocellular carcinoma shows positivity for α_1-antitrypsin, α-fetoproteins, CEA, arginase 1, glypican 3, CAM 5.2 (CD8/CD18), Hep Par 1, Des-gamma carboxyprothrombin (DCP), TTF-1, vitamin B_{12} binding protein and human hepatocyte growth factor and copper.

- Recommended panel of markers is performed to differentiate conventional hepatocellular carcinoma from metastatic carcinoma are arginase 1 + Hep Par 2 + glycpican 3.
- Recommended panel of markers is performed to differentiate hepatocellular carcinoma from nonmalignant lesions are 2 of 3: glypican 3/GPC3 (cytoplasmic and canalicular), HSP70 and glutamine synthetase; adding clathrin heavy chain may be helpful.
- Recommended panel of markers is performed to differentiate hepatocellular carcinoma from nonmalignant lesions: GPS3 plus CD34 complete staining pattern.
- Recommended panel of immunohistochemical markers applied in differential diagnosis of hepatocellular carcinoma and cholangiocarcinoma are given in Table 21.84.

Markers	Expression
Hep Par 1 (hepatocyte paraffin 1)	Positive (80–90%)
α_1-Antitrypsin	Positive (75%)
α-Fetoproteins (AFP)	Positive (60–80%)
CEA (carcinoembryonic antigen) polyclonal	Positive (canalicular pattern 50–90%)
Arginase 1*	Positive (sensitive marker)
Glypican 3	Positive
CAM 5.2 (CD8/CD18)	Positive (50%)
Des-gamma carboxyprothrombin (DCP protein induced by vitamin K absence or antagonist II/PIVKA-2)	Positive
TTF-1	Positive (cytoplasmic staining)
Vitamin B_{12} binding protein	Positive
Human hepatocyte growth factor	Positive
Copper	Positive (7–40%)

**Arginase 1 is a binuclear metalloproteinase enzyme that catalyzes by hydrolysis of arginine to ornithine and urea. It is sensitive and specific marker for hepatocellular carcinoma.*

Neurotensin is expressed by fibrolamellar hepatocellular carcinoma.



Fig. 21.84: Gross morphology of conventional hepatocellular carcinoma. The satellite nodules of this hepatocellular carcinoma represent either intrahepatic spread of the tumor or multicentric origin of the tumor. Such masses may also focally obstruct the biliary tract and lead to an elevated alkaline phosphatase (arrow).

Table 21.83 Histologic grading of conventional hepatocellular carcinoma

Histologic Grade	Liver Architecture	Nuclear Features	Cell Size and Cytoplasmic Features	Other Features
Well-differentiated HCC grade I	Liver plates ≥3 cells, pseudoglandular pattern common	Minimal nuclear atypia	Neoplastic cells relatively smaller in size; fatty change	Well differentiated tumor; with presence of sinusoidal vessels surrounding tumor cells an important diagnostic factor
Moderately-differentiated HCC grade II	Liver plates ≥3 cells, trabecular pattern common	Nuclear atypia more marked	Neoplastic cells relatively larger in size with abundant eosinophilic cytoplasm; giant cells and bile may be demonstrated	Differentiation in tumor >2 cm size
Poorly-differentiated HCC grade III	Compact growth pattern with rare or absent trabeculae	Nuclear atypia marked with enlarged hyperchromatic nuclei	Spindle and small cells; and bile absent	Difficult to recognize hepatocellular origin

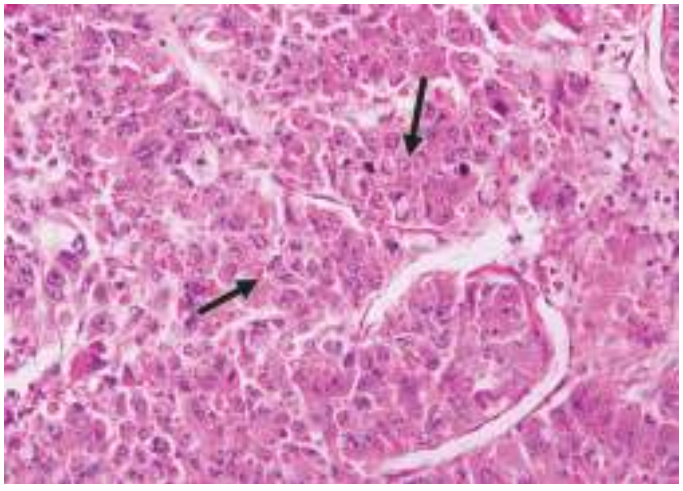


Fig. 21.85: Histology of conventional hepatocellular carcinoma. Tumor is composed of liver cords that are much wider than the normal liver plate that is two cells thick. Tumor cells are arranged in trabeculae. There is no discernible normal lobular architecture, though vascular structures are present. It is associated with elevated α-fetoprotein (arrows) (400X).

Electron Microscopy

Electron microscopy of conventional hepatocellular carcinoma demonstrates numerous mitochondria, microbodies and abundant glycogen. Intracytoplasmic blue products (bile canaliculi, peroxisomes) are present in the tumor cells.

Molecular Genetic Alterations

- **Hepatocyte growth factor increased baseline activity:** Many cancer stem cells acquire the ability to synthesize the same growth factors to which they are responsive, generating an autocrine loop. Increased baseline activity of hepatocyte growth factor (HGF) oncogene product is linked to hepatocellular carcinoma and thyroid carcinoma.
- **Insertional mutagenesis:** It is form of oncogene activation, which occurs due to insertion of viral gene into the mammalian DNA, resulting in genetic dysregulation and development of hepatocellular

Table 21.84 Panel of immunohistochemical markers applied in differential diagnosis of hepatocellular carcinoma and cholangiocarcinoma

Immunohistochemical Markers	Conventional Hepatocellular Carcinoma	Cholangiocarcinoma
Hep Par 1	Positive (95% fairly specific)	Negative
α-Fetoproteins (AFP)	Positive (30–60% not specific)	Negative (positive in rare case)
mCEA	Negative	Positive
p-CEA	Positive (80% canalicular highly specific)	Positive (cytoplasmic)
CD10	Positive (canalicular)	Negative
Villin	Positive (canalicular)	Negative
CK7 (cytokeratin 7)	Negative	Positive
CK19 (cytokeratin 19)	Negative	Positive (70–100%)
CK20 (cytokeratin 20)	Positive (up to 20%)	Positive (usually)

Contd...

Table 21.84 Panel of immunohistochemical markers applied in differential diagnosis of hepatocellular carcinoma and cholangiocarcinoma (*Contd...*)

Immunohistochemical Markers	Conventional Hepatocellular Carcinoma	Cholangiocarcinoma
CK AE1/AE3 (pan-CK)	Positive	Positive
CAM 5.2 (LMWCK) pan-CK	Positive (5–20%)	Positive
CK903 (HMWCK)	Positive	Positive
Glypican 3	Positive (new marker of hepatocellular differentiation)	Negative
Arginase 1 (new marker of hepatocellular differentiation)	Positive	Negative
α_1-Antitrypsin	Positive (up to 50%)	Negative
TTF-1 (thyroid transcription factor 1)	Positive (cytoplasmic staining)	Negative
EMA (epithelial membrane antigen) marker of glandular epithelium	Negative	Negative
MOC-31	Negative (marker of glandular epithelium)	Negative
Mucicarmine	Negative (marker of glandular epithelium)	Negative

Fibrolamellar variant of hepatocellular carcinoma is positive for neurotensin.

carcinoma. The best example of such an event may be found in hepatitis virus-infected hepatocytes.

- **Mutant RAS:** Mutant signal transducing RAS protein has been linked to hepatocellular carcinoma.
- **Translocation and amplification of CCND1/cyclin D1:** It has been demonstrated in conventional hepatocellular carcinoma.
- **Amplification or point mutation of CDK4 (cyclin-dependent kinase):** It has been demonstrated in hepatocellular carcinoma.

Pathology Pearls: Molecular Genetic Alterations in HCC

- Hepatocytic growth factor (HGF) increased baseline activity
- Insertional mutagenesis by viruses
- RAS mutation
- Translocation and amplification of CCND1/cyclin D1
- Amplification or point mutation of CDK4 (cyclin-dependent kinase)

Mode of Spread

- **Lymphatic route:** Conventional hepatocellular carcinoma spreads by lymphatic route to peripancreatic and para-aortic lymph nodes.
- **Hematogenous route:** Hematogenous spread of conventional hepatocellular carcinoma is relatively less frequent. It may spread by hematogenous route by invading portal vein reaching to right side of heart resulting in metastases to lungs, bones, adrenal glands and central nervous system.

Prognostic Factors

Patients with hepatocellular carcinoma measuring ≤5 cm in size treated by surgical excision have five-year survival in 10–50% of cases.

- Death occurs from hepatocellular failure, rupture of malignant tumor, cachexia and gastrointestinal bleeding.
- Favorable prognostic factors are low stage, encapsulated tumor size <5 cm, without lymphovascular invasion, negative surgical margin and low nuclear grade.
- Conventional hepatocellular carcinoma is treated by surgical resection, liver transplantation and radiofrequency ablation/transarterial chemoembolization.

Pathology Pearls: Protocol for Reporting Hepatocellular Carcinoma

Protocol for reporting resection of hepatocellular carcinoma recommended by College of American Pathologists are given as under.

Gross Morphology

- Tumor size
- Tumor focality
- Tumor margins
- Tumor extension
- Lymph nodes study

Histologic Examination

- Histologic type
- Histologic grade
- Lymphovascular invasion
- Perineural invasion

Pathologic Findings

- Grading
- Staging

Ancillary Studies

- Immunohistochemistry technique
- Serum tumors

Pathology Pearls: Protocol for Reporting Lymph Node in Hepatocellular Carcinoma

Protocol for reporting lymph node biopsy specimen recommended by College of American Pathologists is given below.

- Most often, three or four lymph nodes are submitted for histologic examination.
- Lymph node biopsy is obtained from hepatic region, i.e. hilar, hepatoduodenal ligament, inferior phrenic and caval regions. Involvement of these lymph nodes is considered as distant metastases.

Pathology Pearls: Increased Serum α-Fetoprotein (AFP) Tumor Marker in Various Disorders

Serum α-fetoprotein level is increased in 60–80% cases of hepatocellular carcinoma. Serum α-fetoprotein is also raised in cirrhosis, chronic viral hepatitis, hepatoblastoma and yolk sac tumor of gonads.

- Hepatocellular carcinoma
- Hepatoblastoma
- Yolk sac tumors of gonads
- Cirrhosis
- Pregnancy
- Massive liver cell necrosis with regeneration
- Chronic viral hepatitis
- Immunohistochemistry technique in hepatocellular and metastatic carcinomas is given in Table 21.85.

Clinical Features

Patient with conventional hepatocellular carcinoma may present with symptoms of paraneoplastic syndrome such as hypoglycemia, increased red blood cell count, increased serum calcium level, hypercholesterolemia and porphyria.

- Clinical symptoms of conventional HCC may be masked in the background of chronic viral hepatitis or cirrhosis. Patients present with abdominal fullness, upper abdominal pain, weight loss and generalized weakness.
- These patients reach terminal stage characterized by cachexia, gastrointestinal tract or esophageal variceal bleeding, and hepatocellular failure with hepatic coma or rupture of tumor with fatal hemorrhage.
- On clinical examination, liver is enlarged with irregular nodularity.

Table 21.85 Immunohistochemistry technique in hepatocellular and metastatic carcinomas

Markers	Hepatocellular Carcinoma	Metastatic Carcinoma
▪ Arginase 1 ▪ Glypican 3 ▪ *Hep Par 1* (hepatocyte paraffin 1)	Positive	Negative

Specific markers are used to confirm origin of tumors metastasizing to liver.

Laboratory Diagnosis

Conventional hepatocellular carcinoma is screened by ultrasonography, hepatic angiography, MRI and serum AFP and des-gamma carboxyprothrombin markers. MRI demonstrates location, invasiveness and presence or absence of multifocality.

- **Serum α-fetoprotein:** It is a glycoprotein synthesized in the fetus by the yolk sac, liver, and gastrointestinal tract. In adults, an elevated serum level of AFP is a useful indicator of hepatocellular carcinoma and germ cell tumors of the testis. Elevated serum AFP is demonstrated in 60–80% cases of conventional HCC. Its sensitivity is reduced in alcohol-related cirrhosis, tumors arising in noncirrhotic liver and tumor size 2 cm or less. Disorders associated with serum AFP are conventional hepatocellular carcinoma, hepatoblastoma, cirrhosis, massive hepatocellular necrosis with regeneration, chronic hepatitis, yolk sac tumor of gonads and pregnancy. Disorders associated with elevated AFP are given in Table 21.86.
- **Serum des-gamma carboxyprothrombin:** Its positivity is demonstrated in 75–90% cases with large tumor. Small tumors do not show its positivity. Serum des-gamma carboxyprothrombin marker (DCP protein induced by vitamin K absence or antagonist II/PIVKA-2) is more specific marker than serum AFP for poor prognosis. Abnormal des-gamma carboxyprothrombin is synthesized by hepatocellular carcinoma associated with early portal vein invasion, intrahepatic metastasis and capsular invasion.
- **Imaging techniques:** Imaging techniques include ultrasonography, hepatic angiography, CT scan and MRI. MRI demonstrates location, invasiveness and presence or absence of multifocality.

Table 21.86 Disorders associated with elevated AFP

Conventional hepatocellular carcinoma
Hepatoblastoma
Cirrhosis
Massive hepatocellular necrosis with regeneration
Chronic viral hepatitis
Yolk sac tumor of gonads
Pregnancy

- **Fine needle aspiration cytology (FNAC):** FNAC is cost effective diagnostic tool for early diagnosis.
- **Histologic examination:** It is performed to confirm the diagnosis of conventional hepatocellular carcinoma, which should be differentiated from hepatocellular adenoma and focal nodular hyperplasia. Gross and histologic features of hepatocellular tumors are given in Table 21.87.
- **Immunohistochemistry:** Panel of markers is used to study the expression of hepatocellular carcinoma and metastases.

Prognosis

Without aggressive surgical resection, abalative therapy or liver transplantation hepatocellular carcinoma results in hepatocellular failure and eventually death.

FIBROLAMELLAR HEPATOCELLULAR CARCINOMA

Fibrolamellar hepatocellular carcinoma, a variant of hepatocellular carcinoma is a rare primary malignant tumor of liver. It affects adolescents with female predominance. It occurs without chronic liver disease (hepatitis B virus infection).

- Tumor is composed of large polygonal neoplastic cells arranged in nests and cords. Collagen fibers are arranged in lamellar fashion around tumor cells. It is diagnosed by imaging techniques and histologic examination. It is associated with better prognosis than conventional hepatocellular carcinoma.
- Tumor appears to be more common in Western countries and accounting for 6% of liver cancers. It usually affects younger patients with slightly female predominance.

Molecular Genetic Alterations

The recent studies revealed the presence of DNAJB-1-PRKACA chimeric transcript resulting from a 400 kb somatic deletion on chromosome 19 in cases of fibrolamellar hepatocellular carcinoma.

Clinical Features

Initially, the patient is asymptomatic. Cases are diagnosed in advanced disease.

Table 21.87 Gross and histologic features of hepatocellular tumors

Parameters	Conventional Hepatocellular Carcinoma	Fibrolamellar Hepatocellular Carcinoma	Hepatocellular Adenoma	Focal Nodular Hyperplasia of Liver
Gross morphology				
Tumor number	Solitary > multiple	Solitary pale brown tumor	Solitary tumor in subcapsular region	Well circumscribed lesion without capsule
Background liver morphology	Fibrotic or cirrhotic background	Normal background features	Normal background features	Normal background features
Histologic features				
Architecture	Tumor cells (cell platelets) arranged in trabecular, acinar, pseudoglandular architecture with mitoses, bile in tumor cells	Neoplastic cells arranged in clusters enmeshed in dense collagen	Neoplastic cells with normal-appearing hepatocytes with thickened plates	Nodules composed of normal hepatocytes
Quality of fibrous stroma of tumor	Absent or minimal stroma, fibrous in fibrolamellar variant	Dense collagenous stroma	Fibrous septa but absence of portal tracts	Radiating septa with ductular proliferation present
Cytomorphological features	Uniform tumor cells resembling hepatocytes; pleomorphism in high-grade tumor	Neoplastic cells with abundant cytoplasm and nucleus with macronucleoli; cytoplasm containing small intracytoplasmic hyaline globules and pale bodies	Neoplastic cells with normal-appearing hepatocytes with thickened plates; and naked blood vessels, cholestasis and bile plugs	Nodules composed of normal hepatocytes with thickened arteries within fibrous stroma
Mitoses	Easily demonstrated	Less common	Absent	Absent

- Patient presents with epigastric mass, vague abdominal pain and fullness, nausea, vomiting, malaise, weight loss. Rare presentation is Budd-Chiari syndrome, hypoglycemia, obstructive jaundice and gynecomastia.
- Later, patient develops jaundice, ascites, fulminant hepatocellular failure, encephalopathy, thrombophlebitis of lower extremities, recurrent deep vein thrombosis and anemia.
- Serum aminotransferases (ALT, AST) and serum AFP may be within normal range or mildly elevated. Serum des-gamma carboxyprothrombin (DCP) level is increased.
- About 70% of cases have lymph node metastasis at presentation, in contrast to conventional HCC, which seldom involves lymph nodes.

Surgical Pathology: Fibrolamellar Hepatocellular Carcinoma

Gross Morphology

- Fibrolamellar hepatocellular carcinoma is single large, hard scirrhous tumor with fibrous bands running through it. Sometimes tumor may show encapsulation.
- Cut surface of tumor reveals multiple nodules.
- Bcakground liver shows no evidence of cirrhosis.
- Bile stain is demonstrated on cut surface of tumor.

Light Microscopy

- Collagen fibers are arranged in lamellar fashion around the large polygonal tumor cells arranged in nests and cords (Fig. 21.86A to C).
- Differences between fibrolamellar hepatocellular carcinoma and conventional hepatocellular carcinoma are given in Table 21.88.

Immunohistochemistry Technique

Neurotensin is tumor marker for fibrolamellar variant of hepatocellular carcinoma.

Mode of Spread

Fibrolamellar hepatocellular carcinoma spreads by lymphatic route to draining lymph nodes.

Prognosis and Treatment

Prognosis of fibrolamellar hepatocellular carcinoma is better than conventional hepatocellular carcinoma.

- Plasma neurotensin and vitamin B_{12} binding globulins are increased in fibrolamellar hepatocellular carcinoma. These parameters are useful in monitoring the disease and detecting recurrence after surgery.

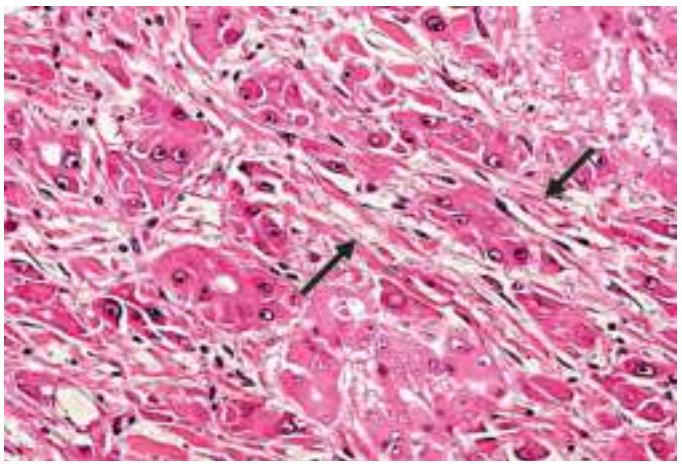


Fig. 21.86A: Fibrolamellar hepatocellular carcinoma shows tumor cells surrounded by fibrocollagenous tissue (arrows) (400X).

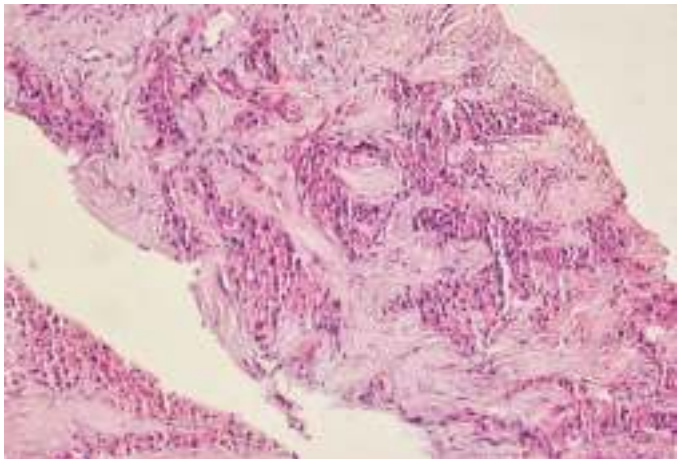


Fig. 21.86B: Fibrolamellar hepatocellular carcinoma on hematoxylin and eosin-stained section. Tumor is composed of large polygonal neoplastic cells with well-defined borders arranged in sheets, cords and trabeculae with abundant granular eosinophilic cytoplasm and nuclei with open chromatin and prominent nucleoli in background of dense collagen bundles. Histologically, fibrous bands coalesce into central scar (400X).

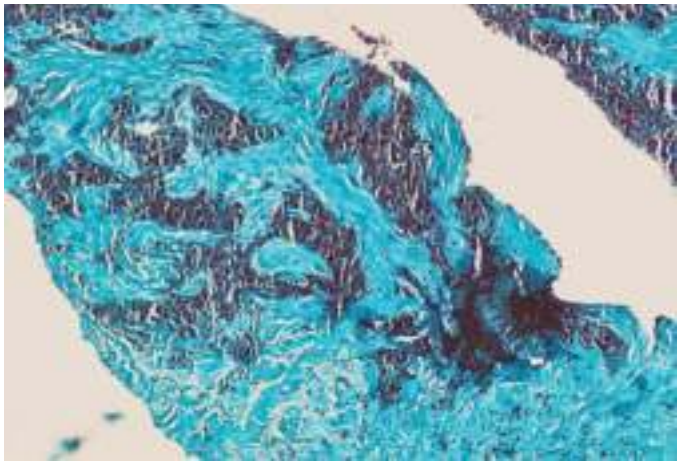


Fig. 21.86C: Masson trichrome stain demonstrates collagen fibers in fibrolamellar hepatocellular carcinoma (400X).

Table 21.88 Differences between fibrolamellar hepatocellular carcinoma and conventional hepatocellular carcinoma

Clinical Manifestations	Fibrolamellar Hepatocellular Carcinoma	Conventional Hepatocellular Carcinoma
Epidemiology		
Incidence	1–5%	95–99%
Age group	Peak incidence in third decade	Peak incidence in sixth to seventh decade
Sex predilection	Women > men	Men > women
Serum des-gamma carboxy-prothrombin (DCP)	100%	60–90%
Serum AFP	10–15%	60–80%
Imaging analysis and tumor resectibility		
Calcification	40–80%	≤5%
Central scarring	50–70%	Rare
Resectability	50–75%	10–80%
Gross morphology		
Solitary or multifocal lesion	Solitary (75%) left lobe > right lobe	Usually multifocal, solitary uncommon
Fibrous septa, scarring	10–60%	Absent
Hemorrhage necrosis	Occasional but may be present in large tumor	Common
Associated cirrhosis in the background	<10%	60–90%
Histologic features		
Cell size, shape	Large	Smaller cell size
Cytoplasm abundance	More	Less
Cytoplasmic granularity	More	Less
Pale bodies in cytoplasm	50%	10%
Hyaline globules	50%	10–50%
Fibrous stroma	Prominent lamellar bands	Scan
Overall histologic features	▪ Collagen fibers are arranged in lamellar fashion around large polygonal tumor cells arranged in nests and cords ▪ Lamellar stromal strands around tumor cells present ▪ Presence of granular cytoplasm in tumor cells common ▪ Hyaline/pale bodies in cytoplasm of tumor cells common	▪ Tumor cells are polyhedral containing hyperchromatic nuclei and prominent nucleoli arranged in solid, tubular and trabecular patterns ▪ Lamellar stromal strands around tumor cells absent ▪ Presence of granular cytoplasm in tumor cells uncommon ▪ Hyaline/pale bodies in cytoplasm of tumor cells uncommon
Electron microscopy		
Mitochondria on electron microscopy	Numerous	Less common
Immunohistochemistry		
Immunomarkers	Neurotensin	▪ Hep Par 1 (hepatocyte paraffin 1), positive (80–90%) ▪ α_1-Antitrypsin, positive (75%) ▪ α-Fetoprotein (AFP), positive (60–80%) ▪ CEA (carcinoembryonic antigen) polyclonal ▪ hCG ▪ Cytokeratin

Contd...

Table 21.88 Differences between fibrolamellar hepatocellular carcinoma and conventional hepatocellular carcinoma (*Contd...*)

Clinical Manifestations	Fibrolamellar Hepatocellular Carcinoma	Conventional Hepatocellular Carcinoma
		▪ Glypican 3 ▪ EMA ▪ Vimentin ▪ Glypican 3, positive ▪ CAM 5.2 (CD8/CD18), positivity (50%) ▪ Des-gamma carboxyprothrombin (DCP protein induced by vitamin K absence or antagonist II/ PIVKA-2), positive
Five-year survival		
Overall survival	25–90%	0–7%
Resected tumor	55–75%	20–50%

- In view of its recurrence nature of fibrolamellar hepatocellular carcinoma, periodic follow-up is essential by use of imaging modality and plasma level of neurotensin and vitamin B_{12} binding globulins.
- Death occurs due to gastrointestinal bleeding and hepatocellular failure.

CHOLANGIOCARCINOMA

Cholangiocarcinoma is most often associated with primary sclerosing cholangitis, which is less common than hepatocellular carcinoma. It is never associated with HBV infection or cirrhosis.

Origin of Tumor

According to WHO convention, the term cholangiocarcinoma is reserved for carcinoma arising in the intrahepatic bile ducts. Cholangiocarcinoma arises from epithelium lining intrahepatic biliary ducts at the porta hepatis to the smallest bile ductules at the periphery of the hepatic lobules.

Locations

- Extrahepatic cholangiocarcinoma is most common type seen in 90% of cases. Perihilar type is most common type seen in 60% of cases and known as Klatskin tumor located at the junction of left and right hepatic ducts. It may be located in distal bile ducts in 20–30% of cases.
- Intrahepatic cholangiocarcinoma is less common type seen in 10% of cases.

Risk Factors

- ***Clonorchis sinensis* (liver fluke):** It is transmitted through consumption of raw or undercooked vegetables. It resides in the biliary tree resulting in cholangiocarcinoma in far East population.
- ***Opisthorchis viverrini*:** It may cause cholangiosarcoma of the bile ducts.
- **Thorotrast contrast medium:** Sometimes, it occurs as a late complication of thorium dioxide (thorotrast) administration.
- **Primary sclerosing cholangitis:** It is associated with ulcerative colitis. There is increased incidence of cholangiocarcinoma.
- **Caroli's disease:** It is also known as choledocal cyst. It is congenital fibropolycystic disease of biliary system, which increases risk for cholangiocarcinoma.
- **Chronic alcoholic disease:** There may be increased risk for development of cholangiocarcinoma.
- **Hepatitis C virus (HCV):** Patient with hepatitis C infection may develop cholangiocarcinoma.

Molecular Genetic Alterations

Upregulation of IL-6 activates AKT results in overexpression of MCL-1 (antiapoptotic protein) leading to cholangiocarcinoma.

K-RAS gene mutation and alteration of p53 tumor suppressor gene play role in development of cholangiocarcinoma.

Clinical Features

Patient presents with painless lump, jaundice, pruritus, weight loss and acholic stools.

Surgical Pathology: Cholangiocarcinoma

Gross Morphology

- Extrahepatic cholangiocarcinoma is either firm gray nodules within bile duct wall or diffusely infiltrative (2%), which often extends into adjacent structures. Tumors may be papillary, multifocal and friable.
- Intrahepatic cholangiocarcinoma is usually large, noncapsulated, well-demarcated, firm (due to desmoplastic reaction), white-tan to gray and nodular mass. It is more frequent in the right lobe. Calcification is common in the tumor.

Light Microscopy
▪ Extrahepatic cholangiocarcinoma is composed of small cuboidal cells with vesicular nuclei and prominent nucleoli arranged in glandular pattern. Mucin is always present within cytoplasm of tumor cells and glandular lumina. ▪ Intrahepatic cholangiocarcinoma consists of infiltrating well-formed glands in abundant fibrous stroma. Malignant glands are lined by cells with varying degrees of atypia and intrapleomorphism (Fig. 21.87A and B).

Immunohistochemistry Technique	
The tumor cells express CK7, CK19 and claudin 4.	
Markers	**Expression**
▪ CK7 ▪ CK19 ▪ Claudin 4	▪ Positive ▪ Positive ▪ Positive

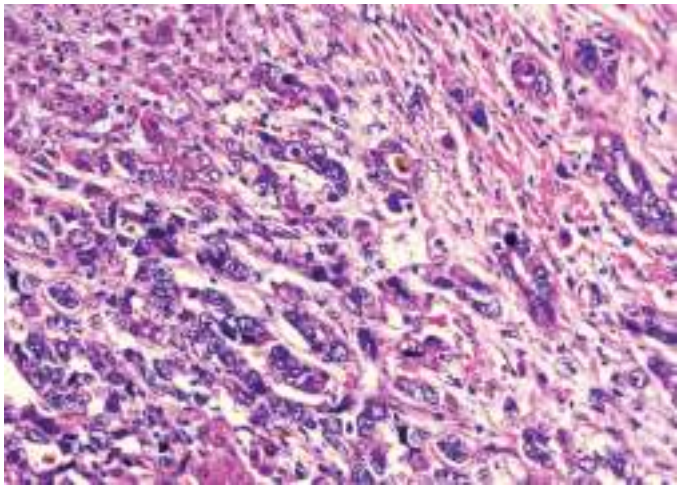


Fig. 21.87A: Cholangiocarcinoma shows glandular appearance. It does not make bile, but the cells do make mucin, and they can be almost impossible to distinguish from metastatic adenocarcinoma on biopsy or fine needle aspirate (400X).

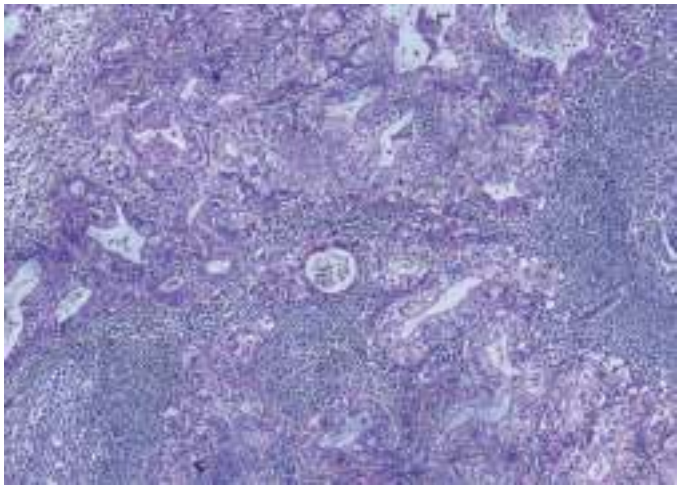


Fig. 21.87B: Cholangiocarcinoma matastasis in lymph node.

Differential Diagnosis

Tumor cells of cholangiocarcinoma are not bile stained, as bile is synthesized by hepatocytes. It is worth mentioning that hepatocellular carcinoma tumor cells show bile. It does not make bile, but the cells do make mucin, and they can be almost impossible to distinguish from metastatic adenocarcinoma on biopsy or fine needle aspirate. Fibrolamellar hepatocellular carcinoma consists of fibrous septa but negative for CK7 and CK19.

Mode of Spread

Like hepatocellular carcinoma, cholangiocarcinoma has a propensity for early invasion of vascular channels.

HEPATOBLASTOMA

Hepatoblastoma is uncommon malignant liver cancer in infants and children <3 years of age. Tumor is composed of tissue resembling fetal liver cells or bile ducts. It is diagnosed during first year of childhood.

Origin of Tumor

Hepatoblastoma originates from immature liver precursor cells. The tumor is usually unifocal affecting right lobe of liver more often than left lobe.

Predisposing Factors

Although the exact etiology of hepatoblastoma is unknown. Genetic disorders associated with an increased risk for developing hepatoblastoma include familial adenomatous polyposis, hemihypertrophy and Beckwith-Wiedemann syndrome.

Molecular Genetic Alterations

β-Catenin has been demonstrated in 70% cases of hepatoblastoma. Activation of Wnt signaling pathway may play an important role in the pathogenesis of hepatoblastoma.

Clinical Features

Patient with hepatoblastoma presents with abdominal mass and failure to thrive. It is usually associated with nephroblastoma, cleft palate, hemihypertrophy, renal or cardiac malformations.

Surgical Pathology: Hepatoblastoma
Gross Morphology
Tumor is solitary with pseudocapsule. It shows no underlying cirrhosis.

Light Microscopy

Histologic pattern of hepatoblastoma includes epithelial type, e.g. fetal, embryonal, macrotrabecular and small cell undifferentiated patterns, and mixed epithelial–mesenchymal type, e.g. teratoid and nonteratoid.

- **Fetal variant of hepatoblastoma:** Tumor is composed of polygonal cells with central round nuclei and prominent nucleoli. The 2–3 cells thick plates are arranged in sheets, trabeculae and solid groups. There may be foci of hematopoiesis.
- **Embryonal variant of hepatoblastoma:** Tumor cells are arranged in sheets, ribbons, acinar and rosettes.
- **Macrotrabecular variant of hepatoblastoma:** Tumor is composed of >10 cells thick plates arranged in trabecular fashion.
- **Small cell (undifferentiated) variant of hepatoblastoma:** Tumor has more primitive histology with predominance of small to medium-sized cells arranged in loose clusters.
- **Epithelial–mesenchymal variant of hepatoblastoma:** Tumor consists of teratoid features, comprising of spindle or oval cells with osteoid, cartilage or other sarcomatoid characteristics. It is worth mentioning that there is absence of portal tracts in hepatoblastoma. The tumor cells show pleomorphism and mitoses in comparison to minimal pleomorphism and low mitotic figures. Histology of hepatoblastoma is shown in Fig. 21.88.

Immunohistochemistry Technique

Hepatoblastoma shows positivity of α-fetoproteins (AFP), Hep Par 1, human chorionic gonadotropin (hCG), cytokeratin, carcinoembryonic antigen (CEA), glycipan 3, epithelial membrane antigen and vimentin.

Markers	Expression
α-Antitrypsin	Positive
AFP	Positive
Hep Par 1	Positive
hCG	Positive
Cytokeratin	Positive
CEA	Positive
Glycipan 3	Positive
EMA	Positive
Vimentin	Positive
Arginase	Positive

Prognostic Factors

Prognosis of hepatoblastoma is better than the hepatocellular carcinoma. The α-fetoprotein (AFP) levels are commonly elevated. When AFP is not raised at diagnosis, the prognosis is poor.

Patient with <1 year of age, histologic variants, i.e. macrotrabecular and small cell types of hepatoblastoma are associated with poor prognosis.

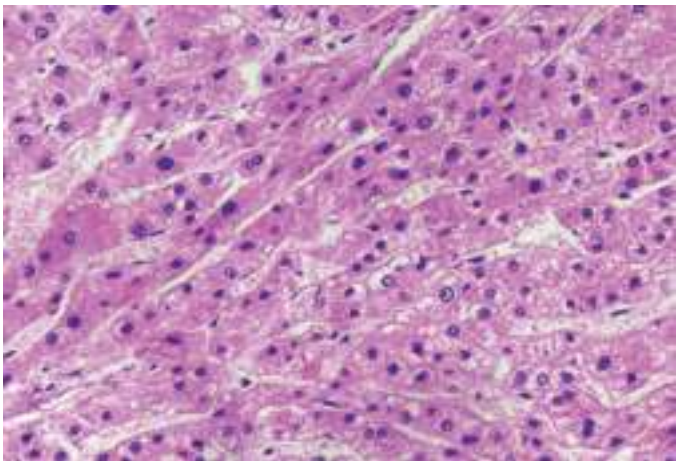


Fig. 21.88: Hepatoblastoma. Tumor is composed of polygonal cells with central round nuclei and prominent nucleoli. The 2–3 cells thick plates are arranged in sheets, trabeculae and solid groups. There may be foci of hematopoiesis (400X).

Children with fetal variant have better prognosis with survival for an average of 9 years. Complete surgical resection remains key treatment for achieving long-term survival.

HEPATIC ANGIOSARCOMA

Hepatic angiosarcoma is a rare malignant vascular tumor of liver. It most often occurs in adults. It may also affect children.

Risk Factors

Predisposing factors for development of hepatic angiosarcoma include macronodular cirrhosis, hemochromatosis and prolonged exposure to drugs, e.g. thorotrast, polyvinyl chloride (PVC), arsenic and androgens.

Clinical Features

Patient with hepatic angiosarcoma presents with abdominal distension and discomfort, weight loss and fatigue. Presence of jaundice, ascites and hepatomegaly is usually associated with advanced disease.

Surgical Pathology: Hepatic Angiosarcoma

Light Microscopy

- Hepatic angiosarcoma tumor is composed of anastomosing vascular channels lined by atypical and proliferative active endothelial cells.
- Tumor may vary from well differentiated to poorly differentiated anaplastic lesions.

Immunohistochemistry Technique

Tumor cells express positivity for immunohistochemical markers factor VIII related antigen, CD31, CD34, p53 and vimentin.

Markers	Expression
▪ Factor VIII related antigen (F VIII R:Ag)	▪ Positive (100%)
▪ CD31	▪ Positive (100%)
▪ CD34	▪ Positive (100%)
▪ p53	▪ Positive (100%)
▪ Vimentin	▪ Positive (100%)

Differential Diagnosis

Hepatic angiosarcoma should be differentiated from Kaposi sarcoma on immunohistochemistry. Tumor cells of Kaposi sarcoma show positivity for human herpesvirus 8 (HHV-8) and BCL-2.

Prognosis

Hepatic angiosarcoma metastasizes to regional lymph nodes, lungs, bones and other regions. Mortality is high in these patients.

HEPATIC EPITHELIOID HEMANGIOENDOTHELIOMA

Hepatic epithelioid hemangioendothelioma is a low-grade malignant vascular tumor, which arises from small blood vessel. It may rarely present as an intravascular neoplasm. Various sites are skin, deep soft tissue, bone, lung and liver. It affects children and adults.

Clinical Features

Patient presents with vascular thrombosis and painful lump in superficial or deep soft tissue. Patient with bone lesion presents with asymptomatic osteolytic lesion. Involvement of lung and liver by tumor may be asymptomatic.

Surgical Pathology: Epithelioid Hemangioendothelioma

Gross Morphology

Epithelioid hemangioendothelioma neoplasm excised from superficial and deep soft tissue is white, firm and sometimes calcified. Lesion from lung may show thrombosed blood vessel lesion.

Light Microscopy

- Epithelioid hemangioendothelioma is composed small uniform epithelioid to bland spindled neoplastic cells without mitoses, arranged in nests, cords and single-file arrays. These neoplastic cells are embedded in dense myxochondroid or myxohyaline matrix. Necrotic debris, hyaline collagen and thrombosed material may be demonstrated within the blood vessel (Fig. 21.89A).
- Approximately 30% of epithelioid hemangioendothelioma shows anaplastic features, i.e. high nuclear grade, numerous mitoses and necrosis with aggressive clinical course referred as malignant or high-grade epithelioid hemangioendothelioma.

Immunohistochemistry Technique

Hepatic epithelioid hemangioendothelioma shows positivity for CD31, CD34, FLI-1, vWF and low molecular weight cytokeratins (25%). Epithelioid hemangioendothelioma shows positivity for CD34 (Fig. 21.89B and C).

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ FLI-1	▪ Positive
▪ vWF	▪ Positive
▪ Cytokeratins (LMW)	▪ Positive (25%)

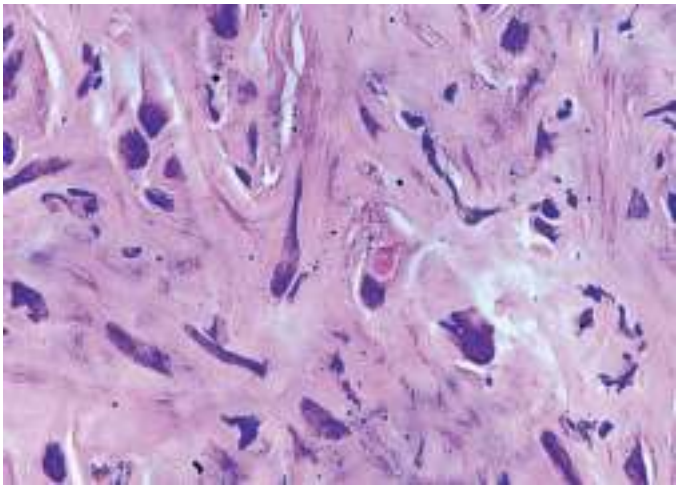


Fig. 21.89A: Hepatic epithelioid hemangioendothelioma. It is composed of small uniform epithelioid to bland spindled neoplastic cells without mitoses, arranged in nests, cords and single-file arrays. Tumor cells are embedded in dense myxochondroid or myxohyaline matrix. Necrotic debris, hyaline collagen and thrombosed material may be demonstrated within the blood vessel (400X).

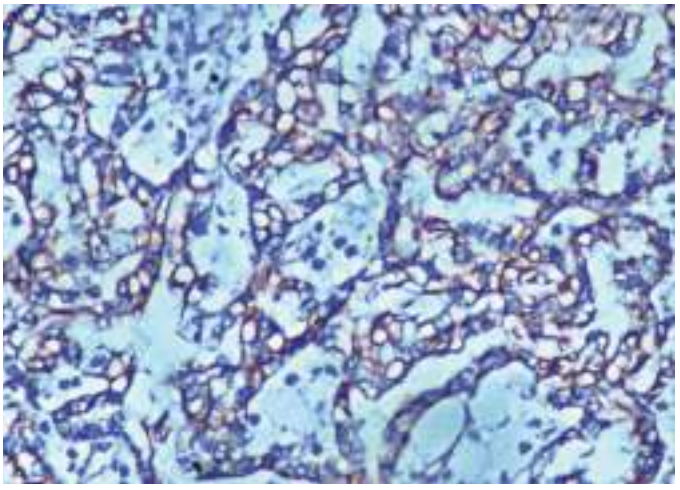


Fig. 21.89B: Hepatic epithelioid hemangioendothelioma. It shows positivity for CD34 (100X).

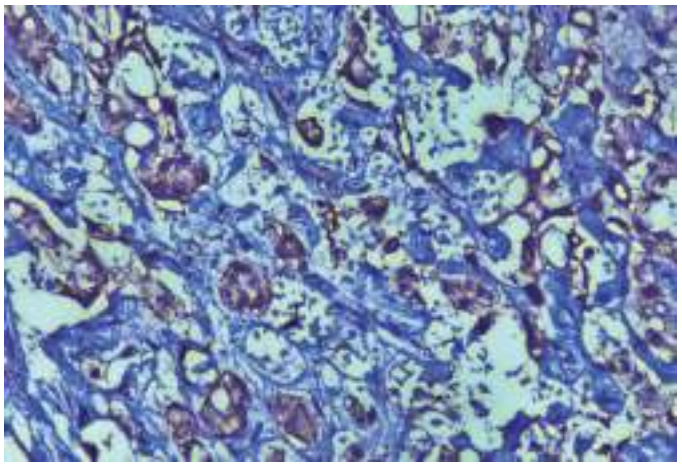


Fig. 21.89C: Hepatic epithelioid hemangioendothelioma. It shows positivity for CD34 (400X).

Differential Diagnosis

Hepatic epithelioid hemangioendothelioma should be differentiated from epithelioid hemangioma, epithelioid angiosarcoma, and metastatic carcinoma especially lobular carcinoma of breast. Distinguishing features of hepatic epithelioid hemangioendothelioma and metastatic adenocarcinoma are given in Table 21.89.

Prognosis and Treatment

Hepatic epithelioid hemangioendothelioma is treated by wide surgical excision. Adjuvant radiotherapy may be in selected cases. Hepatic epithelioid hemangioendothelioma should be treated by liver transplantation. It may metastasize to distant sites in 17% of cases with poor prognosis. Classic low-grade and high-grade epithelioid hemangioendothelioma have risk of local recurrence and distant metastasis. Death occurs due to hepatocellular failure.

METASTATIC TUMORS (LIVER METASTASES)

Despite all the foregoing material, hepatic metastatic malignancies are commonest. Such metastases may be present in 40% of adults with malignant disease elsewhere.

- Metastatic deposits are visible on the capsular surface of the liver. These deposits show multiple nodules of variable sizes with central depression as a result of tumor necrosis.
- Liver metastases arising from epithelial, mesenchymal and round cell tumors are shown in Fig. 21.90.

Immunohistochemistry technique in malignant tumors metastasizing to liver is given in Table 21.90. Gross and histologic features of primary and secondary malignancies of the liver are given in Table 21.91.

MODE OF METASTASES TO LIVER

- **Direct spread:** Liver may be involved by direct spread from malignant neoplasms in the vicinity such as pancreas, extrahepatic bile duct, stomach and gallbladder.

Table 21.89 Distinguishing features of hepatic epithelioid hemangioendothelioma and metastatic adenocarcinoma

Features	Hepatic Epithelioid Hemangioendothelioma	Metastatic Adenocarcinoma
Clinical features		
Patient age	Mean 40 years	Mean 60 years
Gross morphology		
Underlying liver disease	Absent	Absent
Unicentric or monocentric	▪ Multiple or solitary ▪ Well-circumscribed tumor	▪ Multiple > solitary lesions ▪ Ill-defined
Histologic features		
Gland formation	Absent	Present
Tumor cell morphology	Epithelioid morphology	Epithelioid morphology
Cytoplasmic vacuoles	Necrotic debris, hyaline collagen and thrombosed material within the blood vessel	Intracytoplasmic lumina containing mucin
Nuclear features	Mild to moderate atypia	Severe atypia
Mitoses	Occasional mitosis	Frequent mitoses
Stroma of tumor	Myxoid or hyalinized stroma	Desmoplastic stroma
Vascular invasion	Multicentric invasion in large veins	Lymphovascular invasion around portal tracts

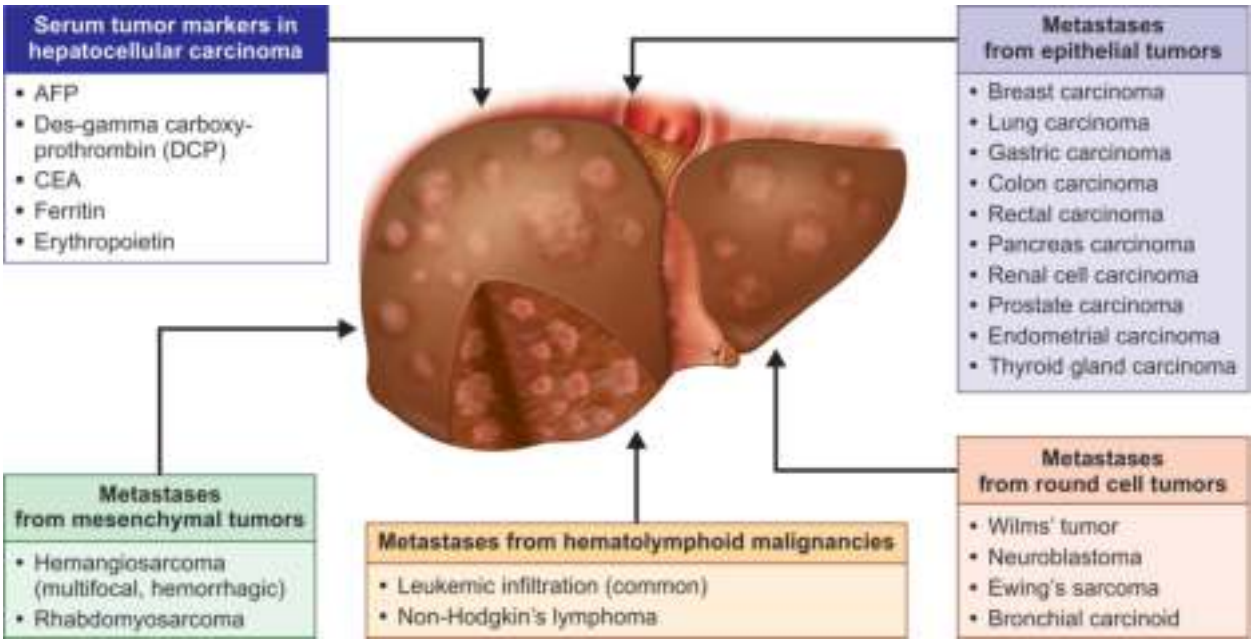


Fig. 21.90: Liver metastases from epithelial, mesenchymal and round cell tumors.

Table 21.90 Immunohistochemistry technique in malignant tumors metastasizing to liver

Tumor	Immunohistochemical Markers
Liver metastases from epithelial tumors	
Breast carcinoma	ER, PR, HER2/neu, CK7, GCDFP-15 (gross cystic disease fluid protein 15) and mammoglobulin, Ki-67, eK5, NY-BR1, E-cadherin
Lung carcinoma	EMA, CK7 and TTF-1
Gastric carcinoma	Cytokeratin, EMA and CEA
Colorectal carcinoma	CK20, CDX2, CEA and β-catenin
Pancreas carcinoma	Vimentin, α_1-antitrypsin and α_1-antichymotrypsin
Renal cell carcinoma	Coexpression of cytokeratin and vimentin
Prostate carcinoma	Prostate-specific antigen (PSA), prostate-specific alkaline phosphatase (PSAP), NXK31, prostein and p63
Endometrium carcinoma type 1	ER, PR and PTEN
Thyroid gland carcinoma	TTF-1, thyroglobulin, CA 125 and cell surface glycoprotein
Liver metastases from lymphoid cell malignancies	
NHL B cell (common)	CD10, CD19 and CD20
NHL T cell (common)	CD3
Leukemias* (common)	Cytochemistry, flow cytometry and cytogenetics
Liver metastases from mesenchymal tumors	
Rhabdomyosarcoma	Desmin, myogenin, myoglobin, MyoD1 and vimentin
Metastases from round cell tumors	
Wilm's tumor	WT1 and WT2
Neuroblastoma	Nonspecific enolase (NSE), neurofilament, chromogranin, synaptophysin
Ewing sarcoma	CD99 (MIC2 specific marker), vimentin and NSE
Bronchial carcinoid tumor	NSE, chromogranin, synaptophysin and cytokeratin

**Hemangiosarcomas (multifocal, hemorrhagic) may also metastasize to liver.*

Table 21.91 Gross and histologic features of primary and secondary malignancies of the liver

Gross and Histologic Features	Conventional Hepatocellular Carcinoma	Metastatic Adenocarcinoma	Metastatic Melanoma	Metastatic Endocrine Tumor
Gross morphology				
Tumor number	Solitary > multiple	Multiple > solitary	Multiple	Multiple
Background liver morphology	Cirrhotic background	Unremarkable	Unremarkable	Unremarkable
Histologic features				
Architecture	Neoplastic cells arranged in trabecular, acinar, pseudo-glandular architecture with mitoses, bile in neoplastic cells	Neoplastic cells form glandular structures with mucin in lumen and cytoplasm	Neoplastic cells arranged in sheets and nests	Neoplastic cells arranged in trabecular and forming glandular structures
Quality of stroma of tumor	Absent or minimal stroma, fibrous in fibrolamellar variant	Desmoplastic stroma	Minimal stroma	Hyalinized stroma
Cytomorphological features	Uniform neoplastic cells resembling hepatocytes; pleomorphism in high-grade tumor	Neoplastic cells pleo-morphic and signet ring cells	Neoplastic cells with eosinophilic cytoplasm containing melanin, eccentric nuclei with macronucleoli	Neoplastic round cells with eosinophilic cytoplasm and stippled chromatin
Mitoses	Easily demonstrated	Easily demonstrated	Easily demonstrated	Rare

- **Hematogenous route:** Most common malignancies metastasizing to liver in adults include colon, lung, breast, kidney, stomach and malignant melanoma. Metastases confined to liver are more probable in association with tumors drained by portal vein. Blood-borne spread from colorectal carcinoma is most often seen in the liver. Multiple metastases in liver are shown in Fig. 21.91. Solitary liver metastasis is shown in Fig. 21.92.

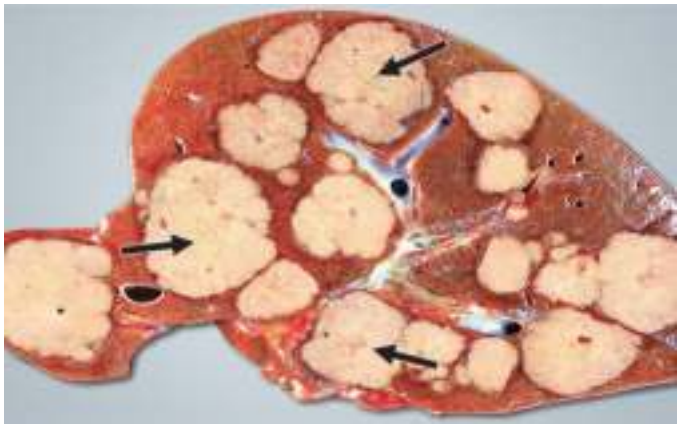


Fig. 21.91: Multiple metastases in liver. Note the numerous mass lesions that are of variable size. Some of the larger ones demonstrate central necrosis. The masses are metastases to the liver. The obstruction from such masses generally elevates alkaline phosphatase, but not all bile ducts are obstructed, so hyperbilirubinemia is typically not present. Also, the transaminases are usually not greatly elevated (arrows).

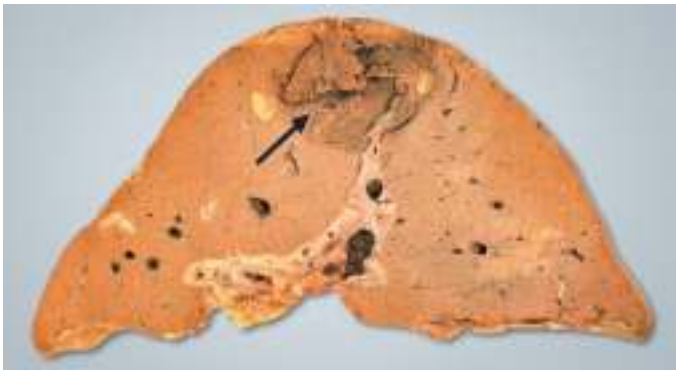


Fig. 21.92: Solitary metastasis in liver (arrow).

CLINICAL FEATURES

Patient presents with massive hepatomegaly with nodules at the free edges, jaundice at a later stage as a result of massive liver involvement and obstruction of major hepatic ducts. There is unexplained increase in the serum concentration of alkaline phosphatase.

IMAGING TECHNIQUE

Tumors metastasizing to liver are most often multiple, however solitary metastatic deposit can be demonstrated by imaging technique. Computed tomographic (CT) scan with contrast of the abdomen in transverse view demonstrates metastases in liver (Fig. 21.93).

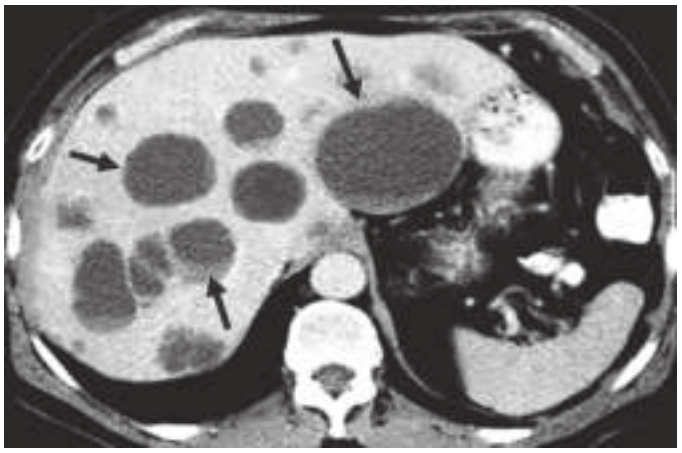


Fig. 21.93: Computed tomographic (CT) scan with contrast of the abdomen in transverse view demonstrates multiple mass lesions representing metastases from a colonic adenocarcinoma. A normal spleen appears at the lower right in the image (on the patient's left) (arrows).

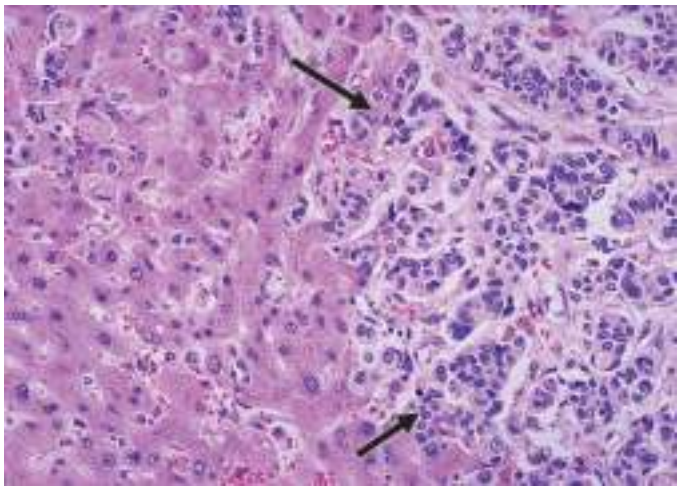


Fig. 21.94: Liver metastases from breast carcinoma. Microscopically, metastatic infiltrating ductal carcinoma from breast is seen on the right, with normal liver parenchyma on the left (arrows) (400X).

Surgical Pathology: Liver Metastases

Light Microscopy

Histologic examination is done on biopsy obtained from liver metastatic deposits. Histologic examination of metastatic deposit in liver from breast carcinoma and from squamous cell carcinoma is shown in Figs 21.94 and 21.95 respectively.

PROGNOSIS

Median survival in untreated cases with liver metastases varies between 4 and 15 months. Patient with solitary liver metastasis has longer survival. Solitary metastasis in liver may be treatable by wedge resection, giving months/years symptom-free remission.

Multiple liver metastases are not resectable. These may respond to chemotherapy.

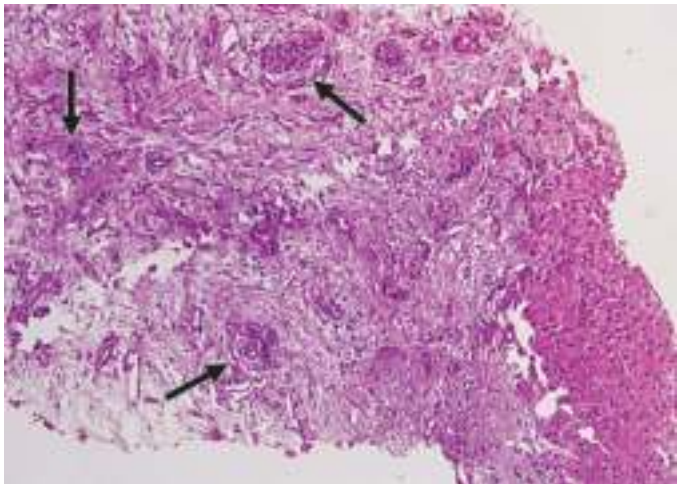


Fig. 21.95: Liver metastasis from squamous cell carcinoma (arrows) (100X).

DISORDERS OF GALLBLADDER

GALLBLADDER: OVERVIEW

ANATOMY

Gallbladder consists of fundus, body and neck, which is covered by serosa except the portion in the liver fossa which merges with liver parenchyma. It is located in the right upper quadrant, under the costal margin at the level of the 9th costal cartilage.

The extrahepatic ducts include the right and left hepatic ducts, which join to form common hepatic duct in the porta hepatis. Common hepatic duct is joined by cystic duct to form common bile duct, which form with pancreatic duct to join and open into the duodenum as the common hepatopancreatic duct guarded by a sphincter called the **sphincter of Oddi**.

HISTOLOGY

Gallbladder consists of a layer of folded columnar epithelium and lamina propria of loose connective tissue that directly rests on muscularis propria and irregularly arranged smooth muscle bundles. External surface of gallbladder is covered by layer of serosa.

PHYSIOLOGY

Bile juice is composed of **bile salts**, water (95%), bilirubin, porphyrins, vitamins, sodium taurocholate and sodium glycolate, cholesterol and phospholipids secreted by the hepatocytes passes through the hepatic ducts stored and concentrated by gallbladder.

Stimuli are received from vagus nerve, secretin, mucus, water and electrolytes for synthesis of bile juice. Bile salts are necessary for emulsification of fats as well as their absorption. Bile juice also activates lipase.

DIAGNOSTIC APPROACH

Cholecystectomy is usually performed for cholelithiasis. **Porcelain gallbladder** is uniformly firm and associated with flattened mucosal surface and submitted to rule out adenocarcinoma.

Endoscopic retrograde cholangiopancreatography (ERCP) is performed to take biopsy for stricture and overt neoplasm. Frozen section is done to evaluate bile duct margins during pancreatoduodenectomy or liver resection for bile duct adenocarcinoma.

NON-NEOPLASTIC DISORDERS

Non-neoplastic disorders of gallbladder are acute and chronic cholecystitis, cholesterolosis, mucocele, choledochal cyst and cholelithiasis.

- Chronic cholecystitis is associated with cholelithiasis in >90% of cases. Classical signs of cholecystitis result from obstruction of the cystic duct by gallbladder stones, which leads to enlarged painful gallbladder distension. Abnormal cholesterol metabolism and bile salts play an important role in the pathogenesis of gallbladder stones.
- Acute cholecystitis is most common during middle age; the chronic cholecystitis occurs most often in elderly people. Classification of cholecystitis is given in Table 21.92.

ACUTE CHOLECYSTITIS

Acute cholecystitis is characterized by full thickness edema, congestion and serosal fibrinopurulent exudate, and most often caused by pyogenic bacteria. It may also occur due to poor or absent blood flow to the gallbladder. Stone impacted in neck or cystic duct results in obstruction of bile flow into the duodenum.

Clinical Features

Patient presents with nausea, vomiting, fever, jaundice (if stone in bile duct), and leukocytosis associated with right upper quadrant and epigastric pain and tenderness. **Murphy sign** is associated with acute cholecystitis.

Table 21.92 Classification of cholecystitis

Acute Cholecystitis
Calculous cholecystitis
Acalculous cholecystitis
Emphysematous cholecystitis
Chronic Cholecystitis
Calculous cholecystitis
Calculous cholecystitis, not otherwise specified
Hyalinizing cholecystitis
Xanthogranulomatous cholecystitis
Acalculous cholecystitis
Lymphoeosinophilic cholecystitis
Eosinophilic cholecystitis
Granulomatous cholecystitis
Diffuse lymphoplasmacytic cholecystitis
Lymphocytic cholecystitis

Surgical Pathology: Acute Cholecystitis

Gross Morphology

- The gallbladder lumen is filled with fibrin, frank pus and hemorrhage, which becomes enlarged and tense. Accumulation of pus in lumen is known as **empyema gallbladder**.
- Patient may also develop gangrenous cholecystitis characterized by hemorrhage, transmural necrosis, and perforation. Serosal surface is covered with inflammatory exudates. Subserosal region exhibits bright red areas of hemorrhages to green-black discoloration.

Light Microscopy

Histopathologic examination of acute cholecystitis shows transmural edema, congestion, serous fibrinopurulent exudate composed of fibrin and polymorphonuclear cells.

Treatment

Acute cholecystitis is initially treated with intravenous fluids and antibiotics which lead to a resolution of the acute inflammation in 70–80% of patients.

Complications

Empyema gallbladder occurs due to accumulation of pus seen in 2–3% of patients with acute cholecystitis. Necrosis of gallbladder results in perforation, abscess formation and peritonitis. Complications of acute cholecystitis are shown in Fig. 21.96.

- Acute inflammation of gallbladder causes edema, vascular compromise and superadded infection resulting in empyema gallbladder (a pus-filled gallbladder).

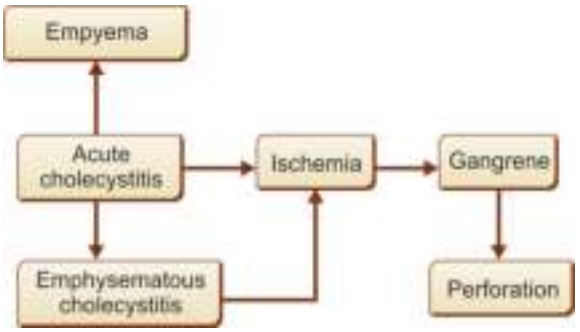


Fig. 21.96: Complications of acute cholecystitis.

- Patient usually experiences severe abdominal pain for more than 7 days.

CHRONIC CHOLECYSTITIS

Chronic cholecystitis is associated with hypertrophy of muscle bundles, fibrosis, chronic inflammatory infiltrate and formation of Rokitansky-Aschoff sinuses. Gallbladder is thickened by fibrosis, which may become rigid.

Etiology

Chronic cholecystitis may develop insidiously or after repeated episodes of acute cholecystitis. It is invariably associated with gallbladder stones.

Clinical Features

Patient may be asymptomatic or symptomatic. Patient with chronic cholecystitis may present with constant or an excruciating colicky pain. Biliary colic and jaundice occur due to obstruction of the biliary system by gallbladder stone.

Infection of static bile causes cholangitis and liver abscess. There is increased risk for development of gallbladder carcinoma in cholelithiasis.

Surgical Pathology: Chronic Cholecystitis

Gross Morphology

- Gallbladder wall is thickened by fibrosis exhibiting gray white in color. The wall is thickened from 2 mm to 1 cm depending on fibrosis.
- Mucosa may be ulcerated. Gallbladder lumen may contain stones impacted in bile (Fig. 21.97).

Light Microscopy

- Epithelium shows formation of '**Rokitansky-Aschoff sinuses**' due to herniation of the lining mucosa into the muscle layers. Rupture of Rokitansky-Aschoff sinuses may lead to '**xanthogranulomatous cholecystitis**' containing lipid-laden macrophages and giant cells mimicking clear cell carcinoma. It is prone to give rise to fistulae. Muscularis mucosa is absent.
- Muscular coat is replaced by **fibrosis**. Muscle bundles are hypertrophied. All the coats are infiltrated by **chronic inflammatory cells** composed of lymphocytes, macrophages and plasma cells. Perimuscular fibrosis is evident.
- Serous coat shows fibrosis. The wall may be markedly thickened, '**porcelain gallbladder**' occurs due to calcification. Light microscopy of chronic cholecystitis is shown in Fig. 21.98.

CHOLESTEROLOSIS (STRAWBERRY GALLBLADDER)

Cholesterolosis (strawberry gallbladder) is characterized by yellow mucosal speckled appearance known as strawberry gallbladder. Cholesterolosis has no association with serum cholesterol elevated level or atherosclerosis. Gross morphology of cholesterolosis is shown in Fig. 21.99.



Fig. 21.97: Gallbladder stones. Cut opened specimen of gallbladder shows multiple faceted white-colored stones. Wall of the gallbladder is thickened.

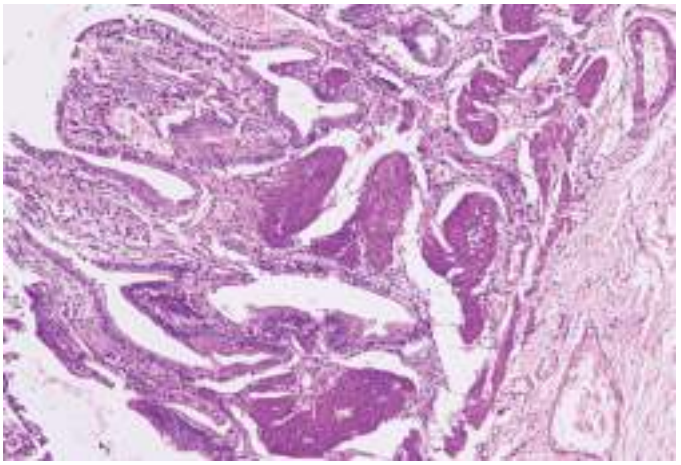


Fig. 21.98: Chronic cholecystitis. It is almost always seen in association with gallbladder stones, though precipitation of bile alone may be sufficient to produce inflammation. There may not be a history of bouts of acute cholecystitis. It shows ulceration of mucosa formation of Rokitansky-Aschoff sinuses due to herniation of the lining mucosa into the muscle layers. There is presence of perimuscular fibrosis (100X).



Fig. 21.99: Cholesterolosis (strawberry gallbladder). Mucosal surface of cut opened gallbladder shows yellow speckled appearance.

Surgical Pathology: Cholesterolosis
Gross Morphology
▪ Cut opened gallbladder specimen shows yellow, flat deposits (focal or diffuse) on mucosal surface. It may have speckled appearance. ▪ Cholesterol polyps may be observed in 20% of cholecystectomy specimens.
Light Microscopy
▪ Histologic examination of cholesterolosis shows presence of cholesterol laden macrophages in the lamina propria of the gallbladder mucosa. ▪ There is no association with inflammatory changes or presence of gallbladder stones. ▪ This finding is incidental without clinical significance (Fig. 21.100).

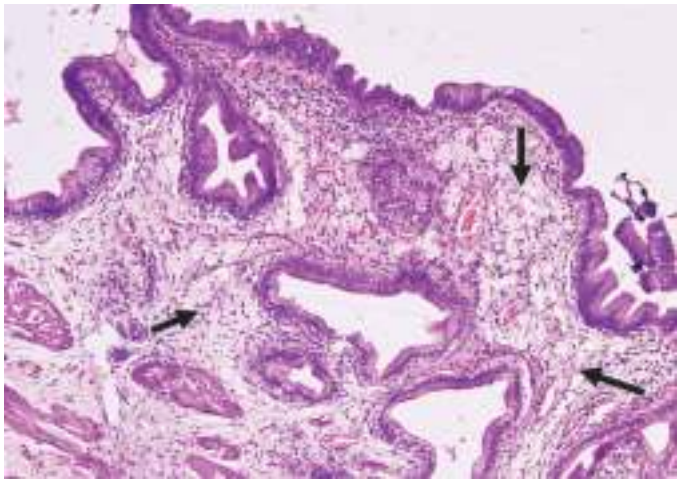


Fig. 21.100: Cholesterolosis (strawberry) gallbladder. Mucosa shows presence of cholesterol laden foamy macrophages in the lamina propria of the gallbladder mucosa (arrows).



Fig. 21.101: Mucocele of gallbladder. It is filled with mucus watery fluid.

MUCOCELE OF GALLBLADDER

Mucocele of gallbladder occurs as a result of sterile long-standing obstruction of the cystic duct by a gallbladder stone. The gallbladder is distended with clear, mucus, watery fluid resulting in flattening of mucosa and thinning of wall. There is absence of bile in gallbladder lumen and inflammatory changes in the wall. Mucocele of gallbladder is shown in Fig. 21.101.

During surgical removal, thin wall of gallbladder demands to avoid spillage of mucus into the peritoneal cavity, so that '**pseudomyxoma peritonei**' is prevented. Pseudomyxoma peritonei becomes seeded with mucus producing epithelial cells and the cavity filled with mucus.

Complications

Mucocele of gallbladder may cause cholangitis, perforation with local abscess, diffuse peritonitis, cholecyst enteric fistula and multiorgan failure.

CHOLEDOCHAL CYST

Choledochal cyst is a form of fibropolycystic disease leading to dilatation of the common bile duct assuming fusiform or spherical shape. Entire lesion should be excised and submitted for histologic examination to exclude biliary intrahepatic neoplasm.

CHOLELITHIASIS (GALLBLADDER STONE DISEASE)

Cholelithiasis is characterized by presence of cholesterol, pigment, or mixed stones in the gallbladder worldwide. It is major cause of morbidity and mortality.

- Risk factors for gallbladder cholesterol stone formation are female sex, obesity, premenopausal and age 40 (**four Fs: female, fat, forty, fertile**). Other risk factors are over age 40, female gender,

high cholesterol diet, increased lipid profile, obesity, sedentary lifestyle, alcohol, tobacco smoking and rapid weight loss.

- Gallbladder stones are usually asymptomatic, however patient may present with severe colicky pain in right upper quadrant with fatty foods, nausea, vomiting and intolerance to fatty food.
- Cholesterol gallbladder stone formation involves four processes: supersaturation of cholesterol, hypomotility of gallbladder, accelerated nucleation of cholesterol in the bile juice and increased synthesis of gel forming mucus. Excess insoluble unconjugated bilirubin precipitates around a nidus forming pigmented stones associated with hemolytic anemia and bacterial infection.
- Complications of gallbladder stones are biliary colic, cholecystitis, common bile duct obstruction, ascending cholangitis, acute pancreatitis, gallbladder stone ileus, mucocele and malignancy. High levels of serum alkaline phosphatase indicate obstructive process by gallbladder stone.
- Cholelithiasis produces histologic changes in the gallbladder mucosa such as acute cholecystitis, chronic cholecystitis, granulomatous inflammation, hyperplasia, cholesterols, dysplasia and gallbladder adenocarcinoma.

BILE JUICE: COMPOSITION

The liver synthesizes bile juice continuously, and enters the gallbladder via hepatic ducts. The gallbladder concentrates and stores it until the duodenum transmits signals to gallbladder to release bile juice into the duodenum for digestion of fat. Bile juice consists of cholesterol, bile salts, bile acids, phospholipids, proteins, bilirubin, sodium bicarbonate and water. Bile juice solutes are shown in Fig. 21.102 and Table 21.93.

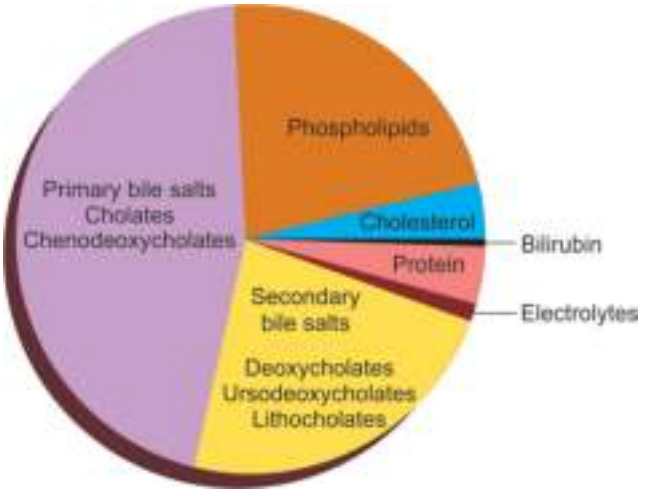


Fig. 21.102: Components of bile juice.

Table 21.93 Bile juice solutes

Bile Solute	% of Solute Present in Bile Solute
Water	97%
Bile salts (cholic acid, chenodeoxycholic acid and deoxycholic acid)	67%
Phospholipids (mainly lecithin)	22%
Protein	4.5%
Cholesterol	4%
Bilirubin (bile pigment)	0.3%
Sodium bicarbonate	Present in small fraction

- **Cholesterol:** Bile contains 4% of cholesterol, which is kept soluble by bile salts and lecithin.
- **Bile salts/acids (cholic acid/chenodeoxycholic acid):** Bile salts are derived from the catabolism of cholesterol and linked with taurine or glycine to form the bile salts. Bile salts constitute 67% of bile, which are mostly reabsorbed in terminal ileum (enterohepatic circulation).
- **Phospholipid (mainly lecithin):** It increases solubility of cholesterol.
- **Proteins:** Bile juice contains 4.5% of proteins.
- **Bilirubin:** It is a by-product of heme degradation.
- **Inorganic salt (sodium bicarbonate):** It keeps bile juice alkaline to neutralize gastric acid in duodenum.
- **Water:** Bile juice contains 97% of water.

GALLBLADDER STONES: CATEGORIES

Vast majority of gallbladder stones are classified into three categories: cholesterol stones (20% cases), pigment stones (5% cases) and mixed stones (75% cases). Depending on chemical constituents in calculus, gallbladder stones are of three types: pure stones, mixed stones and combined stones.

- **Pure gallbladder stones:** Pure gallbladder stone comprises single chemical constituent (**cholesterol** or **calcium bilirubinate** or **calcium carbonate**). Cholesterol stones occur due to disproportion between bile salts/lecithin and cholesterol allows cholesterol to precipitate out of solution and forms stones. Pigment stones occur due to excess of circulating bile pigment (e.g. hemolytic anemia).
- **Mixed gallbladder stones:** Mixed gallbladder stones account for 85% of all gallbladder stones. Most of mixed gallbladder stones are a mixture of cholesterol and calcium salts in variable proportions, which have similar pathophysiology as cholesterol stones.

  Mixed gallbladder stones are multiple and faceted external appearance owing to tight opposition with one other. On cut section, the mixed gallbladder

stones exhibit laminated appearance, which can often be visualized on radiograph due to presence of calcium salts in the stones.

- **Combined gallbladder stones:** Combined gallbladder stones are composed of mixture of salts forming a nucleus surrounded by a shell.

RISK FACTORS

Gallbladder stone disease is a multifactorial disease. Changes in composition of bile juice during synthesis in liver and absorptive ability of gallbladder epithelial lining participate in the pathogenesis of gallbladder stone (cholelithiasis). Risk factors for gallbladder stones are described as under:

- **Race:** Native Americans or Mexican Americans have the highest incidence of gallbladder stones of any specific racial groups.
- **Gender:** Women most common develop gallbladder stones than men especially during the fertile years.
- **Obesity:** Obesity, particularly abdominal or centripetal obesity, is a well-established risk factor for gallbladder stone disease. Obesity is associated with an increased activity of the rate-limiting step in cholesterol synthesis, by hepatic enzyme, 3-hydroxyl-3-methylglutaryl co-enzyme A (HMG-CoA) reductase leading to increased cholesterol synthesis in the liver, its secretion into bile juice and storage in the gallbladder.
- **Female sex hormones:** During pregnancy, female sex hormones are endogenously raised, which adversely influence hepatic bile secretion. Estrogen increases amount of cholesterol in bile and decreases gallbladder activity. These changes allow the cholesterol to crystallize in the gallbladder. Estrogen levels may be related to contraceptive pills, or hormone replacement therapy. Progesterone acts by reducing bile salts secretion and impairing gallbladder emptying leading to stasis.
- **Oral contraceptives:** Oral contraceptives with three months estrogen therapy increase the incidence of gallbladder stone disease in younger women. Estrogen augments functions of the hepatic estrogen receptor-α (ER-α). In the liver, the ER-α receptor stimulates the **SREBP-2** (**sterol regulatory element-binding protein 2**) pathway, promotes cholesterol biosynthesis and hepatic secretion of biliary cholesterol.
- **Rapid weight loss:** Low caloric diet or rapid weight loss exceeding 1.5 kg/week following bariatric surgery increases the risk for gallbladder stone formation especially during the first 6 weeks. These factors reduce gallbladder emptying, so bile becomes concentrated and cholesterol precipitates out into stones.
- **History of gallbladder stones:** People who have gallbladder stones in the past are more likely to develop gallbladder stones in future.
- **Obesity and estrogen imbalance:** Obesity and estrogen imbalance especially in women of 40 years age causes liver to synthesize bile juice that is high in cholesterol or lacking proper concentration of bile salts. When the gallbladder concentrates bile juice, inflammation may occur. Excessive reabsorption of water and bile salts makes the bile less soluble.
- **Diabetes mellitus:** Diabetics have high levels of triglycerides, which increase the risk for gallbladder stones.
- **Liver disease:** Advanced cirrhosis is a well-established risk factor for gallbladder pigmented stones. It is related to altered pigment secretion, abnormal gallbladder motility and/or increased estrogen levels.
- **Crohn's disease:** There is increased risk of development of gallbladder stones in patients with Crohn's disease. Terminal ileum is not able to transport bile acids, so these enter the colon. Biological detergents solubilize unconjugated bilirubin resulting in absorption and return to the liver. The liver then secretes excessive pigment that subsequently precipitates as gallbladder stones. Fasting in Crohn's disease or altered bacterial colonic flora may enhance the deconjugation of bilirubin, which is passively absorbed resulting in increased enterohepatic cycling of the bile pigment.
- **Cystic fibrosis:** Similar to ileal Crohn's disease, cystic fibrosis is associated with bile acid malabsorption due to its binding to undigested dietary nutrients. Gallbladder stone prevalence in cystic fibrosis is 10–30%.
- **Sickle cell disease:** In sickle cell disease, chronic RBCs hemolysis leads to excessive bilirubin excretion with the formation of black pigment stones composed of calcium bilirubinate in gallbladder.
- **Cholesterol lowering drugs:** The drugs that are designed to lower blood cholesterol help to concentrate cholesterol in the gallbladder, thus increasing the risk for forming gallbladder stones.
- **Octreotide drug:** Octreotide drug is a long-acting analogue of somatostatin that inhibits cholecystokinin release, resulting in decreased motility of gallbladder and small intestine. Bile stasis enhances synthesis of deoxycholic acid (bile acid) and cholesterol resulting in precipitation, crystallization of cholesterol and cholelithiasis.
- **Thiazide diuretics:** Thiazide diuretics may increase biliary cholesterol saturation leading to gallbladder stone formation.

PATHOGENESIS OF CHOLELITHIASIS

Bile salts, cholesterol and lecithin are present in the form of mixed micelles and vesicles in gallbladder.

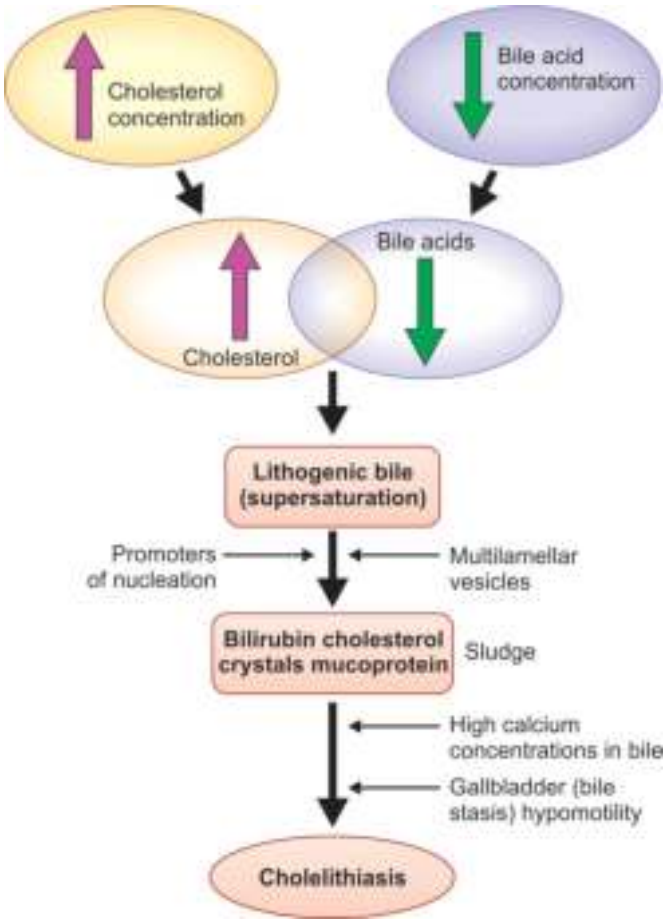


Fig. 21.103: Pathogenesis of gabladder stone formation.

Pathologic changes in gallbladder epithelium may play an important role in the process of gallbladder stone formation. Pathogenesis of gallbladder stone formation is shown in Fig. 21.103.

- **Multilamellar vesicle formation:** Supersaturation of bile juice constituents, i.e. bile salts, cholesterol and lecithin form multilamellar vesicles. The amount of cholesterol in the bile is supposed to increase with age. Hypercholesterolemia is caused by dyslipoproteinemia that results in a linear increase in cholesterol excretion into the bile juice. Cholesterol-7α-hydroxylase is the rate limiting enzyme that regulates the synthesis of bile acids from cholesterol. It is exclusively expressed in the liver. Bile acid synthesis is impared due to deficient cholesterol-7α-hydroxylase.
- **Nucleation:** Gallbladder hypomotility and **cholesterol** nucleation convert multilamellar vesicles to cholesterol monohydrate filaments, which are arranged in plates (nucleation).
- **Promotion:** Promoters, i.e. mucus hypersecretion, gallbladder hypomotility, **calcium salts** act on cholesterol monohydrate plate resulting in accretion (nucleation) and formation of cholesterol stones.

CHOLELITHIASIS: CLINICAL ASPECTS

Asymptomatic/Silent Gallbladder Stones

Most patients are unaware of gallbladder stone disease and remain asymptomatic for life. They never experience biliary pain or complications such as acute cholecystitis, cholangitis, or pancreatitis. Hence, most gallbladder stones are clinically silent, an incidental finding often discovered during abdominal ultrasound being performed for another reason.

Symptomatic Gallbladder Stone Disease

Gallbladder stones can produce a variety of symptoms depending on their anatomical site. These patients require surgical cholecystectomy. Most complications associated with cholelithiasis are related to obstruction of the biliary tree.

Additional rare complications include empyema of the gallbladder, perforation, fistula formation, bile peritonitis, and gallstone ileus. In most cases, gallbladder stones are associated with chronic cholecystitis. Natural history of gallbladder stone disease is given in Table 21.94.

Functional (Acalculous) Gallbladder Disease

Acalculous cholecystitis occurs due to gallbladder ischemia caused by sepsis with hypotension, burns, major trauma and multisystem organs failure. Other causes include diabetes mellitus and infections by cytomegalovirus in immunosuppression state.

- Biliary pain in acalculous gallbladder results from increased intraluminal pressure, as the gallbladder contracts against an obstructed sphincter of Oddi outlet. Biliary colic occurs due to lack of coordination between gallbladder contraction and sphincter of Oddi relaxation, or visceral hypersensitivity.
- A clue to its existence is impaired gallbladder emptying, reliably estimated by cholecystokinin-cholescintigraphy. Ultrasonography shows empty gallbladder lacking gallbladder stone disease.
- Patient presents with acute abdominal pain in the right upper quadrant that may radiate to the back, between the shoulders, or to the front of the chest, colic, nausea and vomiting, chill, low-grade fever,

Table 21.94 Natural history of gallbladder stone disease

Symptom	% of Patients
Asymptomatic	40%
Cholangitis	20%
Jaundice	20%
Biliary colic	15%
Pancreatitis	5%

jaundice, belching, flatulence, indigestion and light headedness.

GALLBLADDER STONES: TYPES

The causes of gallbladder stones are hypercholesterolemia, hyperbilirubinemia, and gallbladder stasis.

GALLBLADDER CHOLESTEROL STONES

Molecules involved in dissolving cholesterol in bile include water-soluble bile salts and water insoluble lecithins.

- More than 75% of gallbladder stones contain significant amount of cholesterol. Cholesterol stones are round or faceted, yellow to tan, and may be single or multiple. The stones are too large to enter the cystic duct or the common bile duct.
- Gallbladder cholesterol stones are radiolucent and not demonstrated on radiograph. But these are demonstrated by ultrasonography (Fig. 21.104).

Composition of Gallbladder Stones

- **Gallbladder cholesterol stone:** Gallbladder cholesterol stone contains 90% of cholesterol, which is most often single, large stone >2.5 cm, pale or white in color. It has soft consistency and can be cut easily. Cut surface has radiating structure made up of cholesterol crystals.
- **Gallbladder mixed stones:** Gallbladder mixed stones contain **50% of cholesterol** and rest mucoproteins, calcium and bilirubin. These are usually multiple but smaller than pure cholesterol stones measuring 0.5–2.5 in diameter. Gallbladder mixed stones account for 85% of all gallbladder stones. These exhibit characteristic faceted appearance.

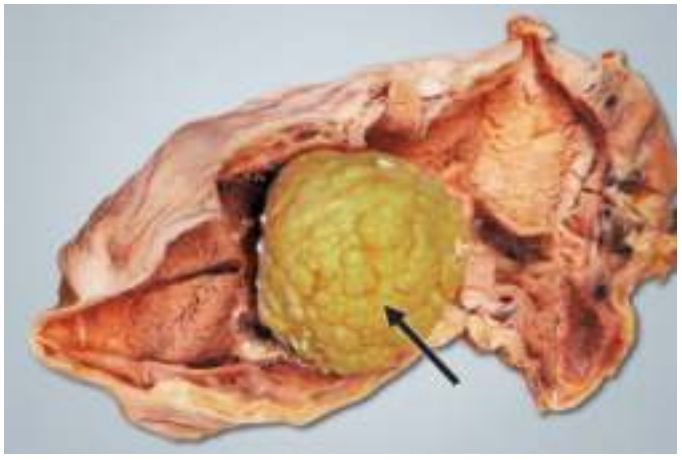


Fig. 21.104: Cholecystectomy specimen shows solitary yellow cholesterol stone (arrow). Cholesterol stone is composed mainly of undissolved cholesterol but may contain other constituents.

Predisposing Factors

During reproductive years, women are up to three times more likely to develop gallbladder cholesterol stones than men. Risk factors for gallbladder cholesterol stones include obesity, female sex, diabetes mellitus, pregnancy, estrogen therapy, hyperlipidemia, bile stasis and inborn disorders of bile acid metabolism.

In obese women, cholesterol secretion by the liver is increased. Impaired gallbladder motor function is another risk factor that leads to gallbladder stone formation. In this way, stasis permits the formation of biliary sludge, which then progresses to macroscopic stones, which are more prevalent in Americans.

Clinical Pearls: Predisposing Factors for Gallbladder Cholesterol Stones

Predisposing factors for development of gallbladder cholesterol stones are described as under:

- Advanced age
- Obesity
- Hyperlipidemia syndrome
- Female sex hormones pregnancy and oral contraceptives
- Bile stasis
- Inborn disorders of bile acid metabolism
- Diabetes mellitus

Pathogenesis

Supersaturation of cholesterol, hypomotility of gallbladder and mucins in gallbladder participate in the pathogenesis of cholesterol stones.

Clinical Pearls: Cholesterol Gallbladder Stone Formation

Cholesterol gallbladder stone formation involves four simultaneous processes as under:

- Supersaturation of cholesterol
- Hypomotility of gallbladder
- Accelerated nucleation of cholesterol in the bile juice
- Increased synthesis of gel forming mucins, e.g. MUC5AC and MUC2.

- **Supersaturation of cholesterol:** If the bile juice contains excess of cholesterol or deficient in bile acids, it becomes supersaturated with cholesterol and precipitates to form stones (lithogenic bile). Cholesterol nucleation is accelerated in bile and mucous hypersecretion in the gallbladder.
- **Gallbladder hypomotility:** Gallbladder motility is decreased in pregnancy, rapid weight loss and long term starvation. Gene polymorphisms increase the rate of cholelithiasis due to impaired gallbladder motility.
- **Mucins in gallbladder:** Mucin-glycoprotein gel is one of the most important and identified pronucleators. Secretory mucins are gel-forming and may increase bile juice viscosity.

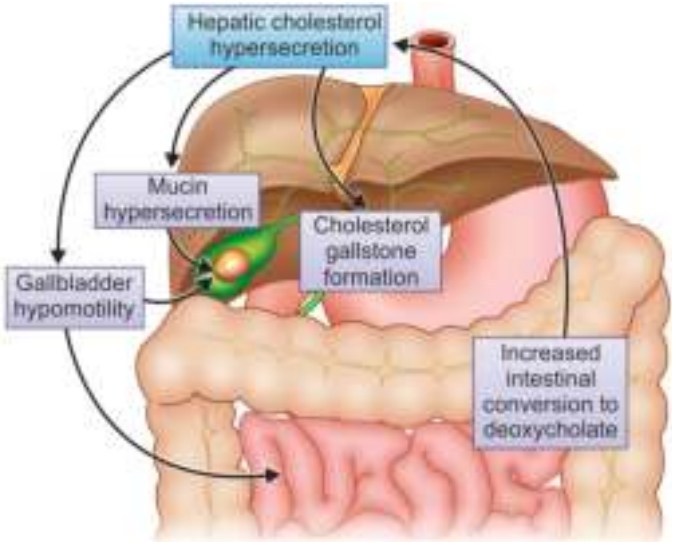


Fig. 21.105: Cholesterol stone formed by hypersecretion leading to formation of cholesterol stone.



Fig. 21.106: Pigmented gallbladder stones. Gallbladder shows multiple faceted yellow tan gallbladder stones. Stone may exit via the cystic duct or common bile duct and obstruct bile flow. It may obstruct at the ampulla of Vater and produce a pancreatitis. Biliary tract obstruction leads to jaundice with increased total and direct bilirubin in serum.

An increased expression of gel-forming mucins such as MUC5AC and MUC2 has been associated with hepatobiliary gallbladder stones, indicating a gene-gene interaction that might affect the accumulation of mucin gel and cholesterol gallstone formation. Pathophysiology of cholesterol stones is shown in Fig. 21.105.

GALLBLADDER PIGMENT STONES

Gallbladder pigment stones occur in 10% of Western population. These are more common in Asian population. Chronic intravascular hemolysis is the cause of gallbladder pigment stones. Precipitation of excess insoluble unconjugated bilirubin results in the formation of pigment stones, which are most often associated with hemolytic anemia and bacterial infection. Gallbladder pigment stones show multiple faceted yellow tan gallbladder stones are shown in Fig. 21.106.

Categories of Gallbladder Pigment Stones

Gallbladder pigment stones are classified into two main categories:

- **Gallbladder black pigment stones:** Gallbladder black pigment stones are most common and associated with hemolysis and cirrhosis. Chronic extravascular hemolytic anemia, e.g. sickle cell disease increases the synthesis of conjugated bilirubin into the bile juice. Increased concentration of bilirubin in bile juice produces formation of calcium bilirubinate resulting in formation of black pigmented gallbladder stones. These are confined to the gallbladder. Bile juice is sterile. Gallbladder pigment stones consist of large amounts of polymerized degradation product of oxidized bilirubin.
- **Gallbladder brown pigment stones:** Gallbladder brown pigment stones are formed due to bacterial contamination resulting in release of microbial β-glucuronidases, which hydrolyzes bilirubin glucuronides resulting in an increase of unconjugated bilirubin in the biliary tree that exceed its solubility. Gallbladder brown pigment stones contain large amount of calcium bilirubinate, which have soft consistency. Cut surface of brown pigment stone shows laminated appearance. These brown pigment stones are formed either in gallbladder or bile ducts. The bile is most often infected.

Predisposing Factors

There is increased frequency of gallbladder pigment stones in Crohn's disease, ileal resection or bypass, cystic fibrosis with pancreatic insufficiency and chronic hemolytic anemia. Gallbladder pigment stones are more common in Asian than Western population.

Predisposing Factors: Gallbladder Pigment Stones

Predisposing factors for development of gallbladder pigment stones are described as under:

- Chronic hemolytic syndrome
- Biliary infection
- Ileal resection or bypass
- Crohn's disease involving ileum
- Cystic fibrosis
- Pancreatic insufficiency

Table 21.95 Differences between various types of gallbladder pigment stones

Parameters	Cholesterol Stones	Black Pigment Stones	Brown Pigment Stones	Biliary Sludge (Microlithiasis)
Composition	Cholesterol (50–100%)	Calcium bilirubinate polymer	Unconjugated bilirubin, calcium soap of fatty acids, cholesterol and mucin	Pigment (calcium bilirubinate) cholesterol microcrystals and mucin
Location	Gallbladder ± common bile duct	Gallbladder ± common bile duct	Bile ducts	Gallbladder
Detection	Ultrasound	Ultrasound	Cholangiography	Abdominal or endoscopic ultrasonography
Clinical association	Family history (genetic), obesity, female sex and aging (excessive cholesterol secretion)	Increased or altered bilirubin excretion in hemolysis, cirrhosis, cystic fibrosis, Crohn's disease and advanced age (excessive bilirubin excretion)	Infection, inflammation and infestation (stasis and strictures)	Fasting total parenteral nutrition (TPN) and pregnancy

Surgical Pathology: Gallbladder Pigment Stones

Gross Morphology

- Gallbladder pigment stones are usually multiple measuring <1.5 cm in diameter found in sterile bile.
- Gallbladder pigment stones are composed of calcium salts of unconjugated bilirubin (calcium bilirubinate), mucin glycoprotein, less cholesterol monohydrate crystals.
- Gallbladder pigment stones are radiopaque in 50–75% of cases (imaging technique). Differences between various types of gallbladder pigment stones are given in Table 21.95.

GALLBLADDER STONES: COMPLICATIONS

Most common complications of cholelithiasis include severe biliary colic due to stone in cystic duct, obstructive jaundice (common bile duct obstruction), ascending cholangitis and acute pancreatitis. Less common complications are acute cholecystitis, empyema, gallbladder perforation, gallstone ileus, porcelain gallbladder, mucocele of gallbladder, squamous cell carcinoma, extension of inflammation in the biliary tree, Mirizzi's syndrome and Bouveret's syndrome.

Clinical Pearls: Gallbladder Stones—Complications

- Severe biliary colic
- Obstructive jaundice
- Ascending cholangitis
- Acute pancreatitis
- Acute cholecystitis
- Empyema gallbladder
- Gallstone ileus
- Porcelain gallbladder
- Mucocele of gallbladder
- Extending inflammation in biliary tree
- Mirizzi's syndrome
- Bouveret's syndrome
- Squamous cell carcinoma

SEVERE BILIARY COLIC

If a gallbladder stone lodges in the cystic duct, the gallbladder contracts but cannot empty. Passage of stones into the cystic duct often causes severe biliary colic. Fat entering the duodenum causes the intestinal mucosa to secrete the hormone cholecystokinin, which stimulates the gallbladder to contract and empty.

Clinical Features

Patient presents with sudden pain in right upper quadrant that may radiate to scapular region, lasts for 2–4 hours following ingestion of fatty meals, alcohol, and caffeine. Pain is accompanied by nausea and vomiting. Mild inflammatory change in the gallbladder wall can occur with biliary colic; with recurrent episodes leading to chronic cholecystitis.

OBSTRUCTIVE JAUNDICE

Lodgement of gallbladder stone in the common bile duct leads to obstructive jaundice, cholangitis, and acute pancreatitis. Patient develops conjugated hyperbilirubinemia, hypercholesterolemia and high alkaline phosphatase level. Biliary narrowing and swelling of the tissue around the stone can also cause irritation and inflammation of the common bile duct.

Clinical Features

Patient presents with yellow discoloration of skin and the eyes (sclera) and skin itch. As the condition worsens, patient develops severe abdominal pain, fever, chills nausea and vomiting.

ASCENDING CHOLANGITIS

Infection in the common bile duct from obstruction is common and serious. Ascending cholangitis results from secondary bacterial infection facilitated by obstructed bile flow as a result of stone in common bile duct.

- Patient presents with Charcot's triad of fever, jaundice and right upper abdominal pain.

- This potentially life-threatening complication requires urgent supportive care, antibiotics and drainage of the biliary tree, usually by endoscopic retrograde cholangiopancreatography (**ERCP**) with either removal of the stone or placement of a stent.

ACUTE PANCREATITIS

Stones in the lower portion of common bile duct obstructing pancreatic duct increases risk for acute pancreatitis.

- Autodigestion of the pancreas occurs by the lipase enzyme, which normally secretes into the duodenum. Autodigestion of pancreas by lipase leads to release of lipids. These lipids then form soaps, i.e. saponification with calcium leading to hypocalcemia.
- Patient presents with rapid onset of upper abdominal pain and vomiting.
- Acute pancreatitis can be a life-threatening disease and requires correction of hypovolemia, administration of antibiotics and the early consideration of endoscopic sphincterotomy via ERCP in severe cases.

ACUTE CHOLECYSTITIS

Acute cholecystitis occurs due to complete obstruction of the cystic duct by a gallbladder stone in 95% of gallbladder stone disease. Acalculous cholecystitis occurs in remainder 5% of cases.

- The most serious complication of acute cholecystitis is infection, which develops in about 20% of cases. Acute cholecystitis is extremely dangerous and life-threatening if it spreads to other parts of the body leading to septicemia and surgery is often required.
- Untreated cases of acute cholecystitis may result in formation of abscesses, gangrene and necrosis, which are encountered in elderly diabetic patients.
- Patient presents with pain abdomen lasting for several hours, nausea, vomiting, fever, chills and passage of clay colored stool. Pain occurs usually in the middle of right side of upper abdomen, which may also radiate to right shoulder or back region.
- Acute cholecystitis is diagnosed by abdominal ultrasonography, hepatobiliary scintigraphy, cholangiography and CT scan.
- Acute cholecystitis is initially treated by antibiotics and intravenous fluids resulting in resolution of acute inflammation of gallbladder in 70–80% of cases.

EMPYEMA GALLBLADDER

Acute cholecystitis in presence of bacteria in the bile juice may progress to suppurative infection of the gallbladder filled with purulent material (pus) in 2–3% of cases. This condition is referred as empyema gallbladder, which develops when the gallbladder neck is obstructed by gallbladder stone in the presence of infection, thus preventing pus draining via cystic duct.

- Necrosis of the gallbladder results in perforation, abscess formation and peritonitis.
- Patient usually experiences severe abdominal pain, which persists for more than 7 days.
- Most patients of empyema gallbladder require aggressive hydration, intravenous antibiotics and surgery within 48 to 72 hours after optimal hydration.

PERFORATED GALLBLADDER WITH PERITONITIS

An estimated 10% of acute cholecystitis results in a perforated gallbladder, and diffuse peritonitis. It is a life-threatening condition. Perforation of the gallbladder is most common in people with diabetes mellitus.

- The risk for emphysematous cholecystitis increases due to infection by gas forming bacteria (*E. coli* and *Clostridium welchii*).
- Once the gallbladder has perforated, pain may temporarily decrease, which is a dangerous and misleading sign, because peritonitis develops afterward.

GALLSTONE ILEUS

In some cases of gallbladder stone disease, gallbladder stone erodes through the gallbladder into the adjacent small bowel forming cholecystoenteric fistula.

- Gallstone enters ileum through the fistulous tract and blocks the intestine known as gallstone ileus.
- Gallbladder stone ileus can be very serious and requires immediate surgery, which primarily occurs in patients over age 65, and can sometimes be fatal.

RISK FOR SQUAMOUS CELL CARCINOMA OF GALLBLADDER

Primary squamous cell carcinoma of gallbladder is most often associated with cholelithiasis in 75% of cases. Presence of gallbladder stones increases risk of development of carcinoma by 4 to 5 folds. Elective cholecystectomy can be preventive measure for gallbladder carcinoma in some patients with chronic cholelithiasis.

- Chronic irritation of gallbladder induced by gallbladder stones leads to squamous metaplasia of columnar mucosa leading to squamous cell carcinoma (exophytic or infiltrative growth).
- Patient is most often asymptomatic until the disease has reached an advanced stage. Patient presents with weight loss, anemia, recurrent vomiting and abdominal lump.

PORCELAIN GALLBLADDER

Gallbladder is referred to as porcelain gallbladder, when their walls become calcified due to calcium deposits appears like porcelain on radiograph. Porcelain gallbladder has been associated with a high risk for primary squamous cell carcinoma of gallbladder. This condition

may develop from a chronic inflammatory reaction that may actually be responsible for the gallbladder cancer risk.

MUCOCELE OF GALLBLADDER

Gallbladder distended with mucus secondary to sterile long-standing obstruction of the cystic duct by a gallstone is known as mucocele of gallbladder. During surgical removal, thin wall of gallbladder demands to avoid spillage of mucus into the peritoneal cavity, so that '**pseudomyxoma peritonei**' is prevented. Pseudomyxoma peritonei becomes seeded with mucus-producing epithelial cells and the cavity fills with mucus.

Surgical Pathology: Mucocele of Gallbladder
Gross Morphology
Gallbladder is distended with clear, mucus and watery fluid.
Light Microscopy
▪ Accumulation of clear, mucus and watery fluid in gallbladder leads to flattening of mucosa and thinning of wall. ▪ There is absence of bile in lumen and inflammatory changes in the wall.
Differential Diagnosis
Mucocele of gallbladder may be mistaken for a mucinous carcinoma. Macarophages may resemble signet ring cells. Immunohistochemistry helps in differentiating mucocele and mucinous carcinoma of gallbladder.

Complications

Mucocele of gallbladder may cause cholangitis, perforation with local abscess, diffuse peritonitis, cholecyst enteric fistula and multiorgan failure.

EXTENDING INFLAMMATION IN BILIARY TREE

Inflammation due to cholelithiasis can progress up the biliary tree into any of the bile ducts, which causes scar tissue, fluid accumulation, cirrhosis, portal hypertension, and bleeding.

MIRIZZI'S SYNDROME

Impaction of gallbladder stones in the gallbladder neck or cystic duct (Hartmann's pouch) can lead to compression of the common hepatic duct producing jaundice, which is termed '**Mirizzi's syndrome**'. It is rare presentation of gallstone disease. It may be complicated by fistula formation (cholecystocholedochal fistula), which usually requires surgical repair.

BOUVERET'S SYNDROME

A large gallbladder stone can rarely erode through the gallbladder wall and stomach resulting in cholecystogastric fistula formation. Gallbladder stone impaction in the stomach or duodenum leads to gastric outlet obstruction; known as 'Bouveret's syndrome'.

Clinical Pearls: Pain in Right Upper Quadrant
Differential diagnosis of pain in right upper quadrant of abdomen includes gallbladder disease (and its related complications), gastritis/duodenitis, peptic ulcer disease/perforated peptic ulcer, acute pancreatitis, right lower lobe pneumonia and myocardial infarction (Table 21.96).

Table 21.96 Differential diagnosis of pain in right upper quadrant of abdomen

Pathological Entity	Clinical History	Clinical Examination	Laboratory Investigations
Biliary colic	Intermittent pain (minutes to hours) in right upper quadrant/radiating to back and right shoulder	Tenderness in right upper quadrant, afebrile and no peritonism	LFT, CRP and WBCs count normal
Acute cholecystitis	Constant pain in right upper quadrant, radiating to back and right shoulder	Tenderness in right upper quadrant, Murphy's sign and febrile	CRP and WBCs raised, LFT normal or mild elevation
Empyema	Constant pain in right upper quadrant, radiating to back or right shoulder	Tenderness in right upper quadrant, peritonism, Murphy's sign +ve, pyrexia, more septic than acute cholecystitis	WBCs and CRP raised, LFT normal or mild elevation
Obstructive jaundice	Yellow discoloration of eyes, passage of pale stool and dark urine, painless or mild pain in right upper quadrant	Jaundice, nontender right upper quadrant, no peritonism, afebrile, Murphy's sign negative	WBCs and CRP normal, LFT (serum bilirubin and alkaline phosphatase raised)
Ascending cholangitis	Beck's triad (pain in right upper quadrant, jaundice and rigors)	Jaundice, tenderness in right upper quadrant, peritonism, spiking high pyrexia, and may develop septic shock	WBCs and CRP normal, LFT (serum bilirubin and alkaline phosphatase raised)
Acute pancreatitis	Severe constant pain in upper abdominal pain, radiating to back and profuse vomiting	Tenderness in right upper abdomen or generalized peritonism and afebrile	WBCs and CRP raised, LFT normal, serum amylase raised and DIC (complication)

Clinical Pearls: Laboratory Diagnosis of Gallbladder Stone Disease

Laboratory investigations carried out in diagnosing gallbladder stone disease include complete hemogram, blood biochemistry and imaging techniques. Approximately 10% gallbladder stones are radiopaque.

Ultrasonography

Ultrasonography is the first line investigation in gallbladder stone disease for diagnosing cholelithiasis. Dilatation of biliary tree calibre suggests stone in common bile duct (normal CBD <8 mm). Thickening of wall of gallbladder suggests chronic cholecystitis.

Magnetic Resonance Cholangiopancreatography (MRCP)

MRCP is noninvasive diagnostic tool much more accurate than ultrasonography. It visualizes biliary tree accurately. It is used to look for biliary dilatation and any stones in biliary tree.

Endoscopic Retrograde Cholangiopancreatography (ERCP)

- ERCP invasive technique has diagnostic and therapeutic utility in biliary obstruction. It visualizes biliary tree dilatation and stones in biliary tree.
- Gallbladder stones can be extracted to unobstruct the biliary tree and perform sphincterotomy. There is increased risk of development of pancreatitis and duodenal perforation.

Percutaneous Transhepatic Cholangiography (PTC)

PTC invasive technique is used to unobstruct biliary tree when ERCP has failed. It has higher complication rate than ERCP.

CT Scan

CT scan is not the first line investigation used if suspicion of gallbladder empyema, gangrene, or perforation and in acute pancreatitis.

COMMON NEOPLASMS AND PRECURSOR LESIONS OF GALLBLADDER

Benign tumors of the gallbladder are rare. The most common primary tumor of the gallbladder is adenocarcinoma, often associated with gallbladder stones.

- Carcinoma of the extrahepatic biliary ducts and the ampulla of Vater is less common than adenocarcinoma of the gallbladder. Modified WHO classification of the gallbladder and extrahepatic bile ducts is given in Table 21.97.
- Patient suffering from adenocarcinoma of gallbladder presents with progressive, relentless obstructive jaundice and palpable gallbladder.
- Tumor obstructing the common bile duct leads to an enlarged, distended gallbladder. Gallbladder adenocarcinoma is almost always adenocarcinoma.

Table 21.97 Modified WHO classification of the gallbladder and extrahepatic bile ducts

Category	Tumors
Epithelial tumors	▪ Preinvasive lesions ▪ Biliary intraepithelial neoplasms (dysplasia) (BilIN) ▪ Intracholecystic papillary-tubular neoplasm (applicable to extrahepatic bile duct), e.g. adenomas, intracystic neoplasms ▪ Mucinous cystic neoplasm ▪ Carcinomas ▪ Adenocarcinoma, pancreaticobiliary type ▪ Adenocarcinoma, intestinal type ▪ Adenocarcinoma, mucinous type ▪ Signet ring (poorly cohesive cell) carcinoma ▪ Adenosquamous carcinoma ▪ Intracholecystic papillary-tubular neoplasm with associated invasive carcinoma ▪ Squamous cell carcinoma ▪ Undifferentiated carcinoma, medullary type ▪ Undifferentiated carcinoma, sarcomatoid type ▪ Neuroendocrine neoplasms ▪ Well-differentiated neuroendocrine tumor (NET grade 1 and grade 2 carcinoid tumors) and goblet cell carcinoma ▪ High-grade neuroendocrine tumor (NET grade 3), large cell neuroendocrine carcinoma and small cell neuroendocrine carcinoma ▪ Mixed adenocarcinoma and high-grade neuroendocrine carcinoma
Mesenchymal tumors	▪ Granular cell tumor ▪ Leiomyoma ▪ Leiomyosarcoma ▪ Rhabdomyosarcoma ▪ Kaposi sarcoma
Lymphomas	Lymphomas rare
Secondary tumors	▪ Lymphomas ▪ Leukemias ▪ Multiple myeloma ▪ Mastocytosis ▪ Metastatic melanoma

GALLBLADDER ADENOMA

Gallbladder adenoma is a rare benign solitary polypoidal lesion. Small adenoma has low malignant potential. Large adenoma may harbor foci of high-grade biliary intraepithelial neoplasia 3 (BilIN3) and invasive carcinoma. Histologic examination of gallbladder adenoma shows tubular or papillary or tubulopapillary appearance.

BILIARY INTRAEPITHELIAL NEOPLASIA

Biliary intraepithelial neoplasia may be grades 1, 2 or 3. Grade 3 biliary intraepithelial neoplasia may be associated with invasive carcinoma. Therefore, thorough sampling of the cystic duct and margins of the lesion should be carried out to exclude malignancy. Cholecystectomy is curative. Grade 3 biliary intraepithelial neoplasia shows overexpression of TP53 gene.

GALLBLADDER ADENOCARCINOMA

Gallbladder adenocarcinoma can develop in obese women, cholelithiasis, porcelain gallbladder and primary sclerosing cholangitis. The prognosis patient with adenocarcinoma of gallbladder is usually poor, because tumor has often invaded and metastasized by the time tumor is discovered.

Pathology Pearls: Risk Factors for Development of Gallbladder Adenocarcinoma

- Gallbladder stones
- Porcelain gallbladder
- Choledochal cyst
- Gallbladder polyps
- Abnormality of the pancreaticobiliary duct

Pathogenesis

- Metaplasia of gallbladder mucosa occurs as a result of irritation by solitary gallbladder stone. It is widely accepted that metaplastic epithelium is more susceptible to malignant transformation.
- Low-grade dysplasia occurs in 40% and high-grade dysplasia in 16% of cases with cholelithiasis. Carcinoma *in situ* has been observed in 3.5% of cholecystectomy specimens. The great majority of *in situ* carcinoma is grossly indistinguishable from cholecystitis and can easily be overlooked on microscopic examination. Dysplasia, carcinoma *in situ* and invasive carcinoma of gallbladder have been associated with multiple gallbladder stones.

Molecular Genetic Alterations

Changes of the E-cadherin/β-catenin complex during cell to cell interactions result in the loss of cell adhesion and may account for the ability of cancer cells to metastasize.

Surgical Pathology: Gallbladder Adenocarcinoma

Gross Morphology

- The thickness of the gallbladder wall may be normal or minimally thickened.
- Mucosal induration mimics chronic cholecystitis. The mucosa may appear granular, slightly nodular or trabecular pattern in carcinoma *in situ* and invasive carcinoma. It gives support to the idea that carcinoma *in situ* grows slowly and considered a precursor lesion of invasive carcinoma of gallbladder (Fig. 21.107).

Light Microscopy

- **Well differentiated adenocarcinoma:** Tumor is composed of columnar cells forming glandular and papillary structures invading gallbladder wall.The mucosal surface shows massive ulceration and necrosis. Perineural invasion, and vascular emboli may be observed. Sometimes acidophilic secretion is demonstrated within the lumen. Histologic features of well differentiated adenocarcinoma of gallbladder are shown in Fig. 21.108.
- **Undifferentiated adenocarcinoma:** Tumor shows presence of clusters of tumor cells with numerous mitoses surrounded by sclerohyaline stroma.
- **Squamous cell carcinoma:** Cholelithiasis causing squamous metaplasia of columnar epithelium leads to squamous cell carcinoma (exophytic or infiltrative growth). It comprises islands of tumor cells with keratinization and keratin pearls. In some islands of tumor cells, partial necrosis lesions and a polymorph inflammatory reaction are observed.
- **Mucin secreting adenocarcinoma:** Tumor is composed of tumor cells arranged in glandular pattern. There is presence of abundant mucus secretion in the cells identified as signet ring cells and outside glands compressing clusters of malignant cells. Numerous tumor emboli in lymphatic channels predict unfavorable prognosis.
- **Papillary adenocarcinoma:** Tumor is composed of exuberant surface proliferation forming villous structures. The tumor cells invade the gallbladder wall. There is presence of perineural invasion by tumor cells. Lymphatic channels show presence of tumor emboli.

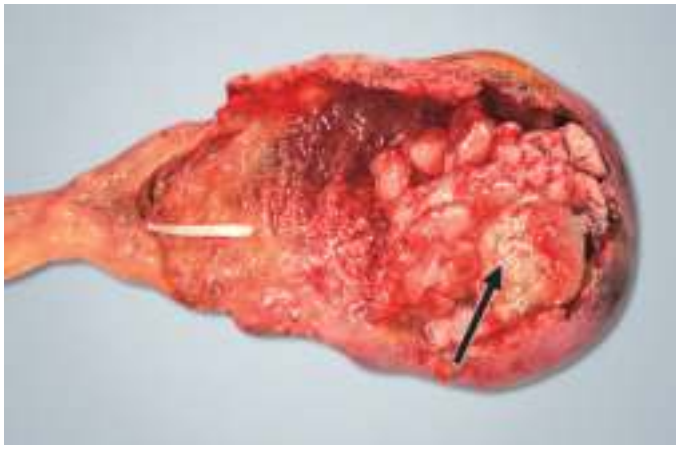


Fig. 21.107: Gallbladder adenocarcinoma. Cholecystectomy specimen shows exophytic and polypoid growth, thickened and indurated gallbladder wall. Cut surface is firm, gritty, tan-yellow to yellow-gray (arrow).

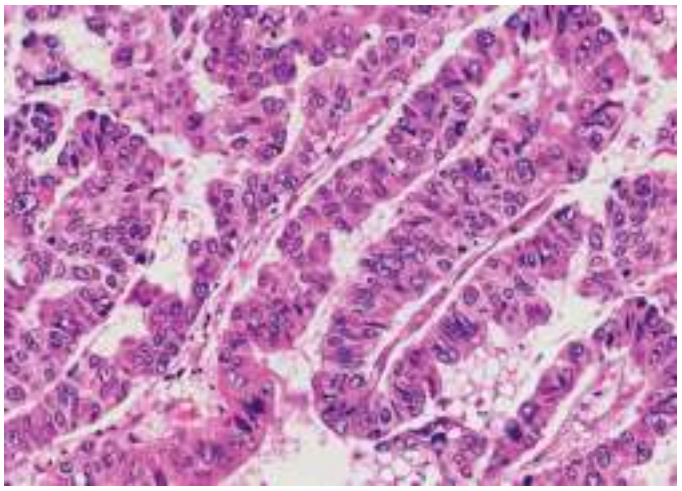


Fig. 21.108: Well-differentiated adenocarcinoma of gallbladder. Tumor is composed of columnar cells forming glandular and papillary structures. The prognosis with adenocarcinoma of the gallbladder is usually poor, because they have often invaded and metastasized by the time they are discovered (400X).

KLATSKIN TUMOR

Klatskin tumor is a slow-growing hilar cholangiocarcinoma, occurring at the confluence of the right and left hepatic ducts.

Predisposing Factors

Etiology of Klatskin tumor is not well defined, which may occur due to acute or chronic biliary tract epithelial injury, primary sclerosing cholangitis, choledochal cysts, caroli's disease and anomalous pancreaticobiliary duct junction. In South East Asia, disease may be caused by *Clonorchis sinensis* and *Opisthorchis viverrini* parasites.

Serum Tumor Markers

Levels of CA 19-9, carcinoembryonic antigen (CEA) and CA 125 are abnormally high in the blood of patients with Klatskin tumor and intrahepatic cholangiocarcinoma.

Imaging Techniques

Ultrasonography detects dilatation of the bile ducts, but more rarely the tumor itself. Magnetic resonance cholangiopancreatography (MRCP) is a good non-invasive technique to detect tumor.

BILIARY ADENOCARCINOMA

Biliary adenocarcinoma may be composed of intestinal, goblet and neuroendocrine cells. Tumor composed of intestinal goblet cells are most often CK20 and CDX2 immunoreactive.

EXTRAHEPATIC BILE DUCT ADENOCARCINOMA

Extrahepatic bile duct adenocarcinoma has been classified as perihilar or distal adenocarcinomas. Each of these entities has its own staging system. These tumors show thickening of the wall. Therefore,

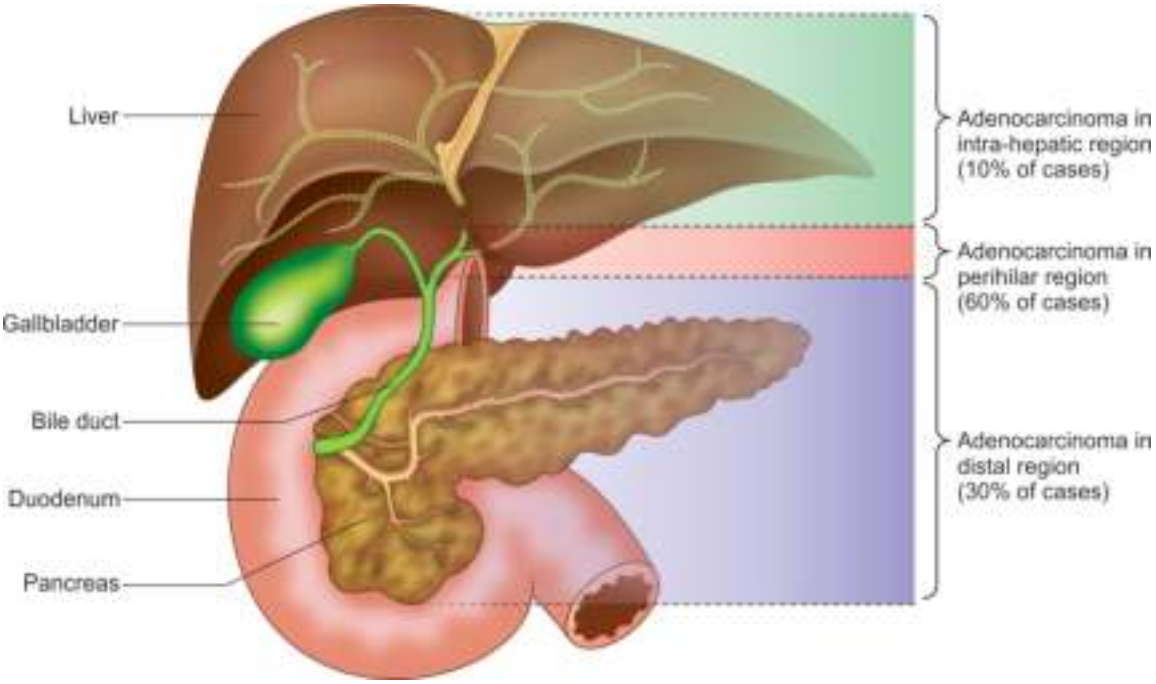


Fig. 21.109: Anatomic boundaries for classification of adenocarcinomas of extrahepatic bile ducts.

thorough sampling of the tumor is required to evaluate margins and local extension.

Perihilar Region of Adenocarcinoma

Perihilar adenocarcinoma originates proximal to cystic duct, which accounts for 60% cases of adenocarcinomas.

Distal Region of Adenocarcinoma

Distal adenocarcinoma arises between the junction of the cystic duct and common bile duct and the ampulla of Vater. It accounts for 30% of adenocarcinomas. Anatomic boundaries for classification of adenocarcinomas of extrahepatic bile ducts are shown in Fig. 21.109.

DISORDERS OF EXOCRINE PANCREAS

EXOCRINE PANCREAS: STRUCTURE

ANATOMY

Pancreas is a large gland located behind stomach and beside the duodenum or upper part of the small intestine. It measures 15–20 cm and weighs 85–120 g and consists of head, neck, body and tail. Pancreas is divided into exocrine portion (acinar and duct system) and endocrine portion (islets of Langerhans).

- The exocrine pancreas forms 85% of the pancreas.
- Exocrine pancreas has duct system. Acinar cells drain into intercalated ducts, interlobular ducts, and main pancreatic duct of Wirsung and accessory duct of Santorini.
- Pancreas receives blood supply from coeliac trunk and superior mesenteric artery. Lymphatic drainage of pancreas occurs to lymph nodes in the region of celiac trunk.
- Annular pancreas is a congenital anomaly in which head of the pancreas surrounds the second portion of the duodenum, which may associated with duodenal atresia. Infants present with feeding disorders and growth retardation.

PHYSIOLOGY

Exocrine pancreas synthesizes digestive enzymes, water, $NaHCO_3$ into the duodenum. The endocrine pancreas synthesizes its hormones (insulin, glucagon) and somatostatin into the bloodstream. In addition, the pH of the pancreatic secretions is alkaline due to very high concentration of $NaHCO_3$. Pathologic disorders of pancreas seen in clinical practice are shown in Fig. 21.110. Composition of pancreatic juice is given in Table 21.98.

PANCREATITIS: OVERVIEW

Pancreatitis is a rare disorder in which the pancreas becomes inflamed. Pancreatic damage occurs when the digestive enzymes are activated and begin attacking the pancreas. In very severe cases, pancreatitis can cause serious damage to pancreas resulting in bleeding into the gland itself, pseudocyst formation and rupture.

- Severe pancreatitis can result in damage to the other vital organs such as heart, lungs and kidneys.

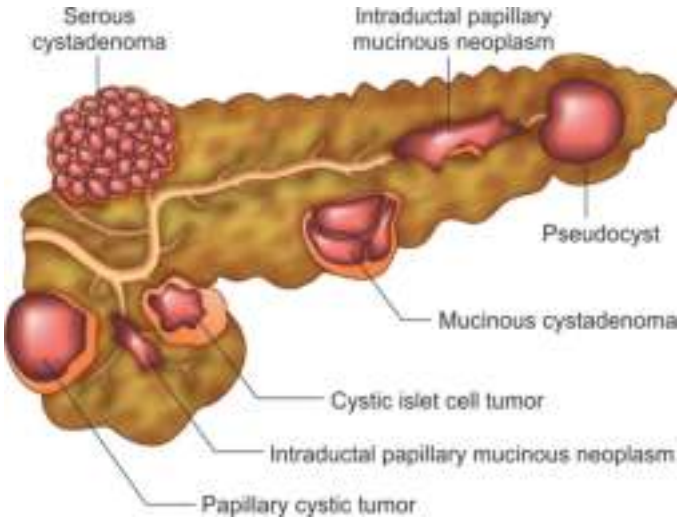


Fig. 21.110: Pathological lesions of pancreas in clinical practice.

Table 21.98 Composition of pancreatic juice

Pancreatic Juice Constituent	Comments
Amylase	Converts starch into maltose and α-dextrins
Lipase	Converts emulsified triglyceride by bile salts into fatty acids and monoglycerides
Trypsin	Enterokinase converts trypsinogen into trypsin, that converts proteins into peptides
Chymotrypsin	Trypsin also converts chymotrypsinogen into chymotrypsin, that proelastase into elastase; and converts proteins into peptides
Carboxypeptidase	Trypsin also converts procarboxypeptidase into carboxypeptidase, that converts terminal amino acid at carboxyl acid end of peptides into peptides and amino acids
Ribonuclease	Coverts ribonucleic acid into nucleotides
Nucleosidase	Converts nucleotides into nucleosides

There are two forms of pancreatitis: acute and chronic.

- Acute pancreatitis occurs suddenly and may lead to life-threatening complications. However, the 80% of patients recover.
- Chronic pancreatitis occurs as a result of long-standing damage to the pancreas in chronic alcoholic persons. Chronic pancreatitis is marked by severe pain abdomen and impairment of pancreatic functions.

- Significant proportion of patients with idiopathic acute and chronic pancreatitis have underlying genetic abnormalities. Laboratory evaluation for acute and chronic pancreatitis is given in Table 21.99.

ACUTE PANCREATITIS

Acute pancreatitis is an inflammatory disease of the exocrine pancreas that results from injury to the acinar cells. It is characterized clinically by acute abdominal pain and increased levels of **serum amylase** and **lipase**. Approximately 89% of the episodes of acute pancreatitis are mild resulting in resolution without serious morbidity. Pancreatic neoplasm may also manifest as acute pancreatitis in an occasional case.

Etiopathogenesis

Alcohol abuse (>80 g daily intake) and obstruction of common bile duct by stones are most important causes of acute pancreatitis in 80% of middle-aged persons.

- Cholelithiasis induced pancreatitis usually affects elderly women as a result of migration of gallbladder stone and subsequent pancreatic duct obstruction.
- Although cholelithiasis is the most important cause of biliary obstruction, yet biliary duct obstruction by neoplasms in periampullary region and head of the pancreas may also provoke acute pancreatitis.
- Pancreatic acinar cell injury induced by injurious agents leads to premature activation protease in pancreatic acinar cells resulting in autodigestion of pancreas.
- Pancreatic acinar cell injury occurs by autoactivation of cathepsin B, which converts cationic trypsinogen to cationic trypsin, anionic trypsinogen to anionic trypsin and mesotrypsinogen to mesotrypsin. These metabolic conversions are inhibited by SPINK1 gene. The cationic trypsin and anionic trypsin convert pro-elastase to elastase, chymotrypsinogen to chymotrypsin, pro-carboxypeptidase A1 to carboxypeptidase A1; and pro-phospholipase A2 to phospholipase A2.
- Autodigestion of pancreas is caused by various enzymes. Phospholipase and lipase cause fat necrosis of pancreas.
  - Elastase enzyme causes damage to the blood vessel resulting in hemorrhage in pancreas.
  - Protease enzyme damages pancreatic acinar cells. Thrombosis in small blood vessels occurs due to activation of coagulation system cascade and complement system.
  - The release of amylase and lipase enzymes from injured pancreas into the blood is a sensitive indicator for monitoring acute pancreatitis. Causes of acute pancreatitis are given in Table 21.100.

Pathogenesis

Genetic Predisposition

Genetic predisposition of acute pancreatitis may occur in minority of cases.

- In recurrent acute pancreatitis, genetic testing demonstrates mutations of **PRSS1** (cationic trypsinogen) gene, **SPINK1** (serine protease inhibitor Kazal type 1) gene, **CFTR** (cystic fibrosis transmembrane conductance regulator) gene and **CTRC** (chymotrypsin C) gene.
- Mutations in PRSS1, SPINK1 and CFTR have been implicated in idiopathic acute and chronic pancreatitis.
- SPINK1 gene is a serine peptidase inhibitor, Kazal type 1. The protein encoded by this gene is a trypsin inhibitor, which is synthesized from pancreatic acinar cells and secreted into the duodenum, which prevents trypsin-catalyzed premature activation of zymogens within the pancreas and the pancreatic duct. SPINK1 gene mutation is associated with hereditary pancreatitis and tropic chronic pancreatitis.

Table 21.99 Laboratory evaluation for acute and chronic pancreatitis

Disorder	Test	Expected Result
Acute pancreatitis	▪ Serum amylase ▪ Serum lipase ▪ Amylase/creatinine clearance	▪ Increased ▪ Increased ▪ Increased
Chronic pancreatitis	▪ Serum amylase ▪ Serum lipase ▪ Amylase/creatinine clearance	▪ Increased, normal or decreased ▪ Increased, normal or decreased ▪ Increased, normal or decreased

Table 21.100 Causes of acute pancreatitis

Category	Causes	
Toxic agents	▪ Alcohol abuse (>80 g daily intake) ▪ Tobacco smoke	▪ Drugs (phenacetin, valproate, thiazide, estrogen, azathioprine)
Obstruction of main pancreatic duct	▪ Cholelithiasis (45%)	▪ Pancreatic neoplasms
Genetic predisposition including hereditary pancreatitis results in recurrent acute pancreatitis	▪ PRSS1 (cationic trypsinogen gene) mutation ▪ SPINK1 (serine protease inhibitor Kazal type 1 gene) mutation	▪ CFTR (cystic fibrosis transmembrane conductance regulator gene) mutation ▪ CTRC (chymotrypsin C gene) mutation
Infectious agents	▪ Human immunodeficiency virus ▪ Mumps virus	▪ Coxsackie virus
Miscellaneous causes	▪ Traumatic injury to pancreas ▪ Hypotensive shock ▪ Post-transplantation	▪ Post-irradiation ▪ Vascular disease (atheroembolism) ▪ Tropical pancreatitis

Alcohol abuse (>80 g daily intake) and obstruction of common bile duct by stones are most important causes of acute pancreatitis in 80% of cases.

- PRSS1 (cationic trypsinogen) gene is located on chromosome 7q34, which provides instructions for making an enzyme called cationic trypsinogen. The cationic trypsinogen enzyme is synthesized in the pancreas and is transported to the small intestine, where it is cleaved to form trypsinogen. PRSS1 gene is associated with hereditary pancreatitis and idiopathic pancreatitis.

Role of Pancreatic Trypsin Role

Activation of trypsinogen to trypsin is a key event in the initiation of pancreatitis. Most investigators believe that acute pancreatitis is caused due to unregulated activation of pancreatic trypsin.

- The trypsin is responsible for autodigestion of pancreas and local inflammation. Enterokinase converts trypsinogen by proteolytic cleavage to trypsin.
- Trypsin also activates other proenzymes in a cascade-like fashion converting chymotrypsinogen into chymotrypsin, that proelastase into elastase.
- Intracellular trypsin may activate other digestive proenzymes, in spite of presence of the trypsin inhibitor SPINK1 and the trypsin-degrading enzyme chymotrypsin C.

Role of Complement System, Kinin System and Cytokines

Complement system and kinin system are also activated, which participate in pathogenesis of acute pancreatitis.

- The inflammation is further propagated by chemical mediators such as IL-1, IL-6 and IL-8 synthesized by neutrophils, macrophages and lymphocytes that reach site of inflamed pancreas.
- Inflamed pancreas shows hemorrhagic fat necrosis and deposition of calcium soaps.
- Autodigestion of the pancreas occurs by the action of various enzymes: (a) phospholipase and lipase produce fat necrosis, (b) elastase damages pancreatic blood vessels resulting in hemorrhage, (c) protease damage pancreatic acinar structures, (d) activated factor XII and complement system cause thrombus formation in the pancreatic blood vessels.
- Pancreatic acinar cell injury results in release of **amylase** and **lipase** in the peripheral blood and their elevated levels are sensitive markers for diagnosing acute pancreatitis.

Clinical Features

Patient presents with acute constant abdominal pain that radiates to the back, nausea and vomiting. Severe acute pancreatitis with retroperitoneal hemorrhage manifests pain in the periumbilical region (**Cullen sign**) and abdominal flank and bluish cutaneous lesions on abdomen. Clinical examination reveals upper abdominal tenderness, tachycardia and hypotension.

Complications

Complications of acute pancreatitis are pseudocyst formation, progression to chronic pancreatitis, diabetes mellitus and abdominal compressional syndrome. Long-term exocrine and endocrine insufficiency may occur. Destruction of islets of Langerhans may result in diabetes mellitus (Fig. 21.111).

- **Pseudocyst formation:** Pseudocyst formation is late complication of acute pancreatitis, which occurs as a result of liquefaction of pancreatic parenchyma by the action of pancreatic enzymes amylase, lipase, elastase and peptidase. Pseudocyst is lined by connective tissue filled with necrotic debris and blood. It may enlarge and obstruct the duodenum. It may become secondarily infected resulting in pancreatic abscess formation.
- **Progression to chronic pancreatitis:** Acute pancreatitis can be imposed on chronic pancreatitis.

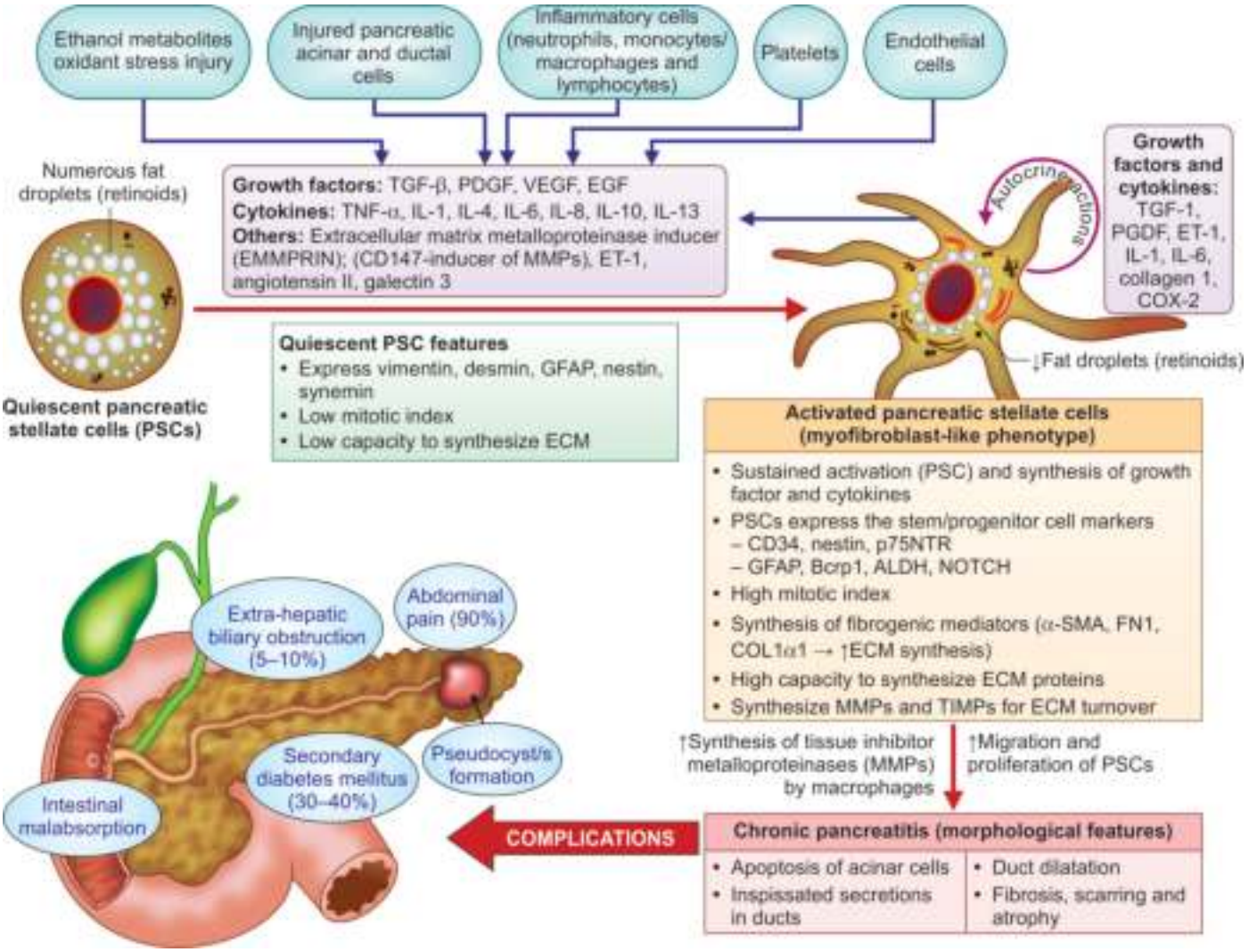


Fig. 21.111: Morphological features and clinical manifestations of chronic pancreatitis.

- **Diabetes mellitus:** Long-term exocrine and endocrine insufficiency may occur in the course of acute pancreatitis. Destruction of islets of Langerhans may result in diabetes mellitus.
- **Abdominal compartment syndrome:** Patient with acute pancreatitis may develop disseminated intravascular coagulation, mesenteric venous thrombosis, bowel infarction, acute respiratory distress syndrome, shock and acute renal failure.

Surgical Pathology: Acute Pancreatitis

- The diagnosis of acute pancreatitis is confirmed by demonstration of elevated serum amylase and lipase.
- Contrast enhanced computed tomography (CT) is performed to confirm the diagnosis of acute pancreatitis, that demonstrates local complications such as fluid collection and necrosis and score the severity of the disease.
- Atlanta classification of acute pancreatitis divides the disease into two grades: mild cases (80%) and severe cases (20%).

Gross Morphology

- The pancreas typically appears swollen and pale. With mild acute pancreatitis, multiple tiny spots of opaque white fat necrosis are observed on the surface of the pancreas.
- In severe cases of acute pancreatitis, large confluent areas of fat necrosis and hemorrhagic areas are observed.

Light Microscopy

- The histologic changes of acute pancreatitis have been demonstrated in autopsy material. In mild form of acute pancreatitis, the disease is concentrated in the interlobular septa of the pancreas. With time, the foci of fat necrosis in the interlobular septa are replaced by fibrosis.
- In more severe form of acute pancreatitis, there is diffuse fat necrosis involving the pancreatic acinar and ductal tissue accompanied by acute inflammatory cells admixed with macrophages. Pancreas shows hemorrhagic necrosis with deposition of calcium soaps. The foci of fat necrosis may liquefy to form a pseudocyst by the action of pancreatic enzymes. The pseudocyst may become infected and associated with high mortality.

Damage to the blood vessels of pancreas results in diffuse hemorrhagic pancreatitis. Well-characterized example of acute pancreatitis triggered by atheroembolism has been reported. Pathogenesis of acute pancreatitis and complications is shown in Fig. 21.112. Fat necrosis of pancreas is shown in Fig. 21.113.

Treatment

Mild form of acute pancreatitis is managed by fluid resuscitation. Severe form of acute pancreatitis requires aggressive fluid resuscitation and antibiotics. Pancreatic fat necrosis and infection are two important local complications, and the infection is responsible for high mortality. When infection is suspected, a fine needle aspiration is done to obtain material for microbiological studies.

CHRONIC PANCREATITIS

Chronic pancreatitis is a pathologic fibroinflammatory disorder of the pancreas and characterized by irreversible morphologic changes such as irregular sclerosis with destruction and permanent loss of exocrine parenchyma, which may be focal, segmental or diffuse and recurrent or persistent abdominal pain.

- Pancreatic duct system shows various degrees of dilatation and its lumen is filled with proteinaceous material and calcification.
- All types of inflammatory cells may be present in varying proportion as well as edema and focal necrosis. Islets of Langerhans may undergo destruction in the later stage resulting in development of diabetes mellitus in these patients.
- Pancreatic insufficiency results in malabsorption syndrome.
- Histologic examination remains the gold standard for diagnosis of chronic pancreatitis. However, biopsy is not feasible in these cases.
- Endoscopic retrograde cholangiopancreatography (ERCP) or biopsy may differentiate inflammatory process from ductal carcinoma of the pancreas.

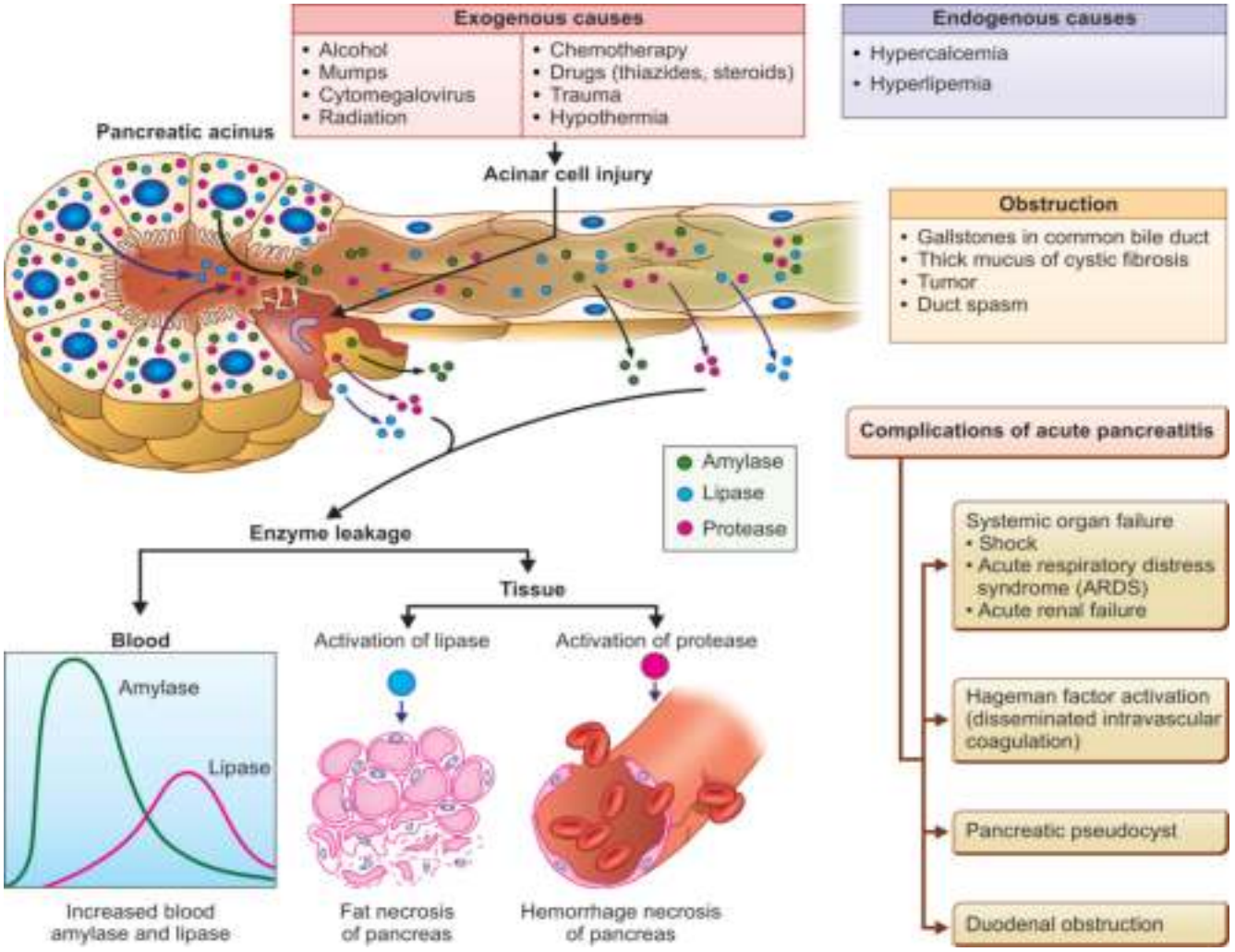


Fig. 21.112: Pathogenesis of acute pancreatitis shows causes of pancreatic injury implicated and complications.

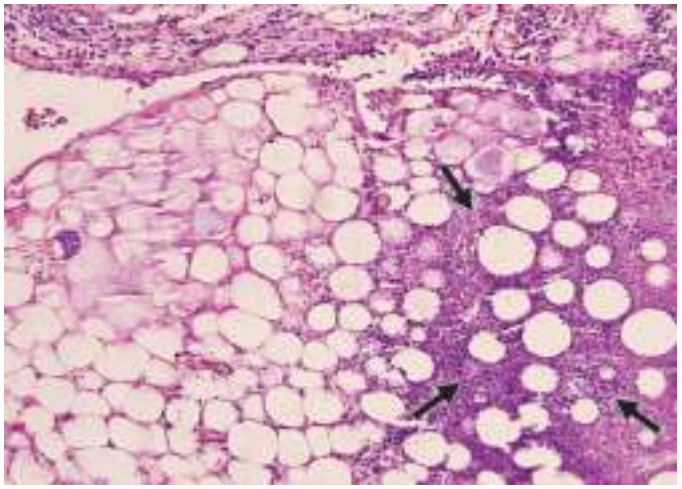


Fig. 21.113: Fat necrosis of pancreas. Microscopic examination reveals irregular islands of necrotic fat cells, which lose their outlines and become indistinct. There is presence of inflammatory cells, macrophages filled with fat and calcium deposits. Deposition of calcium around the periphery of necrotic fat cells gives a basophilic tinge (arrows) (100X).

Etiopathogenesis

In most countries, the most important cause of chronic pancreatitis is alcohol abuse in 60–70% of cases. Some cases suffer from nonalcoholic pancreatitis. Other types of chronic pancreatitis include hereditary pancreatitis, obstructive pancreatitis and pancreatitis associated with hyperparathyroidism.

- Genetic predisposition of acute pancreatitis may lead to chronic pancreatitis in minority of cases.
- In recurrent acute pancreatitis, genetic testing demonstrates mutations of PRSS1 (cationic trypsinogen) gene, SPINK1 (serine protease inhibitor Kazal type 1) gene, CFTR (cystic fibrosis transmembrane conductance regulator) gene and CTRC (chymotrypsin C) gene.
- Mutations in PRSS1, SPINK1 and CFTR have been implicated in idiopathic acute and chronic pancreatitis.
- More recently, mutation in the pancreatic secretory trypsin inhibitor (PST1; N34S located on chromosome 5) has been demonstrated in idiopathic pancreatitis in 25% of cases. Causes of chronic pancreatitis are given in Table 21.101.

Table 21.101 Causes of chronic pancreatitis

Chronic alcoholism
Cholelithiasis
Hypertriglyceridemia
Hypercalcemia
Biliary tract disease
Certain medications
Viral infection

Clinical Features

Clinical features in chronic pancreatitis are variable. Patient may present with recurrent episodic abdominal pain and steatorrhea (foul smelling stool) as a result of pancreatic insufficiency.

- Long-standing malabsorption is accompanied by nutritional deficiency such as anemia, weight loss, bleeding manifestations and osteocalcin (vitamin deficiency).
- The most common local complication of chronic pancreatitis is development of pseudocysts in 25% of cases. These pseudocysts are either retention cysts or that develop following acute exacerbation of chronic pancreatitis. Most pseudocysts regress spontaneously over time.
- Biliary strictures may occur as a complication of chronic pancreatitis.
- Another important complication of chronic pancreatitis is splenic vein thrombosis in 10% of cases. Rare complication is rupture of the main pancreatic duct.

Radiologic Findings

Plain radiograph of the abdomen may demonstrate calcification in chronic pancreatitis. The presence of calcification is best demonstrated by CT scan of the abdomen. Ductal changes are demonstrated by gold standard ERCP and magnetic resonance cholangiopancreatography.

Surgical Pathology: Chronic Pancreatitis

Gross Morphology

Gross morphologic findings in autopsy cases in chronic pancreatitis include irregular scarring, formation of pseudocysts, fat necrosis (autodigestion of pancreatic fat by enzymes) and calcification.

Light Microscopy

- Histologic findings in a case of chronic pancreatitis include presence of extensive fibrosis and inflammatory cells such as lymphocytes, plasma cells and macrophages, perivenular and obliterative thrombophlebitis.
- It is worth mentioning that ductal adenocarcinoma of the pancreas may show histologic features of obstructive pancreatitis distal to the tumor (Fig. 21.114).

AUTOIMMUNE PANCREATITIS

Autoimmune pancreatitis (AIP) is a chronic discrete mass-forming inflammatory lesion in the head of the pancreas, that may mimic pancreatic carcinoma. About 25% of pancreatic resections lack evidence of pancreatic carcinoma. Two subtypes of AIP are now recognized, type 1 and type 2.

- Type 1 AIP is also called IgG4-related disease that often affects multiple organs such as pancreas, bile

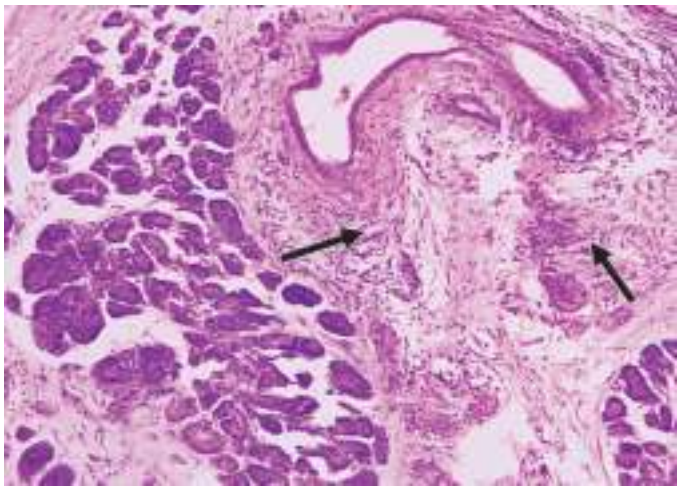


Fig. 21.114: Chronic pancreatitis. It shows infiltration by chronic inflammatory cells. Fibrosis is evident (arrows) (100X).

ducts in the liver, salivary glands, kidney and lymph nodes over age of 40 years. A major milestone in the understanding of the disease is the demonstration of elevated levels of serum IgG4.

- Type 2 AIP is idiopathic, that affects only duct system of pancreas over age of 40 years. Although 33% of persons with type 2 AIP are associated with inflammatory bowel disease.
- AIP can cause pancreatic insufficiency, diabetes mellitus and pancreatic calcification. Differences between type 1 and type 2 AIP are given in Table 21.102.

Clinical Features

Autoimmune pancreatitis is difficult to diagnose. Most patients are asymptomatic. In symptomatic patients, signs and symptoms are similar to those observed in pancreatic carcinoma.

- Patient presents with passage of dark colored urine, pale stool floating in the toilet, yellow discoloration of eyes and skin (jaundice), pain occurring in the upper or middle abdomen, nausea, vomiting, weakness, loss of appetite and weight loss.
- Most common sign of autoimmune pancreatitis in 80% of persons is jaundice as a result of blockage of bile ducts.
- Type 1 AIP responds rapidly to corticosteroid therapy, which is likely to relapse if treatment is discontinued.

Laboratory Investigations

Elevated serum IgG4 is the most robust biomarker for type 1 autoimmune pancreatitis in 80% of cases. The specificity of the test is improved when the threshold of serum IgG4 is set at 280 mg/dl (two times than the normal).

- On imaging, three patterns of AIP are recognized: diffuse (most common), focal and multifocal.
- On ERCP, following four features help in distinguishing AIP from pancreatic adenocarcinoma: (a) a long stricture involving more than one-third of the main pancreatic duct, (b) lack of upstream dilatation, (c) side branches arising from a strictured segment, and (d) the presence of multiple strictures involving the main pancreatic duct.

Surgical Pathology: Autoimmune Pancreatitis

Gross Morphology

- Autoimmune pancreatitis can involve any part of the pancreas. In the active phase, pancreas is enlarged, but in the later phase, pancreas becomes shrunken and fibrotic.
- Gross examination reveals bulky and hard in consistency. Occasionally, a discrete mass is identified, the gross appearance is indistinguishable from pancreatic carcinoma. Main duct of the pancreas is narrow.

Table 21.102 Differences between type 1 and type 2 autoimmune pancreatitis (AIP)

Parameters	Type 1 Autoimmune Pancreatitis	Type 2 Autoimmune Pancreatitis
Age group	Elderly persons during seventh decade of life	Middle aged persons during fifth decade
Sex predilection	Men > women affected	Men = women affected
Clinical presentation	Jaundice (75%) and acute pancreatitis (15%)	Jaundice (50%) and acute pancreatitis (33%)
Systemic disease	Presence of association with system disease	Absence of association with system disease
Elevated serum IgG4	80%	Uncommon
Inflammatory bowel disease	No association	16–30%
Histologic features	Periductal inflammation with one or more of the obliterative phlebitis and storiform fibrosis	Periductal inflammation with one or more of the ductal/lobular abscesses and ductal ulceration with neutrophils
Long-term outcome	Frequent relapse	No relapse

Light Microscopy

- **Type 1 AIP:** It shows periductal inflammation with one or more of the obliterative phlebitis and storiform fibrosis.
- **Type 2 AIP:** It shows periductal inflammation with one or more of the ductal/lobular abscesses and ductal ulceration with neutrophils.

Differential Diagnosis

Autoimmune pancreatitis should be differentiated from pancreatic carcinoma.

CYSTIC FIBROSIS OF PANCREAS

Cystic fibrosis is autosomal recessive disorder of exocrine glands that causes their secretions (mainly mucus, digestive enzymes, bile and sweat) to become abnormally thick and viscous. It is also known as mucoviscidosis or fibrocystic disease of pancreas. It is a lethal disease among White population.

- **Physiologic state:** Normally, transmembrane conductance regulator (CFTr) gene located on long arm of chromosome 7 codes for a membrane protein that participates in the movement of chloride and other ions across membranes.
- **Pathologic state:** Mutation of CFTr gene causes malfunction of exocrine glands results in increased viscosity of mucus and increased chloride concentration in sweat and tears. Pathologic effects in cystic fibrosis are shown in Fig. 21.115.

PATHOLOGICAL LESIONS

Cystic fibrosis affects mostly the lungs but also the pancreas, liver, kidneys and reproductive systems. Systemic manifestations of cystic fibrosis are given in Table 21.103.

- **Respiratory system:** Patient develops recurrent pulmonary infections such as chronic bronchitis, lung abscess, and bronchiectasis. *Pseudomonas aeruginosa* infection is a common cause of death in cystic fibrosis. Other respiratory system changes include development of nasal polyps and chronic sinusitis. Ultimately congestion of lung may alter the pulmonary circulation and to the possibility of right-sided heart failure.
- **Digestive system:** Newborn develops intestinal obstruction caused by thick viscous meconium is known as meconium ileus. In adults, cystic fibrosis interferes with the normal synthesis and drainage of bile juice resulting in gallbladder stones, portal hypertension, splenomegaly, or cirrhosis. Thick mucus also clogs pancreatic duct and hinders the release of pancreatic enzymes. About 90% of cystic fibrosis also develop exocrine pancreatic insufficiency. Later chronic pancreatitis causes diabetes mellitus.
- **Skin:** Cystic fibrosis affects sweat glands in the skin resulting in abnormally thick salty perspiration. There is increased risk of heat stroke and salts depletion especially in infants.
- **Reproductive system:** Almost all men with cystic fibrosis are sterile because epididymis cannot synthesize or incomplete formation of vas deferens. Women with cystic fibrosis often have normal reproductive system and can have successful pregnancies.

DIAGNOSIS AND MANAGEMENT

Sweat chloride test is an important diagnostic tool. Secretion by sweat glands of chloride and sodium is normal, but their reabsorption by sweat ducts is impaired.

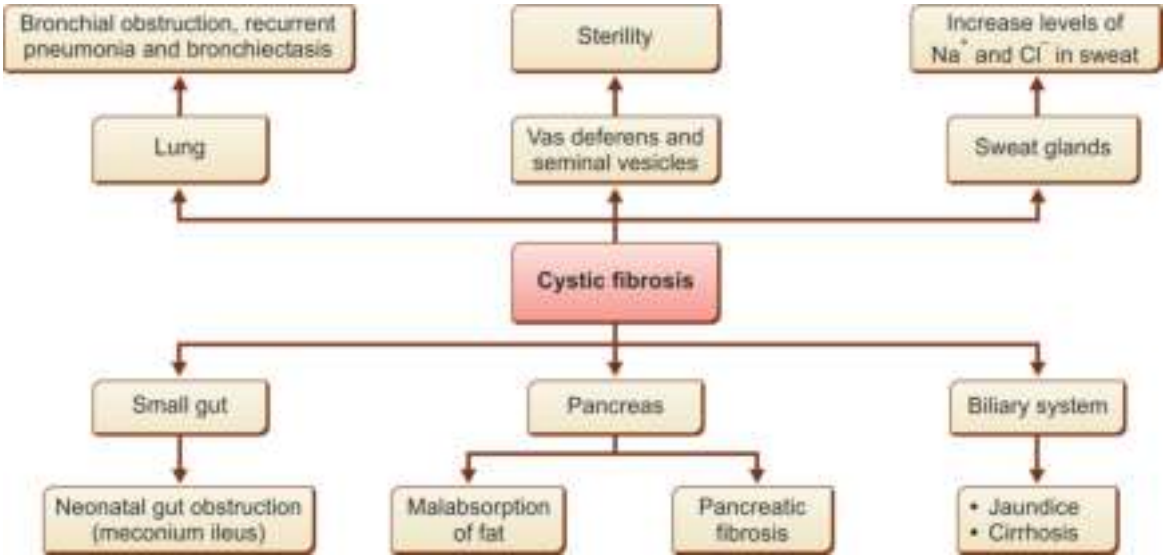


Fig. 21.115: Pathologic effects of cystic fibrosis of pancreas.

Table 21.103 Systemic manifestations of cystic fibrosis

Organs	Secondary Effects
Skin	▪ Salty sweat results in hyponatremia ▪ Acrodermatitis ▪ Dilation of eccrine and apocrine glands
Gastrointestinal lower tract	Meconium ileus resulting in obstruction, perforation, peritonitis, rectal prolapse
Pancreas	▪ Pancreatic duct obstruction causes pancreatic insufficiency (steatorrhea) ▪ Chronic pancreatitis ▪ Failure to thrive ▪ Diabetes mellitus
Liver	▪ Hepatic duct obstruction causes biliary cirrhosis resulting in esophageal varices ▪ Sclerosing cholangitis
Gallbladder	Cholecystitis
Kidney	▪ Nephrolithiasis ▪ Diabetic glomerulopathy ▪ Immune complex-mediated glomerulonephritis
Respiratory system	▪ Bronchiectasis ▪ Recurrent infections ▪ Atelectasis ▪ Allergic bronchopneumonia ▪ Aspergillosis
Heart	▪ Cor pulmonale ▪ Left ventricular fibrosis ▪ Carotid bodies enlarged
Male genital system	Male infertility (obstructive aspermia due to vas deferens atresia in 6% and male infertility in 1–2%)
Female genital system	▪ Multiple ovarian follicular cysts ▪ Cervicitis ▪ Reduced fertility
Musculoskeletal system	▪ Osteoporosis ▪ Muscle wasting ▪ Hypertrophic pulmonary arthropathy ▪ Clubbing
General manifestations	▪ Malnutrition ▪ Vitamin deficiencies (vitamin B_{12}, vitamin K*) ▪ Amyloidosis

Contd...

**Vitamin K deficiency at birth may lead to bleeding.*

- Molecular testing of CFTR gene mutation is another tool. Screening done by using immunoreactive trypsinogen (IrT) assay shows elevated levels in infants with cystic fibrosis.
- Inhalation of bronchodilators reduces resistance in respiratory airways. Inhaled mucolytic agents and saline help in dissolving mucus. Antibiotics are administered to cover infection.

EXOCRINE AND ENDOCRINE PANCREATIC TUMORS

WHO classification of pancreatic neoplasms is given in Table 21.104. Solid and cystic neoplasms of pancreas are given in Table 21.105. Characteristics of epithelial solid neoplasms of pancreas are given in Table 21.106.

Table 21.104 WHO classification of pancreatic neoplasms

Invasive Pancreatic Ductal Adenocarcinoma	
▪ Tubular (conventional) adenocarcinoma	▪ Undifferentiated carcinoma
▪ Colloid (mucinous noncystic) carcinoma	• Anaplastic (giant cell) carcinoma
▪ Medullary carcinoma	• Sarcomatoid carcinoma
▪ Adenosquamous carcinoma	• Carcinosarcoma
▪ Signet ring cell carcinoma	• Undifferentiated carcinoma with osteoclast-like giant cells
▪ Hepatoid carcinoma	▪ Mixed ductal-neuroendocrine carcinoma
Pancreatic Intraepithelial Neoplasia (PanIN)	
▪ PanIN-1A	▪ PanIN-2
▪ PanIN-1B	▪ PanIN-3
Pancreatic Serous Neoplasms	
▪ Microcystic serous cystadenoma	▪ Solid serous cystadenoma
▪ Macrocystic serous cystadenoma	▪ Serous cystadenocarcinoma

Contd...

Table 21.104 WHO classification of pancreatic neoplasms (*Contd...*)

Pancreatic Mucinous Cystic Neoplasms	
▪ Mucinous cystic neoplasm with low-grade dysplasia (mucinous cystadenoma)	▪ Mucinous cystic neoplasm with high-grade dysplasia (carcinoma *in situ*)
▪ Mucinous cystic neoplasm with intermediate dysplasia	▪ Mucinous cystic neoplasm with an associated invasive carcinoma
Pancreatic Intraductal Neoplasms	
▪ Intraductal papillary mucinous neoplasms	▪ Intraductal papillary mucinous neoplasm with an associated invasive carcinoma
▪ Intraductal papillary mucinous neoplasm with low-grade dysplasia	▪ Intraductal oncocytic papillary neoplasm
▪ Intraductal papillary mucinous neoplasm with intermediate grade dysplasia	▪ Intraductal tubulopapillary neoplasm
▪ Intraductal papillary mucinous neoplasm with high-grade dysplasia (carcinoma *in situ*)	▪ Intraductal tubulopapillary neoplasm with an associated invasive carcinoma
Pancreatic Acinar Cell Neoplasms	
▪ Acinar cell cystadenoma	▪ Mixed acinar-ductal carcinoma
▪ Acinar cell carcinoma	▪ Mixed acinar-neuroendocrine-ductal carcinoma
▪ Acinar cell cystadenocarcinoma	
Pancreatic Neuroendocrine Neoplasms	
▪ Neuroendocrine microadenoma	▪ Well differentiated PanNET, nonfunctional
▪ Well differentiated PanNET, functional	• PPoma
• Insulinoma	• Not otherwise specified
• Gastrinoma	▪ Poorly differentiated neuroendocrine carcinoma
• Glucagonoma	▪ Small cell carcinoma
• Somatostatinoma	▪ Large cell neuroendocrine carcinoma
• VIPoma	
Other pancreatic neoplasms	
▪ Pancreatoblastoma	▪ Lymphoma
▪ Solid pseudopapillary neoplasm	▪ Secondary neoplasms
▪ Mesenchymal neoplasms	

PanNET: Pancreatic neuroendocrine tumor; PPoma: Pancreatic polypeptide-secreting tumor; VIPoma: Tumor-secreting vasoactive intestinal polypeptide

Table 21.105 Solid and cystic neoplasms of pancreas

Solid Pancreatic Neoplasms	Cystic Pancreatic Neoplasms
Ductal adenocarcinoma and variants	Serous cystic neoplasms
Acinar cell carcinoma	Mucinous cystic neoplasms
Neuroendocrine neoplasms	Intraductal papillary mucinous neoplasms
Pancreatoblastoma	Solid pseudopapillary neoplasm
	Lymphoepithelial cyst

PANCREATIC ADENOCARCINOMA

Pancreatic ductal adenocarcinoma is the most common malignancy of gastrointestinal tract with high death rate. The majority of pancreatic carcinomas arise from pancreatic duct epithelium. Acinar cell carcinoma is much less common. It is most often silent before widespread dissemination occurs. Death usually results within 1 year. Pancreatic adenocarcinoma elicits an intense desmoplastic response.

Predisposing Factors

- Cigarette smoking is the most important risk factor in the development of pancreatic adenocarcinoma.
- Consumption of smoked meat and fish intake containing polycyclic hydrocarbons increases risk for pancreatic adenocarcinoma. Indirect acting chemical carcinogens associated with pancreatic cancer are given in Table 21.107.

Table 21.106 Characteristics of epithelial solid neoplasms of pancreas

Parameters	Ductal Cell Carcinoma	Acinar Cell Carcinoma	Neuroendocrine Carcinoma
Incidence	More common	Less common	Rare
Desmoplastic response	Marked	Uncommon	Absent
Molecular genetics	K-RAS, TP53, SMAD4, CDKN2A, BRCA2, TGF-β	APC/β-catenin pathway	MEN1 gene, DAXX gene, ATRX gene and mTOR pathway genes
Histologic features	Tumor cells arranged in glandular and papillary structures with mucin in the lumina	Tumor cells arranged in solid sheets, acini, nests with eosinophilic cytoplasm and prominent nuclei with nucleoli	Tumor cells arranged in nests, trabecular and gyriform patterns with uniform salt and pepper chromatin of nuclei
Histochemistry	PAS, mucicarmine positive	d-PAS positive granules	Argyrophil positive granules
Electron microscopic features	Mucigen granules	Zymogen irregular fibrillary granules	Neurosecretory granules
Immunohistochemistry	Glycoprotein markers (carcinoembryonic antigen, CA 19-9, MUC1, TAG72, DUPAN2)	Enzyme markers (chymotrypsin, trypsin, lipase)	Neuroendocrine markers (chymogranin, synaptophysin, CD56)

Table 21.107 Indirect acting chemical carcinogens associated with pancreatic cancer

Chemical Carcinogen	Examples	Associated Cancers
Polycyclic hydrocarbons	▪ Smoked meat and fish intake ▪ Cigarette smoke	▪ Pancreas carcinoma ▪ Oral carcinoma ▪ Esophagus carcinoma (middle and distal) ▪ Lung carcinoma ▪ Renal pelvis transitional cell carcinoma ▪ Urinary bladder transitional cell carcinoma

- *Schistosoma haematobium* also increases risk of pancreatic carcinoma. Parasite associated with pancreatic carcinoma is given in Table 21.108.
- Pancreatic intraepithelial neoplasia and acinar ductal hyperplasia are precursor lesions of pancreatic ductal carcinoma.
- Chronic pancreatitis, diabetes mellitus and alcoholism have been associated with an increased risk for pancreatic carcinoma. Consumption of high fat diet has also been implicated but less common.
- Several familial syndromes increasing the risk for pancreatic adenocarcinoma include Lynch syndrome, Peutz-Jeghers syndrome (STK11/LB1 gene on chromosome 19p13), hereditary pancreatitis (PRSS1 gene), familial atypical mole melanoma syndrome (p16 gene, chromosome 9p21), BRCA2 gene mutation (13q12–q13), familial pancreatic cancer syndrome and Fanconi anemia component genes.

Table 21.108 Parasite associated with pancreatic carcinoma

Parasite	Associated Tumors
Schistosoma haematobium	▪ Pancreatic carcinoma ▪ Squamous cell carcinoma of the urinary bladder

Molecular Genetic Alterations: Pancreatic Adenocarcinoma

Oncogenes, their mode of activation and associated cancers are given in Table 21.109. Tumor suppressor gene mutations associated with pancreatic carcinoma are given in Table 21.110. Genetic progression model of pancreatic carcinogenesis is shown in Fig. 21.116.

- **K-RAS signal-transducing proteins:** Point mutation of K-RAS (chromosome 12p) has been identified in pancreas (90%), colon (50%), and lung (30%).

  SMADA4 tumor suppressor gene (chromosome 18q): SMADA4 tumor suppressor gene is also known as *DPC4*. Mutation of DPC4 (deletion) has been demonstrated in 55% of pancreatic carcinoma.
- **BRCA2 gene (chromosome 13q):** BRCA2 tumor suppressor gene mutation has been associated with pancreatic carcinoma.
- **APC gene (chromosome 5p21):** Mutation of APC tumor suppressor gene (deletion and nonsense) has been demonstrated in sporadic carcinomas (colon, stomach and pancreas) and familial adenomatous polyposis developing colon carcinoma.
- **TGF-β tumor suppressor gene:** TGF-β tumor suppressor gene inhibits G1 to S phase of cell cycle. Mutation of TGF-β has been demonstrated in pancreatic carcinoma.

Table 21.109 Oncogenes, their mode of activation and associated cancers (signal-transducing proteins)

Category	Proto-oncogene	Mode of Activation	Associated Malignancies
GTP-binding (G) proteins	K-RAS	Point mutation	▪ Pancreatic carcinoma ▪ Colon carcinoma ▪ Lung carcinoma

Table 21.110 Tumor suppressor gene mutations associated with pancreatic carcinoma

Gene	Protein	Function	Familial Tumors	Sporadic Cancers
Mitogenic signaling pathway inhibitors				
APC	▪ Adenomatous polyposis protein (locus 5p21) ▪ Mutation by deletion or nonsense	APC inhibits Wnt signaling activator pathway, thus prevents nuclear transcription. It degrades catenin	Familial polyposis coli with malignant transformation	▪ Pancreatic carcinoma ▪ Colon carcinoma ▪ Gastric carcinoma ▪ Thyroid carcinoma ▪ Melanoma
SMADA2 SMADA4	SMADA2 protein SMADA4 protein	Both inhibit TGF-β signaling pathway. These repress CDK4 and MYC. These also induce expression of CDK inhibitor	Juvenile polyposis	▪ Pancreatic carcinoma (55%) ▪ Colon carcinoma
Cell cycle progression inhibitors				
CDKN2A	P16/INK4a and P14ARF	P16 inhibits CDK. P14 indirectly activates p53 tumor suppressor gene	Familial melanoma	▪ Pancreatic carcinoma ▪ Breast carcinoma ▪ Esophagus carcinoma
Angiogenesis inhibitors				
STK11	Liver kinase B1 (LKB1) or STK11	It activates AMPK family of kinases. It suppresses cell growth when cell nutrients and energy levels are low	*Peutz-Jeghers syndrome:* cancers of GIT and pancreas	Diverse carcinomas in 5–20% of cases

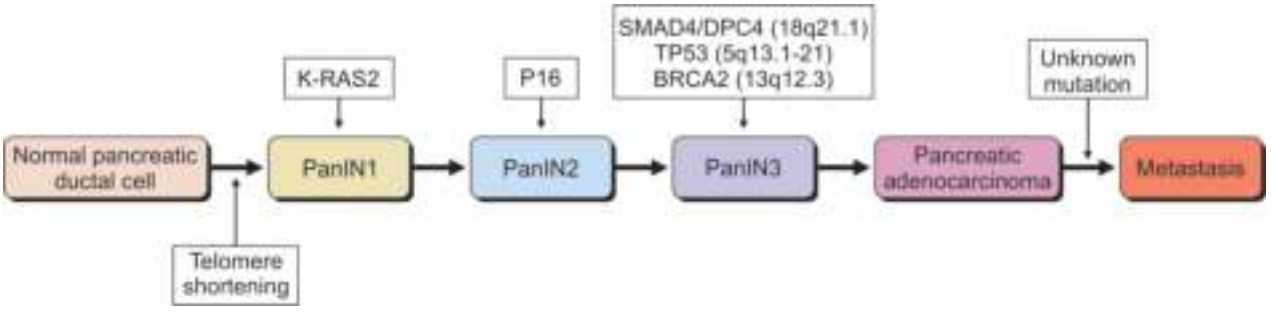


Fig. 21.116: Genetic progression model of pancreatic carcinogenesis.

Pathology Pearls: Evolution of Pancreatic Adenocarcinoma

Pancreatic carcinoma is a genetic disease of inherited or acquired gene mutations. Accumulation of multiple mutations of various genes is most important than their occurrence in a specific order. K-RAS gene mutation and alterations of tumor suppressor genes, i.e. p53, p16, SMADA4/DPC4 play important role in carcinogenesis of pancreatic carcinoma.

- **Early stage of pancreatic adenocarcinoma**: It is thought that telomerase shortening and point mutation of K-RAS (12p) oncogene is an early event in most cases of pancreatic intraductal neoplasia in >90% of cases.
- **Intermediate stage of pancreatic adenocarcinoma**: There is inactivation of the p16 tumor suppressor gene (CDKN2A on chromosome 9p) in >95% of cases.
- **Late stage of pancreatic adenocarcinoma**: Inactivation of p53, SMAD4 (DPC4 on chromosome 18q) in 55% of cases and BRCA2 (13q) tumor suppressor gene occurs in 10%. It results in invasive carcinoma. Other less common gene mutations include LKB1/STK11 (19q), MKK4 (17p) and RB1 (13q).

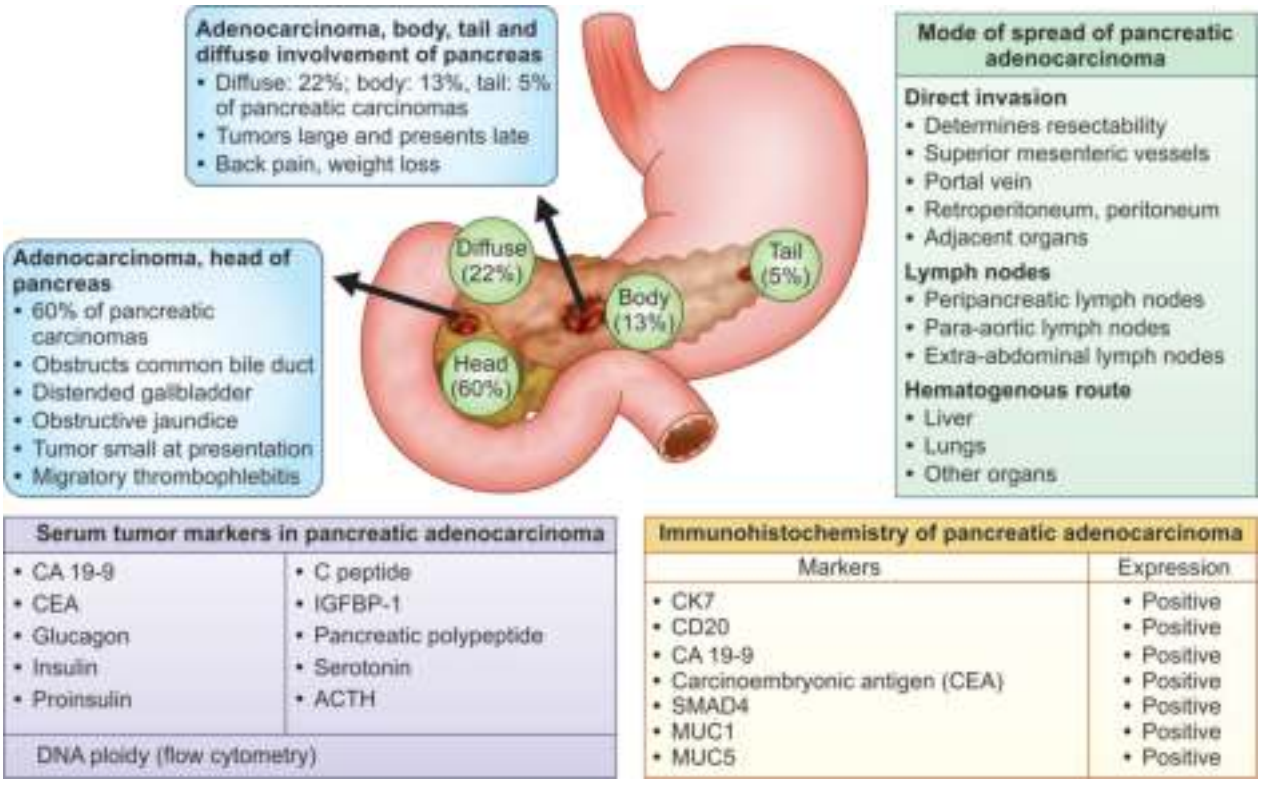


Fig. 21.117: Locations of pancreatic adenocarcinoma and clinical manifestations.

Clinical Features

Patient with pancreatic adenocarcinoma presents with abdominal dull pain constant in nature in upper abdomen radiating to the back, marked loss of weight and anorexia. Patient also mentions passage of dark-colored urine and clay-colored stool. Locations of pancreatic adenocarcinoma and clinical manifestations are shown in Fig. 21.117.

Physical Examination

Physical examination reveals loss of weight, jaundice, and distended palpable gallbladder (Courvoisier sign).

- **Migratory thrombophlebitis (Trousseau sign):** It occurs probably due to release of thrombogenic substances into the circulation (e.g. **serine proteases**) that initiate the coagulation cascade in 10% of cases.
- **Obstructive jaundice (Courvoisier sign):** Carcinoma arising in head of pancreas frequently obstructs common bile duct resulting in painless obstructive jaundice in 20% of cases. It is often accompanied by a distended, palpable gallbladder termed as Courvoisier sign in this setting.
- **Secondary diabetes mellitus:** Carcinoma involving tail of the pancreas can cause islet destruction and secondary diabetes mellitus.

Laboratory Findings

Laboratory tests are consistent with obstructive jaundice, i.e. conjugated hyperbilirubinemia with high 5′-nucleosidase (specific marker for bile duct epithelial cell injury) and alkaline phosphatase. CA19-9 tumor marker is most often elevated (normal range <37 IU/ml) in pancreatic adenocarcinoma.

Surgical Pathology: Pancreatic Adenocarcinoma

Gross Morphology

- Pancreatic adenocarcinoma occurs in various portions of pancreas: head (60%), body (15%) and tail (5%) of the gland. The tumor diffusely involves the entire gland.
- Tumor is usually hard, satellite, poorly circumscribed and gray-white in color. Gross morphology of pancreatic adenocarcinoma is shown in Fig. 21.118.

Light Microscopy

Pancreatic adenocarcinoma is most common histologic variant. Less common histologic variants include acinic cell, adenosquamous and poorly differentiated carcinoma. Morphology of pancreatic adenocarcinoma is described as under.

- Tumor is composed of pleomorphic small to bizarre cuboidal to columnar malignant cells forming abortive glandular structures or nests. Light microscopy of pancreatic adenocarcinoma is shown in Fig. 21.119. Differences between pancreatic adenocarcinoma and chronic pancreatitis are given in Table 21.111.

- Tumor is most often moderately to poorly differentiated adenocarcinoma irrespective of location of tumor in pancreas (well differentiated rare).
- Tumor cells infiltrate the wall resulting in dense stromal fibrosis.
- Perineural invasion and lymphatic invasion may be demonstrated.

Immunohistochemistry Technique

Panel of markers is used to analyze expression in pancreatic adenocarcinoma on tissue sections as given below. Panel of immunohistochemical markers applied in pancreatic tumors is given in Table 21.112.

Markers	Expression
CK-7	Positive
CK-20	Positive
CA 19-9	Positive
DPC4 (SMAD4, MADH4) tumor suppressor gene	Negative (allelic loss)
CEA	Positive
MUC1	Positive
MUC5	Positive
EMA	Negative

Mode of Spread

Pancreatic adenocarcinoma most often metastasizes to regional **lymph nodes, liver, lung, pleura, intestine** and **peritoneum**. Tumor may also metastasize to adrenal glands, bones, gallbladder, diaphragm and kidney.

CT Scan

CT scan technique highlights early detection of pancreatic adenocarcinoma, accurate staging and effective treatment of pancreatic cancer. A minimum of involvement of 15 lymph nodes has been suggested to achieve optimal staging.

Surgical Resection

Surgical excision of pancreatic adenocarcinoma is the only curative treatment option. Only 10–30% cases of pancreatic adenocarcinoma are resectable at the time of presentation with a 5-year survival rate in 18–20% of cases, and a median survival of these patients is 17–21 months. Patient with locally advanced disease has a median survival of 6–10 months.

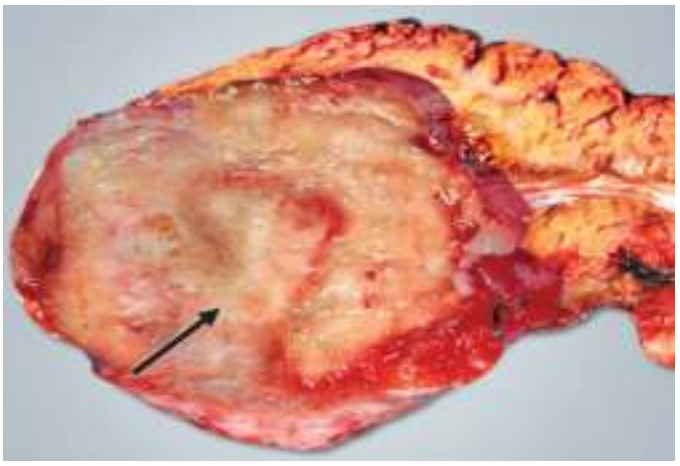


Fig. 21.118: Pancreatic adenocarcinoma. Tumor arising from head of pancreas is gray-white with areas of hemorrhage (arrow).

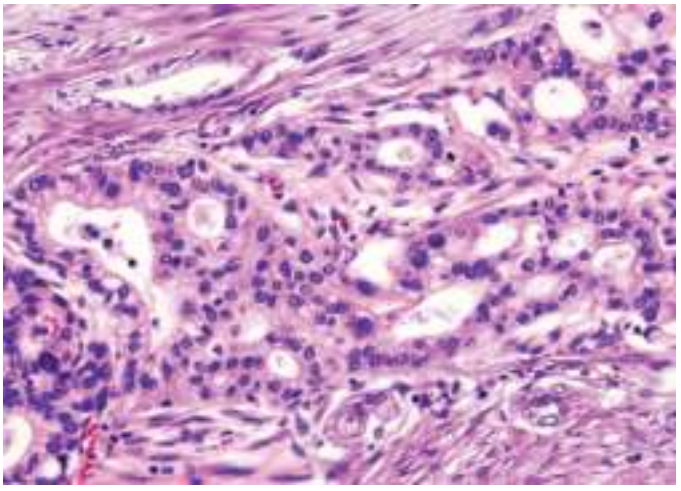


Fig. 21.119: Pancreatic adenocarcinoma. Tumor is composed of pleomorphic small to columnar malignant cells forming abortive glandular structures or nests (400X).

Table 21.111 Differences between pancreatic adenocarcinoma and chronic pancreatitis

Histologic Features	Pancreatic Adenocarcinoma	Chronic Pancreatitis
Histologic pattern	Haphazardly arranged	Lobular arrangement
Ruptured or incomplete glands	Present	Absent
Companion muscular vessel	Present	Absent
Nuclear pleomorphism	Marked in the same glands	Insignificant
Perineural invasion	Present	Absent
Invasion of blood vessels	Present	Absent
Mitoses	Frequent	Infrequent

Table 21.112 Panel of immunohistochemical markers applied in pancreatic tumors

Immunohistochemical Markers	Expression
Pancreatic adenocarcinoma	
CK-7 (cytokeratin 7)	Positive
CK-20 (cytokeratin 20)	Positive
CA 19-9	Positive
DPC4 (SMAD4, MADH4) tumor suppressor gene	Allelic loss
CEA (carcinoembryonic antigen)	Positive
MUC1	Positive
MUC5	Positive
EMA (epithelial membrane antigen)	Negative
Pancreatoblastoma (pancreatic element with ductal, acinar and neuroendocrine differentiation)	
Trypsin	Positive (acinar marker)
Chymotrypsin	Positive (acinar marker)
Lipase	Positive (acinar marker)
Cytokeratin (CK)	Positive (ductal marker)
CEA (carcinoembryonic antigen)	Positive (ductal marker)
Synaptophysin	Positive (neuroendocrine marker)
Chromogranin A	Positive (neuroendocrine marker)
NSE (neuron-specific enolase)	Positive (neuroendocrine marker)
CD56 (neuronal cell adhesion molecule—NCAM)	Positive (neuroendocrine marker)
Pancreatic endocrine neoplasm (formerly known as islet cell tumor)	
Synaptophysin	Positive (neuroendocrine marker cytoplasmic staining)
Chromogranin A	Positive (neuroendocrine marker cytoplasmic staining)
NSE (neuron-specific enolase)	Positive (neuroendocrine marker cytoplasmic staining)
CD56 (neuronal cell adhesion molecule) neuroendocrine marker	Positive (membranous staining)
Cytokeratins (AE1/AE3 and CAM 15.2 epithelial marker)	Positive (cytoplasmic staining)
Insulin hormone	Variable staining
Glucagon hormone	Variable staining
Somatostatin hormone	Variable staining
Mucinous cystic neoplasm	
ER/PR (estrogen/progesterone receptors)	Positive
Inhibin	Positive
Intraductal papillary mucinous neoplasm (IPMN)	
DPC4 intact	Positive
MUC1	Positive
MUC4	Positive
MUC2	Negative
CDX2	Negative

Contd...

Table 21.112 Panel of immunohistochemical markers applied in pancreatic tumors (*Contd...*)

Immunohistochemical Markers	Expression
Pancreatic serous cystadenoma	
CK (cytokeratin)	Positive
EMA (epithelial membrane antigen)	Positive (33%)
Inhibin	Positive
MUC6	Positive
Calponin	Positive
CD31	Positive
Factor VIII	Positive
Lymphoplasmacytic sclerosing (autoimmune) pancreatitis	
IgG4+ plasma cells infiltration	Positive (>20 per high power field)

Serum tumor markers for pancreatic adenocarcinoma include CA 19-9, carcinoembryonic antigen (CEA) glucagon, proinsulin, C-peptide, IGFBP-1, pancreatic polypeptide and serotonin.

PANCREATOBLASTOMA

Pancreatoblastoma is rare malignant pancreatic neoplasm most often affecting children up to 15 years of age.

- Most cases are sporadic, however, it may be associated with Beckwith-Wiedemann syndrome (BWS) or familial adenomatous polyposis (FAP). Patient may present with abdominal lump and pain abdomen, weight loss, diarrhea and nausea. Serum AFP, CEA and ACTH are frequently elevated.
- More than 80% of cases show loss of 11p, mutation in CTNNB1 (50–80%) and mutation in APC gene in FAP.
- Pancreatoblastoma is large (mean size 10 cm), solid and well circumscribed partially encapsulated neoplasm.
- Histologic examination of tumor reveals acinar, ductal, endocrine and squamoid differentiation. There is no cytologic atypia.
- Immunohistochemical analysis reveals tumor cells are positive, i.e. acinar cells (trypsin, chymotrypsin, lipase), neuroendocrine cells (synaptophysin, chromogranin A) and ductal cells (CK7, CK19).
- Pancreatoblastoma can be locally invasive and can metastasize to regional lymph nodes and liver at presentation.
- Prognosis is excellent if tumor is resected before it has metastasized. Overall cure rate is about 50%.

ISLET CELL TUMORS

Mitosis and tumor necrosis are most important in grading a pancreatic endocrine tumor and predicting its malignant potential.

GLUCAGONOMA

Glucagonoma is rare neuroendocrine tumor of pancreatic α cells of pancreas, which results in secondary diabetes mellitus. Patient with glucagonoma presents with necrotizing migratory erythema, mild hyperglycemia, and anemia.

INSULINOMA

Insulinoma is the most common islet cell tumor derived from β cells of pancreas. It is low-grade malignant neoplasm, which synthesizes excess of insulin resulting in hypoglycemia.

- Insulinoma synthesizes insulin by splitting of C-peptide fragment of the proinsulin molecule resulting in increased serum C-peptide level (diagnostic tool).
- Purification of commercial insulin preparations is done by splitting of C-peptide fragment of proinsulin. Hence, exogenous administration of insulin does not increase C-peptide.

Clinical Features

Insulinoma is characterized clinically by Whipple triad: episodic hypoglycemia, central nervous system dysfunction temporally related to hypoglycemia (anxiety, confusion stupor, seizures, paralysis, coma) and dramatic reversal of central nervous system symptoms by glucose administration.

GASTRINOMA

Gastrinoma is a malignant tumor derived from G cells and most often located in the pancreas. It may also occur in duodenum.

- Tumor synthesizes excess of gastrin resulting in increased serum gastrin levels.
- Gastrinoma of pancreas results in Zollinger-Ellison syndrome characterized by gastric hypersecretion of hydrochloric acid, recurrent peptic ulcer disease of the duodenum and sometimes the jejunum, and elevated levels of gastrin in blood.

STOMATOSTATINOMA

Stomatostatinoma is islet cell tumor derived from δ cells of pancreas.

- Normally, delta cells of pancreas secrete somatostatin hormone that inhibits the release of insulin by pancreas. Somatostatin also inhibits the pituitary gland to release of growth hormone. It decreases secretion, motility and absorption in the digestive tract.
- Excess synthesis of somatostatin hormone by tumor results in mild diabetes mellitus, cholelithiasis, steatorrhea, and hypochlorhydria.

VASOACTIVE INTESTINAL PEPTIDE TUMOR

Vasoactive intestinal peptide tumor is marked by secretion of vasoactive intestinal peptide (VIP).

- Vasoactive intestinal peptide stimulates adenylyl cyclase enzyme that leads to the synthesis of large amounts of cAMP. The cAMP causes increased secretion of potassium and water into the intestinal lumen.
- Patient presents with watery diarrhea (5 liters/day), hypokalemia, low levels of chloride in gastric juice (achlorhydria). These symptoms constitute pancreatic cholera.

MULTIPLE ENDOCRINE NEOPLASIA 1 (MEN 1)

Multiple endocrine neoplasia type 1 is also known as Wermer-Morrison syndrome caused by mutation of the MEN 1 tumor suppressor gene.

Wermer-Morrison syndrome comprises pituitary adenoma (acromegaly), parathyroid gland hyperplasia (hypercalcemia) or adenoma and pancreas islet cell tumors (insulinoma and gastrinoma). Gastrinoma produces Zollinger-Ellison syndrome.

CARCINOID TUMORS

Carcinoid tumors of the pancreas are rare malignant neoplasms that closely resemble intestinal carcinoids.

- When carcinoid tumors are confined to the pancreas, serotonin, bradykinin, and histamine synthesized from the tumor into venous blood, induce atypical carcinoid syndrome.
- Hepatic metastases cause the full blown carcinoid syndrome.
- Patient presents with severe facial flushing, bronchial wheezing, watery diarrhea, abdominal colic, hypotension, periorbital edema, and tearing.

METASTATIC PANCREATIC TUMORS

- Most common malignant tumors of lung, breast, thyroid gland, kidney and melanoma (skin) via hematogenous route reach the pancreas.
- Malignant tumors of stomach, duodenum, colon, kidney, lymph nodes and adrenal glands may spread to pancreas by direct extension.

Recent Advances: Liver Biopsy Specimen Handling in Surgical Pathology

Liver biopsy remains the gold standard in the diagnosis of numerous liver pathologies, which has emerged as a useful diagnostic tool to assess severity of hepatocellular damage in patients with abnormal liver functions and/or liver lesion on imaging, treatment plan, monitor response, prognosis, and function of liver transplant. The liver function tests include alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), prothrombin time and international normalized ratio (PT–INR), serum bilirubin, total serum proteins and albumin/globulin ratio. Nearly 50% of liver biopsies are performed by radiologists by using ultrasonography, computed tomography, and magnetic resonance imaging diagnostic tools.

- **Liver biopsy techniques:** Ultrasound-guided liver biopsy specimen may be taken by using an aspiration needle (i.e. Menghini or Jamshidi) and tru-cut needle.
  - Percutaneous, ultrasound-guided liver biopsy (Menghini technique) has become a worldwide standard. Laparoscopic liver biopsy enables macroscopic inspection of the liver surface and provides more information than percutaneous liver biopsy.
  - Transjugular liver biopsy is a reasonable option for patients with severe coagulopathies or massive ascites, but it carries the risk of intraperitoneal hemorrhage while placing jugular catheter in right jugular vein, skin hematoma, pneumothorax, vena cava perforation, and cardiac arrhythmias. Liver biopsies are at least 1.5–2.5 cm in length allow for better interpretation.
- **Handling, processing, and staining of liver biopsy:** Liver biopsy specimen is fixed in 10% neutral buffered formalin, and then dehydrated and embedded in paraffin, before microtome sectioning and mounting the slides. Liver biopsy is fixed in 2% glutaraldehyde for electron microscopy. The evaluation of liver biopsy requires the use of special stains (e.g. Masson trichrome, iron, PAS with diastase, reticulin, Oil red O, Congo red, and rhodamine stain for copper) is used on

liver biopsy tissue sections other than routine hematoxylin and eosin (H&E) to highlight many important histologic features. Immunohistochemistry has diagnostic role in identification of hepatitis B surface antigen (HBsAg).

- **Hematoxylin and eosin stain:** Liver biopsy specimens are initially stained with hematoxylin and eosin (H&E). The hallmark of the lobular pattern is 'lobular disarray' with increased inflammatory cells, in which normal liver architecture undergoes disruption due to injured hepatocytes. In portal pattern, histologic features include portal/periportal inflammation with mononuclear infiltrate and fibrosis. Extension of inflammation to limiting plates is associated with interface hepatitis. In biliary pattern, distortion of the liver parenchyma is associated with inflammation within portal tracts, neutrophilic infiltrate, proliferation of bile ducts and fibrosis or cholestasis. In steatotic pattern, hepatocytes are laden with intracytoplasmic lipid (microvesicular or macrovesicular steatosis) distributed throughout the liver lobules. Steatosis associated with inflammation is known as steatohepatitis
- **Masson trichrome stain:** Masson trichrome stain is used to assess/stage fibrosis, which stains collagen fibers blue against red background of hepatocytes. It stains type 1 collagen fibers normally present in the portal tracts and blood vessel walls, but highlights reactive fibrosis due to hepatocellular injury. Masson trichrome stain highlights characteristic pattern of advanced fibrosis secondary to steatohepatitis. There is scarring around central vein, perisinusoidal fibrosis separating hepatocyte cords, formation of fibrous septum and periportal fibrosis.
- **Reticulin stain:** Reticulin stain uses silver impregnation to highlight reticulin fibers made of type 3 collagen fibers, which appear black against gray/light pink background. Reticulin stain highlights the reticular pattern in acute hepatic necrosis. The hepatocyte cords are focally expanded, band-like of reticulin collapse near central vein highlights the necrosis.
- **Perls iron stain:** Perls iron stain (prussian blue reaction) detects iron stored in the hepatocytes in the soluble form (ferritin) and insoluble form (hemosiderin). Perls iron stain highlights hemosiderin as coarse blue granules, while ferritin is seen in cytoplasmic as faint blue. Perls iron stain shows marked iron deposition in hepatocytes and Kupffer cells in the settings of primary and secondary hemochromatosis. Perls iron stain can be performed on routinely processed formalin-fixed paraffin-embedded (FFPE) liver biopsy tissue section.
- **Periodic acid–Schiff stain:** Periodic acid–Schiff (PAS) stain is useful for detection of glycogen in glycogen storage disease, but removing glycogen with diastase digestion enhances detection of undigested material such as α_1-antitrypsin globules, basement membrane, fungal organisms, and debris within macrophages.
- **Congo red stain:** Congo red stain shows bright orange positivity in areas of amyloid deposition with a characteristic apple-green birefringence under polarizing microscopy.

CHAPTER

22

Kidney and Ureter

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

RENAL EMBRYOLOGY, ANATOMY AND PHYSIOLOGY

- Embryology
- Anatomy and histology
- Physiology

GLOMERULAR DISEASES OF KIDNEY

- Glomerulus structure
  - Glomerular permeability barriers
  - Glomerular filtration rate
- Glomerulonephritis: terminology
- Classification of glomerulonephritis based on etiology, clinical presentation, appearance of glomerular capillary tuft and duration of disease
  - Primary glomerular diseases
  - Secondary glomerular diseases
  - Familial glomerular diseases
- Classification of glomerulonephritis based on integrity of glomerular basement membrane and cellularity of glomeruli
  - Thin basement membrane disease
  - Nonproliferative glomerulonephritis
  - Proliferative glomerulonephritis
- Glomerulonephritis: molecular pathogenesis
  - *In situ* immune complex-mediated glomerulonephritis (circulating antibodies against fixed intrinsic normal glomerular tissue antigen and nonglomerular antigen planted in glomeruli and immune complex deposition glomerular diseases)
  - Circulating immune complex-mediated glomerulonephritis
  - Cytotoxic antibody-mediated glomerulonephritis
  - Antineutrophilic cytoplasmic autoantibody-mediated glomerulonephritis
  - T cell-/NK cell-mediated glomerular injury
  - Complement system-mediated glomerulonephritis
  - Inflammatory cells involved in glomerulonephritis
  - Coagulation/fibrinolysis system in fibrin-mediated glomerulonephritis
  - Platelet secretory products involved in glomerulonephritis
  - Nephritic factors involved in glomerulonephritis
  - Soluble factors involved in glomerulonephritis
- Glomerular diseases: clinical syndromes
  - Asymptomatic microscopic hematuria
  - Asymptomatic proteinuria
  - Nephritic syndrome
  - Nephrotic syndrome
  - Acute kidney disease
  - Chronic kidney disease
- Nephritic syndrome
  - Poststreptococcal glomerulonephritis
  - Alport's syndrome
  - IgA nephropathy (Berger's disease)
  - Goodpasture syndrome
  - Henoch-Schönlein purpura
  - Hemolytic uremic syndrome
- Nephrotic syndrome
  - Minimal change disease
  - Focal segmental glomerulosclerosis
  - Membranous glomerulonephritis
  - Membranoproliferative glomerulonephritis
  - Diabetic nephropathy (diffuse/nodular glomerulosclerosis)
  - Renal amyloidosis
  - Monoclonal immunoglobulin deposition disease of kidney
  - Lupus nephritis
- Rapidly progressive (crescentic) glomerulonephritis
- Chronic kidney disease
  - Chronic kidney disease: laboratory findings and clinical manifestations
  - Chronic kidney disease: medical/surgical management
- Renal biopsy evaluation and interpretation
  - Light microscopy
  - Immunofluorescence microscopy
  - Electron microscopy

TUBULAR DISEASES OF KIDNEY

- Acute tubular necrosis
  - Ischemic acute tubular necrosis
  - Nephrotoxic acute tubular necrosis
- Acute kidney disease

TUBULOINTERSTITIAL DISEASES OF KIDNEY

- Pyelonephritis
  - Acute pyelonephritis
  - Chronic pyelonephritis
  - Xanthogranulomatous pyelonephritis
  - Tubercular pyelonephritis
- Urate nephropathy
- Renal papillary necrosis
- Acute interstitial nephritis
- Light chain cast nephropathy in multiple myeloma
- Renal tubular functional disorders
  - Fanconi syndrome
  - Cystinuria
  - Hartnup disease
- Nephrocalcinosis

UROLITHIASIS

- Molecular pathogenesis of urolithiasis
  - Calcium oxalate and phosphate stones
  - Magnesium, ammonium, calcium phosphate stones (struvite stones)
  - Uric acid stones
  - Cystine stones
  - Xanthine stones
- Complications of urolithiasis
  - Hydronephrosis
  - Urinary tract infection
  - Pyonephrosis
- Laboratory diagnosis of urolithiasis
- Management of urolithiasis

VASCULAR DISEASES OF KIDNEY

- Diffuse renal cortical necrosis secondary to shock
- Renal infarction
- Sickle cell nephropathy
- Nephrosclerosis
  - Benign nephrosclerosis leads to obliteration of glomeruli
  - Malignant nephrosclerosis leads to fatal renal disease
- Thrombotic microangiopathy
- Toxemia of pregnancy involving kidneys
- Vasculitis involving kidneys

CYSTIC DISEASES OF KIDNEY

- Adult polycystic kidney disease
- Infantile polycystic kidney disease
- Solitary renal cyst

- Medullary sponge kidney
- Acquired renal cystic disease

CONGENITAL ANOMALIES OF KIDNEY

- Renal agenesis
- Multicystic renal dysplasia
- Severe congenital anomalies of kidneys and urinary tract
- Ectopic kidney
- Horseshoe kidney
- Ureter anomalies

NEOPLASTIC DISORDERS OF KIDNEY

- Nephroblastic and cystic tumors
  - Wilms' tumor (nephroblastoma)
- Renal oncocytoma
- Renal cell carcinoma
  - Clear cell renal cell carcinoma
  - Multilocular cystic renal cell carcinoma
  - Papillary (chromophil) renal cell carcinoma
  - Chromophobe renal cell carcinoma
  - Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 gene fusion
  - Collecting duct carcinoma of Bellini
  - Urothelial carcinoma of renal pelvis
- Mesenchymal tumors occurring mainly in children
  - Clear cell sarcoma of kidney
- Tumor-like lesions of kidney
  - Renal angiomyolipoma (hamartoma)

RENAL FUNCTION TESTS

- Glomerular filtration rate assessment
  - Exogenous marker of glomerular filtration rate assessment
  - Creatinine clearance
- Tubular reabsorption tests
- Blood biochemistry
  - Blood urea nitrogen
  - Serum creatinine
  - Ratio of blood urea nitrogen to serum creatinine
  - Serum cystatin C
- Novel serum, urine and tissue biomarkers
- Urine analysis

RENAL EMBRYOLOGY, ANATOMY AND PHYSIOLOGY

EMBRYOLOGY

The main functions of the urinary system include: excretion of metabolic waste products such as urea, creatinine and uric acid, maintenance of electrolyte, water and pH balance, regulation of blood pressure, blood volume and erythropoiesis, and production of active form of vitamin D. Development of the urinary system is closely related to the development of the reproductive system, especially during the early stages of embryogenesis, where both develop from the same origin. However, urinary system develops ahead of the reproductive system. The urinary system consists of the kidneys, urinary bladder and urethra. A region of intermediate mesoderm, known as urogenital ridge, which gives rise to these structures (i.e. kidneys, urinary bladder and urethra) during embryogenesis.

DEVELOPMENT OF KIDNEYS

Urogenital ridge forms nephrogenic cord, that gives rise to the urinary system. Nephrogenic cord develops into pronephros, mesonephros and metanephros.

- Pronephros, being nonfunctional regresses completely by five weeks of gestation. Mesonephros differentiates to form mesonephric tubules and mesonephric ducts (Wolffian duct). Metanephros develops into the adult kidney.
- Metanephros located in the sacral region becomes functional at 10 weeks gestation. Ureteric bud undergoes repeated divisions to form the ureters, renal pelvis, major and minor calyces; and collecting ducts.
- The kidneys ascend from the sacral region during fetal life to vertebral levels T12–L3 in the adult. Kidneys migration is accompanied by medial rotation of kidneys by 90°. Supernumerary arteries are formed during ascent.
- A congenital developmental renal malformation occurs resulting from abnormal nephrogenesis due to inability of the ureteric bud to communicate with the metanephric blastema.
- Patients present with bilateral severe renal disease, which is characterized by maternal oligohydramnios, neonatal pulmonary and renal failure.
- Diagnosis of bilateral severe renal disease can be established on antenatal ultrasonography, which reveals small renal size, subcortical cysts, patchy contrast enhancement, architectural disarray, distorted pelvicalyceal system. It may be associated with multiple malformations. Prognosis is worst in bilateral severe renal disease.
- Kidneys are of small size to solid large size cystic and lined by dysplastic epithelium. Dysplastic renal epithelium expresses PAX2, BCL-2 and galectin 3.

DEVELOPMENT OF URINARY BLADDER AND URETHRA

Urinary bladder and urethra develop from the upper end of urogenital sinus, which is continuous with the allantois.

- Allantois becomes the median umbilical ligament (urachus). The lower end of the urogenital sinus forms the female urethra. The transitional and stratified epithelium lining the female urethra are derived from endoderm.
- The prostatic urethra, membranous urethra and proximal part of the penile urethra are also derived from endoderm. Distal part of penile urethra is derived from ectodermal glandular plate.
- The metanephric vesicles differentiate to form glomerulus, Bowman capsule, proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting ducts.
- Ureter, renal pelvis, major calyx, minor calyx and urinary bladder are lined by transitional epithelium. Collecting ducts are lined by simple cuboidal epithelium.

ANATOMY AND HISTOLOGY

The urinary system consists of the kidneys, urinary bladder and urethra. The kidneys and renal pelvises constitute upper urinary tract; the ureters, urinary bladder and urethra form lower urinary tract.

- Urine produced by nephrons within a renal pyramids empties into the orifices of the terminal collecting ducts (Bellini's ducts) into a minor calyx, major calyx and then funnel-shaped renal pelvis. The collecting system comprises renal pelvises, urinary bladder and urethra that carry urine from the kidneys to the urinary bladder and urethra for urination.
- Normal urinary tract health depends on smooth unobstructed urine. Kidneys participate in excretion of waste metabolic products, adjustment of blood pH by excretion of more or less acid, maintenance of plasma concentration of salts, regulation of blood volume, regulation of blood pressure and hematopoiesis.

KIDNEYS

Kidneys are paired bean-shaped encapsulated organs located in the abdomen (T12, L1, L2 and L3) along vertebral column in retroperitoneal region. Kidneys are invested in a layer in perinephric adipose tissue and covered anteriorly with Gerota's fascia, which is a landmark for staging renal cell malignant tumors.

- Each kidney in the human weighs 150 g. Dimensions of each kidney include: 10–12 cm vertically, 5–7 cm transversely and 3 cm in anteroposterior plane. Outer renal surface is divided into anterior surface, posterior surface, upper pole and lower pole. Medial surface of kidney is concave, deeply grooved (renal sinus) and forms renal hilum.
- Renal hilum is a slit-like space on the medial border of kidneys through with runs the ureter, branches of arteritis and veins, nerves and lymphatics.
- Renal sinus is the region on the medial aspect of the kidney that contains renal collecting system, nerves, blood vessels, lymphatics and adipose tissue and important for renal carcinomas to metastasize outside the kidney.
- Renal capsule covers the convex region of the renal cortex. Renal cortex in contact with the renal sinus lacks capsule, therefore allowing renal cancers to involve renal sinus.
- Nephron is the functional unit of the kidney. Each kidney has about one million nephrons. The number of nephrons is established during prenatal development. After birth, no new nephron can develop and a lost nephron cannot be replaced by regeneration following injury. There is gradual decrease in number of nephrons during aging process.
- Each nephron derived from the ureteric bud consists of a glomerulus, proximal tubule, loop of Henle (descending and ascending limb), distal tubule, connecting segment, and collecting duct.
- Kidneys participate in processing of blood and formation of urine. Urine produced by nephrons within a renal pyramid empties into collecting duct, which passes through a papilla into a calyceal system then renal pelvis.

Renal Histology

Kidney is composed of glomeruli, tubules, interstitial; tissue (cortex 10% and medulla 20%) and blood vessels. Disease of any one of these components affects functions of rest of components resulting in impairment of renal functions. Erythropoietin is synthesized in the cortex, while prostaglandins synthesis occurs in the medulla. Renal cortex receives 90% of blood supply. Renal medulla receives 10% of blood supply.

- Renal cortex is one centimeter beneath the renal capsule and extending down between the renal pyramids (columns of Bertini). Cortex contains 85% of nephrons (proximal and distal tubules) and lacks vasa recta.
- Beneath renal cortex, medulla is divided into outer medulla and inner medulla (papillae). At the tips of the papillae in inner medulla, the epithelium of collecting ducts transforms into urothelium. Renal medulla contains 15% of juxtamedullary nephrons and possesses vasa recta. Medulla contains loop of Henle collecting ducts.
- Efferent arterioles of juxtamedullary nephrons send straight branches and deliver isotonic blood to the renal medulla. Along with loop of Henle, these vasa recta play a crucial role in the establishment of the nephrons' countercurrent exchange system. Medulla participates in the formation of urine.

Renal Anatomy

Each kidney is composed of 8–18 lobes. Each lobe of kidney constitutes a conical medullary pyramid capped by cortical tissue. Downward extension of cortical tissue between the pyramids is called '**renal columns of Bertini**'.

- Normal renal cortex is 1.2–1.5 cm thick over the pyramids. Renal columns of Bertini are located where cortical tissue dips into the medulla between the pyramids.
- Base of pyramid is towards cortex and its papilla pointing towards renal pelvis. Comparison between renal cortex and medulla is given in Table 22.1.

Table 22.1 Comparison between renal cortex and medulla

Parameters	Renal Cortex (1.2–1.5 cm)	Renal Medulla
Blood supply	90%	10%
Number of nephrons	85%	15%
Vasa recta	Absent	Present
Nephron's portions	Glomeruli, proximal and distal convoluted tubules	Collecting ducts and loop of Henle
Interstitial tissue	10%	20–30%
Pyramids	Absent	Present
Synthesis of substances	Erythropoietin	Prostaglandins

Clinical Pearls: Papillae in Renal Pyramids

There are two types of papillae in renal pyramids: simple and compound. Schematic representation of renal simple and compound papillae is shown in Fig. 22.1.

- **Compound papillae:** Compound papillae in renal pyramids are concave shaped with valveless straight ducts located at the poles. That is the reason that hydronephrotic changes are marked in the polar regions due to presence of compound papillae resulting from obstruction to the urinary flow.
- **Simple papillae:** Rest of the pyramids contain conical simple papillae, which are obliquely placed ducts having valve-like action.

Renal Blood Supply

Renal pelvis contains renal artery, renal vein, and nerves. Renal artery arises from abdominal aorta enters kidney through the renal hilum and gives rise to segmental arteries and then branches progressively to form interlobar arteries, arcuate arteries (forming loops at the base of pyramids), traversing the renal parenchyma near the corticomedullary junction.

- Arcuate arteries give rise to interlobular arteries and subsequently efferent arterioles that course into the glomerular capillary tufts.
- Distal ends of glomerular capillaries tuft coalesce to form the afferent arterioles, which progress to a second capillary network. The peritubular capillaries surround the renal tubules.
- Renal arteries are the end arteries. Obstruction to renal artery results in renal infarction (pale/white infarct).
- Renal vein drains into interlobular veins, arcuate veins, interlobar veins, segmental veins, inferior vena cava, which opens into right heart.
- Each kidney has dual lymphatic drainage system. Major lymphatic drainage follows the vasculature

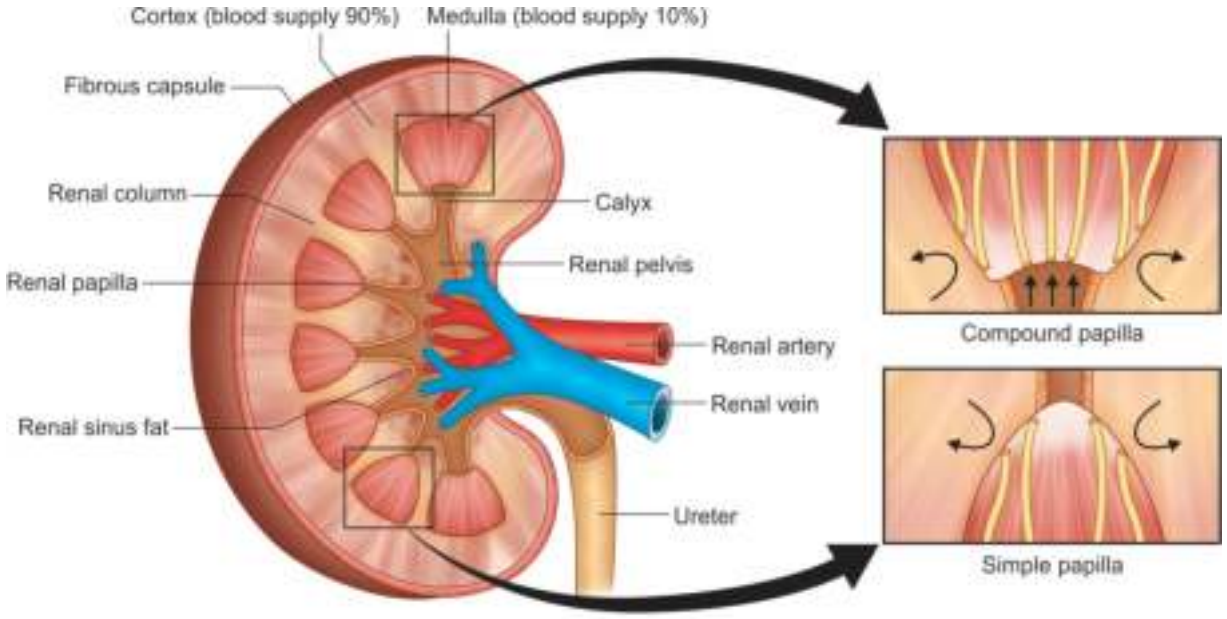


Fig. 22.1: Schematic representation of renal simple and compound papillae. Human kidney contains two types of renal papillae: simple (convex) and compound (concave) papillae. Compound papillae predominate at the polar regions of the kidney, whereas simple papillae are located at nonpolar regions. Approximately, 66% of human papillae are simple (convex) and 34% are compound and are concave (concave) papillae. Obstruction to urine flow first affect compound papillae.

from the renal parenchyma to the renal sinus and terminates in lateral para-aortic lymph nodes. Minor capsular lymphatic drainage from the superficial renal cortex courses along the renal capsule to the renal hilum to join the major lymphatic drainage.

Renal Blood Flow

Kidneys receive 25% of cardiac output: 1200 ml of blood (600 ml of plasma) per minute. The glomeruli filter 120 ml of filtrate per minute (GFR = 120 ml/minute). About 130–180 liters of glomerular filtrate is presented to the renal tubules each day, which results in production of one liter of urine per day. Renal cortex receives 90% and medulla receives 10% of arterial blood.

RENAL PELVIS

Renal pelvis is a funnel-shaped structure present at the hilum of kidney, which contains renal artery, renal vein, and nerves.

- The renal pelvis is the point where the two or three major calyces join together, which is lined by transitional epithelium.
- The renal pelvis functions as a funnel for urine flowing to the ureter. Renal pelvis is site for transitional cell carcinoma and infection.
- The size of the renal pelvis plays a major role in the grading of hydronephrosis. Transverse diameter of renal pelvis measures over 10 mm in adults.

URETERS

The ureters are tube-like structures, which carry urine from the kidneys to the urinary bladder. There are two ureters, each one is attached to each kidney.

- The upper half of each ureter is located in the abdominal region and the lower half is located in the pelvic region. The ureter is about 10–12 inches long in the average adult.
- The ureter has thick walls composed of a fibrous, a muscular, and a mucus coat, which are able to contract. Ureters are lined by transitional epithelium.
- Strong muscular contractions within calyces and renal pelvis propel the urine into the ureters. Musculature of ureter and pelvis is continuous, which allow synchronous peristaltic contractions from kidney to urinary bladder.
- Ureter disorders include duplication of ureter, ureteropelvic junction obstruction and ureterovesical junction obstruction.
- Duplication of the ureter is a congenital anomaly in which two ureters form on the same kidney. Ureteropelvic junction obstruction occurs when the connection between the kidney and ureter is blocked, preventing urine from exiting the kidney.
- Ureterovesical junction obstruction occurs between the ureter and urinary bladder. All these disorders cause obstruction to passage of urine resulting in pyelonephritis, loss of renal functions, and formation of urinary stones.
- Treatment of ureteric obstruction is possible through insertion of a catheter (a special tube), and implantation of a stent (a support to keep vessels or tubes open), or through surgery. If an infection is found, antibiotics are often prescribed.

URINARY BLADDER

The urinary bladder is a muscular sac located in the pelvis, just above and behind the pubic bone, which is lined by transitional epithelium and supplied by sacral nerves (S2, 3, 4).

- When urinary bladder is empty, it is about the size and shape of a pear. Urine produced by kidneys travels down through ureters to the urinary bladder, that stores urine, allowing urination to be infrequent and controlled. Urine is forced out of the bladder and through the urethra.
- Capacity of urinary bladder is 400–600 ml. Potential causes of urinary bladder disorders include overactive urinary bladder, urinary bladder outlet obstruction, interstitial cystitis, urinary tract infections and urinary bladder carcinoma.

URETHRA

Urethra is approximately 3 cm long in females and 20 cm in males. It is a thin fibromuscular tube at the lower opening of the urinary bladder and extends through the pelvis and urogenital diaphragms to the outside of the body, called the external urethral orifice. In males, it consists of prostatic, membranous and penile parts. Women are more prone to urinary tract infection due to short urethra. The urethra also connects to the ductus deferens in males, for the ejaculation of spermatozoa. Females only use their urethra for urination.

PHYSIOLOGY

Homeostasis is defined as a steady state for normal life's metabolic processes. The kidneys regulate the content of blood plasma to maintain the homeostasis of the internal fluid environment within normal limits. Changes in the water content in the cells beyond range may cause death. Approximately >12% of the body water loss in humans may be fatal. Human beings possess readily permeable membranes such as respiratory surfaces and oral membranes. Kidneys perform functions by filtration, reabsorption of essential metabolites, secretion and excretion of waste metabolites.

Table 22.2 Reabsorption of essential metabolites by nephron tubules

Part of Nephron	Reabsorption of Metabolites by Active Process	Reabsorption of Water by Passive Process
Proximal tubule	Na^+, K^+, Cl^-, HCO_3^-, amino acids, glucose and phosphates	Water (80%)
Loop of Henle	▪ Descending limb is only permeable to water only ▪ Ascending limb is impermeable to water, but permeable to salts. Reabsorption of Na^+, chloride, K^+, Ca^+, HCO_3^- and Mg^+	▪ Water (10%) ▪ Water (Nil %)
Distal tubule	Na^+, Cl^-, Ca^+ and Mg^+ (absorption of Na^+, Cl^-, by aldosterone)	Water (5%)
Collecting duct	Water absorption by ADH	Water (5%)

Hydrogen ions and organic acids secreted by proximal tubules enter peritubular network of blood vessels. Hydrogen ions are also secreted by distal tubule.

REABSORPTION OF ESSENTIAL METABOLITES BY TUBULES

Renal tubules absorb essential metabolites by active process such as sodium, potassium, amino acids, glucose, phosphate and bicarbonate. Water is absorbed in proximal tubules by passive process. Sodium is pumped out from tubular cell into interstitial fluid, which drives chloride ions into the interstitial fluid by passive process. Reabsorption of essential metabolites by nephron tubules are given in Table 22.2.

EXCRETION OF HARMFUL PRODUCTS

Kidneys participate in excretion of waste products such as urea, creatinine, uric acid, guanidino compounds, metabolic end products of nucleic acids, aliphatic amines, middle molecules (polypeptides). Normal range of blood urea is 20–40 mg% and serum creatinine 0.6–1.5 mg%. If the renal functions are impaired, accumulation of waste products in the body is termed '**azotemia**'. If the patient develops clinical manifestations, it is denoted as '**uremia**'.

MAINTENANCE OF ACID–BASE HEMOSTASIS

Distal tubules participate in deamination of amino acids resulting in ammonia production that enters the filtrate. Hydrogen and potassium ions also enter distal tubular filtrate in exchange of sodium ions (sodium ions moving out of filtrate). Ammonia combines with hydrogen ions to form ammonium salts.

Pathology Pearls: Urea Cycle

Blood Urea Level

- Normal blood urea is 20–40 mg% and blood urea nitrogen in 10–20 mg/dl.
- Blood urea level is increased due to prerenal causes (myocardial infarction, protein catabolism), renal diseases and post-renal causes (prostatic nodular hyperplasia).
- Blood urea level is decreased due to protein malnutrition, massive liver necrosis, pregnancy and rapid dehydration.

Liver Role in Urea Cycle

- Exogenous and endogenous amino acids reach the liver. Transamination and deamination of amino acids lead to formation of large quantity of ammonia in liver.
- Ammonia enters urea cycle leading to formation of urea 25–35 g per day in liver.
- Approximately 100 g of amino acids produce 30 g of urea. Urea synthesis is decreased in liver diseases.

Kidneys Role in Urea Cycle

Urea formed in the liver reach the kidneys and get filtered passively until it reaches the collecting ducts. Approximately 40% of urea is reabsorbed by nephrons at high rate and 60% at low rate. Most of this urea finds its way back into the descending loop of Henle. The reabsorbed urea plays important role in the maintenance of the medullary solute content.

Pathology Pearls: Creatinine Metabolism

- Creatinine is derived from high energy phosphate compound phosphocreatine, which maintains the level of ATP in muscle.
- Phosphocreatine loses its phosphate to form creatinine. Creatinine is excreted by kidneys. Small quantity of creatinine is reabsorbed by tubules.
- Plasma creatinine level depends two factors: rate of production and rate of excretion. Rate of production is related to skeletal muscle mass.
- Creatinine level is more in young persons with larger muscle mass, and low in children and elderly persons. Creatinine levels are increased in cases of reduced glomerular filtration rate.

REGULATION OF WATER AND ELECTROLYTES

Kidneys regulate water by concentrating and diluting urine and also control sodium reabsorption in the proximal and distal collecting tubules. The functioning of the kidneys is efficiently monitored and regulated by hormonal feedback mechanisms involving hypothalamus, juxtaglomerular apparatus and to a certain

extent, the heart. Renin-angiotensin-aldosterone system (RAAS), antidiuretic hormone (ADH), and atrial natriuretic factor (ANF) provide an elaborate system of checks and balances that regulate body fluid osmolarity, salt concentrations, blood pressure and blood volume. Human substances that affect renal functions are given in Table 22.3. Schematic representation of regulation of fluid and electrolytes is shown in Fig. 22.2.

Juxtaglomerular Apparatus (JGA) and Renin-Angiotensin-Aldosterone System

Renin is a central hormone enzyme in the regulation of blood pressure, electrolyte balance via renin-angiotensin-aldosterone system. Main source of renin is the juxtaglomerular cells of the juxtaglomerular apparatus (JG appratus), which release renin from stored granules in the juxtaglomerular cells.

Table 22.3 Human substances that affect renal functions

Human Substance	Source	Actions
Aldosterone	Zona glomerulosa of adrenal cortex in response to hypotension	Promotes reabsorption of sodium and water in kidneys to increase plasma volume and blood pressure
Atrial natriuretic factor (ANF)	Atrial myocardial cells in response to hypertension	Promotes excretion of sodium and water in kidneys to decrease plasma volume and blood pressure
Antidiuretic hormone (ADH)	Hypothalamus and concentrated in posterior pituitary gland in response to hypotension	Promotes water absorption from distal tubules and collecting ducts to conserve water to concentrate the urine
Renin	Kidney in response to hypotension	Renin stimulates liver to synthesize angiotensinogen
Angiotensin	Renin stimulates liver to synthesize angiotensinogen, which gets converted to angiotensin I and then angiotensin II in lungs	Angiotensin II stimulates synthesis of aldosterone by adrenal cortex, which also causes vasoconstriction and increases peripheral resistance

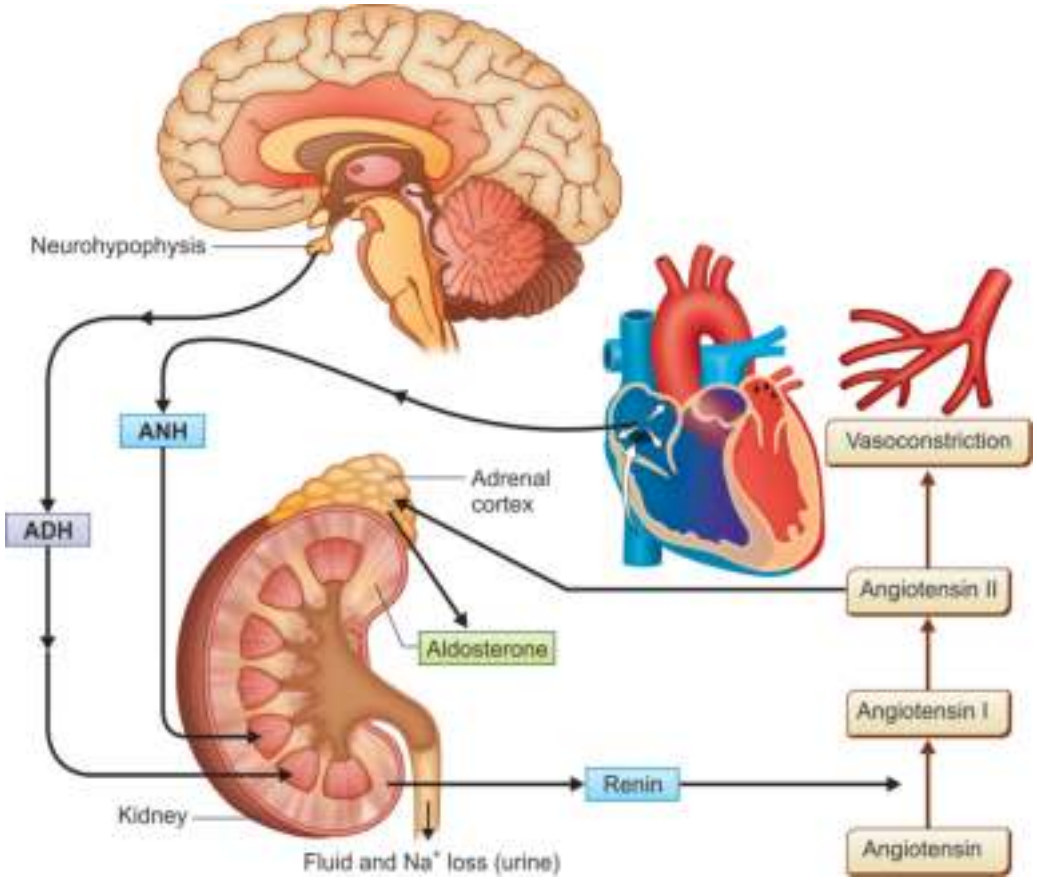


Fig. 22.2: Schematic representation of juxtaglomerular apparatus (JG apparatus). Juxtaglomerular apparatus is closely related to glomerulus, where afferent arteriole enters. JG apparatus is formed by juxtaglomerular cells in the glomerular afferent arteriole, distal convoluted tubule (macula densa, specialized epithelial cells), and nongranular lacis cells. JG apparatus regulates blood pressure and rate of glomerular filtration rate through renin-angiotensin-aldosterone system.

Juxtaglomerular Apparatus (JGA)

Juxtaglomerular apparatus is specialized structure located near the vascular pole of the glomerulus and formed by the distal convoluted tubule (macula densa), glomerular afferent arteriole and nongranular lacis cells. Schematic representation of juxtaglomerular apparatus is shown in Fig. 22.3.

- **Juxtaglomerular cells:** These are modified smooth muscle cells with granular cytoplasm present in the media of afferent arterioles. Juxtaglomerular cells synthesize renin when the blood pressure falls. Renin hormone increases blood pressure via the renin-angiotensin-aldosterone system. Lacis cells, also called extraglomerular mesangial cells, are flat and elongated cells located near the macula densa, which stores renin.
- **Macula densa:** Macula densa is a collection of specialized epithelial cells in the distal convoluted tubule, that detect sodium concentration of the filtrate in the tubular cells in response to elevated sodium. Macula densa cells trigger contraction of the afferent arteriole, reducing blood flow to the glomerulus and the glomerular filtration rate. Main function of macula densa is to regulate blood pressure and the filtration rate of glomerulus.
- **Nongranular lacis cells:** Nongranular lacis cells are present in area bounded by afferent arterioles, macula densa and glomerulus. Function of lacis cells remains unclear.

Renin-Angiotensin-Aldosterone System

- Renin secreted by juxtaglomerular apparatus (JGA) stimulates liver to synthesize angiotensinogen, which gets converted to angiotensin I, and then angiotensin II in the lungs by angiotensin converting enzyme (ACE).
- Angiotensin II is a vasoconstrictor, that increases the peripheral resistance. Angiotensin II also stimulates adrenal zona glomerulosa cells to synthesize aldosterone, which acts on distal convoluted tubule resulting in absorption of sodium and water and expansion of blood volume.
- Aldosterone acts on distal convoluted tubule and helps in absorption of Na^+ and water and completion of the feedback circuit by supporting the release of renin.
- Distal convoluted tubule helps in regulation of blood pH by the reabsorption of an important buffer bicarbonate (HCO_3^-).

Antidiuretic Hormone (ADH)

Osmoreceptors in the hypothalamus are activated by changes in blood volume, body volume and ionic concentration.

- Excess of loss of fluid from the body leads to synthesis of antidiuretic hormone (ADH) by hypothalamus via posterior pituitary gland.
- ADH circulates in blood and acts on collecting ducts to absorb water. ADH also acts on the principal cells of collecting ducts and helps absorption of water.

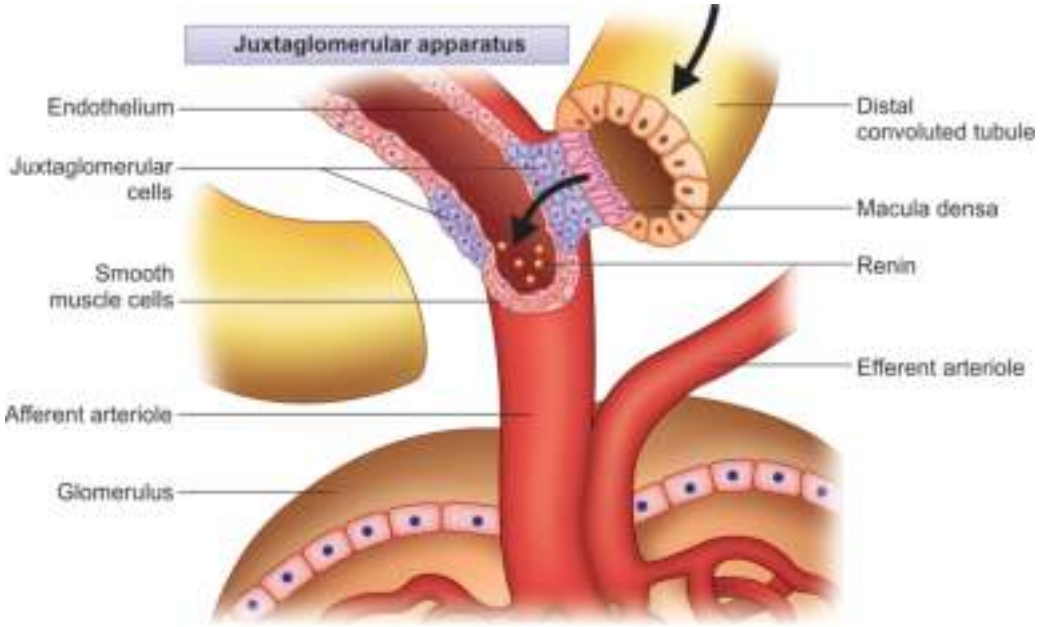


Fig. 22.3: Schematic representation of juxtaglomerular apparatus. Juxtaglomerular apparatus is closely related to glomerulus, where afferent arteriole enters. It is formed by juxtaglomerular cells in the glomerular afferent arteriole, distal convoluted tubule (macula densa, specialized epithelial cells), and nongranular lacis cells. Main function of juxtaglomerular apparatus is to regulate blood pressure and the filtration rate of glomerulus. Juxtaglomerular apparatus releases renin into the blood to raise blood pressure through renin-angiotensin-aldosterone system.

Atrial Natriuretic Factor

Atrial natriuretic factor (ANF) is a peptide hormone, which opposes the regulation by renin-angiotensin-aldosterone system (RAAS).

- The walls of both atria of the heart release atrial natriuretic factor in response to an increase in blood volume and pressure.
- Atrial natriuretic factor inhibits the release of renin from the juxtaglomerular apparatus (JGA), and thereby, inhibits NaCl reabsorption by the collecting duct and reduces aldosterone release from adrenal gland.

MAINTENANCE OF VASCULAR TONE

Kidneys play an important role in maintaining vascular tone by synthesis of angiotensin II and prostaglandins. Angiotensin II synthesized in response to renin production by juxtaglomerular cells causes vasoconstriction of peripheral afferent arterioles and efferent arterioles.

SYNTHESIS OF PRODUCTS

Erythropoietin synthesized in the endothelial cells in the peritubular capillaries (cortex) plays key role in hematopoiesis. Prostaglandins synthesized by renal medulla cause vasodilation of the afferent arterioles. Kidneys participate in the conversion of vitamin D_2 to D_3 by second hydroxylation of vitamin D.

Erythropoietin Synthesis

Erythropoietin is a glycoprotein synthesized in the endothelial cells in the peritubular capillaries of renal cortex (89–90%) and liver (<15%) in response to cellular hypoxia.

- Plasma concentration of erythropoietin is 2–25 IU/L. Erythropoietin is generally increased except in anemia of renal disease. Erythropoietin stimulates BFU-E and CFU-E to divide and mature.
- Erythropoietin increases rate of mRNA and protein (hemoglobin) synthesis. It increases rate of extrusion of nucleus from normoblasts. It stimulates early release of bone marrow reticulocytes. It decreases maturation time of normoblasts.
- Osteoclasts release enzyme alkaline phosphatase, that participates in hydrolysis of pyrophosphate and other inhibitors of calcium-phosphate crystallization, resulting in bone mineralization.

Prostaglandin Synthesis

Prostaglandin synthesis occurs in the cortex by arteries and arterioles as well as the glomerulus. Prostaglandins are important in maintaining blood flow and glomerular filtration rate (GFR) especially during enhanced vasoconstriction activity.

Calcium Homeostasis

Proximal renal tubular cells synthesize enzyme 1α-hydroxylase, that participates in conversion of vitamin D_2 (25-hydroxycholecalciferol) to vitamin D_3 (1, 25-dihydroxycholecalciferol). Vitamin D_3 is the active form of vitamin D.

- Vitamin D increases reabsorption of calcium and phosphorus by gastrointestinal tract. Vitamin D_3 along with parathormone participate in calcium absorption by distal convoluted tubules, resulting in bone mineralization. It increases the production of osteoclasts from macrophage stem cells.
- Osteoclasts release enzyme alkaline phosphatase, which participates in hydrolysis of pyrophosphate and other inhibitors of calcium phosphate crystallization, resulting in bone mineralization.

URINE FORMATION: MECHANISM

Nephrons regulate water and electrolytes, mainly sodium, in the loop of Henle, distal tubule and collecting ducts according to the body's needs. Urine is formed by glomerular filtration, tubular reabsorption, tubular secretion, and concentration of urine by the countercurrent mechanism. Schematic representation of tubular processing of the glomerular filtrate in urine formation is shown in Fig. 22.4.

Glomerular Filtration

Glomerular filtration is the first step in urine formation. Blood pressure inside the glomeruli forces water and dissolved substances into the glomerular Bowman's capsule. Blood cells and proteins remain behind in the blood.

- Glomeruli possess size and charge-dependent barriers. Glomeruli exclude all molecules ≥3.5 nm in size and negatively charged albumin with 70,000 molecular weight.
- The smaller diameter of the efferent arteriole as compared with that of afferent arteriole maintains high hydrostatic pressure. Depending on the extent of injury to the glomeruli, excretion of red blood cells and proteins in urine occurs.

Reabsorption of Essential Metabolites by Nephron

Tubular reabsorption moves essential metabolites back into the blood, while keeping waste products in the nephrons to be eliminated in the urine. Schematic representation of tubular reabsorption of essential metabolites by nephron is shown in Fig. 22.5. Schematic representation of mechanism of sodium reabsorption and potassium secretion by principal cells of the distal and collecting tubules is shown in Fig. 22.6.

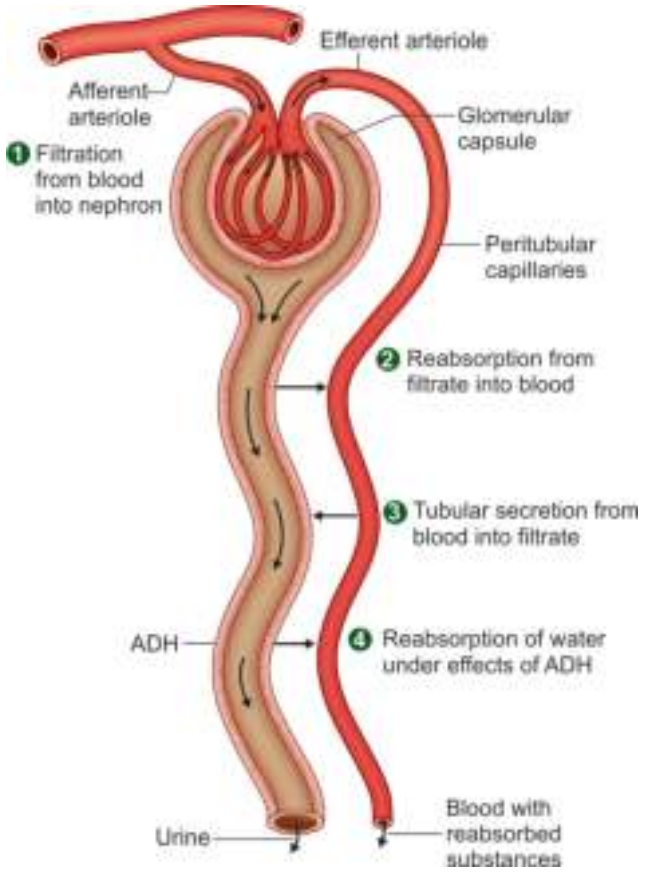


Fig. 22.4: Schematic representation of tubular processing of the glomerular filtrate and formation of urine. Normally, the tubules completely reabsorb essential substances such as glucose and amino acids from the glomerular filtrate. The tubules reabsorb variable quantity of water to adjust plasma osmolarity and secrete variable quantity of hydrogen and electrolytes to adjust plasma pH and electrolyte concentration. Toxins, urea, and creatinine are excreted into urine.

- **Proximal tubules:** Proximal tubules process the filtrate and regulate water and electrolytes. Proximal tubules contain abundant mitochondria, ATPase pump, sodium transporter proteins, aquaporins (water channels) and claudins and membrane proteins in tight junctions.
  - Proximal tubules reabsorb essential metabolites by active process such as sodium (50–55%), potassium, amino acids, glucose, phosphates (100%) and bicarbonate (80%). Water is absorbed in proximal tubules by passive process.
  - Sodium ions are pumped from tubular cell to interstitial fluid. Proximal tubules drive chloride ions into the interstitial fluid by passive process. Proximal tubules synthesize hydrogen ions, that participate in the maintenance of acid–base equilibrium.
- **Loop of Henle:** Loop of Henle participates in absorption of sodium and chloride by active process and water by passive process. Descending limb of loop of Henle is permeable to water only. Ascending limb of loop of Henle is impermeable to water but permeable to essential metabolites such as sodium, potassium, chloride, calcium, bicarbonate and magnesium. Hydrogen ions are also secreted by ascending limb of loop of Henle.
- **Distal tubules:** Distal tubules reabsorb essential metabolites such as sodium, potassium, potassium chloride, bicarbonate, calcium and magnesium, which regulate acid and base equilibrium; and maintain of fluid and electrolyte balance. Several hormones such as aldosterone and natriuretic factor affect tubular reabsorption. Both hormones have opposite action.
- **Collecting duct:** Collecting ducts are permeable to urea, but impermeable to salt. Urea diffuses out of collecting ducts and enters into interstitial fluid contributing to high osmolarity in medulla. Collecting ducts participate in absorption of water by ADH action on collecting ducts. As the urine flows through the collecting ducts, ADH increases the permeability of the collecting duct, and pushes out water from filtrate into interstitial fluid.

Tubular Secretion

Tubular secretion moves additional metabolites from the blood into the nephrons for elimination. Movement of hydrogen ions is one way by which the pH of body fluids is balanced. Distal tubules participate in deamination of amino acids resulting in ammonia production that enters the filtrate. Hydrogen and potassium ions also enter filtrate in exchange of sodium ions (sodium ions moving out of filtrate). Ammonia combines with hydrogen ions to form ammonium salts.

Countercurrent Mechanism and Urine Concentration

Human kidneys can produce nearly four times concentrated urine than the initial glomerular filtrate formed. Proximity of loop of Henle and vasa recta play an important role in production of concentrated urine by countercurrent mechanism. Fluid is flowing in opposite direction between loop of Henle and vasa recta, and thus forming countercurrent system.

- Countercurrent mechanism concentrates the urine and reduces the volume excreted. There is increasing concentration of the interstitial fluid moving from the cortex to the medulla.
- Urine concentration is regulated by means of exchange of water and electrolytes, mainly sodium, in the loop of Henle, distal tubule and collecting ducts. As urine flows through the collecting ducts, water is drawn and hypertonic interstitial fluid moving from cortex to medulla thus concentrating the urine. ADH increases the permeability of the collecting duct, and pushes out water from filtrate into interstitial fluid.

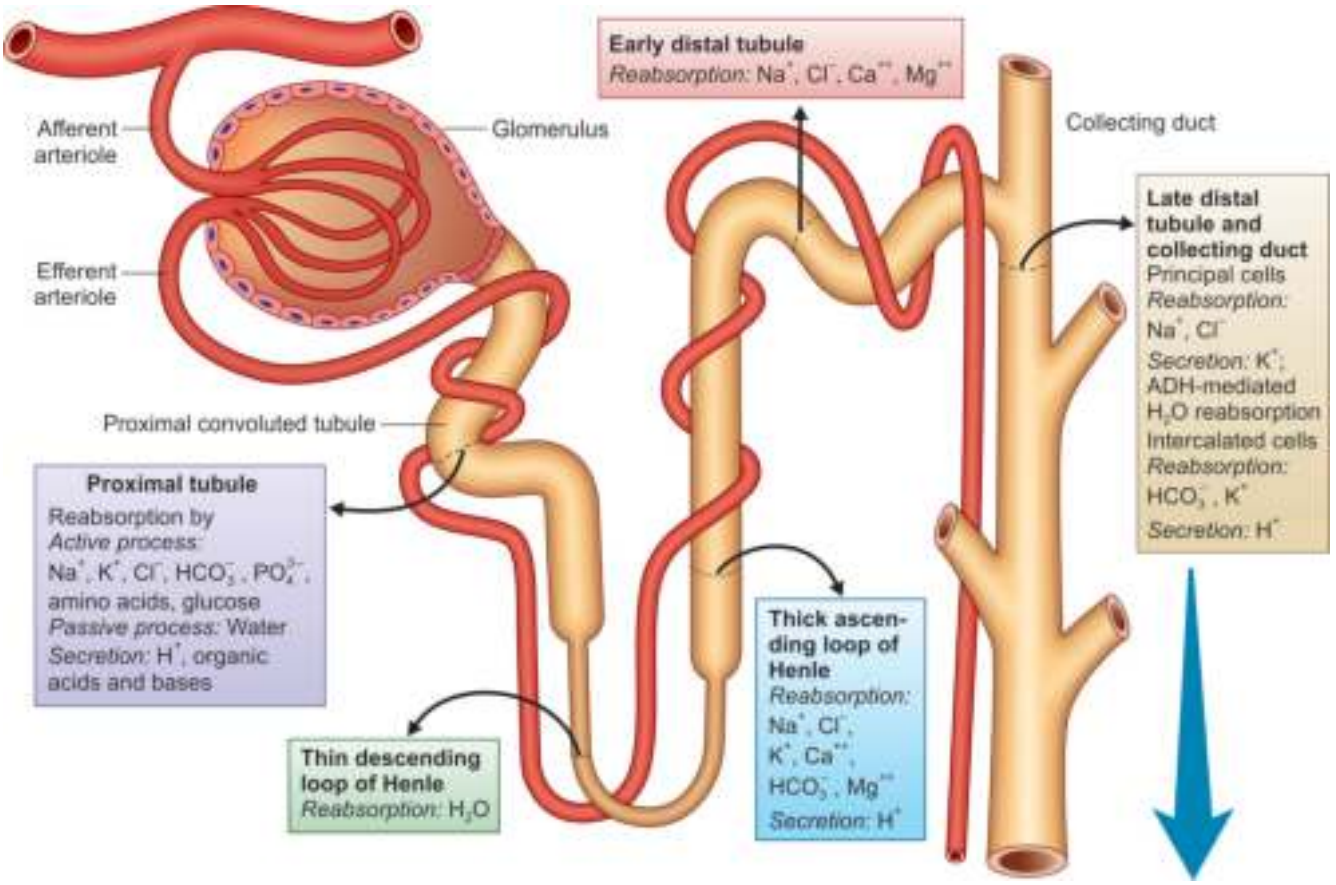


Fig. 22.5: Schematic representation of tubular reabsorption of essential metabolites by nephron. In renal physiology, tubular reabsorption is the process by which the nephron removes water and solutes from the tubular fluid and returns them to the blood circulation. Essential metabolites in the tubular fluid include water (H_2O), glucose, amino acids, Na^+ (sodium), Cl^- (chloride), HCO_3^- (bicarbonate), K^+ (potassium), Ca^{++} (calcium), and Mg^{++} (magnesium), organic acids and bases, H^+ (hydrogen), and K^+ secretion.

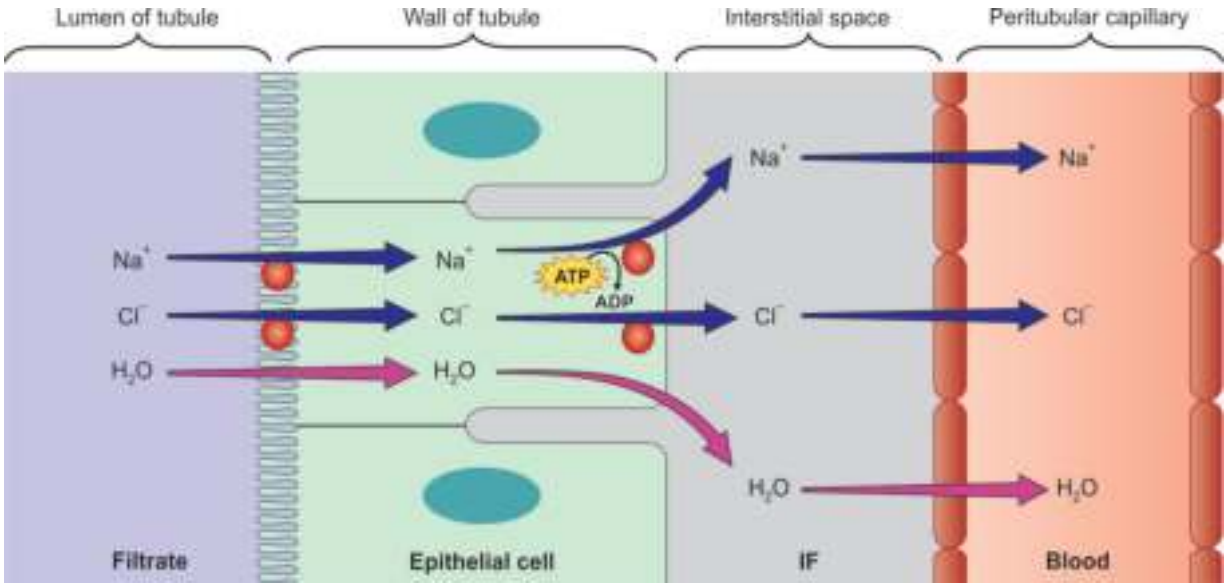


Fig. 22.6: Schematic representation of mechanism of sodium reabsorption and potassium secretion by principal cells of the late distal and collecting tubules. It is well known that sodium reabsorption and aldosterone play key roles in potassium secretion by aldosterone-sensitive principal cells of the late distal and collecting tubules of nephron. Aldosterone exerts its action by increasing the activity of the Na^+/K^+ ATPase pump that transports sodium outward through the basolateral membrane of the cell and into the blood at the same time it pumps potassium into the cell. Aldosterone also increases the permeability of the luminal membrane for potassium.

- Collecting ducts are permeable to urea, but impermeable to salt. Urea diffuses out of collecting ducts and enters into interstitial fluid contributing to high osmolarity in medulla. As the urine flows through the collecting ducts, ADH increases the permeability of the collecting duct, and pushes out water from filtrate into interstitial fluid.
- Schematic representation of concentration of urine by the countercurrent mechanism is shown in Fig. 22.7.

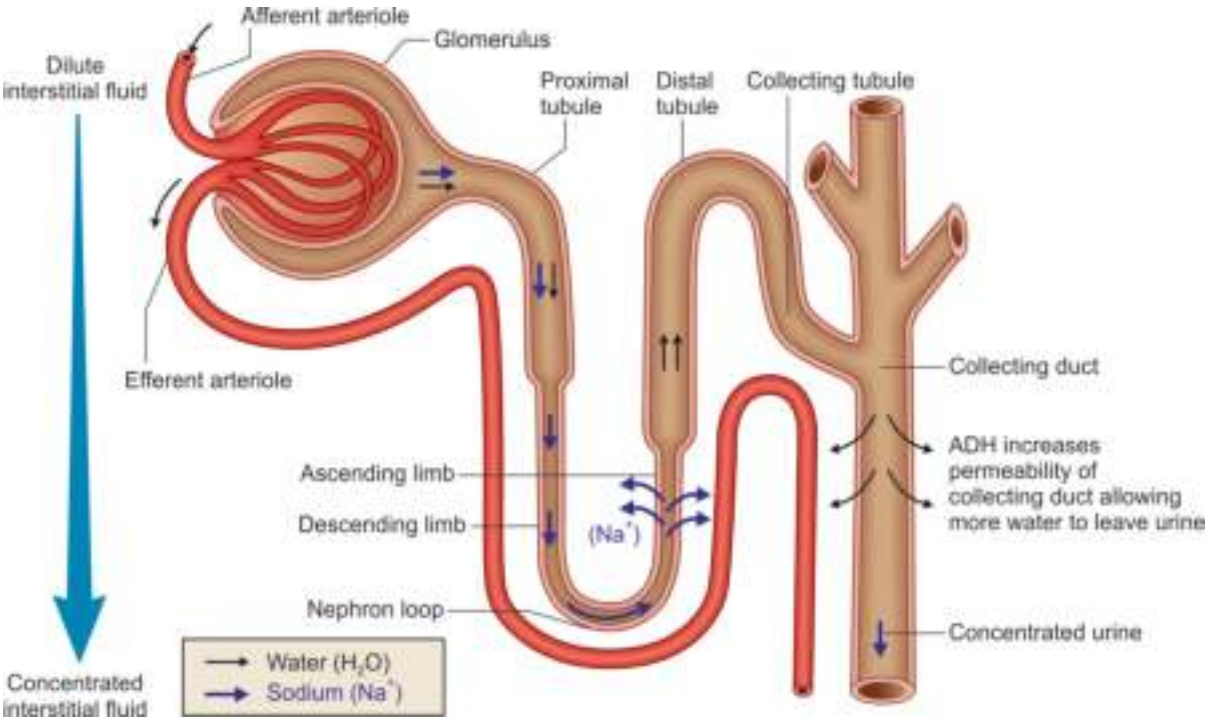


Fig. 22.7: Schematic representation of concentration of urine by the countercurrent mechanism. There are two countercurrent mechanisms in the kidneys: Loop of Henle and the vasa recta. Urine concentration is regulated by exchange of water and electrolytes mainly sodium in the loop of Henle, distal tubule and collecting duct. As urine flows through the collecting ducts, water is drawn out by the hypertonic interstitial fluid, thus concentrating the urine. Antidiuretic hormone (ADH) increases the permeability of the collecting ducts, allowing more water to leave the urine.

GLOMERULAR DISEASES OF KIDNEY

GLOMERULUS STRUCTURE

Glomerulus is composed of bunch of capillaries are lined by thin layer of fenestrated endothelial cells, and glomerular basement membrane. Capillaries are lined by thin layer of fenestrated endothelial cells being about 70–100 nm in diameter.

- The glomerular basement membrane is synthesized from the proteins synthesized by visceral cells of Bowman capsule (podocytes) and the closely apposed capillary endothelium, resulting in a double-thick layer of matrix proteins that is essential part of the glomerular blood filter.
- Glomerular basement membrane (GBM) is composed of collagen fiber types IV and V, laminin, fibronectin, proteoglycans and fibroblasts. Proteoglycans consist of glycosaminoglycans linked to protein and calcium. GMB regulates connective tissue structure permeability.
- Bowman's capsule (lined by visceral and parietal epithelial cells) and mesangial cells, size and charge on GBM determine protein filtration.
- Water, salts, glucose and other small molecules cross glomerular basement membrane from blood into Bowman's space as glomerular filtrate except red blood cells, proteins and other large molecules.
- Schematic representation of glomerulus is shown in Fig. 22.8. Schematic representation of functional parts of the glomerulus is shown in Fig. 22.9. Basic structure and function of the glomerulus is given in Table 22.4.

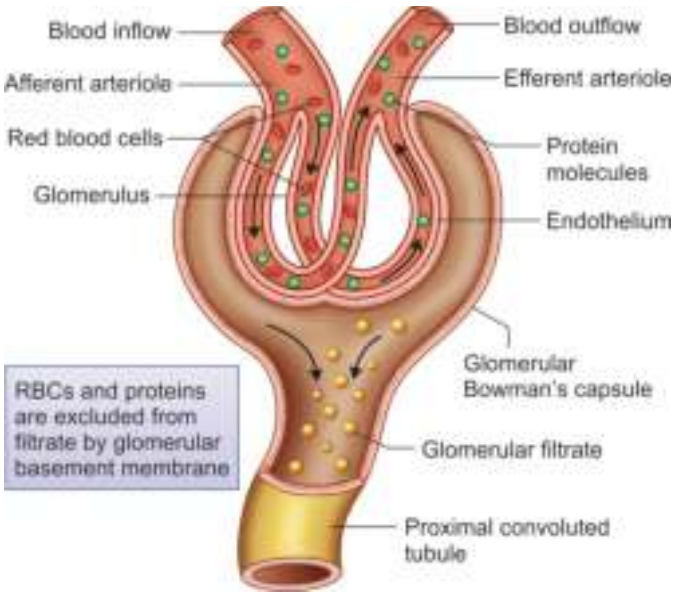


Fig. 22.8: Schematic representation of glomerulus. The glomerulus is a tuft of capillaries supported by mesangial cells and located within Bowman's capsule. Afferent and efferent arterioles are branches of renal artery. The capillaries are lined by delicate fenestrated endothelia with a central lumen. Afferent arteriole carries blood to the glomerulus, whereas efferent arteriole drains blood away from the glomerulus. Diameter of lumen of efferent arteriole is smaller than afferent arteriole, hence glomerular filtration occurs as a result of small lumen's diameter of efferent arteriole. Hydrostatic pressure in the glomerulus is >60 mm Hg. Blood flow to kidneys is 1200 ml/minute (blood plasma 600 ml/minute). Glomerular filtration is 10% of the total renal blood flow. RBCs and proteins are excluded from glomerular filtrate by GBM.

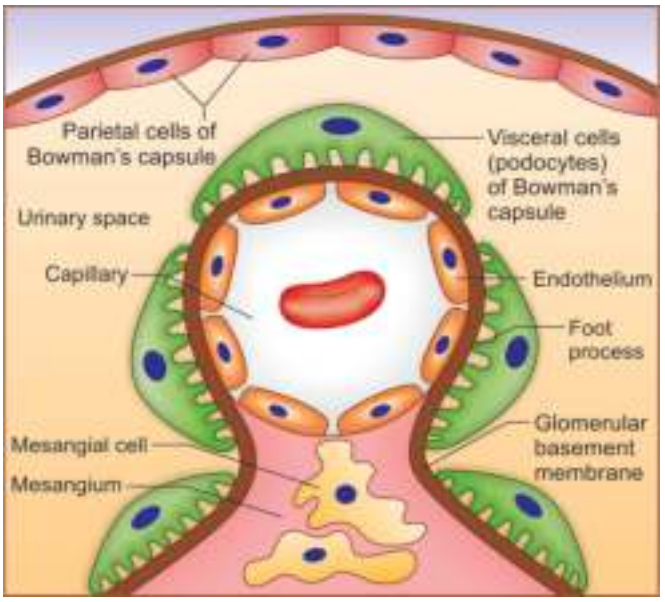


Fig. 22.9: Schematic representation of functional parts of the glomerulus. Glomerulus is the filtering unit of the kidney, which has tuft of capillaries lined by delicate fenestrated endothelia, which in contact with inner surface of glomerular basement membrane (GBM) and specialized visceral epithelial cells of Bowman's capsule. Visceral cells of the Bowman's space form the slit diaphragms between foot processes. Endothelial pores act as size-dependent barrier that excludes blood cells. GBM acts as size-dependent barrier that excludes large macromolecules. Podocytes act filtration slit diaphragm which acts as size-dependent barrier. Outer layer of the Bowman's capsule is lined by parietal epithelial cells. GBM also acts as charge-dependent barrier. Negative charge on the GBM excludes negative charged albumen in the glomerular filtrate.

Table 22.4 Basic structure and function of the glomerulus

Components	Functions
Glomerular capillary endothelial cells	
Endothelial cells lining glomerular capillaries	Exclude molecules ≥3.5 nm diameter
Glomerular basement membrane (GBM)	
GBM structure	▪ Collagen IV fibers provide strength to GBM ▪ Laminin helps in the attachment of endothelial cells and visceral cells of Bowman's capsule to the matrix ▪ Fibronectin binds to collagen fibers, heparan, and proteoglycans, fibrin, and other cells via integrin receptors. It is involved in interactions of cells with the extracellular matrix.
GBM layers	▪ Lamina rara interna is innermost layer of GBM towards glomerular capillary endothelial cells ▪ Lamina densa is middle layer of GBM ▪ Lamina rara externa is outermost layer of GBM towards visceral epithelial cells
GBM charge	Negative charge on GBM is due to polyanionic proteoglycans and anionic sialoglycoproteins exclude negative charged molecules like albumin
Bowman's capsule	
Visceral epithelial cells of Bowman's capsule	▪ Visceral epithelial cells line inner layer of Bowman's capsule form podocytes ▪ Slit pores are present between podocytes prevent filtration of large size molecules
Parietal epithelial cells of Bowman's capsule	Parietal epithelial cells lining outer layer of Bowman's capsule
Mesangial cells	
Mesangial cells present in mesangial region	Mesangial cells provide mechanical support to the glomerular capillaries and regulate glomerular filtration rate

Pathology Pearls: Components of the Glomerulus

Glomerular Capillaries

Glomerular capillaries are lined by thin layer of fenestrated endothelial cells being about 70–100 nm in diameter.

Glomerular Basement Membrane

- The glomerular basement membrane is a 250–350 nm thick ribbon-like extracellular matrix that lies between the fenestrated endothelium and podocyte foot processes, i.e. visceral layer of Bowman's capsule. The mesangium contains mesangial cells and their associated mesangial matrix. Schematic representation of glomerular basement membrane (GBM) is shown in Fig. 22.10.
- GBM consists of central electron dense layer, the lamina densa, thinner electron-lucent lamina rara interna (towards endothelial cells), and thinner electron-lucent lamina rara externa (towards visceral layer of Bowman's capsule).
- GBM is composed of collagen fiber type IV, laminin, fibronectin, proteoglycans and fibroblasts. Proteoglycans consist of glycosaminoglycans linked to protein and calcium. It regulates connective tissue structure permeability.
- There is negative charge on endothelial cells, glomerular basement membrane and visceral epithelial cells (podocytes).
- As albumin also possesses negative charge, therefore, it is repelled and not filtered in the glomerular filtrate.

Collagen IV

The major component of glomerular basement membrane is known to be type IV collagen, which provides structural strength to the capillary. It is resistant to collagenase enzymes.

Laminin

- Laminin is a cross-shaped molecule distributed along glomerular basement membrane's lamina rara interna and externa. It comprises long arm and short arm. Long arm binds heparan sulfate and short arm with globular ends to collagen. Laminin has high affinity for collagen and proteoglycans. Laminin is believed to mediate attachment of endothelium and visceral epithelium to connective tissue matrix substrates.

Fibronectin

- Fibronectin is a multifunctional glycoprotein consisting of two dimers held together by disulphide bonds. It is synthesized by fibroblasts, monocytes, endothelial cells and other cells.
- Fibronectin binds to collagen, heparan sulfate and proteoglycans, fibrin, and other cells via integrin receptors. It is involved in the interactions of cells with the extracellular matrix.

Polyanionic Proteoglycans

- Polyanionic proteoglycans comprise heparan sulfate hyaluronic acid, chondroitin, dermatan, and keratin, linked covalently to protein core.
- Heparan sulfate is present in the form of clusters in lamina densa and along lamina rara interna and externa, which retain fluid and maintain normal shape and structure of GBM.
- Proteoglycans play diverse role in regulating connective tissue, structure, charge dependent permeability barrier and modulating cell differentiation.

Anionic Sialoglycoproteins

Anionic sialoglycoproteins coat glomerular capillary endothelial cells and the visceral epithelial cells. These are responsible for charge dependent glomerular filtration barrier.

Fibroblasts

- Fibroblasts synthesize glycosaminoglycans comprising of hyaluronic acid, chondroitin, dermatan, keratin and heparan sulfate.
- Fibroblasts synthesize elastic fibers. In inflammation, elastase synthesized by bacteria, polymorphonuclear cells degrade elastic fibers resulting in formation of elastic myofibrils and elastin fibers.
- Elastin fibers are abundant amorphous substance comprising of tropoelastin (polypeptide), desmosine, iododesmosine and tropoelastin.

Bowman's Capsule

Bowman's capsule is a portion of the nephron that forms a cup-like sac surrounding the glomerulus. Bowman's capsule encloses a space called 'Bowman's space', which represents the beginning of the urinary space and is contiguous with the proximal convoluted tubule of the nephron.

Visceral Epithelial Cells of Bowman's Capsule

- Visceral epithelial cells of Bowman's capsule are primarily responsible for production of the glomerular basement membrane, which contain podocytes (foot-like processes).
- Slit pores between the podocytes 20–30 nm wide are bridged by a thin diaphragm, which serve as a distal major filtration barrier for preventing protein loss in the urine. Proteins linking podocytes include nephrin, podocin and CD2 associated proteins (CD2AP). Schematic representation of structure of kidney slit diaphragm adaptor CD2 associate protein (CD2AP) is shown in Fig. 22.11.
  - CD2AP is a multidomain scaffolding protein that has a critical role in renal function. CD2AP is expressed in glomerular podocytes at the slit diaphragm and links nephrin and podocin to phosphoinositide-3-OH kinase; this complex has cell signaling properties.
  - Nephrin and podocin are slit diaphragm proteins identified in families with congenital nephrotic syndrome.
  - Integrins span the membrane and anchor the actin cytoskeleton to the collagen type IV in the lamina rara externa of the basement membrane.
  - Effacement of podocytes seen in minimal change disease is responsible for nephrotic syndrome.

Parietal Epithelial Cells of Bowman's Capsule

Parietal epithelial cells line the Bowman's capsule. Due to increased permeability of glomeruli, fibrinogen leaks and stimulates the parietal cells, which undergo proliferation forming crescents resulting in destruction of the glomeruli, and impairment of renal functions.

Mesangial Cells

- Normally, mesangial cells are present in small number in mesangial matrix.

- Mesangial cells provide mechanical support to the glomerular capillaries.
- Mesangial cells participate in processing plasma proteins and immune complexes.
- Mesangial cells maintain glomerular basement membrane and matrix elements.
- Mesangial cells participate in modulation of glomerular filtration rate.
- Mesangial cells synthesize prostaglandins and cytokines, which participate in cell proliferation and chemotaxis of inflammatory cells.
- Mesangial cells release inflammatory mediators in IgA nephropathy.

GLOMERULAR PERMEABILITY BARRIERS

Glomeruli consist of size and charge dependent barriers, which prevent RBCs, protein, and other large molecules from leaving blood and passing into urine. Alteration in size and charge dependent barriers of glomeruli leads to leakage of proteins and red blood cells in urine.

- Glomerulopathy causes impairment of charge dependent barrier resulting in protein loss. Normally, about 300 mg of albumin is excreted in urine per 24 hours.
- Selective proteinuria reveals presence of only albumin in the urine. Nonselective proteinuria refers to loss of both albumin and globulin in urine. Glomerular permeability size and charge barriers are given in Table 22.5.

GLOMERULAR FILTRATION RATE

Kidneys receive 25% of cardiac output, i.e. 1200 ml of blood (600 ml of plasma) per minute. The normal GFR for an adult male is 90–120 ml per minute.

- Glomerular filtration occurs through endothelial cells (85%) and mesangial region (15%). Almost all of the glomerular filtrate is reabsorbed by renal tubules. Mesangial cells regulate glomerular filtration rate (GFR) and have phagocytic activity. Glomeruli participate in filtration of substances.
- Glomerular filtration is the first step in urine formation. Blood pressure inside the glomeruli forces water and dissolved substances into the glomerular Bowman's capsule. Red blood cells and proteins remain behind in the blood. The smaller

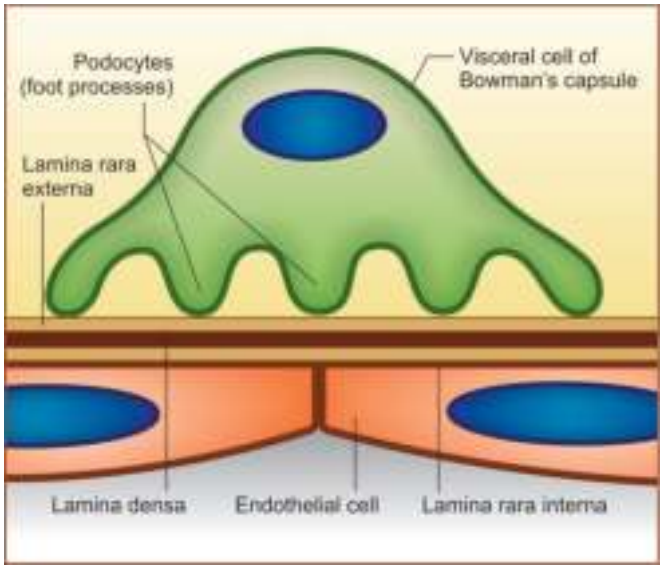


Fig. 22.10: Schematic representation of glomerular basement membrane (GBM). Glomerular basement membrane is a ribbon-like extracellular matrix that lies between the endothelium and podocyte foot processes. The mesangium contains mesangial cells and their associated matrix. GBM is composed of lamina densa (middle portion), lamina rara interna towards endothelial cells and lamina rara externa towards visceral cells of Bowman's capsule. There is a negative charge on the endothelial cells, GBM and visceral cells. These act as charge dependent barrier for negative charged albumin. Outer layer of the Bowman's capsule is lined by parietal epithelial cells.

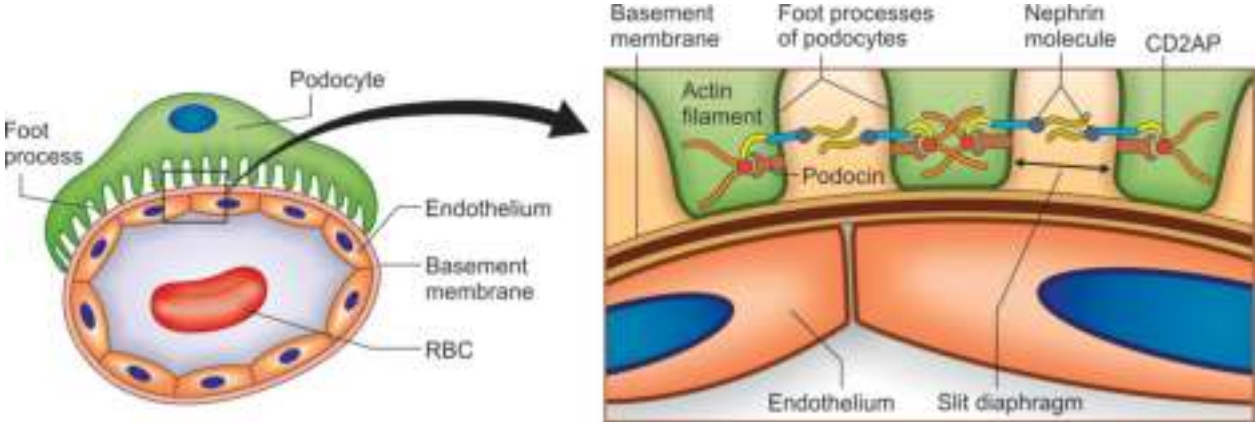


Fig. 22.11: Schematic representation of structure of kidney slit diaphragm adaptor CD2 associate protein (CD2AP). CD2-associated protein (CD2AP) is a multidomain scaffolding protein that has a critical role in renal function. Nephrin and podocin are slit-diaphragm proteins identified in families with congenital nephrotic syndrome.CD2AP is expressed in glomerular podocytes at the slit diaphragm and links nephrin and podocin to phosphoinositide 3-OH kinase; this complex has cell signaling properties.

Table 22.5 Glomerular permeability size and charge-dependent barriers

Size-dependent Glomerular Permeability Barrier
In health, the glomerular filtration size and charge dependent barriers function as a highly organized semipermeable membrane preventing the presence of the majority of proteins into the urine
Glomerular filtration size-dependent permeability barrier excludes ≥3.5 nm diameter molecules such as proteins and red blood cells
Filtration slit diaphragms are present between the podocytes of visceral epithelium of Bowman's capsule. The filtration slits are comprised of matrix proteins of glomerular basement membrane
Glomerular capillary wall and surrounding glomerular visceral epithelial cells of Bowman capsule regulate fluid movement from the glomerular capillary into Bowman's capsule space and also regulate the movement of macromolecules
Charge-dependent Glomerular Permeability Barrier
There is negative charge on the glomerular capillary endothelial cells, glomerular basement membrane and visceral epithelial podocytes of Bowman's capsule. Hence, negatively charged albumin is excluded of glomerular filtrate
Glomeruli consist of negatively charged molecules such as polyanionic glycosaminoglycans (heparan sulfate and sialoproteins), which provide negative charge on endothelial cells, glomerular basement membrane and epithelial cells
Glomeruli allow greater penetration of neutral and cationic molecules than anionic molecules of the same size. These exclude negatively charged albumin from glomerular filtrate

diameter of the efferent arteriole as compared with that of afferent arteriole maintains high hydrostatic pressure.

- Glomerular filtration rate depends upon: (a) renal blood flow, (b) hydrostatic pressure in glomeruli (normal 60 mm Hg due to smaller lumen of efferent arterioles), (c) number of functional glomeruli, (d) blood colloidal pressure (normal 25 mm Hg), and (e) back pressure in tubules (normal 15 mm Hg).
- Filtration pressure is necessary to drive fluid across the glomerular basement membrane. It is equal to the difference between the blood pressure in the glomeruli (60 mm Hg) and sum of oncotic pressure exerted by plasma proteins (27 mm Hg) and intratubular pressure (15 mm Hg). Filtration pressure = Blood pressure in the glomeruli – (oncotic pressure 27 mm Hg in glomeruli + intratubular pressure 15 mm Hg).
- Water distribution in the body is regulated by osmotic and oncotic pressure of plasma. Lower plasma oncotic pressure induces intracellular edema, while lower plasma oncotic pressure induces extracellular edema.
- Normally, renal tubules completely reabsorb from glomerular filtrate such as glucose and amino acid. Almost all of the glomerular filtrate is reabsorbed by renal tubules and returned to blood. The tubules reabsorb variable amount of water to adjust plasma osmolality and secrete variable quantity of hydrogen and electrolytes to adjust plasma pH and electrolyte concentration. Toxins, creatinine and urea pass unaltered into urine.

GLOMERULONEPHRITIS: TERMINOLOGY

Terminology used to describe morphologic lesions of kidney is given in Table 22.6. Schematic diagram of structure of distribution of glomerulonephritis disease in kidney based on glomerular involvement as shown in Fig. 22.12.

Table 22.6 Terminology used to describe morphologic lesions of kidney

Terminology	Features
Light microscopy	
Focal glomerulonephritis	▪ Involving <50% of glomeruli in both kidneys ▪ Example: Henoch-Schönlein purpura, IgA nephropathy, Goodpasture syndrome, infective endocarditis, vasculitis and connective tissue disorders
Diffuse glomerulonephritis	▪ Involving >50–100% glomeruli in both kidneys ▪ Example: Post-infectious glomerulonephritis (glomeruli are hypercellular due to proliferation of glomerular cells and leukocytic infiltration and immune complex deposits in glomeruli. It is termed as exudative/necrotizing glomerulonephritis)
Segmental glomerulonephritis	▪ Involving part of glomerular tuft ▪ Example: Diabetes mellitus (increase in extracellular matrix (hyalinization) within the mesangial region)
Global glomerulonephritis	▪ Involving whole glomerular tuft ▪ Example: Lupus nephritis (renal involvement in cases of systemic lupus erythematosus)

Contd...

Table 22.6 Terminology used to describe morphologic lesions of kidney (*Contd...*)

Terminology	Features
Membranoproliferative glomerulonephritis	Combined capillary wall thickening and mesangial or endocapillary hypercellularity
Lobular glomerulonephritis	Lobular appearance of capillary loop architecture due to endocapillary proliferation seen in membranous glomerulopathy
Diffuse glomerulosclerosis	Obliteration of capillary loop and increased mesangial matrix
Nodular glomerulosclerosis	Relatively acellular areas of mesangial matrix expansion seen in diabetic nephropathy
Crescentic glomerulonephritis	▪ Extracapillary hypercellularity other than the epithelial hyperplasia of collapsing variant of focal segmental glomerulosclerosis (FSGS) ▪ In crescentic glomerulosclerosis, inflammatory chemical mediators cause proliferation of parietal epithelial cells in Bowman's syndrome resulting in proliferation of glomerular tuft, e.g. rapidly proliferative glomerulonephritis such as Goodpasture's syndrome
Spikes and domes appearance of glomeruli	Projections of glomerular basement membrane intervening between subepithelial immune complex deposits seen in membranous glomerulopathy
Endocapillary hypercellularity	Increased cellularity internal to the glomerular basement membrane (GBM) composed of leukocytes, endothelial cells and/or mesangial cells filling up and distending capillary lumen seen in proliferative lupus nephritis
Extracapillary hypercellularity	Increased cellularity in Bowman's space, i.e. >1 layer of parietal or visceral epithelial cells on monocytes/macrophages
Fibrinoid necrosis	Destruction of glomerular cells and matrix with deposition of acidophilic fibrin-rich material
Hyaline change	Glassy acidophilic extracellular material
Sclerosis	Increased collagenous extracellular matrix that is expanding the mesangium, obliterating capillary lumens or forming adhesions to Bowman's capsule
Mesangium	Mesangium is stalk region of capillary loop with mesangial cells surrounded by matrix
Mesangial hypercellularity	Presence of 4 or more nuclei in a peripheral mesangial matrix segment
Subepithelial region	Region between glomerular basement membrane and podocytes
Subendothelial region	Region between glomerular basement membrane and endothelial cell
Tram-track appearance	Double contour of glomerular basement membrane due to deposits and/or circumferential interposition
Wire loop appearance	Thick rigid appearance of capillary loop due to massive subendothelial deposits
Activity of lesion	Treatment sensitive lesions such as extent of cellular infiltrate, crescents, necrosis and proliferation
Chronicity of renal disease	Irreversible renal lesions, i.e. extent of tubular atrophy, interstitial fibrosis, fibrous crescents, sclerosis
Immunofluorescence microscopy staining pattern	
Diffuse linear staining of GBM	Smooth continuous staining seen along capillary loops in ant-GBM antibody mediated glomerulonephritis or along tubular basement membrane in Goodpasture's syndrome
Granular lumpy bumpy deposits in glomeruli	Discontinuous flecks of staining producing granular pattern seen in membranous glomerulopathy, Poststreptococcal glomerulonephritis, IgA nephropathy and lupus nephritis
Irregular (fluffy) deposits	Irregular (fluffy) staining is seen in monoclonal light chains (AL) and amyloid associated protein (AA)
Subendothelial deposits	Immune complex deposits in subendothelial region in lupus nephritis in systemic lupus erythematosus
Subepithelial deposits	Granular lumpy bumpy (large size) immune complex deposits are demonstrated in poststreptococcal glomerulonephritis and membranous glomerulonephritis
Mesangial region deposits	Immune complex deposits (IgA1 and complement) in IgA nephropathy
Intramembranous region deposits	Immune complex deposits in membranoproliferative glomerulonephritis and lupus nephritis

Contd...

Table 22.6 Terminology used to describe morphologic lesions of kidney (*Contd...*)

Terminology	Features
Electron microscopy	
Electron dense immune complex deposits	▪ Immune complex deposits in intramembranous and subepithelial region in membranous glomerulonephritis ▪ Immune complex deposits in mesangial region in IgA nephropathy ▪ Immune complex deposits in subendothelial region in lupus nephritis
Effacement of foot processes (podocytes)	Flattening of foot processes so that cover the glomerular basement membrane with loss of slit diaphragms seen in minimal change disease
Microvillous transformation	Small extensions of visceral epithelial cells with villus-like appearance
Circumferential interposition	Extension of mesangial cell or infiltrating monocyte cytoplasm with interposition between endothelial and basement membrane, often with underlying new basement membrane formation seen in membranoproliferative glomerulonephritis
GBM thickening	Diabetic glomerulosclerosis
Collagen matrix deposit	Focal segmental glomerulosclerosis
Fibrillary deposit	Fibrils 14 nm diameter without organization seen in amyloid nephropathy

Immunofluorescence microscopy demonstrates patterns and location of immune complex deposits of IgG, IgA, IgM, complement (C1q, C3), fibrin, light chains, and albumin).

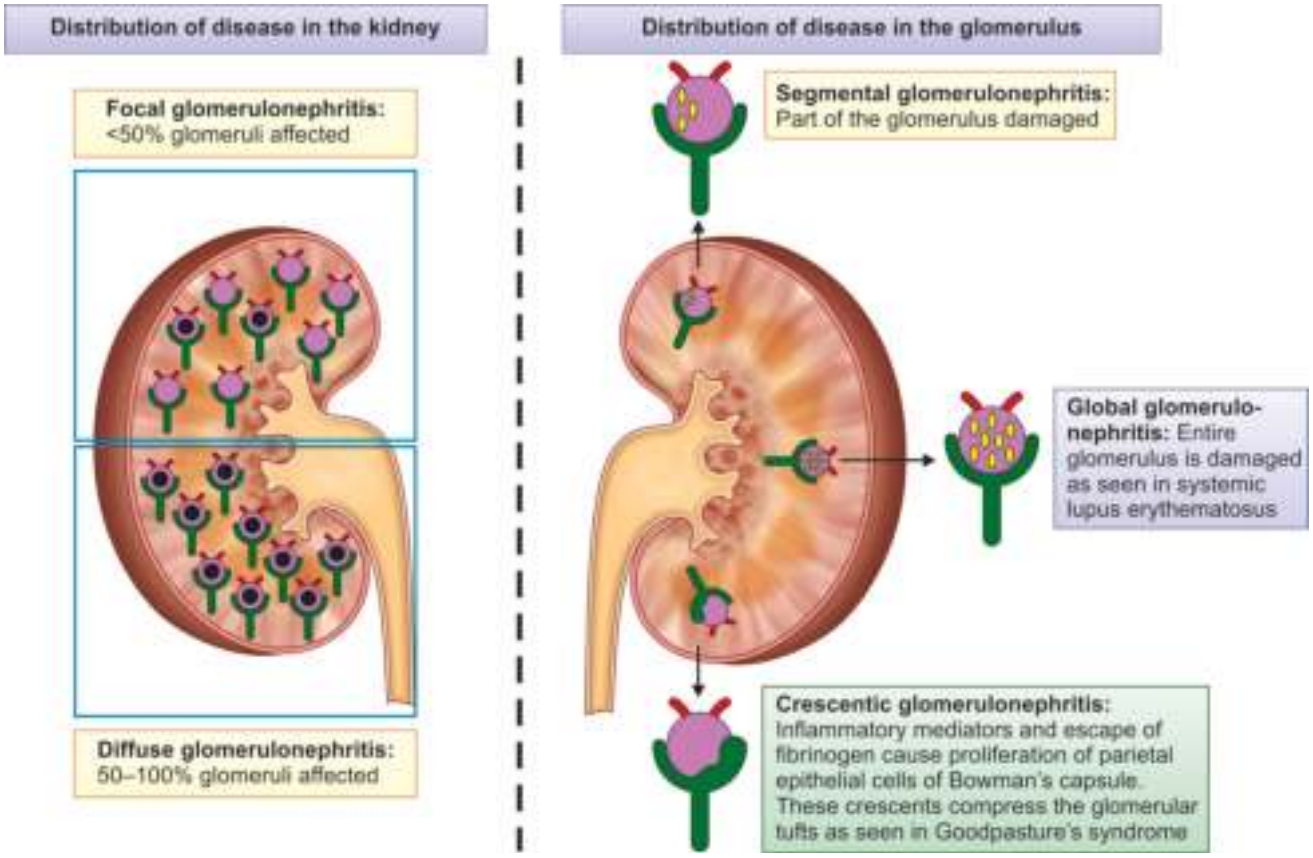


Fig. 22.12: Schematic diagram of structure of distribution of glomerulonephritis disease in kidney based on glomerular involvement. It depicts: (a) diffuse glomerulonephritis (bilateral glomeruli involvement >50–100%), (b) focal glomerulonephritis (bilateral glomeruli involvement <50%), (c) global glomerulonephritis (all the capillaries involved within glomerulus), (d) segmental glomerulosclerosis (segment of glomeruli involved with hyalinization within the mesangial region), (e) crescentic glomerulonephritis is an immune-mediated disease, which causes severe glomerular inflammation and injury, and often results in irreversible renal failure. Inflammatory mediators stimulate proliferation of parietal cells of Bowman's capsule leading to formation of crescents, which compress glomerular tufts and thus adversely affect glomerular filtration.

CLASSIFICATION OF GLOMERULONEPHRITIS BASED ON ETIOLOGY, CLINICAL PRESENTATION, APPEARANCE OF GLOMERULAR CAPILLARY TUFT AND DURATION OF DISEASE

Glomerular diseases are caused by damage to structural components of glomeruli, i.e. endothelial cells lining glomerular capillaries, glomerular basement membrane, mesangial cells, mesangial matrix and visceral epithelial cells. Visceral epithelial cells of Bowman capsule are in contact with the outer surface of the glomerular basement membrane via a series of foot processes known as podocytes. Almost all primary glomerular diseases occur due to autoimmunity. Immunologic injury to glomerular basement membrane activates complement system that attract inflammatory cells. Proteolytic enzymes released by inflammatory cells cause glomerular injury with or without interruption of the filtration barrier.

- **Classification of glomerulonephritis (GN) on the basis of etiology:** Glomerulonephritis is classified according to etiology, i.e. involvement of glomeruli either due to intrinsic cause (primary GN) or systemic disorders (secondary GN) and familial glomerulopathy. Primary glomerulonephritis is initiated by immunogenic mechanisms: (a) **antibody-mediated glomerular injury**, e.g. antibody directed against intrinsic glomerular antigen and *in situ* immune deposit, circulating antigen planted in glomeruli, and immune complex deposits, circulating immune complex deposits, or cytotoxic antibodies and (b) In secondary glomerulonephritis, T cell mediated glomerular injury. glomeruli may be damaged in the course of systemic diseases due to immune complex deposits, metabolic disorders, hematological and vascular disorders. Immune complex mediated glomerulonephritis includes systemic lupus erythematosus, Henoch-Schönlein purpura and infective endocarditis. Etiological classification of glomerular diseases is given in Table 22.7.

Table 22.7 Etiological classification of glomerular diseases

Etiology	Glomerular Diseases
Primary glomerular diseases	
Antiglomerular basement disease	▪ Goodpasture's syndrome (kidney and lung involvement) ▪ Antiglomerular basement disease without lung involvement
Proliferative glomerular diseases (immune-mediated mechanism)	▪ Diffuse proliferative glomerulonephritis ▪ Focal proliferative glomerulonephritis ▪ Membranoproliferative glomerulonephritis (MPGN) ▪ Crescentic glomerulonephritis, i.e. rapidly progressive glomerulonephritis (RPGN) ▪ Mesangial IgA nephropathy
Nonproliferative glomerulonephritis (immune-mediated mechanism)	▪ Membranous glomerulonephritis (MGN) ▪ Minimal change disease (MCD) ▪ Focal segmental glomerulosclerosis (FSGS)
Secondary glomerular diseases	
Immune-mediated glomerulopathy	▪ Lupus nephritis (glomeruli involvement in systemic lupus erythematosus) ▪ Glomerulopathy (glomeruli involvement in infective endocarditis) ▪ Glomerulopathy (glomeruli involvement in Henoch-Schönlein purpura)
Metabolic diseases induced glomerulopathy	▪ Diabetic glomerulopathy (diffuse or nodular glomerulosclerosis) ▪ Amyloid nephropathy ▪ Myeloma kidney (renal involvement in multiple myeloma)
Vascular and hematologic disorders induced glomerulopathy	▪ Polyarteritis nodosa (PAN) ▪ Microscopic polyarteritis ▪ Wegener's granulomatosis ▪ Idiopathic thrombocytopenic purpura (ITP) ▪ Disseminated intravascular coagulation (DIC)

Contd...

Table 22.7 Etiological classification of glomerular diseases (*Contd...*)

Etiology	Glomerular Diseases
Hereditary glomerular diseases	
Hereditary glomerulopathy	▪ Alport's disease ▪ Fabry's disease ▪ Thin membrane disease ▪ Nephrosialidosis ▪ Congenital nephrotic syndrome due to mutation of NPHS1 (nephrin molecules) gene and NPHS2 (podocin) gene ▪ Congenital membranoproliferative glomerulonephritis (complement factor-H, PPARγ) ▪ Focal segmental glomerulosclerosis (FSGS) due to gene mutation of ACTN4 (actinin-α) podocyte binding protein or calcium regulating TRPC6 (transient receptor potential cation channel-6)

- **Classification of GN on basis of clinical presentation:** Glomerulonephritis can also be classified on the basis of clinical presentation, which can range from heavy proteinuria ≥3.5 g/24 hours and anasarca (generalized edema), hypoalbuminemia, hyperlipidemia and lipiduria qualify for nephrotic syndrome. Nephritic syndrome is characterized by proteinuria ≤3.5 g/24 hours, hematuria, passage of smoky colored urine, hypertension and periorbital edema. The renal functions should be assessed by analyzing blood urea and serum creatinine, and urine.
- **Classification of GN according to appearance of glomerular tuft:** Glomerulonephritis is classified according to the appearance of glomerular tuft, i.e. proliferative or nonproliferative, and according to histologic patterns such as focal, diffuse, global, segmental, minimal change disease, membranous and membranoproliferative patterns.
- **Classification of GN according to duration of disease:** Glomerulonephritis is classified according to duration of renal disease such as acute, subacute and chronic.

Pathology Pearls: Causes of Nephritic and Nephrotic Syndrome

Nephritic Syndrome: Causes

- Poststreptococcal glomerulonephritis
- IgA nephropathy
- Anti-glomerular basement membrane (GBM disease), e.g. Goodpasture syndrome
- Lupus nephritis
- Idiopathic rapidly progressive (crescentic) glomerulonephritis
- Hepatitis C infection
- Polyarteritis nodosa (PAN)
- Henoch-Schönlein purpura
- Granulomatosis with angiitis
- Thin basement membrane disease
- Wegener granulomatosis
- Essential cryoglobulinemia
- Idiopathic immune complex proliferative glomerulonephritis
- Pauci-immune/antineutrophil cytoplasm antibody glomerulonephritis.

Nephrotic Syndrome: Causes

- Minimal change disease (MCD)
- Membranous glomerulonephritis (MGN)
- Membranoproliferative glomerulonephritis (MPGN) type 1 and type 2 (dense deposit disease)
- Focal segmental glomerulosclerosis
- Renal amyloidosis
- Diabetic nephropathy
- HIV associated nephropathy
- C1q nephropathy
- Hemolytic uremic syndrome
- Fibrillary glomerulonephritis
- Monoclonal immunoglobulin deposition disease
- Pre-eclampsia/eclampsia glomerulopathy
- Infections (malarial parasite, hepatitis B virus, *Treponema pallidum*)
- Drugs (penicillamine, heroin, gold, captopril)

PRIMARY GLOMERULAR DISEASES

Primary glomerular diseases are intrinsic to the kidney and mediated by immune complex mechanism. Primary glomerulonephritis is classified according to mechanism of glomerular injury.

In situ Immune Complex Mediated Glomerulonephritis

In situ immune complexes are seen in anti-GBM antibody-mediated glomerulonephritis. Antibodies circulating in blood attach to the native fixed antigen on the glomerular basement membrane (GBM).

- Antibodies can cross-react *in situ* with previously planted nonglomerular antigens such as DNA, IgG and bacterial products. Antibodies deposition shows a linear pattern of immunoglobulin deposition in GBM on immunofluorescence microscopy.
- Anti-GBM can also cross-react with the alveolar basement membrane resulting in simultaneous kidney/lung lesions as obtained in Goodpasture syndrome, characterized by rapidly progressive glomerulonephritis accompanied by pulmonary hemorrhage caused by antibodies to the glomerular and alveolar basement membranes.

Circulating Immune Complex Mediated Glomerulonephritis

Immune complexes formed elsewhere in the settings of systemic lupus erythematosus, HBV infection, *Plasmodium falciparum*, syphilis, and streptococcal infection, circulate in blood and get deposited in the glomerular basement membrane, which leads to complement system activation, leukocytes recruitment, coagulation system cascade activation and inflammation. Examples of immune-complex mediated glomerulonephritis are proliferative glomerulonephritis (focal or diffuse), membranous glomerulonephritis, membranoproliferative glomerulonephritis, focal segmental glomerulosclerosis (FSGS) and crescentic glomerulonephritis (rapidly progressive glomerulonephritis).

SECONDARY GLOMERULAR DISEASES

Glomeruli may be damaged in the course of systemic diseases caused by immune complex mediated, metabolic disorders, hematologic and vascular disorders. Immune complex mediated glomerulonephritis includes systemic lupus erythematosus, Henoch-Schönlein purpura and infective endocarditis.

- Glomerulopathy may be caused by metabolic disorders such as diabetes mellitus, renal amyloidosis and multiple myeloma.
- Glomerulopathy can be caused in settings of various hematologic and vascular disorders such as hypertension, polyarteritis nodosa, Wegener's granulomatosis, hemolytic uremic syndrome, idiopathic thrombocytopenic purpura and disseminated intravascular coagulation.

FAMILIAL GLOMERULAR DISEASES

Familial glomerular diseases include congenital nephrotic syndrome (Finnish type), Alport's syndrome, familial segmental glomerulosclerosis, familial nondiabetic end stage disease, familial membranoproliferative glomerulonephritis, Fabry's disease and nephrosialidosis. Genetic basis of familial glomerulopathy is given in Table 22.8.

Congenital Nephrotic Syndrome

Congenital nephrotic syndrome is an autosomal recessive disorder due to mutations in NPHS1 (encoding nephrin molecules) and NPHS2 (encoding podocin). This means that each parent must pass on a copy of the defective gene in order for the child to have the disease.

- Normally, NPHS1 (encoding nephrin molecules) and NPHS2 (encoding podocin) regulate slit pores of glomerular basement membrane (GBM).
- Newborn has congenital nephrotic syndrome at birth but manifestations appear at three months of life. There is no cure for congenital nephrotic syndrome.

Alport's Syndrome

Alport's syndrome occurs due to defects in collagen IV synthesis in glomerular basement membranes in kidney,

Table 22.8 Genetic basis of familial glomerulopathy

Gene	Chromosome Location	Encoding Protein	Glomerulopathy
NPHS-1 gene	19q13	Nephrin protein (key component of the slit diaphragm) regulates glomerular filtration rate	Congenital nephrotic syndrome (Finnish type)
NPHS-2 gene	1q	Podocin protein presents in the slit diaphragm	Steroid resistant nephrotic syndrome in children or autosomal recessive focal segmental glomerulosclerosis
α-Actin 4 protein (ACTN4)	Not designated	Podocyte binding protein regulates calcium channels in podocytes	Autosomal dominant focal segmental glomerulosclerosis
TRPC6 (transient receptor potential cation channel 6) gene	Not designated	Calcium channel proteins present on podocytes regulating calcium channels	Adult-onset focal segmental glomerulosclerosis
Apolipoprotein L1 gene (APOL1)	22	Apolipoprotein	Focal segmental glomerulosclerosis and renal failure

inner ear, eye caused by mutations in COL4A3, COL4A4 and COL4A5 genes. Patient presents with hereditary nephritis, subtle nerve deafness (55%), eye disorders (15–30%, anterior lens dislocation, posterior cataracts, corneal dystrophy). It may share genetic defects and clinical features with thin glomerular basement membrane disease.

Familial Focal Segmental Glomerulosclerosis

Recently, researchers have identified a large number of families from all over the world in which two or more family members suffer from familial focal segmental glomerulosclerosis (FSGS). This is considered familial FSGS. Several genes, mainly involved in podocyte cytoskeleton regulation, have been implicated in familial forms of primary focal segmental glomerulosclerosis. Various studies identified a homozygous missense mutation in the TTC21B gene in seven families with FSGS. TRPC6 mutation causes familial focal segmental glomerulosclerosis.

Nephrosialidosis

Nephrosialidosis occurs due to deficiency of N-acetylneuraminic acid hydrolase causing nephrosialidosis. Patient progresses to end stage renal disease. Renal biopsy demonstrates histologic features of focal segmental glomerulosclerosis.

Familial Nondiabetic End Stage Renal Disease

Familial nondiabetic end stage renal disease occurs due to second hit mutation in apolipoprotein L1 (APOL1) gene in 70% of first-degree relatives of African American patients. Patients develop overt proteinuria. Serum cystatin levels are high reflecting decreased glomerular filtration rate.

Familial Membranoproliferative Glomerulonephritis

Genetic factors have been implicated in the pathogenesis of certain cases of familial membranoproliferative glomerulonephritis (MPGN).

- Genetic defects in the regulation of complement pathways appear to be at the root of many hereditary forms of MPGN. Peroxisome proliferator-activated receptor gamma (PPARγ or PPARG) gene is located on the 8p11–12 locus. Mutation in PPARγ gene causes familial membranoproliferative glomerulonephritis.
- Familial membranoproliferative glomerulonephritis (MPGN) describes a histologic lesion, characterized by mesangial hypercellularity and thickening of the glomerular capillary wall on histologic examination. It most often causes progressive chronic kidney disease, although the clinical expression is variable.

Familial Nephropathy in Fabry Disease

Fabry's disease is lysosomal storage X-linked disease due to deficiency of α-galactosidase deficiency resulting in familial glomerulopathy. Fabry's syndrome is characterized by painful extremities, red hyperkeratotic papules on skin, proteinuria, and renal failure.

CLASSIFICATION OF GLOMERULONEPHRITIS BASED ON INTEGRITY OF GLOMERULAR BASEMENT MEMBRANE AND CELLULARITY OF GLOMERULI

Glomerulonephritis is classified based on integrity of glomerular basement membrane and cellularity of glomeruli. Glomerulonephritis includes thin basement membrane disease, non-proliferative glomerulonephritis and proliferative glomerulonephritis. Classification of glomerulonephritis based on integrity of glomerular basement membrane and cellularity of glomeruli is given in Table 22.9.

Pathology Pearls: Endothelial, Epithelial and Mesangial Cell-induced Glomerulonephritis

Endothelial Cell Injury-induced Glomerulonephritis

- Postinfectious glomerulonephritis
- Mesangiocapillary glomerulonephritis
- Lupus nephritis class III and class IV
- Antiglomerular basement membrane disease
- Vasculitis and cryoglobulinemia
- Hemolytic uremic syndrome

Epithelial Cell Injury-induced Glomerulonephritis

- Membranous glomerulonephritis
- Minimal change disease
- Focal segmental (crescentic) glomerulosclerosis
- Lupus nephritis class V
- Diabetic nephropathy

Mesangial Cell-induced Glomerulonephritis

- IgA nephropathy
- IgM nephropathy
- Mesangioproliferative glomerulonephritis
- Lupus nephritis class II
- Diabetic nephropathy

THIN BASEMENT MEMBRANE DISEASE

Thin basement membrane disease (TBMD) is an autosomal dominant disorder that mainly affects glomeruli (tiny tufts of capillaries) in the kidney. Electron microscopy demonstrates thin basement membrane disease of glomeruli.

Table 22.9 Classification of glomerulonephritis based on integrity of glomerular basement membrane and cellularity of glomeruli

Glomerulonephritis	Features
Thin membrane disease	
Thin basement membrane disease	▪ Persistent hematuria ▪ Mild proteinuria ▪ Prognosis is excellent
Nonproliferative glomerulonephritis	
Minimal change disease	Effacement of visceral epithelial foot processes
Glomerulosclerosis	Obliteration of glomerular capillaries by matrix (subdivided into global and segmental sclerosis)
Focal segmental glomerulosclerosis	Thickening and wrinkling of glomerular basement membrane, mild increase in mesangial cells and lack of immune complex deposits
Nodular sclerosis	A nodular increase in mesangial matrix with patent glomerular capillary loops
Mesangial sclerosis	An increase in mesangial matrix with parent glomerular capillary loops
Membranous glomerulonephritis	Diffuse thickening of the GBM without any proliferative change
Proliferative glomerulonephritis	
Endocapillary proliferation	Increased cellularity of glomerular capillaries, due to marginating neutrophils or monocytes, or proliferation of endothelial cells of glomeruli
Extracapillary proliferation (cellular crescent)	Increased cells within Bowman's space, usually due to parietal epithelial cell proliferation extending into Bowman's capsule and infiltrating macrophages
Mesangial proliferation	Increased mesangial cellularity (>3 mesangial cells/mesangial area) with patent glomerular capillary loops
Exudative/necrotizing glomerulonephritis	Glomeruli showing infiltration by leukocytes and necrosis
Membranoproliferative glomerulonephritis	Glomerular basement membrane (GBM) thickening with proliferation of endothelial and mesangial cells of glomeruli (hypercellular glomeruli)
Crescentic glomerulonephritis (rapidly progressive glomerulonephritis)	>80% glomeruli involved with crescent formation in Bowman's capsular space around glomeruli due to proliferation of the parietal cells

- Mutations in COL4A3 and COL4A4 affecting type IV collagen of glomerular basement membrane have been demonstrated in some families.
- Patient presents with persistent hematuria and mild proteinuria but overall prognosis is excellent. Patient may develop hypertension and swelling over body.
- Renal functions are impaired. Serum creatinine is raised. Urine analysis shows proteins and red blood cells. Thin basement membrane disease is diagnosed on renal biopsy examined under electron microscope.

NONPROLIFERATIVE GLOMERULONEPHRITIS

Injured glomeruli lack increased glomerular cellularity in nonproliferative glomerulonephritis. Examples of nonproliferative glomerulonephritis are minimal change disease, focal segmental glomerulosclerosis and membranous glomerulonephritis, which are important causes of nephrotic syndrome.

PROLIFERATIVE GLOMERULONEPHRITIS

The injured glomeruli exhibit increased glomerular cellularity in proliferative glomerulonephritis, which occurs either due to proliferation of glomerular cells (endothelial cells, mesangial cells) and/or influx of inflammatory cells (neutrophils, macrophages) in glomeruli. Majority of cases of glomerulopathy over 70% belong to the group of proliferative glomerulonephritis.

- Proliferative glomerulonephritis can be categorized based on histologic appearance, e.g. diffuse proliferative glomerulonephritis, focal proliferative glomerulonephritis, exudative glomerulonephritis, membranoproliferative glomerulonephritis, crescentic glomerulonephritis, i.e. rapidly progressive glomerulonephritis (RPGN), Goodpasture syndrome and mesangial IgA nephropathy. However, it is worth mentioning that categorization is not diagnostic but rather describing a pattern glomerulopathy caused by glomerular insults.
- Proliferation of glomerular cells may be either endocapillary or extracapillary regions resulting in proliferative glomerulonephritis. More than 100 nuclei are demonstrated in affected glomeruli in diffuse or focal glomerulonephritis.
- Recent study establishes that mesangial cell proliferation in glomerulonephritis is mediated by

complement system and platelets. Mesangial cell proliferation is accompanied by increased expression genes encoding platelet-derived growth factor (PDGF) and PDGF receptor proteins resulting in an autocrine mechanism of mesangial cell proliferation.

Diffuse Proliferative Glomerulonephritis

Diffuse proliferative glomerulonephritis occurs in setting of poststreptococcal glomerulonephritis in majority of cases. Almost all the glomeruli (>50–100%) of both kidneys show pathologic change. There is diffuse global pattern of acute inflammation of glomeruli due to deposition of immune complex in glomeruli. Diffuse proliferative glomerulonephritis occurs in setting of poststreptococcal glomerulonephritis in majority of cases. Glomeruli become hypercellular due to proliferation of glomerular cells and leukocytic infiltration, which is termed as exudative/necrotizing glomerulonephritis.

Focal Segmental Glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) refers to pathologic change in some glomeruli (<50% of glomeruli) of both kidneys, whereas others are normal. FSGS occurs in the settings of mesangial IgA nephropathy. Goodpasture syndrome, Henoch-Schönlein purpura, infective endocarditis, vasculitis and connective tissue disorders.

Global Glomerulosclerosis

Global glomerulosclerosis is defined as glomerular impairment involving >50% of any one glomerulus manifested as scarring lesion or hyaline deposition in the glomeruli during advancing age and lupus nephritis in systemic lupus erythematosus (SLE) cases.

Segmental Glomerulosclerosis

Segmental glomerulosclerosis refers to an increase in extracellular matrix (hyalinization) within the mesangial region, involving only a segment of the glomerulus, which occurs in diabetes mellitus.

Rapidly Progressive Glomerulonephritis

Rapidly progressive glomerulonephritis (RPGN) is also known as crescentic glomerulonephritis. RPGN occurs in the settings of poststreptococcal glomerulonephritis (small percentage of cases), Goodpasture's syndrome and other forms of glomerular disease. Rapidly progressive glomerulonephritis is characterized by formation of cellular crescent-shaped masses within Bowman space. Severe injury to the glomeruli results in leakage of fibrinogen and inflammatory mediators into Bowman space, that stimulate parietal epithelial cells of the Bowman capsule and thus resulting in formation of '**crescents in the glomeruli**'.

Membranoproliferative Glomerulonephritis

Membranoproliferative glomerulonephritis (MPGN) is also known as mesangiocapillary glomerulonephritis, which exhibits diffuse global pattern of glomerulonephritis with features of both proliferation of glomerular cells and thickening of glomerular basement membrane. MPGN is categorized into primary and secondary types. Primary MPGN type 1 accounts for 90% of cases and MPGN type 2 accounts for 10% of cases. Secondary MPGN is seen in the setting of systemic lupus erythematosus, infective endocarditis, malarial parasite and infected ventricular cerebrospinal shunts.

GLOMERULONEPHRITIS: MOLECULAR PATHOGENESIS

Central to the pathogenesis of glomerulonephritis is most often immune-mediated glomerular injury. Glomeruli possess three properties that make then vulnerable to immune-mediated injury: (a) glomeruli filter and trap immune complexes, (b) glomeruli contain immunologically competent cells capable of processing antigen, and (c) glomeruli have constituents like glomerular basement membrane (GBM) and mesangial cells, which can become targets to antibodies. Glomerulonephritis occurs by three mechanisms: *in situ* complex-mediated, immune complex-mediated and ANCA-mediated mechanisms.

- Soluble factors play important role in the pathogenesis of glomerulonephritis. Examples of soluble factors are cytokines, growth factors, vasoactive chemical mediators, reactive oxygen species (ROS), complement system, specific proteases, proteins in the coagulation system cascade. The soluble factors promote recruitment and activation of inflammatory cells, activation of resident glomerular cells, which directly induce glomerular injury and stimulate production of matrix proteins, which forms the basis of glomerular scarring.
- Schematic representation of pathogenesis of glomerulonephritis is shown in Fig. 22.13. Schematic representation of pathogenesis of inflammatory glomerulonephritis is shown in Fig. 22.14. Exogenous and endogenous antigens inducing glomerulonephritis is given in Table 22.10. Pathogenesis of glomerulonephritis is given in Table 22.11.

IN SITU IMMUNE COMPLEX-MEDIATED GLOMERULONEPHRITIS

In situ immune complex deposition glomerular diseases encompass a diverse group of inflammatory conditions characterized by antigen–antibody deposition and activation of complement system in the settings

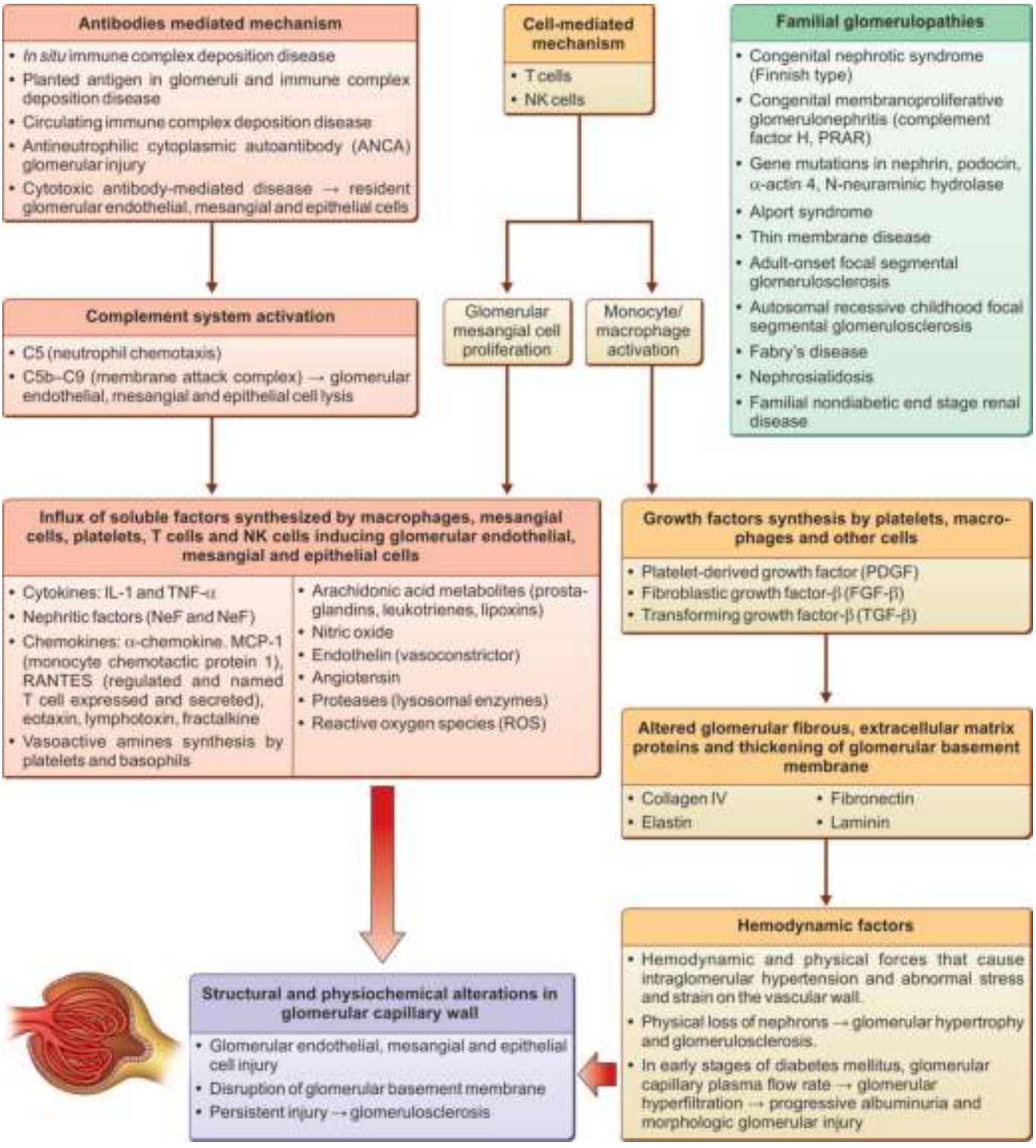


Fig. 22.13: Schematic representation of pathogenesis of glomerulonephritis

of glomerulonephritis, rheumatoid arthritis, scleroderma and Sjögren's syndrome.

- *In situ* complex-mediated glomerulonephritis is characterized by formation of antibodies against fixed intrinsic or planted antigen. *In situ* immune complex deposit formation may occur in the subendothelial, subepithelial and mesangial regions of the glomeruli.
- Type 2 hypersensitivity reaction plays an important role in the pathogenesis of *in situ* immune complex-mediated glomerulonephritis.

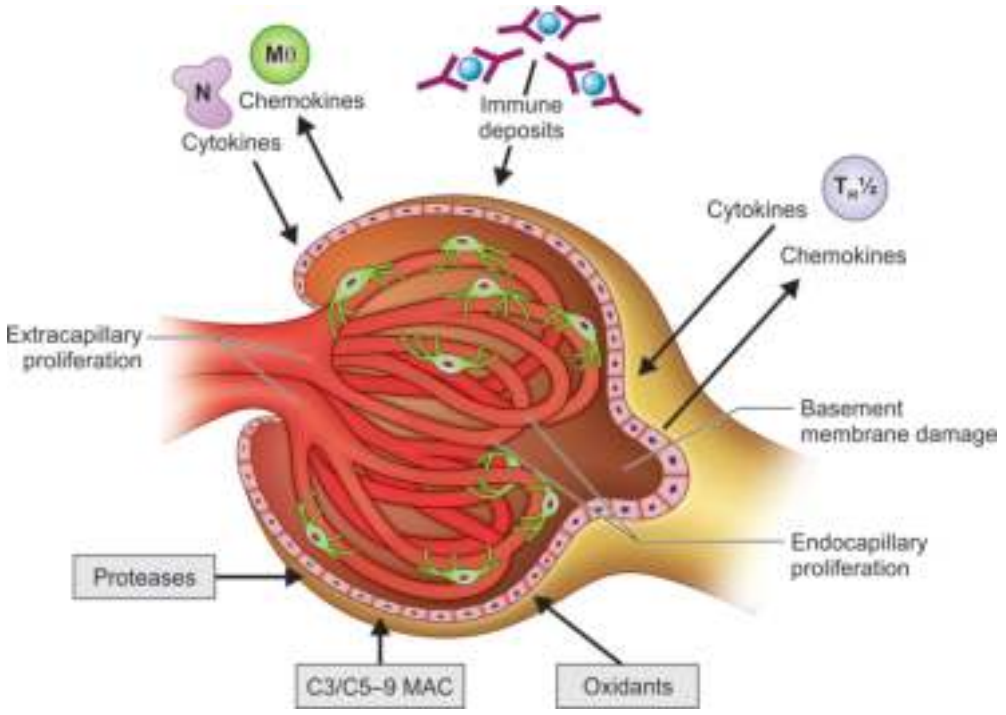


Fig. 22.14: Schematic representation of pathogenesis of inflammatory glomerulonephritis. Inflammatory is mediated by circulating inflammatory cells (neutrophils, macrophages, platelets) and/or resident glomerular mesangial cells. Neutrophil mediated injury involves local release of glomerular basement membrane (GBM) proteases or GBM halogenation induced by interaction of neutrophil-derived myeloperoxidase with H_2O_2 and a halide. Neutrophil induced glomerular injury is enhanced by platelet. Mesangial cell proliferation in glomerulitis is mediated by complement and platelets. Mesangial cell proliferation is accompanied by increased expression of platelet-derived growth factor (PDGF) receptor proteins and genes for these proteins resulting in an autocrine mechanism of cell proliferation.

Table 22.10 Exogenous and endogenous antigens inducing glomerulonephritis

Exogenous Antigens
Bacteria
Viruses (HBV, HCV)
Parasites (malarial parasite—falciparum)
Tumor antigens (colon carcinoma, lung carcinoma and lymphomas)
Drugs (heroin, gold, penicillamine)
Autoimmune disorder (systemic lupus erythematosus)
Endogenous Antigens
Goodpasture's antigen in kidney and lung
Heymann antigen: megalin antigen complex GP-330 on basal surface of visceral epithelial cells and proximal tubules

Contd...

Table 22.11 Pathogenesis of glomerulonephritis

Genetic Factors Linked to Glomerulopathy	
▪ Congenital nephrotic syndrome ▪ Gene mutations in nephrin, podocin, α-actin 4, N-neuraminic hydrolase	▪ Alport syndrome ▪ Thin basement membrane disease ▪ Familial segmental glomerulosclerosis (FSGS)
Antibody-mediated Glomerular Injury	
▪ Antibody directed against intrinsic glomerular antigen and *in situ* immune complex deposition in the glomeruli ▪ Circulated antigen planted in glomeruli and immune complex deposition in the glomeruli ▪ Circulating antigen–antibody immune complex deposition in the glomeruli	▪ Antineutrophilic cytoplasmic autoantibody-mediated glomerulonephritis with paucity of immunoglobulin staining in <2% of untreated cases ▪ Cytotoxic antibody-mediated injury to glomerular endothelial, mesangial and epithelial cells
Complement System-mediated Glomerular Injury	
▪ C5a (neutrophil chemotaxis) ▪ C5b–C9 (membrane attack complex) → glomerular cells lysis	▪ Examples: lupus nephritis, membranoproliferative glomerulonephritis type 1 and 2, and postinfectious glomerulonephritis

Contd...

Table 22.11 Pathogenesis of glomerulonephritis (*Contd...*)

Antineutrophilic Cytoplasmic Autoantibody (ANCA) Glomerulonephritis

- Cell-mediated glomerular injury
  - CD8+ cytotoxic T cells induced glomerular mesangial cell injury
  - NK cells induced glomerular injury

Influx of Circulating Inflammatory Cells

- Neutrophils
- Monocytes/macrophages
- T cells/NK cells
- Platelets
- Inflammatory cells release proteolytic lysosomal enzymes and synthesis of chemical mediators to induce glomerulopathy

Coagulation System

Glomerular endothelial injury activates clotting factors leading to formation of microthrombi and hence reduction in glomerular filtration rate (GFR)

Soluble Factors Synthesis by PMN Cells, Macrophages, Mesangial Cells, Platelets, T Cells and NK Cells

- Cytokine (IL-1 and TNF-α) and chemokines participate in recruitment of inflammatory cells at site of glomerular injury. Chemokines include α-chemokine. MCP-1 (monocyte chemotactic protein 1), RANTES (regulated and named T cell expressed and secreted), eotaxin, lymphotoxin, fractalkine
- Nephritic factors (NeF and NeF) are IgG autoantibodies are demonstrated in C3 glomerulopathy, membranoproliferative glomerulonephritis and systemic lupus erythematosus
- Proteolytic lysosomal enzymes $\rightarrow$ glomerular injury
- Vasoactive amines synthesis by platelets and basophils $\rightarrow$ increased glomerular permeability
- Eicosanoids, nitric oxide (vasodilator), endothelin synthesized by endothelial cells (vasoconstrictor), and angiotensin II regulate blood flow through the inflamed glomeruli. PGD2 promotes platelets aggregation and increases glomerular permeability
- Reactive oxygen species (ROS) act both as a signaling molecule and mediator of inflammation

Activation of Resident Glomerular Cells

- Glomerular endothelial cells
- Glomerular mesangial cells
- Glomerular epithelial cells lining Bowman space

Growth Factors Synthesis by Platelets, Macrophages and other Cells

- Platelet-derived growth factor (PDGF) $\rightarrow$ proliferation of mesangial cells
- Fibroblastic growth factor-β (FGF-β) $\rightarrow$ ECM deposition and hyalinization of glomeruli
- Transforming growth factor-β (TGF-β) $\rightarrow$ ECM deposition and hyalinization of glomeruli

Hemodynamic Factors

- Hemodynamic and physical forces that cause intraglomerular hypertension and abnormal stress and strain on the vascular wall
- Physical loss of nephrons $\rightarrow$ glomerular hypertrophy and glomerulosclerosis
- In early stages of diabetes mellitus, glomerular capillary plasma flow rate $\rightarrow$ glomerular hyperfiltration $\rightarrow$ progressive albuminuria and morphologic glomerular injury

Altered Glomerular Extracellular Matrix Proteins

- Collagen IV deposition
- Elastin
- Fibronectin
- Laminin

Circulating Antibodies against Fixed Intrinsic Normal Glomerular Tissue Antigen

Circulating antibodies are directed against fixed intrinsic normal glomerular antigens in NC1 domain of collagen type IV with linear pattern of immune deposition on immunofluorescence microscopy, and podocyte. Heymann antigen or mesangial antigen with granular pattern of immune deposition is observed on immunofluorescence microscopy.

- Antibodies (IgG) may cross-react with non-collagenous domain of type IV collagen fibers of glomerular basement membrane (GBM) COL4-A3 antigen (Goodpasture antigen) and alveolar basement membrane leading to Goodpasture syndrome.
- Over 90% of patients with anti-GBM glomerulonephritis develop Goodpasture syndrome, which is characterized by rapidly progressive (crescentic) glomerulonephritis with interstitial fibrosis, tubular atrophy and pulmonary hemorrhage. Patient develops rapidly progressive renal failure, hemoptysis and nephritic syndrome.
- Glomeruli become hypercellular with crescents in Bowman's space and influx of macrophages. Antibodies directed against the glomerular basement membrane cause rupture of GBM and extravasation

of red blood cells and inflammatory cells into the urinary space (i.e. the space between Bowman's capsule and the glomerular capillary tufts).

- Fibrinogen stimulates proliferation of parietal cells of Bowman's capsule resulting in crescents formation (crescentic glomerulonephritis). Approximately 80% of cases show presence of 'crescents' obliterating the Bowman's space. Crescentic glomerulonephritis can also occur in the settings of Wegener granulomatosis or polyarteritis nodosa.
- Renal biopsy should be considered, which has a better yield. Immunofluorescence microscopy shows diffuse linear deposition of IgG-1 and complement (C3) and fibrin along the glomerular basement membrane in >90% of cases. Serologic testing for ELISA for circulating anti-GBM antibodies should be done immediately on suspicion of the glomerulopathy. Schematic representation of pathogenesis of Goodpasture syndrome is shown in Fig. 22.15.

Nonglomerular Antigen Planted in Glomeruli and Immune Complex Deposition Glomerular Disease

Nonglomerular antigens from bacterial products, viral products (HBV, HCV), DNA, drugs (penicillamine, gold or heroin), malignant tumors of colon, lung, and *Plasmodium falciparum* are deposited in glomerular basement membrane (GBM) from the blood circulation and then circulating antibody and antigen forms immune complex, which gets deposited in the glomeruli and giving granular pattern of immune complex deposition on subepithelial region of glomeruli on immunofluorescence microscopy.

CIRCULATING IMMUNE COMPLEX-MEDIATED GLOMERULONEPHRITIS

Immune complexes formed elsewhere in the settings of systemic lupus erythematosus, HBV and HCV infection, *Plasmodium falciparum*, syphilis, and streptococcal infection, circulate in blood and get deposited in the GBM, which leads to complement activation, leukocytes recruitment, coagulation system cascade and glomerular inflammation.

- Short-lived antigen–antibody complexes are degraded by polymorphonuclear cells, monocytes, macrophages, mesangial cells and endogenous proteases. Repeated cycle of formation of antigen–antibody complexes in circulation, are trapped in the glomerular components and causing glomerular injury.

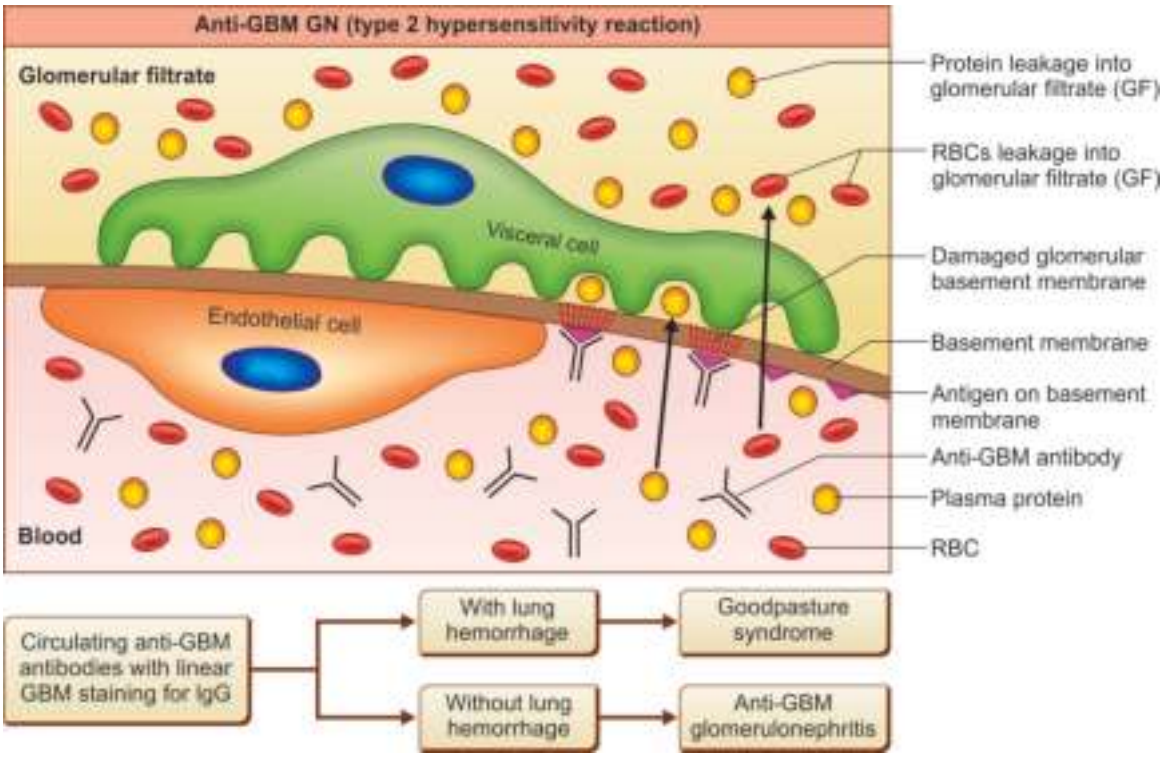


Fig. 22.15: Schematic representation of pathogenesis of Goodpasture syndrome. Goodpasture syndrome is also referred to as anti-glomerular basement membrane (anti-GBM)-mediated glomerulonephritis. Anti-glomerular basement membrane (anti-GBM) disease is a small vessel vasculitis in which circulating antibodies are directed against an antigen intrinsic to the glomerular basement membrane (GBM) and alveolar basement membrane resulting in complement activation and tissue injury. Patient develops rapidly progressive glomerulonephritis involving bilateral kidneys and/or alveolar hemorrhage. Renal biopsy should be considered, which has a better yield. Immunofluorescence shows the linear deposition of IgG-1 and complement C3 along the basement membrane. Serologic testing for enzyme-linked immunosorbent assay (ELISA) for circulating anti-GBM antibodies should be done immediately on suspicion of the disease.

- Factors influencing antigen trapped in glomeruli depend on size, electrical charge, solubility, molecular configuration and carbohydrate content of antigens.
- Antigens can be exogenous (*Plasmodium falciparum*, HBV, HCV, drugs, streptococci) or endogenous (DNA, nuclear proteins, IgA).
- Since the preformed immune complexes represent large protein aggregates that cannot pass through the GBM, they are trapped in glomeruli. Type III hypersensitivity reaction plays an important role in the immune complex-mediated glomerulonephritis.
- Immune complexes can be deposited in subendothelial region, intramembranous within glomerular basement membrane, subepithelial (between epithelial cells and GBM) and mesangial region (within).
- Examples of immune complex-mediated glomerulonephritis are given in Table 22.12. Schematic representation of immune complex-mediated glomerulonephritis is shown in Fig. 22.16.

Table 22.12 Examples of immune complex-mediated glomerulonephritis

▪ IgA nephropathy	▪ Polyarteritis nodosa
▪ Henoch-Schönlein purpura	▪ Bacterial endocarditis
▪ Lupus nephritis	▪ *Mycobacterium leprae*
▪ Poststreptococcal glomerulonephritis	▪ Hepatitis B and hepatitis C infection
▪ Membranous glomerulonephritis	▪ Malarial parasite
▪ Membranoproliferative glomerulonephritis	

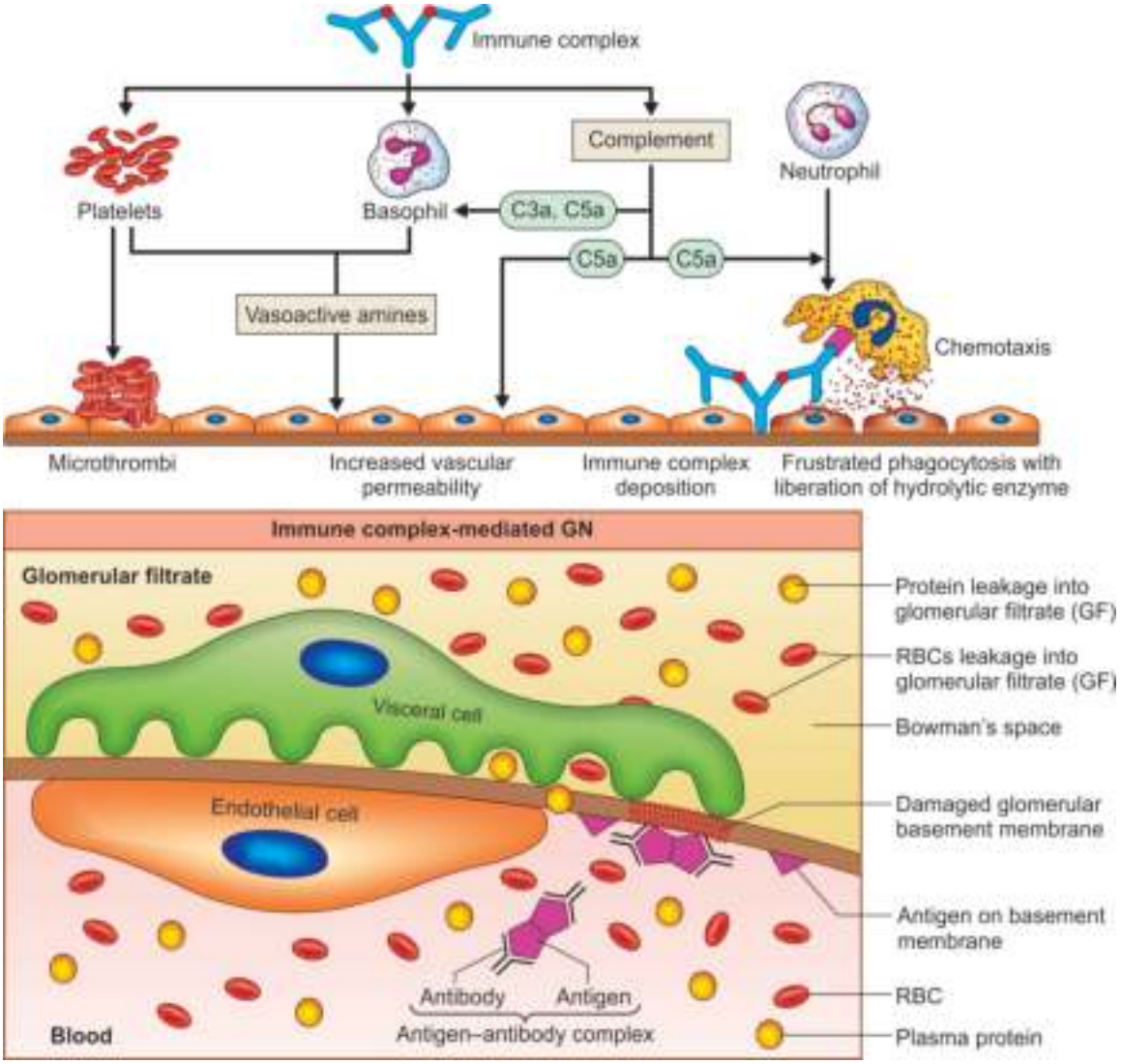


Fig. 22.16: Schematic representation of Immune complex-mediated glomerulonephritis. Immune complex-mediated proliferative glomerulonephritis is caused by the deposition of immune complexes in the glomeruli. Immune complexes activate complement system and cause tissue injury. Immune complex-mediated glomerulonephritis is characterized by proteinuria, red blood cell casts and impaired l renal functions with elevated serum creatinine and blood urea nitrogen (BUN). Renal biopsy shows granular deposits of immunoglobulin visible by immunofluorescence microscopy and electron-dense deposits visible by electron microscopy.

Pathology Pearls: Immune Complex-mediated Glomerulonephritis

Pathophysiology

- Immune complexes activate complement system. C3a and C5a increase glomerular permeability and C5a is chemotactic for neutrophils.
- Neutrophils phagocytose immune complexes. Frustrated phagocytosis by neutrophils cause to liberation of hydrolytic enzymes leading to glomerular damage.
- Platelets, basophils, complement and inflammatory cells play an important role in the pathogenesis of immune complex-mediated glomerulonephritis.
- Platelets form microthrombi resulting in decreased glomerular filtration rate. Basophils and platelets also release vasoactive amines leading to increased glomerular permeability.
- Depending on extent of glomeruli injury, patient develops clinical manifestations such as proteinuria, hematuria and uremia.
- DNA and anti-DNA complexes are formed in systemic lupus erythematosus by forming granular deposits along the glomerular basement membrane in systemic lupus erythematosus (SLE).

Location of Immune Complex Deposits in Glomeruli

- Highly anionic macromolecules are excluded by glomerular basement membrane hence trapped in subendothelial region of kidney.
- Highly cationic immunogens tend to cross subepithelial region and bind to anionic sites, DNA nucleosome and other nuclear proteins.
- Neutral charged molecules are trapped in mesangial region. Large circulating complexes are not usually nephritogenic, which are cleared by reticuloendothelial system.

CYTOTOXIC ANTIBODY-MEDIATED GLOMERULONEPHRITIS

Cytotoxic antibody-mediated glomerular injury is initiated by antigen–antibody complex formation, within the glomerulus. CD4+ helper T cells differentiate into Th2 cells and synthesize IL-4, IL-5, IL-10 and IL-13 cytokines, which activate B cells and regulate humoral immune response.

- Cytotoxic antibody-mediated glomerulonephritis is characterized by injury to resident glomerular endothelial cells, mesangial cells and epithelial cells (podocytes).
- Endothelial cells maintain glomerular perfusion, prevent leukocyte adhesion and inhibit platelet aggregation. Injured endothelial cells induce vasoconstriction, leukocytes adhesion, influx of leukocytes inducing focal/diffuse glomerulonephritis and intravascular microthrombi in glomeruli, thrombotic microangiopathies and reduction in glomerular filtration rate.
- Mesangial cells regulate glomerular filtration rate. Antibodies directed against mesangial cells result in mesangial cell proliferation or mesangiolysis. Membranoproliferative glomerulonephritis is mesangial related disease.
- Visceral epithelial cells (podocytes) prevent plasma protein filtration. Antibodies directed against visceral epithelial cells result in proteinuria. Minimal change disease and focal segmental glomerulosclerosis are examples of visceral epithelial cells related diseases.

ANTINEUTROPHILIC CYTOPLASMIC AUTOANTIBODY-MEDIATED GLOMERULONEPHRITIS

Antineutrophilic cytoplasmic autoantibody (ANCA) induces small vessel vasculitis involving capillaries, venules, and arterioles. It is characterized by complete absence or paucity of immunoglobulin staining (<2%) distinct from anti-GBM disease. Approximately 85% of active untreated cases show ANCA in the circulation.

Pathophysiology

Resting neutrophils sequestrate ANCA antigens within the primary granules. Microbes, cytokines, complement system and other inflammatory products activate neutrophils. Complement system C5a initiates priming, activation and chemotaxis of neutrophils at the site of injury.

- Activated neutrophils induce glomerular endothelial injury by release of reactive oxygen species (ROS), hydrolytic enzymes and damaged associated molecular patterns (DAMPs) within endothelial cells. Membrane attack complex (C5b, C6, C7, C8, C9) results in glomerular endothelial cell lysis.
- ANCA antigen is expressed on surface of neutrophil and released in extracellular space. Vicious cycle between neutrophil activation occurs by ANCA, NETosis, ANCA antigens exposure and further ANCA production.
- Neutrophil extracellular traps (NETs), known as NETosis, are triggered by microorganisms and endogenous stimuli such as damaged associated molecular patterns (DAMPs) and immune complexes. NETosis is unique form of cell death that is characterized by the release of decondensed chromatin and granular contents into extracellular space.
- The major clinicopathologic expression of pauci-immune and ANCA-associated glomerulonephritis and vasculitis include crescentic glomerulonephritis, ANCA glomerulonephritis without pulmonary hemorrhage, microscopic angiitis without granulomas and bronchial asthma, Wegener's granulomatosis with granulomas and polyangiitis; and without bronchial asthma and Churg-Strauss syndrome (eosinophilic granulomatosis with polyangiitis).

- Histologic examination of renal biopsy in ANCA-mediated GN shows fibrinoid necrosis and crescent formation in glomeruli. Glomerulonephritis may be accompanied by necrotizing arteritis (interlobular and vasa recta) in <10% of cases. Pathogenetic role of antineutrophilic cytoplasmic antibody-mediated glomerulonephritis and vasculitis is shown in Fig. 22.17.

T CELL-/NK CELL-MEDIATED GLOMERULAR INJURY

T cell is sensitized to endogenous or exogenous antigen present in the glomeruli recruit macrophages resulting in nonimmune complex deposition glomerulonephritis. Disease occurs through delayed type of hypersensitivity reaction initiated by CD4+ helper T cells and activating monocytes/macrophages. These cells synthesize powerful inflammatory cytokines such as IL-2, IL-12, IFN-γ and TNF-α, which induce glomerular injury. CD8+ cytotoxic T cells act by their cytotoxic ability. Examples of T cell-mediated glomerular injury include minimal change disease and focal segmental glomerulosclerosis.

COMPLEMENT SYSTEM-MEDIATED GLOMERULONEPHRITIS

Complement proteins provide an important line of defense against bacteria, fungi, and viruses. Complement system enhances the ability of antibodies, phagocytic cells to clear microbes and damaged cells, promote inflammation and attack the pathogen's cell membrane.

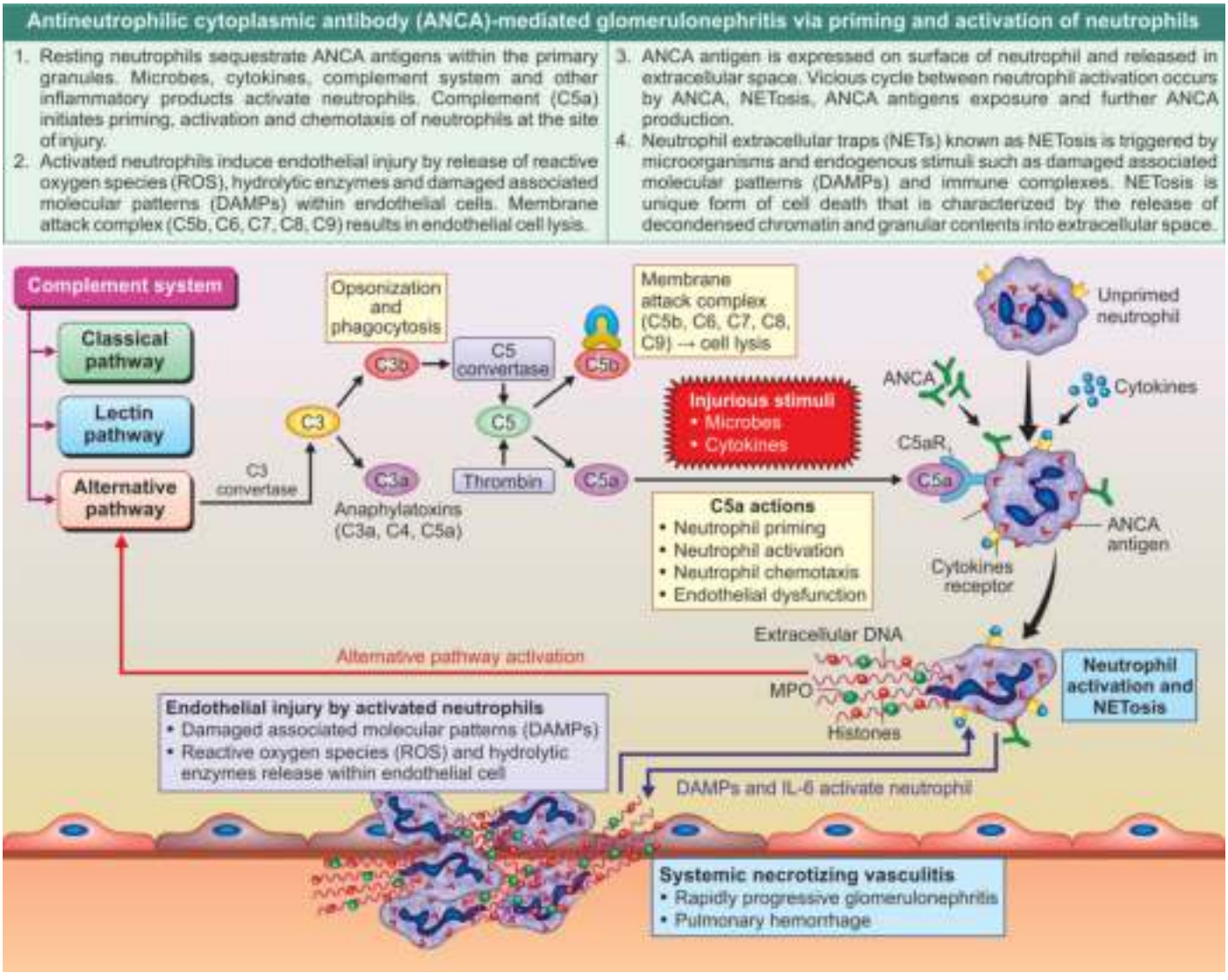


Fig. 22.17: Pathogenetic role of antineutrophilic cytoplasmic antibody-mediated glomerulonephritis and vasculitis. Antineutrophilic cytoplasmic autoantibody (ANCA) glomerulonephritis is an autoimmune disease that affects capillaries of the glomeruli due to activation of neutrophils and subsequently release inflammatory cytokines, reactive oxygen species and lytic enzymes. In addition, this excessive activation of neutrophils by ANCAs induces formation of neutrophil extracellular traps (NETs).

Complement system involves three pathways, namely classical pathway (activated by antigen–antibody, i.e. complement fixing IgG and IgM complexes, bacterial products, viruses), alternative pathway (spontaneous activation by microorganisms and IgA), and mannose-binding lectin pathway. All three pathways of complement system yield C3 convertase. Three pathways of complement system are shown in Fig. 22.18. Biological effects of complement system are given in Table 22.13.

Classical Pathway of Complement System

The classical pathway of complement system involves C1, C2 and C4, which is triggered by immune complexes (specific immune response). When antibody (IgG and IgM) binds antigen, a subtle conformational change occurs that renders the antibody molecule capable of interacting with C, which itself has subcomponents C1q, C1r and C1s. The classical pathway forms C3 convertase, C4b2a, which cleaves C3 into two fragments: (a) larger fragment C3b can covalently bind to the surface of microbial pathogens and opsonize them, and (b) small fragment C3a activates mast cells causing the release of vasoactive chemical mediators such as histamine, which increases glomerular permeability.

Alternative Pathway of Complement System

The alternative pathway of complement system is a component of the innate immune system, a natural

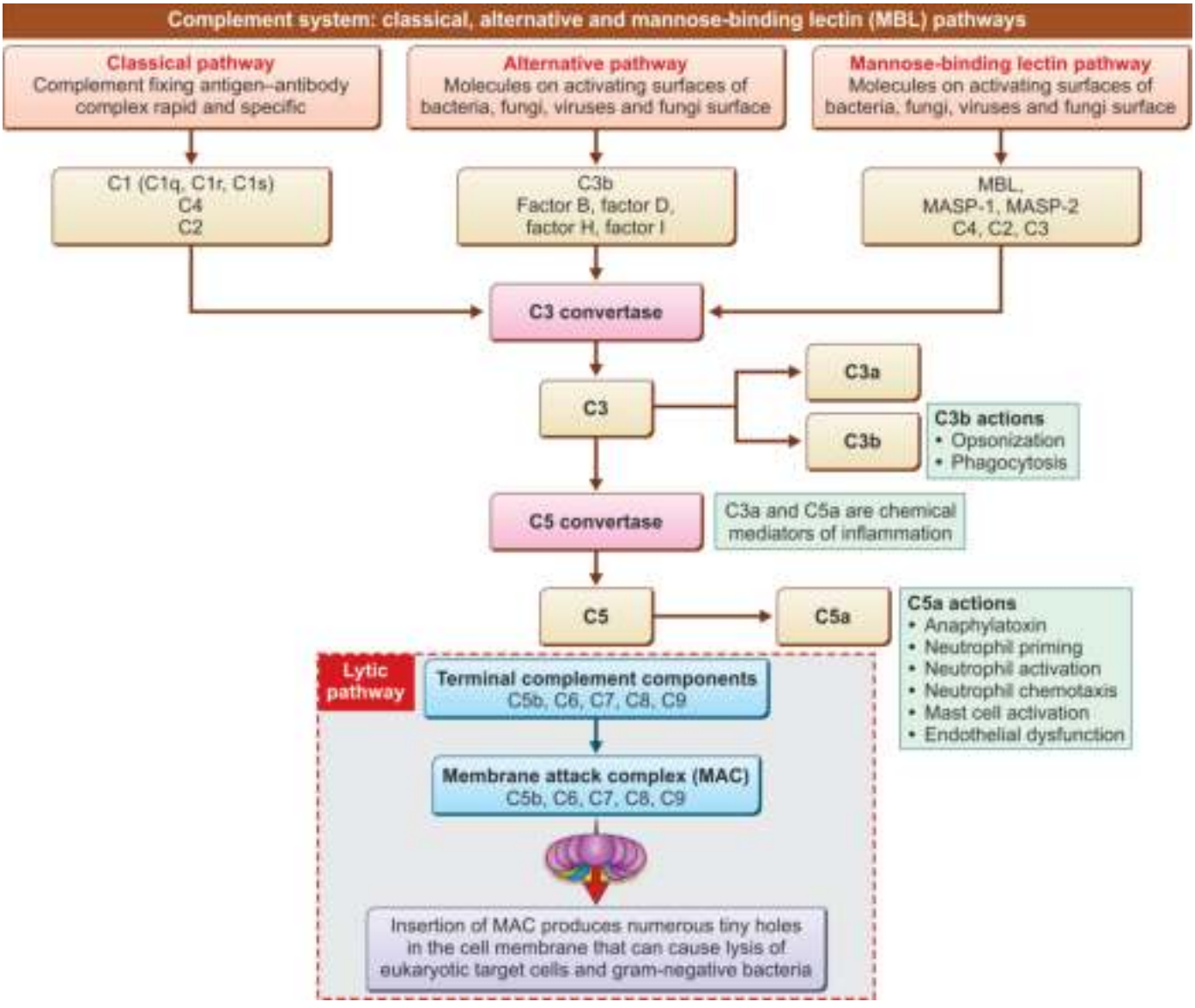


Fig. 22.18: Three pathways of complement system. Complement system involves classical, alternative and mannose-binding lectin pathways. Common aim of complement system pathways is to yield C3 convertase, which facilitates the cleavage of C3, which in turn cleaves C5 to yield C5b. C5b hence forms a complex with C6, C7, C8 and C9 to form C5b–C9, also known as the membrane attack complex (MAC) that leads to lysis of pathogen plasma membrane and target cells.

Table 22.13 Biological effects of complement system

C3a Complement Actions	
▪ Increased capillary permeability ▪ Opsonization of bacteria and chemotaxis and phagocytosis by leukocytes	▪ Contraction of smooth muscle ▪ Mast cell degranulation
C5a Complement Actions	
▪ Increased capillary permeability ▪ Chemotaxis of neutrophils	▪ Phagocytosis of bacteria by leukocytes ▪ Mast cell degranulation
C3a and C5a Complement Actions	
▪ Increased capillary permeability ▪ Contraction of smooth muscle	▪ Mast cell degranulation
C5b to C9 (Membrane Attack Complex or MAC) Complement Actions	
MAC produces hundreds of tiny holes in the bilipid layer cell membrane and results in pathogens or target cell lysis.	

defense system against infections. It is one of the three complement pathways that opsonize and kill pathogens.

- The alternative pathway of complement system is activated by IgA, bacteria, viruses, fungi, parasites, cobra venom and polysaccharide. Initiation of alternative pathway is not dependent on the presence of immune complexes. The alternative pathway involves various factors, B, D, H & I, which interact with each other, and with C3b, to form C3 convertase. C3 convertase facilitates the cleavage of C3 into C3a and C3b. Now C3b binds to C5 convertase, which in turn cleaves C5 to C5a/C5b.
- Both C3a and C5a are potent mediators of inflammation, which act by increasing glomerular permeability, contraction of smooth muscle and mast cell degranulation.
- Complement components C3a, C5a and C5, 6, 7 increase capillary permeability, attract polymorphonuclear cells and later macrophages, which cause glomerular damage by releasing proteolytic lysosomal enzymes, and by generating reactive oxygen species (ROS) and arachidonic acid metabolites. Terminal complement components C5b–C9, membrane attack complex (MAC) induces glomerular basement membrane (GBM) injury.

Mannose-binding Lectin (MBL) Pathway

The mannose-binding lectin (MBL) pathway of complement system is activated following the recognition of mannose residues and binding of pathogen-associated molecular patterns (PAMPs) on the surface of pathogen. This in turn activates MBL associated serine proteases, MASP1 and MASP2, which activate C4 and C2 to form the C3 convertase, and C3b2a.

Membrane Attack Complex Lytic Pathway

Membrane attack complex lytic pathway is initiated by the splitting of complement C5 to C5a and C5b. C5b fragment polymerizes with complement proteins C6, C7, C8 and C9 to form complex C5b–C9, also known as membrane attack complex (MAC), that produces hundreds of tiny holes in the bilipid layer of cell membrane and results in target cell lysis.

Complement System Dysregulation-related Glomerulopathy and other Disorders

Complement system has a paradoxical role in the development of lupus nephritis. Complement protein deficiencies, particularly of components of the classical pathway, are strong risk factors for the development of systemic lupus erythematosus due to defective clearance of nuclear antigens released by injured and apoptotic cells.

- C3 glomerulopathy is a recently described disorder caused by dysregulated alternative pathway activation. It is diagnosed by demonstration of C3 deposits in the glomeruli in the absence of immunoglobulin.
- Membranoproliferative glomerulonephritis type 2, also called **dense deposit disease (DDD)**, is considered a subset of C3 glomerulopathy, in which electron dense deposits are demonstrated within the glomerular basement membrane by electron microscopy. It may have a worse prognosis than C3 glomerulopathy in the absence of dense deposits.
- In immune complex-mediated glomerulonephritis, glomerular immune complexes activate the complement proteins (C1q, C4 and C3) of classical pathway. C3 fragments are demonstrated in nearly 100% of renal biopsies from patients with membranoproliferative glomerulonephritis type 1, lupus nephritis and postinfectious glomerulonephritis. Low serum C3 complement levels in patients with various renal diseases are given in Table 22.14. Normal serum C3 complement levels in various renal diseases are given in Table 22.15.

Table 22.14 Low serum C3 complement levels in patients with low various renal diseases

Glomerulonephritis	Low Serum Complement
Primary glomerulonephritis	
Poststreptococcal glomerulonephritis	90%
Membranoproliferative glomerulonephritis type 1	70%
Membranoproliferative glomerulonephritis type 2 (dense deposit disease)	80%
C3 glomerulonephritis	80%
Secondary glomerulonephritis	
Lupus nephritis III	75%
Lupus nephritis IV	90%
Lupus nephritis V	60%
Subacute bacterial endocarditis	90%
Cryoglobulinemia	90%
Hemolytic uremic syndrome	50%

Table 22.15 Normal serum C3 complement levels in various renal diseases

Glomerulonephritis	Serum Complement
Primary glomerulonephritis	
IgA nephropathy	Normal
Antiglomerular basement disease	Normal
Renal limited ANCA-associated vasculitis	Normal
Secondary glomerulonephritis	
Polyarteritis nodosa	Normal
Henoch-Schönlein purpura	Normal
Microscopic angiitis	Normal
Wegener granulomatosis with polyangiitis	Normal
Goodpasture syndrome	Normal

INFLAMMATORY CELLS INVOLVED IN GLOMERULONEPHRITIS

Neutrophils, lymphocytes, monocytes/macrophages and natural killer cells participate in the pathogenesis of glomerulonephritis. Neutrophils and macrophages synthesize proteases, reactive oxygen species (ROS), and arachidonic acid metabolites decrease glomerular filtration rate (GFR).

- Reactive oxygen species (ROS) include superoxide inion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl ions (–OH) and singlet oxygen. ROS synthesized by neutrophils and released in cells act both as a signaling molecule and chemical mediators of inflammation. ROS accumulate in the target cells, react and damage biomolecules (proteins, lipids, carbohydrates, nucleic acids) leading to oxidative damage of plasma membrane and cell death.
- On activation of macrophages, T cells and natural killer cells (NK cells) produce biologically active molecules, which participate in the pathogenesis of glomerulonephritis.

COAGULATION/FIBRINOLYSIS SYSTEM IN FIBRIN-MEDIATED GLOMERULONEPHRITIS

Recent clinical observations revealed that the fibrin deposition within glomeruli is an important pathogenetic mechanism in the series of events leading to progressive destruction of glomerular capillaries.

- Fibrin deposition can occur in any injured blood vessels and, if not rapidly dealt with fibrinolysis, and result in endothelial alterations, development of microthrombi (trapped platelets in fibrin) and organization of glomerular microthrombi leading to reduced glomerular filtration rate. It seems that glomerular capillaries are especially vulnerable to fibrin deposition. Macrophages participate in deposition of fibrin in the glomeruli.
- Plasminogen activator inhibitor 1 (PAI-1) inhibits degradation of fibrin and glomerular extracellular matrix proteins; thereby causing microthrombi formation and glomerulosclerosis.
- Resident glomerular cells and monocytes/macrophages, synthesis of proinflammatory cytokines and accumulation of inflammatory chemical mediators seem to act in concert to ensure the long-term consolidation of local inflammatory tissue injury, microthrombi (fibrin trapped platelets), organization of fibrin deposits, glomerular obliteration, reduced glomerular filtration, and progression toward irreversible glomerulosclerosis and irreversible renal failure.
- Fibrin also acts as a stimulus to form crescents in glomeruli due to proliferation of parietal cells of Bowman's capsule. Fibrin deposition is a prominent feature of rapidly progressive (crescentic) glomerulonephritis and hemolytic uremic syndrome.

PLATELET SECRETORY PRODUCTS INVOLVED IN GLOMERULONEPHRITIS

As extensively documented, platelets degranulate and release within glomeruli vasoactive, chemotactic and

mitogenic products that interact with a number of soluble chemical mediators generated by renal resident cells or inflammatory cells and contribute to amplify glomerular injury resulting in increase in glomerular permeability to proteins.

- Platelet-activating factor (PAF) and other platelet secretory products, polycationic macromolecules, platelet factor 4 and β-thromboglobulin alter glomerular permeability to proteins and enhance immune-mediated glomerular injury.

NEPHRITIC FACTORS (NE-AP AND NE-CP) INVOLVED IN GLOMERULONEPHRITIS

Normally, C3 convertase enzyme causes break down of complement proteins. While properdin opposes the action of C3 convertase.

- Nephritic factors (NeF and NeF) are IgG autoantibodies specific to C3 convertase (C42b2a or C3bBb) or C5 convertase that stabilize these enzyme complexes and prevent normal regulatory control.
- Nephritic factors (NeF and NeF), i.e. IgG autoantibodies have been mainly associated with renal diseases like C3 glomerulopathy and immune complex-mediated membranoproliferative glomerulonephritis. These IgG autoantibodies have been demonstrated in systemic lupus erythematosus.

SOLUBLE FACTORS INVOLVED IN GLOMERULONEPHRITIS

Soluble factors are synthesized by macrophages, mesangial cells, platelets, T cells and NK cells, which induce injury to glomerular endothelial, mesangial and epithelial cells. Soluble factors include cytokines (IL-1 and TNF-α), nephritic factors (NeF and NeF), chemokines, e.g. α-chemokine, MCP-1 (monocyte chemotactic protein 1), RANTES (regulated and named T cell expressed and secreted), eotaxin, lymphotoxin, fractalkine; vasoactive amines synthesis by platelets and basophils, arachidonic acid metabolites (prostaglandins, leukotrienes, lipoxins), nitric oxide (NO), endothelin, angiotensin, proteases (lysosomal enzymes) and reactive oxygen species (ROS).

Cytokines and Chemokines Involved in Glomerulonephritis

Cytokines (interleukin 1) and chemokines participate in the recruitment of inflammatory cells and their activation, which can directly contribute to the glomerular injury and glomerular permeability.

- Mesangial cells also synthesize various substances, i.e. CXC, MCP-1 and RANTES, which initiate inflammatory response in the absence of leukocytic infiltration. CXC chemokine (α-chemokine participates in activation and chemotaxis of neutrophils.
- Neutrophils liberate hydrolytic enzymes resulting in glomerular injury. MCP-1 (monocyte chemotactic protein 1) synthesized by mesangial cells attracts monocytes. RANTES (regulated and named T cell expressed and secreted) synthesized by mesangial cells attracts lymphocytes. Chemokines involved in glomerulonephritis are given in Table 22.16.

Prostaglandins, Nitric Oxide and Endothelin Mediators of Inflammation Involved in Glomerulonephritis

Prostaglandins, nitric oxide and endothelin chemical mediators act on glomerular endothelial, mesangial, and epithelial cells and may play key role in regulating the blood flow through the inflamed glomeruli.

- Nitric oxide can accumulate in high concentration and can react with oxygen-derived free radicals thereby setting up two mechanisms of cell death: oxidative injury and ATP depletion. The mitochondria can lose their membrane potential due to high concentration of nitric oxide, which can halt ATP production all together. This process can cause glomerular endothelial damage resulting in stimulation of the inflammatory response.
- PGD2 promotes platelets aggregation and causes vasodilation, and increased vascular permeability of glomeruli.

Table 22.16 Chemokines involved in glomerulonephritis

Chemokines	Examples	Actions
CXC chemokine	α-chemokine	Neutrophils activation and recruitment
CC chemokine (β-chemokine)	▪ MCP-1 (monocyte chemotactic protein 1) ▪ RANTES (regulated and named T cell expressed and secreted) ▪ Eotaxin	▪ Monocytes recruitment ▪ Lymphocytes recruitment ▪ Eosinophils and basophils recruitment
C-chemokine (γ-chemokine)	Lymphotoxin	Lymphocytes recruitment
CX3C chemokine	Fractalkine	Monocytes and T cells recruitment and strong adhesion between monocytes and T cells

Table 22.17 Role of growth factors in the pathogenesis of glomerulonephritis

Growth Factor	Source/Synthesis	Action
Platelet derived growth factor (PDGF)	▪ Macrophages ▪ Smooth muscle cells ▪ Endothelial cells ▪ Keratinocytes	Proliferation of mesangial cells
Fibroblastic growth factor-β (FGF-β)	▪ Macrophages ▪ T cells ▪ Endothelial cells ▪ Platelets	Deposition of extracellular matrix and hyalinization of glomeruli
Transforming growth factor-β (TGF-β)	▪ Macrophages ▪ T cells ▪ Endothelial cells ▪ Platelets	Deposition of extracellular matrix and hyalinization of glomeruli

- PGD_2 and PGE_2 modulate leukocytic phagocytic activity. Nitric oxide causes vasodilatation by relaxing vascular smooth muscle cells. Endothelin is potent vasoconstrictor synthesized by endothelial cells leading to decreased glomerular filtration rate (GFR).

Growth Factors Involved in Glomerulonephritis

Platelets and many other cells produce many growth factors such as platelet-derived growth factor, transforming growth factor-β (TGF-β) and fibroblast growth factor-β (FGF-β), which mediate progression of glomerulonephritis via their chemotactic activity by influx of leukocytes and their effect on extracellular matrix synthesis by resident glomerular cells.

Platelet-derived growth factor (PDGF) also causes proliferation of mesangial cells. Identification of specific platelet inhibitors or interventions specific for platelet secretory products and their target cells will be crucial for understanding the exact role of platelets and their products involved in glomerulopathy. Role of growth factors in the pathogenesis of glomerulonephritis is given in Table 22.17.

GLOMERULAR DISEASES: CLINICAL SYNDROMES

Glomerular diseases produce various clinical syndromes: asymptomatic hematuria, asymptomatic proteinuria, acute nephritic syndrome, nephrotic syndrome, rapidly progressively glomerulonephritis, chronic renal failure and end stage renal disease. Clinical syndromes of glomerular disease and underlying causative renal disease are given in Table 22.18. Progression of glomerulopathy and clinical manifestations is given in Table 22.19. Classification of primary glomerulonephritis by predominant clinical presentation is given in Table 22.20. Comparison between nephritic syndrome and nephrotic syndrome is given in Table 22.21.

- **Asymptomatic microscopic hematuria:** Asymptomatic microscopic hematuria is defined as the presence of three or more red blood cells per high power field visible in a properly collected urine specimen without evidence of infection. Idiopathic hematuria is found in 95% of cases and significant pathology on assessment in 5–10% of cases such as exercise-induced hematuria, IgA nephropathy, polyarteritis nodosa (PAN, bacterial endocarditis and Henoch-Schönlein purpura.
- **Asymptomatic proteinuria:** Asymptomatic proteinuria can be transient in many persons. It can be caused by illness with fever, dehydration, exercise, and urinary tract infections. **Orthostatic proteinuria** can also occur in urine produced in an upright position, but the same patient's urine might test negative in the supine position. Asymptomatic proteinuria can be present in the initial stage of focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), systemic lupus erythematosus (SLE), polyarteritis nodosa (PAN), bacterial endocarditis and Henoch-Schönlein purpura.
- **Nephritic syndrome:** Nephritic syndrome is characterized by hematuria (passage of smoky colored urine), hypertension, oliguria (urine output <400 ml/24 hours), and periorbital edema. The major underlying pathology is inflammation of the glomerulus that results in nephritic syndrome. It is often caused by immune response triggered by an infection or other disease.
- **Nephrotic syndrome:** Nephrotic syndrome is a clinical syndrome defined by massive proteinuria (≥3.5 g/24 hours) responsible for hypoproteinemia resulting with hyperlipidemia, generalized edema and various complications. Nephrotic syndrome occurs due to damage to glomeruli as a result of either structural change or deposition of excessive mesangial matrix in glomeruli in the settings of primary or secondary diseases.
- **Acute kidney disease:** Acute kidney disease occurs within a few hours/days resulting in failure to excrete waste metabolic products, concentrate urine and conserve body electrolytes in settings of prerenal, renal and postrenal etiology. Diseases other than glomerulonephritis that cause acute kidney disease include: thrombotic microangiopathy, acute tubulointerstitial nephritis, acute tubular necrosis, atheroembolization, light chain nephropathy and cortical necrosis.

Table 22.18 Clinical syndromes of glomerular disease and underlying causative renal disease

Asymptomatic Microscopic Hematuria
- Exercise-induced hematuria
- IgA nephropathy, polyarteritis nodosa (PAN)
- Bacterial endocarditis
- Henoch-Schönlein purpura

Asymptomatic Proteinuria
- Febrile illness
- Dehydration
- Exercise
- Urinary tract infection
- Initial stage of focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), systemic lupus erythematosus (SLE), polyarteritis nodosa (PAN), bacterial endocarditis and Henoch-Schönlein purpura

Diseases that Cause Nephritic Syndrome

Primary diseases
- Postinfectious acute diffuse proliferative glomerulonephritis
- Antiglomerular basement membrane antibody glomerulonephritis (e.g. Goodpasture syndrome)
- Rapidly progressive glomerulonephritis (RPGN)
- IgA nephropathy
- Thin basement membrane disease
- Alport syndrome

Secondary diseases
- Lupus nephritis (renal involvement in systemic lupus erythematosus)
- Polyarteritis nodosa (PAN)
- Wegener granulomatosis
- Henoch-Schönlein purpura
- Essential cryoglobulinemia
- Idiopathic immune complex proliferative glomerulonephritis
- Pauci-immune/antineutrophil cytoplasm antibody glomerulonephritis

Diseases that Cause Nephrotic Syndrome

Primary diseases
- Minimal change disease
- Membranous glomerulonephritis
- Membranoproliferative glomerulonephritis (MPGN) type 1
- Membranoproliferative glomerulonephritis type 2 (dense deposit disease)
- Focal proliferative glomerulonephritis
- Focal segmental glomerulosclerosis (FSGS)
- C1q nephropathy
- Fibrillary glomerulonephritis
- Monoclonal immunoglobulin deposition disease
- Immunotactoid glomerulopathy
- Collagenofibrotic glomerulopathy

Secondary diseases
- Immune complex mediated (e.g. systemic lupus erythematosus, Henoch-Schönlein purpura)
- Metabolic disorders (e.g. diabetic glomerulosclerosis, amyloidosis nephropathy)
- Vascular disorders (polyarteritis nodosa, Wegener granulomatosis, hemolytic uremic syndrome)
- Pre-eclampsia/eclampsia glomerulopathy
- Infections (malarial parasite, hepatitis B virus, *Treponema pallidum*)
- Drugs (penicillamine, heroin, gold, captopril)

Diseases other than Glomerulonephritis that Cause Acute Renal Failure
- Thrombotic microangiopathy
- Acute tubulointerstitial nephritis
- Acute tubular necrosis
- Atheroembolization
- Light chain cast nephropathy
- Cortical necrosis

Diseases that Cause Chronic Renal Failure
- Renal arteriolosclerosis
- Chronic sclerosis glomerulonephritis
- End stage renal disease, not otherwise specified (NOS)
- Primary and secondary diseases or disorders involving kidneys except minimal change disease and exercise-induced hematuria

Contd...

- **Chronic kidney disease:** Chronic kidney disease (chronic glomerulonephritis) is characterized by irreversible and progressive glomerular and tubulointerstitial fibrosis, ultimately resulting in reduction in the glomerular filtration rate (GFR) and retention of uremic toxic metabolic products.
  - Chronic renal failure (CRF) involves a gradual loss renal functions (estimated glomerular filtration rate <60 ml/minute/1.73 square meter for three months or more irrespective of the etiology), ultimately resulting in the need for renal transplantation or dialysis.
  - Chronic renal failure can be caused by primary and secondary renal diseases (arteriolosclerosis, chronic sclerosing glomerulonephritis, and end stage renal disease, not otherwise specified).
  - Signs and symptoms of chronic renal failure include: water retention/swelling of legs and feet, nausea, vomiting, loss of appetite, shortness of breath, insomnia, cramps, muscle twitches, oliguria/anuria, drowsiness and fatigue.

NEPHRITIC SYNDROME

Nephritic syndrome is characterized by inflammatory rupture of the glomerular capillaries with resultant red blood cells into the urinary space proteinuria (<2 g/24 hours), hypertension and mild periorbital edema.

Table 22.19 Progression of glomerulopathy disease and clinical manifestations

Disease	Mechanism	Clinical Manifestations
Nephritic syndrome	Glomerular perfusion failure (usually transient)	▪ Hematuria ▪ Oliguria (<400 ml/24 hours) ▪ Proteinuria <2 g/24 hours ▪ Periorbital edema ▪ Hypertension
Nephrotic syndrome	Glomerular permeability	▪ Massive proteinuria ≥3.5 g/24 hours ▪ Hypoproteinemia ▪ Hyperlipidemia ▪ Anasarca (generalized edema) ▪ Lipiduria
Rapidly progressive glomerulonephritis (RPGN)	Crescents formation (Bowman's capsule parietal cells proliferation) as a result of leakage of fibrinogen	Nephritic syndrome progressing to renal failure
Chronic renal failure (GFR <20% of normal)	Chronic irreversible nephron failure both glomerular and tubular failure. There is progressive parenchymal destruction	Azotemia progressing to uremia over months to years
End stage renal disease (GFR <5% of normal)	Glomeruli are markedly sclerosed, tubular atrophy with thyroidization, interstitial fibrosis and vascular thickening	Terminal stage renal disease with uremic manifestations

Table 22.20 Classification of primary glomerulonephritis by predominant clinical presentation

Primary Glomerulopathy	Nephritic Syndrome	Nephrotic Syndrome
Minimal change disease	Nil	++++
Focal segmental glomerulosclerosis	+	++++
Membranous glomerulonephritis	+	++++
IgA nephropathy	++++	++
Membranoproliferative glomerulonephritis	++	++++
Acute post-infectious glomerulonephritis	++++	+
Crescentic glomerulonephritis	++++	+

- Patient with nephritic syndrome presents with hypertension, hematuria with dysmorphic RBCs, RBC casts (<2 g/24 hours), oliguria and periorbital edema.
- Nephritic syndrome occurs due to poststreptococcal infection (most important cause in children), nonstreptococcal organisms, IgA nephropathy (most common cause in adults), Goodpasture's syndrome, systemic lupus erythematosus (diffuse proliferative type), Wegener's granulomatosis, microscopic polyarteritis, Henoch-Schönlein purpura, and essential cryoglobulinemia. Glomerular diseases commonly associated with nephritic syndrome are given in Table 22.22.

POSTSTREPTOCOCCAL GLOMERULONEPHRITIS

Poststreptococcal glomerulonephritis (PSGN) is an immunologically-mediated disease following

Table 22.21 Comparison between nephritic syndrome and nephrotic syndrome

Parameters	Nephritic Syndrome	Nephrotic Syndrome
Clinical history		
Definition	History of sore throat, periorbital edema, headache and passage of smoky colored urine, proteinuria (2 g/24 hours) and low urinary output (<400 ml/24 hours)	Massive proteinuria ≥3.5 g/24 hours, markedly decreased serum proteins with reversed albumin to globulin ratio and severe generalized edema (pitting edema legs, pleural effusion, ascites)
Hypertension	Present	Less frequent
Biochemical findings		
Serum proteins (total and albumin to globulin ratio)	Total serum proteins decreased up to lesser extent Albumin : globulin ratio normal	Total serum proteins markedly decreased Albumin : globulin ratio reversed (normal albumin/globulin is 2:1)

Contd...

Table 22.21 Comparison between nephritic syndrome and nephrotic syndrome *(Contd...)*

Parameters	Nephritic Syndrome	Nephrotic Syndrome
Serum lipids	Normal	Hyperlipidemia common due to increased synthesis of lipoproteins by liver to compensate protein loss in urine
Serum complement concentration	Low	Most often normal
Urine analysis		
24 hours urine output	Oliguria (urine output <400 ml/24 hours)	Normal urine output
Color	Smoky-colored urine due to hematuria	Milky or frothy due to lipiduria
Specific gravity	Increased due to mild to moderate proteinuria	Increased due to massive proteinuria (sugar in diabetic nephropathy also increases specific gravity)
Proteinuria	<2 g/24 hours	Massive proteinuria ≥3.5 g/24 hours
Heat and acetic acid test for proteins	Positive	Positive
Sugar	Negative	Positive in diabetic nephropathy
Light microscopy	Urinary sediment shows dysmorphic RBCs and RBC casts	Urinary sediment shows hyaline casts (pus cells in diabetic pyelonephritis)
Etiology and prognosis		
Etiology	▪ Poststreptococcal glomerulonephritis ▪ Post-nonstreptococcal glomerulonephritis ▪ IgA nephropathy ▪ Goodpasture's syndrome ▪ Wegener's granulomatosis ▪ Henoch-Schönlein purpura	▪ Minimal change ▪ Membranous glomerulonephritis ▪ Membranoproliferative glomerulonephritis ▪ Focal segmental glomerulosclerosis ▪ Diabetic nephropathy ▪ Amyloid nephropathy
Prognosis	Recovery occurs in 95% cases of post-streptococcal glomerulonephritis. A few cases develop rapidly progressive glomerulonephritis	Prognosis is excellent in minimal change disease, as it responds to corticosteroid therapy. Prognosis is variable in other disorders

Table 22.22 Glomerular diseases commonly associated with nephritic syndrome

Light Microscopy	Immunofluorescence Microscopy	Electron Microscopy	Prognosis
Poststreptococcal glomerulonephritis			
Diffuse proliferative glomerulonephritis: All cells of glomeruli are increased. Glomeruli are infiltrated by neutrophils	Granular pattern: IgG, IgM, C3 (fibrin absent)	Subepithelial deposits or humps	Recovery occurs in 95% children and 60% adults. Rarely patients may develop rapidly progressive glomerulonephritis and chronic glomerulonephritis
Lupus nephritis (systemic lupus erythematosus involving kidneys)			
Diffuse proliferative glomerulonephritis: Endocapillary and/or extracapillary proliferation in 50% or more glomeruli (wire-loop lesion)	Granular pattern: IgG, C3, fibrin in mesangial and glomerular loop	Subendothelial deposits and also in mesangial region	90% patients survive at 10 years
IgA nephropathy			
Focal proliferative glomerulonephritis	Granular pattern: IgA and C3 deposition in mesangial region	Electron dense deposits in mesangial region	Patient may develop chronic renal failure and survival rate in 80–90% patients at 10 years
Rapidly progressive glomerulonephritis			
About 50% of glomeruli are compressed by crescents filling glomerular spaces	Linear pattern: IgG, C3, fibrin	Goodpasture's syndrome: No electron dense deposits	Majority (90%) progress to chronic renal failure

pharyngitis caused by nephrogenic strains of group A β-hemolytic streptococcal infections. PSGN is characterized by reduction in glomerular filtration rate (GFR) and rapid deterioration of renal functions due to an inflammatory response. PSGN most frequently occurs in children between the ages of 2 and 14 years especially boys. It may also affect adults >40 years of age and infants. PSGN affects the glomeruli and the small blood vessels of the kidneys, which returns to normal within months.

- Patient presents with nephritic syndrome usually 1 to 2 weeks after a history of sore throat, or 6 weeks after a skin infection (impetigo). When symptomatic, PSGN typically presents with features of the nephritic syndrome such as macroscopic hematuria (smoky colored urine), oliguria (<400 ml/24 hours), hypertension (reduced glomerular filtration rate and renin-angiotensin-aldosterone system activation), and facial and orbital edema especially in the morning, generalized weakness or anorexia. Less commonly presentation can mimic nephrotic syndrome with significant proteinuria.
- Diagnostic features of poststreptococcal glomerulonephritis include exudative proliferative glomerulonephritis with numerous polymorphonuclear cells infiltration on light microscopy, deposits (IgG, complement) on immunofluorescence microscopy; decreased serum complement (C3 than C4) and hump-type subepithelial deposits on electron microscopy. Schematic representation of poststreptococcal glomerulonephritis (PSGN) is shown in Fig. 22.19.

Etiology

Nephrogenic streptococci infection precedes poststreptococcal glomerulonephritis (PSGN), which initially affects skin or oropharynx.

- More recently, PSGN is associated with skin infections (impetigo after 1–2 weeks by Streptococcus β-hemolyticus group A sero M types 47, 49, 55, 2, 60 and 57) more frequently than throat infections (pharyngitis after 2–6 weeks by Streptococcus β-hemolyticus group A sero M types 1, 2, 4, 3, 25, 49 and 12).

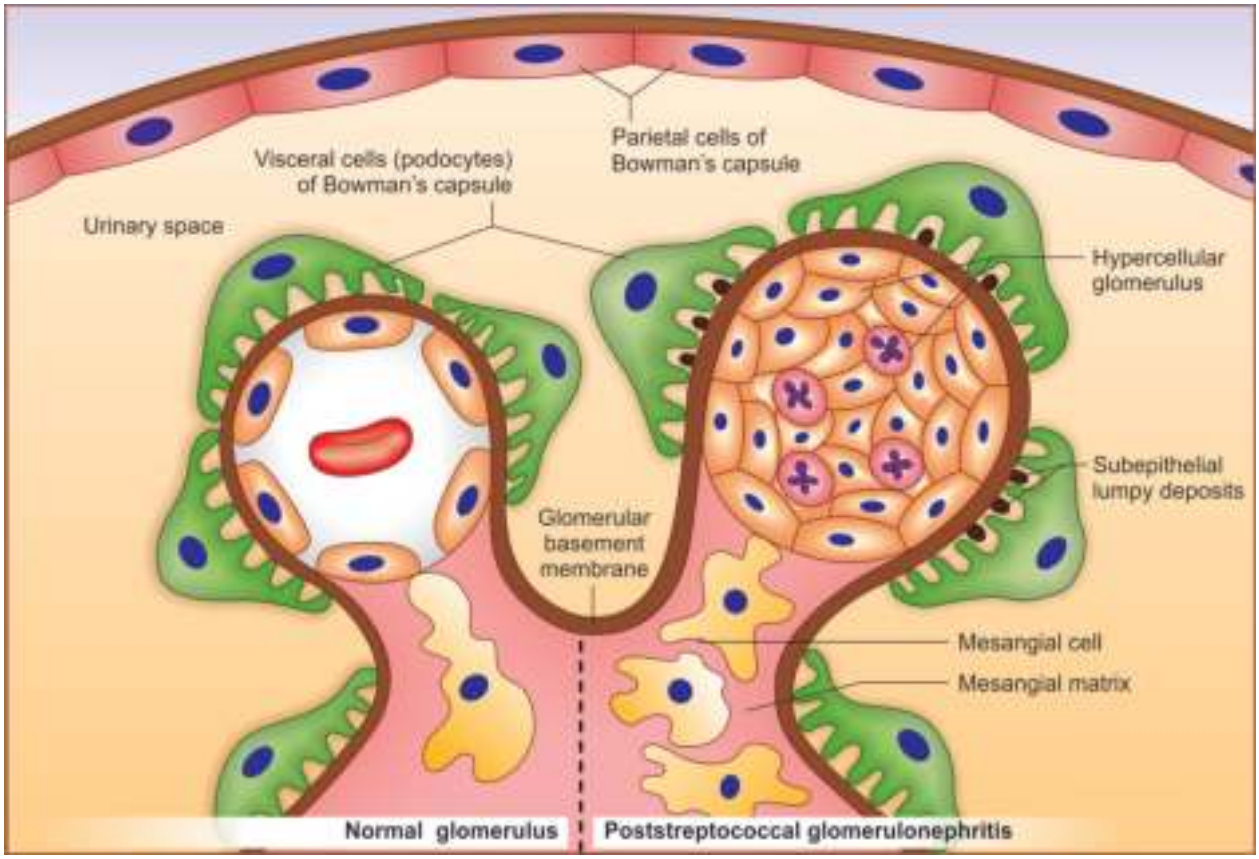


Fig. 22.19: Schematic representation of poststreptococcal glomerulonephritis (PSGN). PSGN is an immunologically-mediated disease following pharyngitis caused by nephrogenic strains of group A streptococcal infections. PSGN is characterized by rapid deterioration of renal functions due to an inflammatory response. Left half of schematic representation shows a normal glomerulus, whereas right glomerulus is hypercellular due to proliferation of glomerular endothelial cells and infiltration by neutrophils. Glomerular lumen is obliterated leading to reduction in glomerular filtration rate. Immune complexes are seen in subepithelial region.

- Rare causes of postinfectious glomerulonephritis include other bacterial or viral infections and malaria. Poor hygiene, overcrowding, and low socioeconomic status are important risk factors for streptococci outbreaks, and this explains the higher incidence of PSGN in the underdeveloped countries.
- Genetic factors are expected to predispose to the condition since almost 40% of patients with focal segmental glomerulosclerosis (FSGS) have a positive family history. There is no specific gene found to cause PSGN.

Pathophysiology

Poststreptococcal glomerulonephritis (PSGN) is an immunological disorder; representing a type 3 hypersensitivity reaction.

- The exact mechanism of pathogenesis of PSGN is not fully determined. The body responds to nephrogenic streptococcal infection by forming immune complexes containing the streptococcal antigen with a human antibody. Some authors suggest that these immune complexes reach via circulation and get deposited in renal glomeruli.
- Other authors claim that the PSGN results from an '*in situ*' formation of antigen–antibody immune complex within the renal glomeruli. This '*in situ* immune complex formation' occurs either due to a reaction against streptococci antigens deposited in the glomerular basement membrane; or according to other theories due to an antibody reaction against glomerular components that cross-react with streptococcal antigen.
- Nephritis-associated plasmin receptor (NAPlr) is released into the blood circulation and deposited on glomerular tufts resulting in plasmin related endocapillary inflammation. Antigen–antibody complex is deposited in the subepithelial region.
- Immune complex formation activates complement system pathway, which leads to the generation of additional inflammatory chemical mediators and recruitment of inflammatory cells in the glomeruli and ending in the destruction of renal glomeruli.
- Early in the course of PSGN, resident glomerular endothelial and mesangial cells undergo excessive proliferation and is accompanied by influx of polymorphonuclear cells and monocytes in glomeruli.
- Macrophages are effector cells that cause cellular proliferation of glomeruli. In addition to the streptococcal antigens, some patients may also have cryoglobulins, rheumatoid factor and antineutrophilic cytoplasmic serum antibodies in the involved glomeruli. Pathophysiology of poststreptococcal glomerulonephritis is shown in Fig. 22.20.

Clinical Features

Approximately 50% of children with PSGN remain asymptomatic. These cases are discovered as incidental finding during routine urine examination. Usually 2–5 years old child presents with history of sore throat with latent period of ≤3 weeks followed by symptoms: (a) passage of smoky-colored urine (hematuria) due to glomerular injury along with dysmorphic RBCs, (b) abrupt onset of oliguria due to reduced glomerular filtration rate (<400 ml/day urine output), (c) periorbital edema, (d) hypertension due to salt retention and increasing peripheral resistance (renin-angiotensin-aldosterone system), and (e) systemic symptoms of headache, malaise, fever, anorexia, nausea, vomiting, pallor and flank pain (due to stretching of renal capsule by inflamed hypercellular glomeruli) in 50% of cases.

- **Hematuria:** Patient presents with hematuria (often described their urine as smoky, cola colored or tea colored or rusty) due to damaged glomeruli. Frank hematuria may occur in 30–50% of cases. Urine analysis contains dysmorphic RBCs with irregular membranes due to passing through inflamed glomeruli wih immune complex deposition. Presence of RBCs cast in urinary sediment is a key finding.
- **Oliguria:** Patient presents with oliguria with urine output is <400 ml/24 hours in 30–50% of patients, which is usually indicative of severe crescentic form of renal disease (normal urine output is 600 to 2500 ml/24 hours). Inflamed glomeruli reduce glomerular filtration rate (GFR). Tubular function is intact. Oliguria is transient and improves in 7–14 days.
- **Periorbital edema (facial puffiness):** Patient develops periorbital puffiness (edema) as a result of proteinuria in nephritic range (<2 g/24 hours) and salt retention in the loose skin in periorbital area. It is most prominent in the morning hours and tends to resolve at the end of the day.
  - Activation of renin-angiotensin-aldosterone system enhances retention of sodium and water absorption resulting in increase in the hydrostatic pressure.
  - Edema may be migratory, appearing in eyelids in the morning, disappearing in the afternoon, and reappearing around the ankle in ambulatory patients by the end of the day.
  - In some cases, edema starts in the eyelids and face then lower and upper limbs and then generalized.
- **Hypertension:** Patient presents with headache due to mild to moderate hypertension. Inflamed glomeruli reduce glomerular filtration rate (GFR) and stimulate juxtaglomerular cells to synthesize renin resulting in activation of renin-angiotensin-aldosterone system.

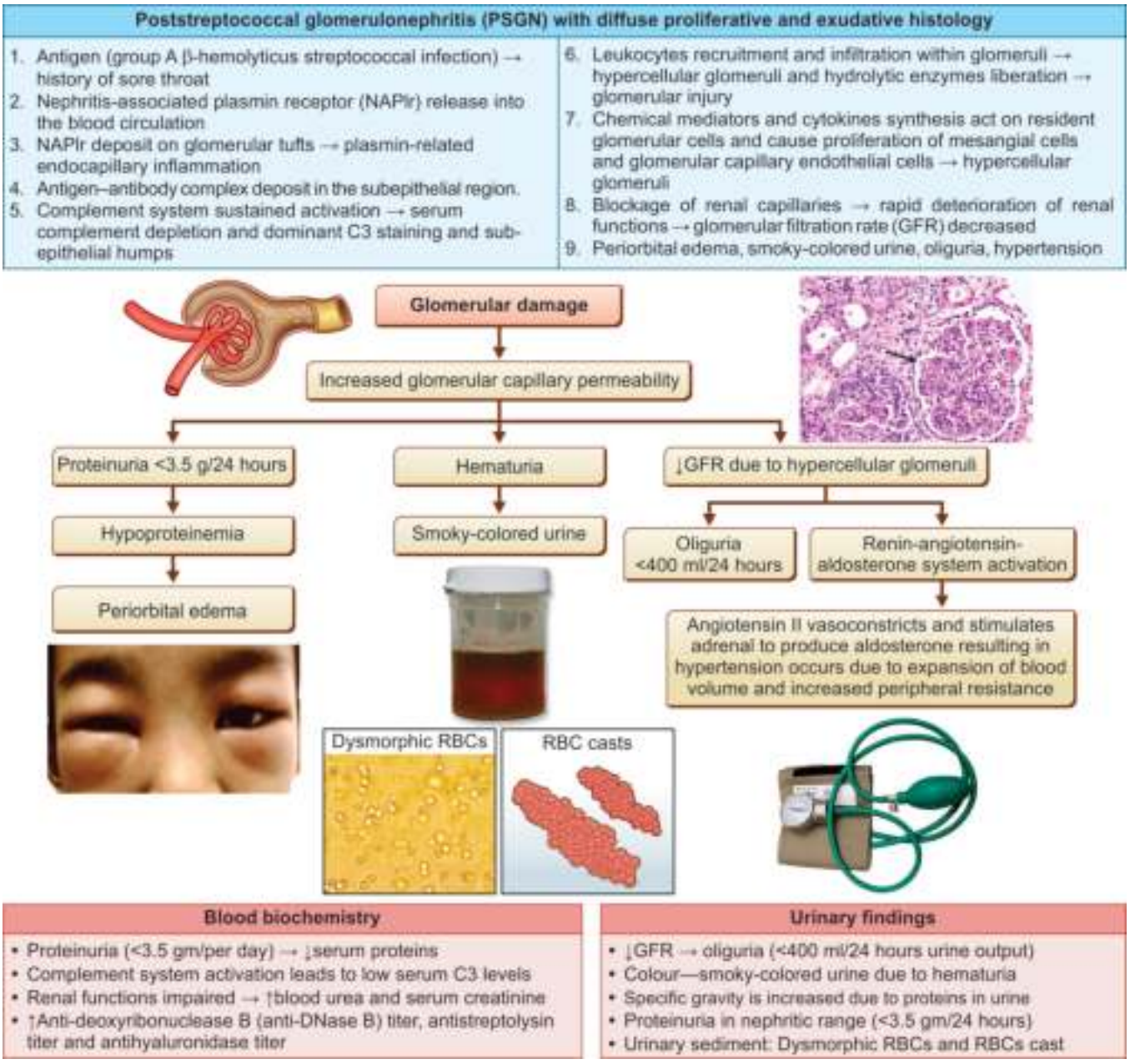


Fig. 22.20: Schematic diagram of pathophysiology of poststreptococcal glomerulonephritis (PSGN). PSGN most often affects children. Patients present with edema (often pronounced facial and orbital edema) especially in the morning, hypertension (due to decreased glomerular filtration rate and activation of renin-angiotensin-aldosterone system), proteinuria in nephritic range, macroscopic hematuria, generalized weakness or anorexia.

- Angiotensin II stimulates adrenal cortex to synthesize aldosterone, that facilitates sodium and water absorption. Angiotensin II acts as vasoconstrictor resulting in increasing peripheral resistance.
- Absorption of sodium and water and increased peripheral resistance cause hypertension. Severe cases may develop hypertensive encephalopathy and acute pulmonary edema.

- **Acute renal failure:** Patient develops acute renal failure due to glomerular injury and/or formation of microthrombi in the glomeruli. Patient may develop acute tubular obstruction due to formation of hyaline casts in the tubules.
- **Flank pain:** Patient may present with flank pain due to stretching of renal capsule by inflamed hypercellular glomeruli in 50% cases.

Imaging Techniques

Ultrasonography demonstrates enlarged kidney up to 50%. The patients may develop acute abdominal pain due to stretching of renal capsule by the enlarged kidneys. Chest radiograph may demonstrate features of heart failure.

Pathology Pearls: Poststreptococcal Glomerulonephritis (PSGN)

Renal biopsy is not recommended for diagnosing patients with PSGN and is performed only when other glomerulopathies are suspected. Renal biopsy is indicated when (a) renal functions are worsening, (b) patient has anuria, (c) there is no latent period between the acute glomerulonephritis and streptococcal infection, (d) normal serum complement level, (e) there is no rise in antistreptococcal antibodies, and (f) there is persistent hypertension.

Light Microscopy

- Hematoxylin and eosin-stained renal tissue section shows hypercellular glomeruli due to proliferation of mesangial cells; and infiltration by PMN cells and macrophages (also called 'exudative' glomerulonephritis). Mesangial cell proliferation causes widening of mesangial region.
- Glomerular endothelial cells do not proliferate but are swollen. Swelling of glomerular endothelial cells and presence of inflammatory cells obstruct capillary lumina resulting in reduction in glomerular filtration rate, and activation of renin-angiotensin-aldosterone system and hypertension. Slight mononuclear infiltrate and edema are present in the interstitium of glomeruli.
- Glomerular basement membrane is not thickened. Tubules lumina contain red blood cells, portentous material and red blood cell casts.
- Interstitial edema and inflammation may be present, indicating that the entire kidney is reacting to the immunologic injury to the glomeruli. These findings are nonspecific and can be present in other glomerulopathies. Histology of poststreptococcal glomerulonephritis (PSGN) is shown in Figs 22.21 and 22.22.

Immunofluorescence Microscopy

Immunofluorescence microscopy shows lumpy bumpy (granular) deposition of IgG, IgM, C3, and C5b–C9 in subepithelial region of peripheral glomerular loops and also in subendothelial region. However, fibrin is absent.

Electron Microscopy

On electron microscopy, large electron-dense immune deposits are most often seen on the epithelial side of basement membrane. Electron-dense immune deposits have a characteristic 'dome' shape. There may be small deposits of variable shapes in other locations.

Poststreptococcal Glomerulonephritis: Case Evaluation

Poststreptococcal glomerulonephritis (PSGN) should be suspected in all children with hypertension and heart failure, even if they do not complain of hematuria or give a history of a preceding sore throat or pyoderma. Laboratory investigations are the most useful in PSGN assessment.

- **Preceding streptococcal infection evidence:** Evidence of a preceding streptococcal infection is determined by measuring antistreptolysin O (ASO) titer, and antinicotinamide-adenine dinucleotidase (anti-NAD), which tends to rise following pharyngitis.

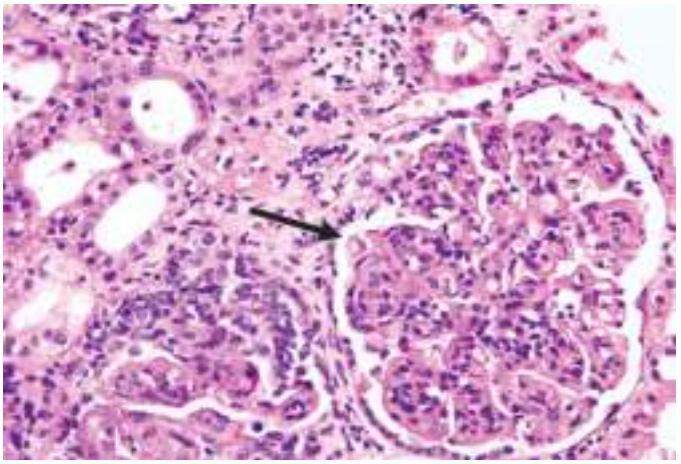


Fig. 22.21: Histology of poststreptococcal glomerulonephritis (PSGN). Hematoxylin and eosin-stained renal biopsy section shows diffuse and global thickening of glomerular basement membrane due to neutrophils and macrophages and proliferation of mesangial and endothelial cells (also called exudative glomerulonephritis). Swelling of endothelial cells and presence of inflammatory cells obstruct capillary lumina. There is sight mononuclear leukocytic infiltrate and edema in the interstitium. Tubules contain red blood cells. Immunofluorescence microscopy demonstrates hump-like structures in subepithelial region and also in subendothelial region leading to proliferation of endothelial cell and decrease in glomerular filtration rate. PSGN returns to normal within months (arrow) (400X).

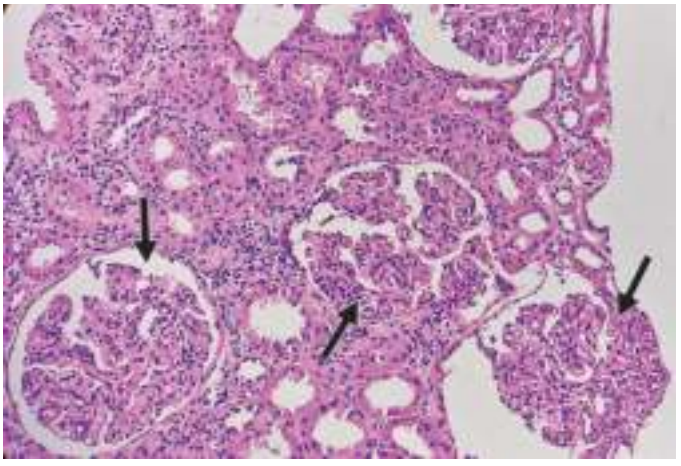


Fig. 22.22: Histology of poststreptococcal proliferative glomerulonephritis (PSGN). Hematoxylin and eosin-stained renal tissue section in PSGN shows hypercellular glomeruli (arrows) resulting from infiltration by neutrophils and macrophages and proliferation of endothelial and mesangial cells. This pattern of PSGN is called exudative glomerulonephritis. Swelling of endothelial cells and presence of inflammatory cells obstruct glomerular capillary lumina resulting in glomerular filtration reduction and activation of renin-angiotensin-aldosterone system and hypertension. Slight mononuclear infiltrate and edema are present in the interstitial tissue. Tubules contain red blood cells (400X). (*Courtesy*: Dr. Neha Kumari and Dr. Tushar Kalonia)

- Elevated serum ASO titer appears 1–3 weeks after group A β-hemolytic Streptococcal infection. It peaks at 3–5 weeks of infection. 50% of patients may show no rise in ASO titer. Height of the titer does not reflect the severity of renal diseases.
- Other antibodies such as anti-DNase B and anti-hyaluronidase are usually elevated after pharyngitis and skin infections. Antihyaluronidase titer is raised in 40% of cases. However, most sensitive is the streptozyme test; which measures the titers of all the antibodies mentioned above.

- **Serum proteins:** Serum proteins are decreased due to proteinuria (<2 g/24 hours) in poststreptococcal glomerulitis.
- **Renal function tests:** Blood urea nitrogen (BUN) and serum creatinine are elevated during the acute phase of PSGN. These values usually return to normal later. Ratio of blood urea to serum creatinine is >15. Tubular function is intact in acute phase of PSGN. In patients with heart failure, in PSGN and levels of NT-proBNP will be elevated.
- **Serum complement C3 concentration:** Serum complement levels (C3) concentration is usually low within 24 hours before the onset of hematuria due to its consumption in the inflammatory reaction. Mostly, the decrease in C3 concentration occurs before serum ASO has risen. Complement levels usually return to normal levels in 6–8 weeks unless the case is complicated.
- **Urine analysis:** Urine analysis shows oliguria (<400 ml/24 hours), smoky-colored urine (macroscopic or microscopic hematuria with dysmorphic red blood cells), mild proteinuria (<2 g/24 hours), raised specific gravity estimated by Vogal's urinometer; and RBC casts, hyaline casts and cellular casts. About 5% of children and 20% of adults have proteinuria in the nephrotic range.
- **Novel serum cystatin C and creatinine biomarkers:** Serum cystatin C and creatinine are novel biomarkers to assess glomerular filtration rate. These are upregulated in these patients.

Clinical Course

In children, prognosis is excellent in 95% of cases, who recover in 2–3 months. But 50% of glomeruli. These represent sign of severe glomerular injury.

- But <1% of cases may progress to rapidly progressive glomerulonephritis (RPGN) and chronic glomerulonephritis in 1–2%.
- Approximately 60% of adults recover from disease, but 3–5% of cases may develop rapidly progressive glomerulonephritis. With rapidly progressive glomerulonephritis (RPGN), the glomerular damage is so severe that fibrinogen leaks into Bowman's space, leading to proliferation of the parietal epithelial cells of glomeruli and formation of crescents.
- Crescents are demonstrated in >50% of glomeruli, which represent sign of severe glomerular injury. Prognosis is good in 60% of adult cases. Many adults may develop RPGN.

Treatment

Poststreptococcal glomerulonephritis (PSGN) is a self-limiting disease in most cases, and thus only symptomatic treatment is needed. Supportive treatment aims at controlling the complications of volume overload such as hypertension and edema, which are prominent during the acute phase of the disease.

- **General measures:** Patient is advised complete bed rest in the first few days. Salt and water restriction are essential to control edema.
- **Therapeutic drugs:** Patients are put on intravenous diuretic frusemide, antihypertensive drugs (angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), or calcium channel blockers (CCBs); antibiotics in case of persistent streptococcal infection; and immunosuppressive corticosteroid therapy in progressing renal failure.
- **Dialysis:** Dialysis is only performed when serum creatinine and potassium levels are critically high.
- **Throat cultures:** Throat cultures should be performed of patient and family members. All members of family should be treated with antibiotics.

ALPORT'S SYNDROME

Alport's syndrome is sex-linked disorder affecting children (M>F) described by Alport in 1927, which most often occurs in males than females.

- Alport's syndrome occurs due to defects in collagen synthesis due to mutations in COL4A3, COL4A4 and COLA5 genes encoding the α5-chain of collagen type IV, which results in interference with the assembly and architecture of collagen type IV, and causes nephritis (glomeruli proliferative and sclerosing), subtle nerve deafness (55% cases of adults) and ocular defects (15–30% of cases, anterior lens dislocation, posterior cataracts, corneal dystrophy).
- Alport's syndrome usually affects 5–20 years of age, usually males (or mosaic females). Males express the full syndrome and present with hematuria and proteinuria.
- Females are carriers, but manifest with hematuria. Rare autosomal recessive and autosomal dominant pedigree also exist, in which males and females are equally susceptible to the full Alport's syndrome.

Pathogenesis

Normally, type IV collagen fibers in glomerular basement membrane give structural strength. Mutation in COL4A3, COL4A4 and COL4A5 genes encoding the α5-chain of collagen type IV results in interference with the assembly and architecture of collagen type IV, which ultimately causes defective synthesis of glomerular basement membranes in kidneys, eye and inner ear leading to nephritis, subtle nerve deafness in adults (55%), ocular disorders in 15–30% cases (corneal dystrophy, anterior lens dislocation, posterior cataracts).

Clinical Features

Patient presents with gross or microscopic hematuria, red blood cell casts, often mild proteinuria, progressive renal failure, and hypertension later in the course of the disease. End stage renal failure occurs in second or third decade of life.

Pathology Pearls: Alport's Syndrome

Light Microscopy

- Hematoxylin and eosin-stained section of renal biopsy shows segmental proliferation of glomeruli, GBM thickening, increased mesangial matrix or mesangial cells causing mesangial widening of glomerular basement membrane, fetal type of glomeruli, and foam cells in glomeruli or tubules.
- Similar alterations may be seen in the tubular basement membrane with a distinctive basket weave appearance.
- As the disease progresses, patient develops focal segmental glomerulosclerosis, vascular sclerosis, tubular atrophy and interstitial fibrosis. Renal tubular cells acquire foamy appearance owing to the accumulation of neutral fats and mucopolysaccharides (foam cells).

Histochemistry

Segmental proliferation or sclerosis of glomeruli, increased mesangial matrix or mesangial cells are causing mesangial widening are demonstrated by Jones methenamine silver stain or periodic acid–Schiff (PAS) stain.

Immunofluorescence Microscopy

Immunofluorescence microscopy shows deposition of IgM and C3 with no definite pattern in glomeruli.

Electron Microscopy

Glomerular basement membrane shows lucency.

IGA NEPHROPATHY (BERGER'S DISEASE)

IgA nephropathy, also called Berger's disease, is a type of diffuse mesangioproliferative glomerulonephritis in adults (HLA-DR4) and children. Disease occurs due to IgA deposit in the mesangial region resulting in glomerulopathy and impaired renal functions. IgA nephropathy is often initiated by infections (bacteria and viruses) of the respiratory or gastrointestinal tracts.

- Patients present with macroscopic/microscopic hematuria (persistent/recurrent) with or without proteinuria. About 80% of cases have been associated with good prognosis. About 25–50% of cases progress to end stage disease over 20 years. Serum polymeric IgA is elevated in 30–50% of cases.
- Four processes come together to induce IgA nephropathy: (a) aberrant glycosylation of IgA1, (b) synthesis of antibodies against galactose-deficient IgA, (c) binding of the galactose-deficient IgA1 by anti-glycan/glycopeptide antibodies to form IgA immune complexes, and (d) deposition of immune complexes in the glomerular mesangial region, activation of alternative complement pathway and possibly the lectin pathway leading to glomerular injury.
- Immunofluorescence microscopy reveals IgA mesangial staining in sclerotic glomeruli.
- **Dominant IgA mesangial deposit (100%) is only defining and consistent feature of the disease.** Predominance of J chain contains IgA1 and λ light chains.
- The diagnostic feature IgA nephropathy is mesangial staining that is more intense for IgA1 and complement C3. IgA nephropathy is shown in Fig. 22.23. Pathogenesis of IgA nephropathy is shown in Fig. 22.24.

Clinical Features

IgA nephropathy is most common form of primary glomerulonephritis worldwide, which usually affects males of 10–30 years of age. Patient presents with recurrent gross or microscopic hematuria after respiratory infection without systemic disease early in life. IgA nephropathy causes slowly progressive end stage renal failure over 10 years in many persons in 30–50% of cases. Disease can recur in renal allografts in 20–50% of cases. Prognosis is poor due to prominent renal arteriolar hyalinization, older age, heavy proteinuria and hypertension.

Pathology Pearls: IgA Nephropathy

Light Microscopy

- Hematoxylin and eosin-stained renal biopsy section shows diffuse proliferation of mesangial cells, and extracellular matrix without significant involvement of capillary walls or lumina.
- Mesangial region involvement is often uneven, that resembles focal and segmental glomerulosclerosis without endocapillary or extracapillary hypercellularity. Histology of IgA nephropathy is shown in Fig. 22.25.

Immunofluorescence Microscopy

Immunofluorescence microscopy shows granular intense staining for IgA and C3 in the mesangial region of glomeruli. IgA is also demonstrated in the capillaries of dermis, lungs, liver and intestine. Immunofluorescence microscopy in IgA nephropathy is shown in Fig. 22.26.

Electron Microscopy

Electron dense deposits are demonstrated in the mesangial region of all the glomeruli.

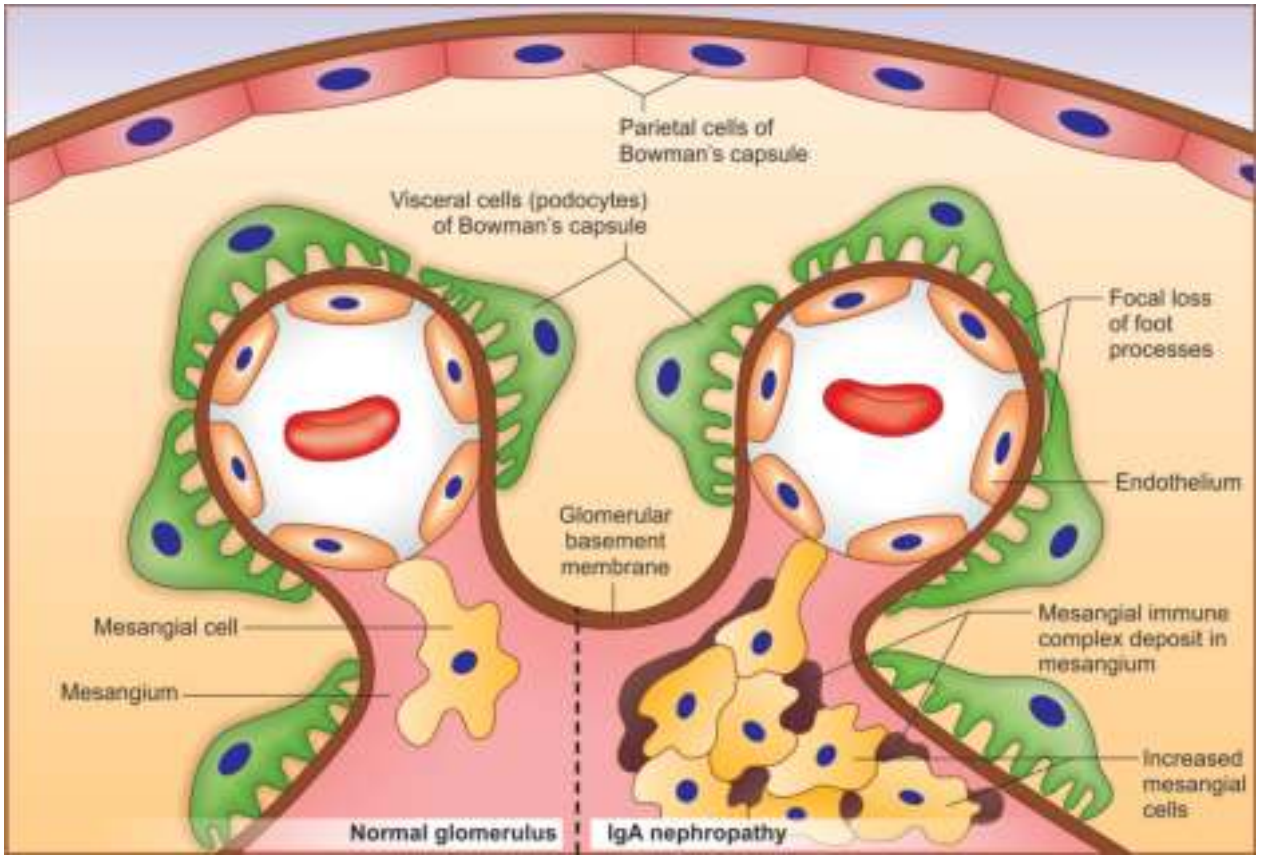


Fig. 22.23: Schematic representation of IgA nephropathy. IgA nephropathy, also called Berger's disease occurs due to IgA deposit in the mesangial region resulting in glomerulopathy and impaired renal functions. Patients present with macroscopic/microscopic hematuria with or without proteinuria. About 80% cases have good prognosis. About 25–50% cases progress to end stage disease over 20 years. Serum polymeric IgA is elevated in 30–50% of cases. Dominant IgA mesangial deposit (100%) is only defining and consistent feature of the disease.

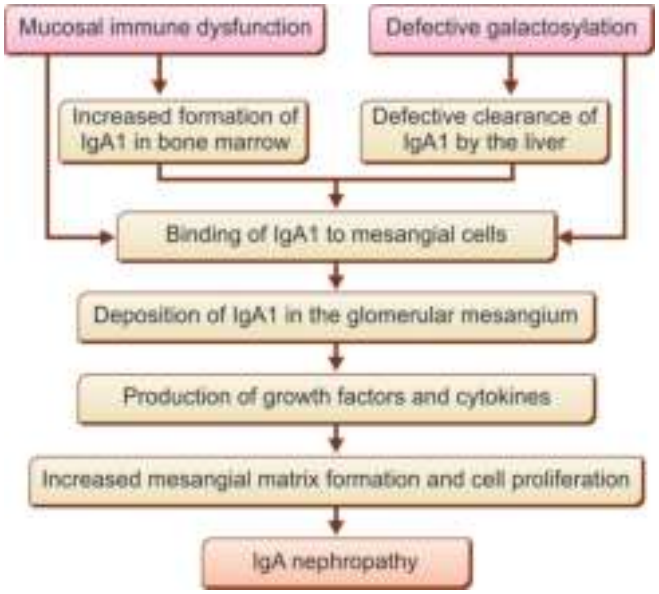


Fig. 22.24: Schematic representation of pathogenesis of IgA nephropathy. Four processes come together to induce IgA nephropathy: (a) aberrant glycosylation of IgA1, (b) synthesis of antibodies against galactose-deficient IgA, (c) binding of the galactose-deficient IgA1 by anti-glycan/glycopeptide antibodies to form immune complexes, and (d) accumulation of these complexes in the glomerular mesangium to initiate renal injury.

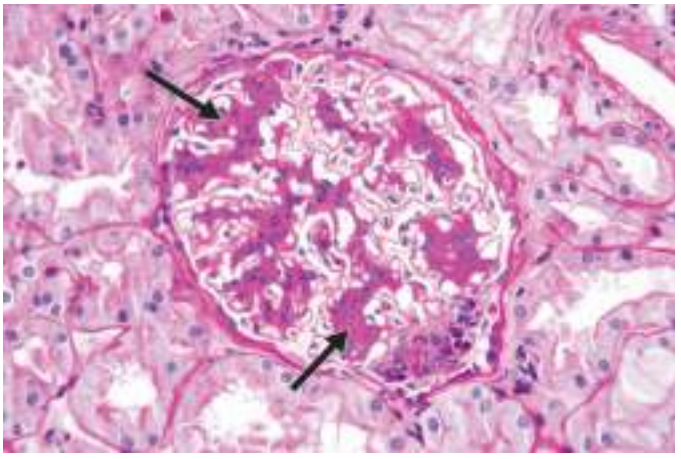


Fig. 22.25: Histology of IgA nephropathy. Confirmation of the IgA nephropathy diagnosis relies on the renal biopsy examination on histology, electron microscopy, and immunofluorescence microscopy. Hematoxylin and eosin-stained renal biopsy section of IgA nephropathy has variable presentation from minimal mesangial expansion to diffuse proliferation of mesangial cells, may have sclerosis and/or crescents resulting from IgA deposit in the mesangium (arrows). Glomeruli lacks hypercellularity in the endocapillary or extracapillary regions. Clinically the most common findings are microscopic hematuria (persistent/recurrent) and episodic macrohematuria. Blood tests might show higher levels of the waste product creatinine or the protein cystatin C (400X).

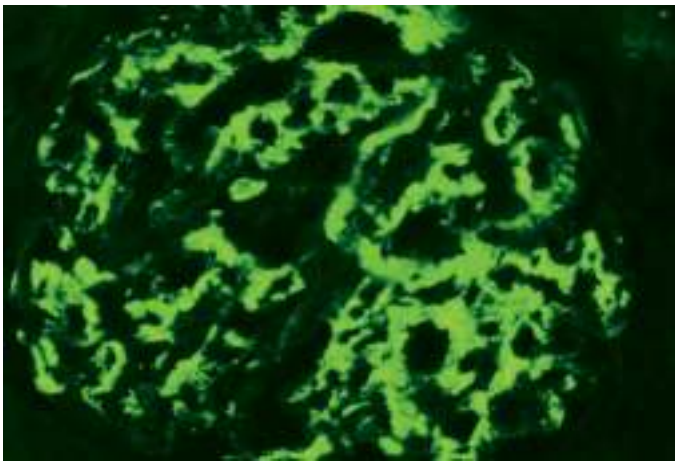


Fig. 22.26: Immunofluorescence microscopy in IgA nephropathy. Immunofluorescence microscopy reveals IgA mesangial staining in sclerotic glomeruli. Dominant IgA mesangial deposit (100%) is only defining and consistent feature of the disease. Predominance of J chain contains IgA1 and lambda light chains.

Treatment

Controlling proteinuria and blood pressure reduces impaired kidney functions in IgA nephropathy. Tonsillectomy in children improves clinicopathologic features in relatively severe form of pediatric IgA nephropathy, especially with early surgery. Patient is treated with corticosteroid therapy. Refractory cases are treated with tacrolimus (fujimycin) immunosuppressive drug.

GOODPASTURE SYNDROME

Goodpasture syndrome is an autoimmune *in situ* mediated immune complex disorder involving kidneys and lungs. Deposition of IgG in the basement membrane of glomeruli and lungs is a feature of Goodpasture's syndrome. The antibodies are formed against basement membranes of the glomeruli (noncollagenous domain of type IV collagen) and the pulmonary alveoli. Injury to glomeruli and lung leads to hematuria and hemoptysis respectively. Patient presents with dyspnea, cough, fatigue, hemoptysis, hematuria and rapidly progressive renal failure.

Pathology Pearls: Goodpasture Syndrome

Light Microscopy

- Over 90% of patients with anti-GBM glomerulonephritis develop **crescentic glomerulonephritis**.
- Antibodies directed against the glomerular basement membrane causes rupture of GBM, extravasation of blood and inflammatory cells into the urinary space, i.e. the space between Bowman's capsule and the glomerular capillary tufts.
- Fibrinogen stimulates proliferation of Bowman's capsule parietal cells resulting in crescents formation (crescentic glomerulonephritis).
- It is worth mentioning that crescentic glomerulonephritis also occurs in Wegener's granulomatosis or polyarteritis nodosa.

Immunofluorescence Microscopy

Diffuse linear immunofluorescence for IgG is seen along the glomerular basement membrane.

WEGENER'S GRANULOMATOSIS

Wegener's granulomatosis is a systemic necrotizing granulomatous vasculitis of unknown etiology, which involves blood vessels of respiratory tract such as nose, sinuses and lungs (90–95%). But renal glomeruli are involved in most cases.

- Wegener's granulomatosis mainly affects 50–60 years of age group. Males are more affected than females. Patients are usually positive for ANCA. It is probably caused due to inhalation of some infectious agents.
- Antineutrophil cytoplasmic antibodies (c-ANCA type) play pivotal role in its pathogenesis. Immune complexes are deposited in small vessels of upper and lower respiratory tract, kidneys and other organs, which cause necrotizing vasculitis, early infiltration by neutrophils, subsequent mononuclear (macrophage) cell infiltration, and fibrosis in chronic cases. Granuloma formation and giant cells in tissues are prominent histologic feature.
- Patient may also develop necrotizing glomerulonephritis, which results in acute renal failure. Cyclophosphamide plus corticosteroid is useful treatment. In untreated patients, outcome is fatal within one year.

HENOCH-SCHÖNLEIN PURPURA

Henoch-Schönlein purpura is the most common type of childhood immune complex-mediated renal vasculitis. The glomerular lesion is identical with that of IgA nephropathy, which may be precipitated by exogenous antigens such as drugs, foods, or infectious organisms. Henoch-Schönlein syndrome follows an upper respiratory streptococcal infection. IgA deposition occurs in blood vessels of skin often similar to IgA nephropathy.

Clinical Features

Patient presents with nephritic syndrome, skin rashes on extensor surfaces of the arms, legs, and buttocks, painful joints, and gastrointestinal tract involvement (colicky abdominal pain with melena). Most patients do well, but a few progress to end stage renal disease. In adults, a rapidly progressive (crescentic) glomerulonephritis can occur.

HEMOLYTIC UREMIC SYNDROME

Hemolytic uremic syndrome (HUS) is characterized by hemolytic anemia, acute renal failure (uremia), and thrombocytopenia. HUS is the most common cause of acute renal damage in children.

Etiopathogenesis

Ingestion of contaminated food with Shiga toxin strain of *Escherichia coli* is most important cause of hemolytic uremic syndrome. Toxin is injurious to glomerular endothelial cells resulting in thrombotic microangiopathy. Subendothelial electron-lucent material causes narrowing of the lumen of glomerular capillaries. Swelling of the endothelial cells also contributes to narrowing of the lumen of glomerular capillary. Patient presents with hemorrhagic diarrhea and rapidly progressive renal failure. Schematic representation of hemolytic uremic glomerulopathy is shown in Fig. 22.27.

NEPHROTIC SYNDROME

Nephrotic syndrome is characterized by heavy proteinuria (≥3.5 g protein/24 hours), hypoalbuminemia resulting from heavy proteinuria, hyperlipidemia (compensatory synthesis of lipoproteins by liver), generalized edema resulting from decreased plasma oncotic pressure), hypercholesterolemia and lipiduria. Hematuria, hypertension or azotemia may or may not be present.

- Nephrotic syndrome occurs due to increased glomerular basement membrane permeability, permitting the urinary loss of plasma proteins, particularly low molecular weight proteins, such as albumin.
- Heavy proteinuria in nephrotic syndrome occurs due to damage to glomeruli by the following mechanisms: (a) damage to the integrity of the epithelial cells, as in minimal change disease or focal segmental glomerulosclerosis, (b) immune-complex deposition occurs within the capillary wall, as in membranous glomerulonephritis, (c) damage to the integrity of the basement membrane by excessive accumulation of abnormal membrane-like material demonstrated in diabetes mellitus, (d) deposition of extraneous substances (e.g. amyloid) occurs within the blood vessels, as in amyloid nephropathy.
- Patients excrete various essential metabolic products in the urine such as proteins, sugar, ketone bodies, immunoglobulins (recurrent infection), transferrin bound iron loss (iron deficiency anemia), antithrombin III, antiplasmin (renal vein thrombosis), thyroxine binding proteins, zinc binding proteins, and copper binding proteins.
- Urine analysis demonstrates heavy proteinuria (albumin, globulins), immunoglobulins, thyroxine binding protein, transferrin bound iron, vitamin D binding protein, zinc binding proteins, copper binding protein, antithrombin III, and antiplasmin.

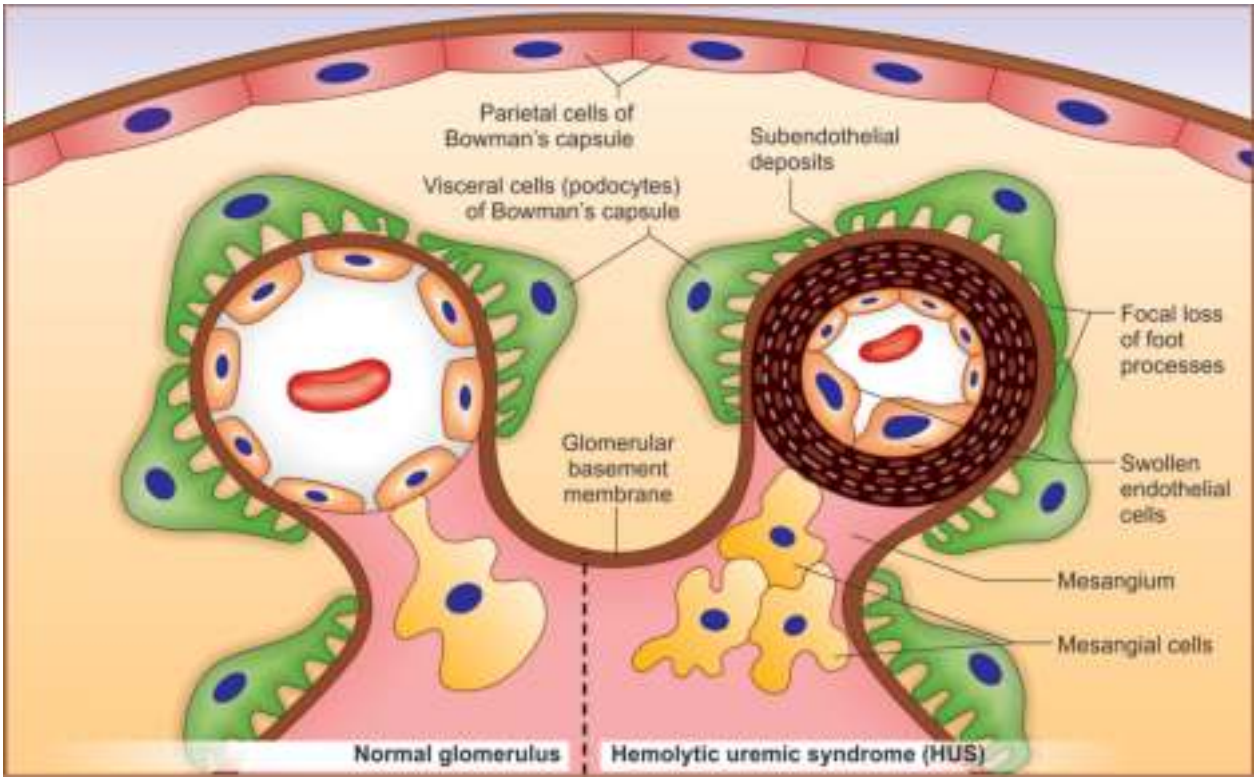


Fig. 22.27: Schematic representation of hemolytic uremic syndrome glomerulopathy. Hemolytic uremic syndrome (HUS) is characterized by progressive renal failure in children and adults that is associated with microangiopathic hemolytic anemia (nonimmune and Coombs' negative) and thrombocytopenia. Hematoxylin and eosin-stained section of renal biopsy shows capillary endothelial swelling, fibrin deposits in lumina and luminal narrowing within glomerulus. Focal loss of foot processes is present.

Light microscopy of urine reveals hyaline casts, oval fat bodies or pus cells.

- Oval fat bodies are lipid containing renal tubular epithelial cells. When urinary sediment examined under polarized light, the lipids in casts are seen as being doubly refractile or birefringent and displaying a symmetric 'Maltese cross pattern'.
- Nephrotic syndrome can be caused by primary glomerular diseases such as minimal change disease, focal segmental glomerulosclerosis, membranous glomerulonephritis membrano-proliferative glomerulonephritis, and systemic diseases (diabetes mellitus and systemic lupus erythematosus).
- Patients should be investigated by analyzing total serum proteins, albumin/globulin ratio, lipid profile, blood urea and serum creatinine, serum testing (HbsAg, HIV). Etiology of nephrotic syndrome varies in adults and children.
- Pathogenesis of nephrotic syndrome is shown in Fig. 22.28. Main causes of nephrotic syndrome in adults and children are given in Table 22.23. Glomerular diseases associated with nephrotic syndrome and its pathogenesis are given in Table 22.24.

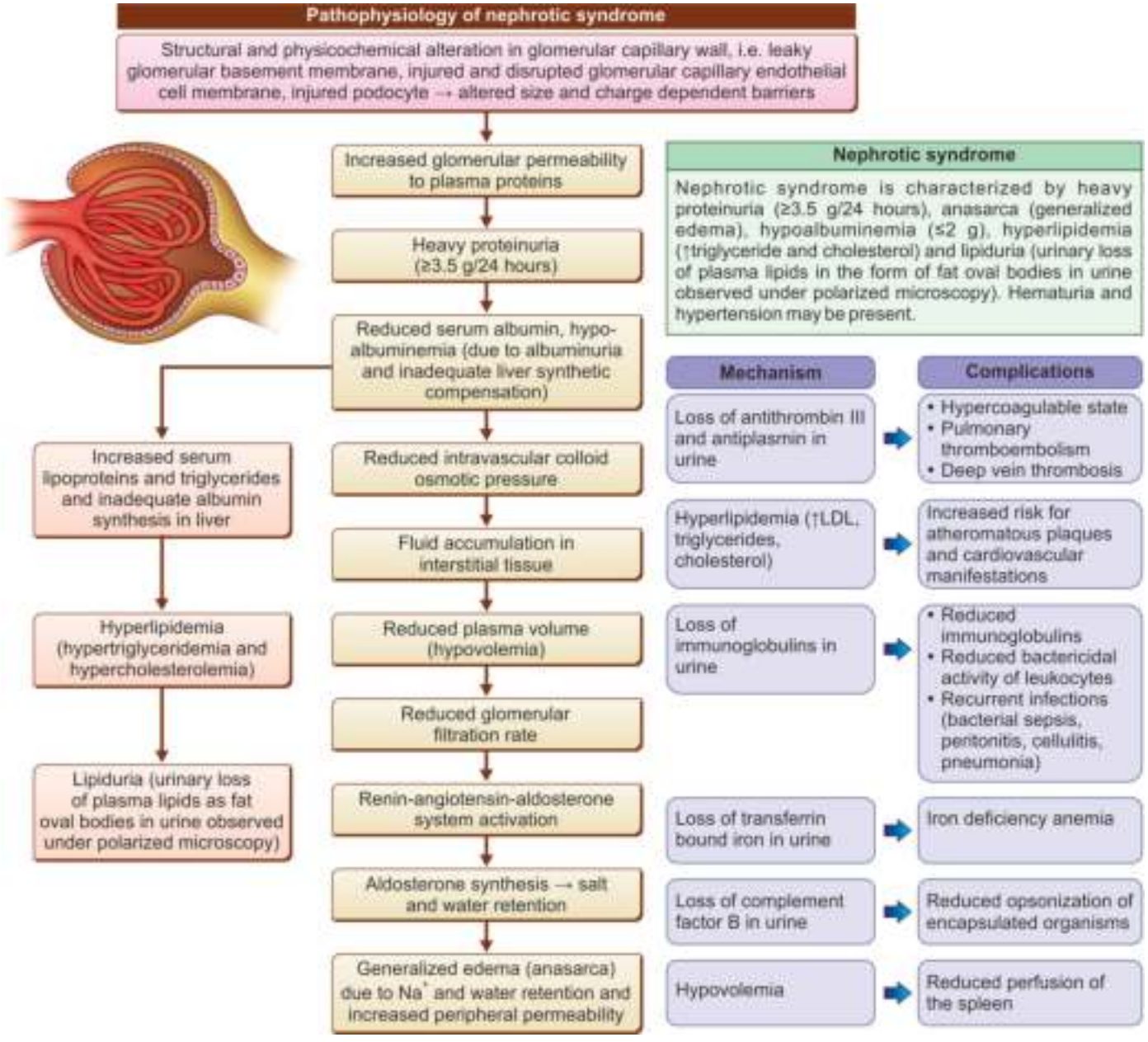


Fig. 22.28: Schematic representation of pathophysiology of nephrotic syndrome. The nephrotic syndrome is characterized by generalized edema, massive proteinuria (≥3.5 gm per day), hypoalbuminemia, hyperlipidemia and hypercholesterolemia. Hematuria, hypertension or azotemia may or may not be present. Nephrotic syndrome can be caused by primary diseases such as minimal change disease, focal segmental glomerulosclerosis, membranous glomerulonephritis; and systemic diseases (diabetes mellitus and systemic lupus erythematosus).

Table 22.23 Main causes of nephrotic syndrome in adults and children

Glomerular Diseases in Adults	Frequency (%)
Minimal change disease	10%
Membranous glomerulonephritis	20%
All forms of proliferative glomerulonephritis	15%
Focal glomerulosclerosis	10%
Membranoproliferative glomerulonephritis	5%
Diabetic nephropathy	40%
Glomerulopathy secondary to systemic disorders (amyloidosis and systemic lupus nephritis)	Relatively common

Glomerular Diseases in Children	Frequency (%)
Minimal change disease	60%
Membranous glomerulonephritis	5%
All forms of proliferative glomerulonephritis	10%
Focal glomerulosclerosis	10%
Membranoproliferative glomerulonephritis	Rare
Diabetic nephropathy	Uncommon
Glomerulopathy secondary to systemic disorders (amyloidosis and systemic lupus nephritis)	5%

Table 22.24 Glomerular diseases commonly associated with nephrotic syndrome

Light Microscopy	Immunofluorescence Microscopy	Electron Microscopy	Prognosis
Minimal change disease (MCD)			
Glomeruli appear normal (histochemistry positive for fat stains in glomeruli and tubules)	No pattern	Effacement of the podocytes of visceral layer of Bowman's capsule	Responsive to corticosteroid therapy with excellent prognosis
Membranous glomerulonephritis (MGN)			
Diffuse thickening of GBM, Jones methenamine silver stain demonstrates spikes and domes due to immune complex deposition in subepithelial region of glomeruli	Granular pattern: IgG and C3	Subepithelial deposits and fusion of podocytes of visceral layer of Bowman's capsule	Prognosis is poor as a patient progressing to end stage disease (40–50%)
Focal segmental glomerulosclerosis (FSGS)			
Detachment of epithelial cells from GBM is the hallmark of disorder	IgM and C3 in some cases	Fusion of podocytes, but no electron dense deposits	Poor prognosis as progresses to end stage disease within 10 years in 50–80% of cases
Membranoproliferative glomerulosclerosis (MPGS)			
Type 1 MPGN: Diffuse thickening of GBM (Jones silver stain shows tram-track appearance due to ingrowth of mesangial cells between endothelial cells and GBM)	Granular pattern (IgG, IgM, C3, C1q)	Electron dense deposits in subendothelial region	Prognosis is poor. About 50% of patients develop chronic renal failure within 10 years
Type 2 MPGN: Tram-track pattern not prominent with Jones silver stain	Granular pattern (IgG, IgM, C3, C1q)	Electron dense deposits within GBM	
Diabetic nephropathy (diffuse/glomerulosclerosis and Kimmelstiel Wilson disease)			
Diffuse glomerulosclerosis and nodular glomerulosclerosis	Trapping of plasma proteins	No electron dense deposits, but shows fusion of podocytes and thickening of GBM	Untreated patients progress to end stage disease
Amyloid nephropathy			
Thickening of GBM due to amyloid deposit, demonstrated by Congo red and apple green birefringence similar to color of Granny Smith apple	Nonspecific trapping of proteins	Amyloid fibrils in mesangial region, GBM and renal tubules	Renal failure is common cause of mortality
Eclampsia of pregnancy			
Glomeruli are bloodless, endothelial cells swollen	Nonspecific deposits of IgG and IgM, fibrin	Endothelial cells show vacuolation, widening of spaces between endothelial spaces	Reversible disorder after delivery of women

Contd...

Table 22.24 Glomerular diseases commonly associated with nephrotic syndrome (*Contd...*)

Light Microscopy	Immunofluorescence Microscopy	Electron Microscopy	Prognosis
Lupus nephritis (systemic lupus erythematosus)			
Type IV lupus nephritis shows endocapillary and/or extracapillary proliferation in ≥50% of glomeruli (wire-loop lesion)	Deposits in mesangial and glomerular loop	Deposits in mesangial and endothelial region	Nephrotic syndrome progresses to chronic renal failure
Type V lupus nephritis shows thickening of GBM and capillary loops; and increased cellularity of mesangial cells	Deposits in mesangial and glomerular loop	Numerous deposits in sub-epithelial region, and scattered deposits in mesangial regions	Nephrotic syndrome progresses to chronic renal failure
Alport's syndrome			
Foam cells are present in glomeruli and tubules. There is increased mesangial matrix. There is increased thickening of GBM with splitting	No definite pattern. Deposition of IgM, C3	No electron dense deposits. GBM shows lucency	End stage renal failure in second or third decade

MINIMAL CHANGE DISEASE

Minimal change disease (MCD) is glomerular disease that causes nephrotic syndrome in 70–90% of young children (peak 2–3 years with M > F) and adults (peak 80–90% with M = F) in 10–15% of cases.

- Minimal change disease in adults can also occur either primary or secondary to Hodgkin's disease (nodular sclerosis), chronic lymphocytic leukemia, or atopic allergies. Rarely familial minimal change disease occurs due to mutation in NPHS2 and dysferlin genes.
- Essential diagnostic features of minimal change disease include normal appearance of glomeruli on light and immunofluorescence microscopy, extensive effacement of foot processes (podocytes) on electron microscopy (hallmark of disease), highly selective proteinuria, mainly albuminuria and response to corticosteroid therapy in 90–95% of cases.
- Lipid accumulation is present in renal tubular cells referred to as lipoid nephrosis. Minimal change disease is also known as idiopathic nephrotic syndrome, nil disease, lipoid nephrosis and foot process disease. Urinary CD80 biomarker level is significantly elevated in MCD. Minimal change disease is shown in Fig. 22.29.

Pathophysiology

Extensive effacement of foot processes (podocytes) occurs due to epithelial injury with loss of glomerular anionic charge in minimal change disease.

- Minimal change disease is usually idiopathic in children. Secondary minimal change disease occurs due to upper respiratory viral infection, atopic allergy, altered immune response (type 4 hypersensitivity reaction with synthesis of lymphokines by T cells), immunological diseases and neoplasam, e.g. Hodgkin's disease (nodular sclerosis), non-Hodgkin's lymphoma and chronic lymphocytic leukemias in all age groups.
- Lymphokines synthesized by T cells cause ballooning of visceral epithelial cells resulting in effacement of foot processes (podocytes) and loss of charge dependent barrier of glomerular basement membrane. Loss of polyanionic proteoglycans leads to massive proteinuria (≥3.5 g/24 hours).

Clinical Features

Patient with minimal change disease presents with selective massive proteinuria ≥3.5 g/24 hours, causing hypoalbuminemia, lipiduria and severe generalized edema. Hypercholesterolemia occurs due to increased synthesis of lipoproteins by liver to compensate massive proteinuria in these cases. Patients usually do not develop hypertension, hematuria and azotemia. Adult patients can present with acute renal insufficiency.

Pathology Pearls: Minimal Change Disease

Gross Morphology

In minimal change disease, enlarged, waxy yellow cortex is observed due to lipid accumulation in proximal tubules.

Light Microscopy

Light microscopy of renal biopsy reveals normal glomeruli. Minimal change disease lacks cell proliferation in glomerular cells and alterations in glomerular basement membrane. Protein reabsorption droplets are observed in tubules. Histology of minimal change disease is shown in Fig. 22.30.

Histochemistry

Fat stains demonstrate presence of lipid in the tubules and glomeruli.

Immunofluorescence Microscopy

Immunofluorescence study is insignificant in minimal change disease. Occasionally, it shows minimal amounts of IgM in the mesangial region.

Electron Microscopy

- Electron microscopy of renal biopsy demonstrates extensive effacement of podocyte foot processes.
- Foot processes retract into cell bodies, not actually fusion occurs associated with weakening of slit pore membranes. This retraction of foot processes presumably results from extensive cell swelling and occurs in virtually all cases of proteinuria in the nephrotic range.
- Microvillous transformation of epithelial cells and cyst formation are demonstrated.

Treatment and Prognosis

Primary responders are minimal change disease patients, who have complete remission after a single course of corticosteroid therapy. Approximately 90–95% of children have complete remission after 8 weeks of steroid therapy, and 80–85% of adults will achieve complete remission after corticosteroid therapy course of 20–24 weeks.

- Immunosuppressive agents are administered in steroid resistant cases (cyclophosphamide, rituximab, abatacept). Relapses are common and often lead to corticosteroid dependence in these patients.
- Rarely, if ever, minimal change disease leads to end stage renal disease without development of focal segmental glomerulosclerosis. Adults may develop acute renal failure, and usually recover.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis (FSGS) is the histologic pattern of glomerulosclerosis affecting some glomeruli and only part of glomerular capillary tuft involving >50% of glomeruli and associated with nephrotic syndrome (heavy proteinuria ≥3.5 g/24 hours, hypoproteinemia, hypercholesterolemia and generalized edema) in adults (40%) and children (20%) and progressive renal failure. FSGS is more common in people over 45 years old and in men than in women.

- Glomeruli are involved without inflammation due to immune complex deposits. Glomeruli appear to adhere to Bowman's capsule. FSGS begins in cortico-medullary region.

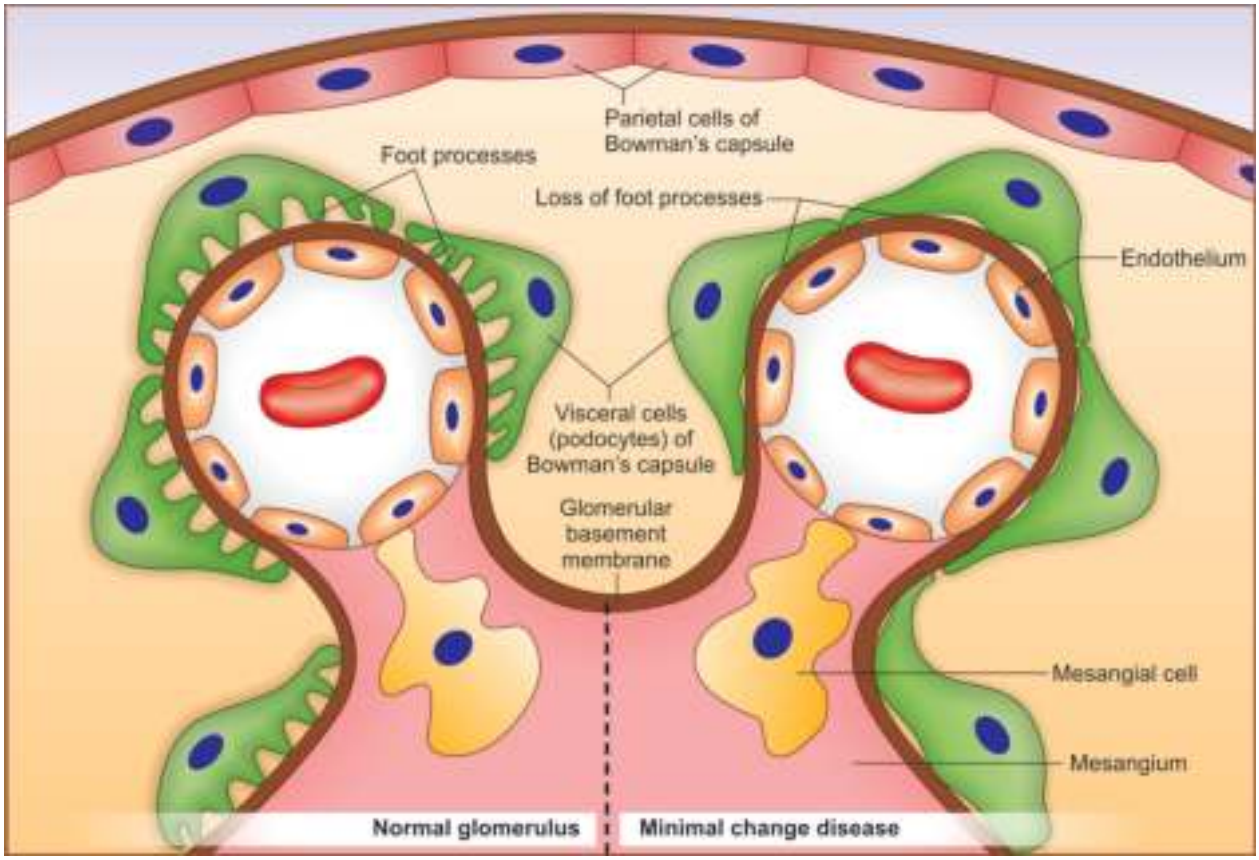


Fig. 22.29: Schematic representation of minimal change disease (MCD). Minimal change disease, also known as lipoid nephrosis or nil disease. Hematoxylin and eosin-stained renal biopsy section reveals normal glomeruli. Immunofluorescence microscopy is typically negative. Three hallmarks of minimal change disease are seen on electron microscopy: diffuse loss of podocyte foot processes of visceral epithelial cells of Bowman's capsule, vacuolation and appearance of microvilli. Patient presents with signs and symptoms of nephrotic syndrome much quicker than they would with other glomerular diseases. Nephrotic syndrome is characterized by massive proteinuria, generalized edema, hypoproteinemia, hypercholesterolemia in children but it can occur in adults. On laboratory testing, profound proteinuria, oval fat bodies may be observed.

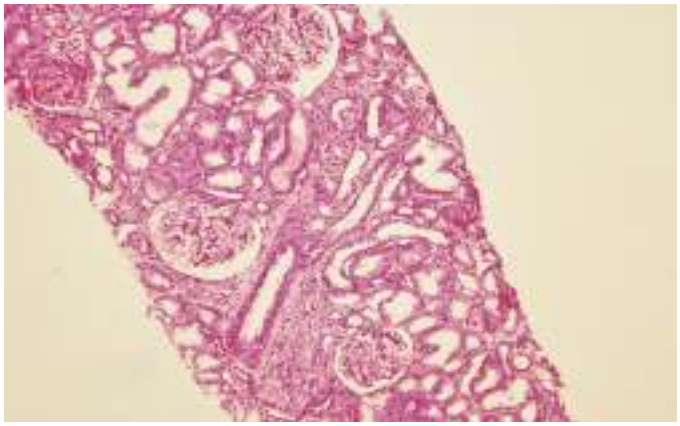


Fig. 22.30: Histology of minimal change disease. Those with minimal change disease experience the signs and symptoms of nephrotic syndrome much quicker than they would with other glomerular diseases. Hematoxylin and eosin-stained renal biopsy section reveals normal glomeruli. Immunofluorescence microscopy is typically negative. Three hallmarks of minimal change disease seen on electron microscopy: diffuse loss of podocyte foot processes of visceral epithelial cells of Bowman capsule, vacuolation and appearance of microvilli (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

- FSGS is characterized by detachment of visceral epithelial cells of Bowman capsule from glomerular basement membrane (hallmark of disease), hyalinosis, adhesions formation, segmental scarring (segmental obliteration of glomerular capillaries), involving a part of the glomerulus (segmental) at tubular pole, and some glomeruli but not all glomeruli sampled observed in H&E stained renal biopsy section. Focal tubular atrophy with interstitial fibrosis, hyaline thickening of afferent arterioles are observed.
- Other histologic features of FSGS include intramembranous hyaline deposits (PAS+, Masson's trichrome stain and Jones methenamine silver stain negative), endocapillary foam cells or lipid droplets in focal glomeruli, mild mesangial hypercellularity, that becomes hypocellular in advanced lesion. It is worth mentioning that the defining glomerular lesions in FSGS cases may not be sampled in renal needle core biopsy due to focal nature of FSGS.
- Patient can present with the clinical manifestations of nephrotic syndrome or associated with hematuria, hypertension and/or renal insufficiency.
- Tip lesion histologic variant of FSGS manifests with lower baseine serum creatinine associated with excellent response to treatment and lowest rate of progression of FSGS.

Etiology

Focal segmental glomerular sclerosis occurs due to adaptative response to several stimuli resulting in glomerular hypertension, hyperfiltration and eventual glomerular hypertrophy in the settings of diabetes mellitus, hypertension, obesity, renal aplasia, hypoplasia or dysplasia, reflux nephropathy, renal artery stenosis, cholesterol emboli, and vascular disease. On histologic examination, glomeruli are large with predominance of perihilar scarring, and partial foot process effacement.

- **Gene mutations:** Mutations in several genes encoding diaphragm proteins, cell membrane proteins, cytoskeleton proteins, mitochondrial proteins, nuclear proteins; and lysosomal proteins lead to loss of integrity of glomerular filtration barrier resulting in FSGS.
- **Circulating glomerular permeability factors/cytokines:** Primary FSGS occurs due to the presence of circulating glomerular permeability factors/cytokines such as cardiotrophin-like cytokine factor 1, apoA1b, anti-CD40 antibodies and suPAR (soluble urokinase-type plasminogen activator receptor); which cause effacement of foot processes and massive proteinuria.
- **Viruses role:** FSGS can be caused by HIV, parvovirus B19, cytomegalovirus, Epstein-Barr virus, hepatitis C virus (HCV), and Simian virus 40.
- **Nephrotoxic drugs:** FSGS can be caused by drugs such as heroin, lithium, pamidronate, interferon, mTOR inhibitors; and anabolic steroids can induce focal segmental glomerulosclerosis.

Pathogenesis

The pathogenesis of focal segmental glomerular sclerosis involves a complex interplay of several cell types including podocytes, glomerular endothelial cells, and the glomerular basement membrane resulting in massive proteinuria and subsequent development of focal sclerosing lesions.

- Podocytes are terminally differentiated cells that provide structural support to the glomerulus and are essential in maintaining an intact glomerular filtration barrier essential to prevent nephrotic range proteinuria.
- FSGS is broadly categorized into primary (idiopathic) and secondary forms, and such distinction carries both prognostic and therapeutic implications. Renal biopsy from uninvolved glomeruli sometimes leads to a misdiagnosis of FSGS.
- Patients progress to end stage renal disease in less than a year. In reflux nephropathy, reduction in renal mass causes adaptive stress on the reduced number of nephrons resulting in increased glomerular pressure and filtration and enlargement of glomeruli due to functional overwork of glomeruli.
- Possible mechanisms include T cell-mediated circulating permeability factor, TGF-β mediated cellular proliferation and mesangial matrix synthesis, and vacuolization of visceral epithelial cells (podocytes), associated with genetic mutations.

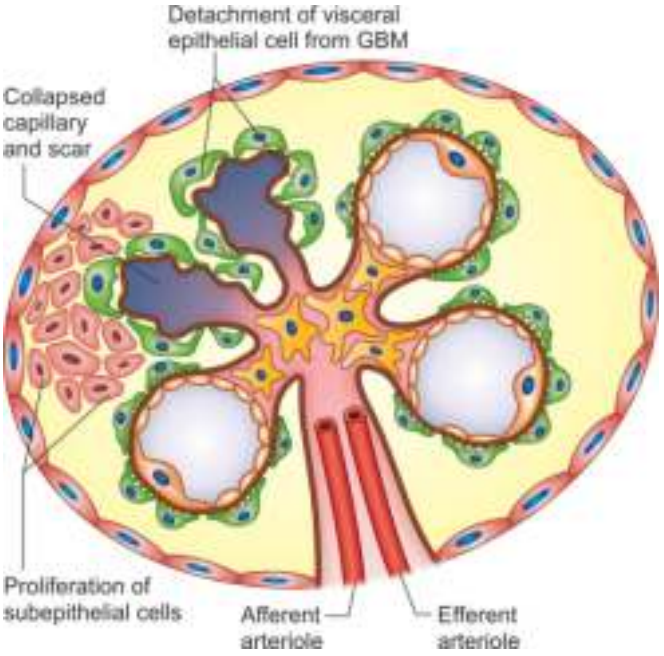


Fig. 22.31: Schematic representation of focal segmental glomerulosclerosis (FSGS). Detachment of epithelial cells from glomerular basement membrane (GBM) is hallmark of focal segmental glomerulosclerosis observed in hematoxylin and eosin-stained renal biopsy section. In FSGS, some but not all of the glomeruli (focal) are affected in one part of the glomerulus (known as a segment (segmental) with formation of scar (sclerosis) resulting in the name focal segmental glomerulosclerosis. FSGS affects both children and adults but is more common in people over 45-year-old. It occurs more often in men than in women.

Diagrammatic representation of focal segmental sclerosis is shown in Fig. 22.31.

Clinical Course

Patient presents with nephrotic syndrome in adults (30%) and children (10%) and renal insufficiency. FSGS may be associated with hematuria, and hypertension, which does not respond well to corticosteroids treatment. Approximately 60–80% of FSGS patients develop end stage renal disease (renal failure) within 10 years. Recurrence of FSGS is common after renal transplant. Approximately 60–80% of patients develop end stage renal disease (renal failure) within 10 years. Renal biopsy is essential to establish diagnosis.

Pathology Pearls: Focal Segmental Glomerulosclerosis

Light Microscopy

Hematoxylin and eosin-stained renal biopsy/tissue section in focal segmental glomerulosclerosis (FSGS), glomerulus shows foam cells in the mesangium. **Detachment of visceral epithelial cells from GBM is hallmark of disease**. Glomerular basement membrane shows thickening and wrinkling. Collapse and hyalinization of glomeruli lead to focal segmental glomerulosclerosis. Working classification of FSGS is given in Table 22.25. Histology of FSGS is shown in Figs 22.32 and 22.33.

- **FSGS perihilar variant:** The sclerosing lesion in perihilar variant is located at the vascular pole of the glomerulus and usually demonstrated in adaptive FSGS due to increased pressure in the glomerulus, which is located in close proximity to the afferent arteriole. Effacement of foot processes is mild and resulting in proteinuria in subnephrotic range and relatively normal serum albumin levels.
- **FSGS tip lesion variant:** The sclerosing lesion in tip lesion variant is located at the tubular pole of the glomerulus with effacement of foot processes, which usually affects Caucasians. Patient presents with abrupt onset of nephrotic syndrome with lower baseline serum creatinine level and lowest progressive disease, and responds well to corticosteroids treatment and associated with excellent prognosis. Histology of tip lesion variant of FSGS is shown in Fig. 22.34.
- **FSGS cellular variant:** This is the least common variant of FSGS, characterized by hypercellular glomerulus including endocapillary and glomerular epithelial cell hyperplasia. Patient presents with diffuse foot process effacement and full-blown nephrotic syndrome.
- **FSGS collapsing variant:** Focal segmental glomerulosclerosis, collapsing variant is characterized by hyperplasia and hypertrophy of podocytes (visceral glomerular epithelial cells) resulting in collapse of the glomerular tuft.
  - Focal segmental glomerulosclerosis, collapsing variant occurs in the setting of as infections (HIV, parvovirus B19, HCV, cytomegalovirus), drugs (bisphosphonates, heroin, alpha and beta interferons, anabolic steroids), vascular and metabolic disease (thrombotic microangiopathy, severe diabetic nephropathy, severe vascular and microvascular disease), genetic disorders (APOL1 high risk alleles, mitochondrial abnormalities), autoimmune disorders (post-transplant, recurrent and *de novo*).
  - Patient presents with heavy proteinuria with the lowest rate of remission, and associated with worst prognosis.
- **FSGS, not otherwise specified (NOS):** Focal segmental glomerulosclerosis, not otherwise specified is the most common subtype of FSGS and does not fit into any other morphologic forms of FSGS. It presents with a variable degree of effacement and proteinuria.

Histochemistry

Periodic acid–Schiff (PAS) stain highlights acellular, hyaline material distributed in segmental manner.

Immunofluorescence Microscopy

Glomeruli usually lack immune complex deposits. However, IgM and C3 deposits can be demonstrated in some cases of focal segmental glomerulosclerosis.

Electron Microscopy

On electron microscopy, glomeruli show fusion of podocytes but lack electron dense deposits. Retraction of foot processes of visceral epithelial cells is present.

Table 22.25 Working classification of focal segmental glomerulosclerosis (FSGS)

Working Classification of FSGS	Histologic Features	Possible Prognostic Implication
FSGS perihilar variant	Sclerosis and hyalinosis of glomeruli at vascular pole	May reflect a secondary type of FSGS
FSGS tip lesion variant	Sclerosis/adhesion of glomeruli at proximal tubule pole	Better prognosis
FSGS cellular variant	Endocapillary proliferation, often visceral hyperplasia	Early-stage lesion related to tip
FSGS collapsing variant	Collapse of glomerular tuft, visceral epithelial hyperplasia	Poor prognosis
FSGS, NOS (not otherwise specified)	Segmental sclerosis of glomeruli	Typical course

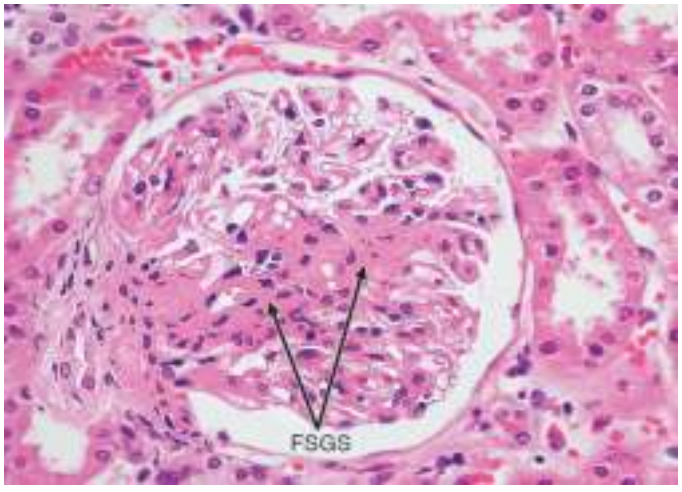


Fig. 22.32: Histology of focal segmental glomerulosclerosis (FSGS). In hematoxylin and eosin-stained renal biopsy section, FSGS appears to adhere to Bowman's capsule, which begins in corticomedullary region, intramembranous hyaline deposits (PAS+, trichrome stain and Jones methenamine silver stain negative), mesangial sclerosis in lobules, endocapillary foam cells or lipid droplets in focal glomeruli, mild mesangial hypercellularity, that becomes hypocellular in advanced lesion. Focal tubular atrophy with interstitial fibrosis, hyaline thickening of afferent arterioles. It is worth mentioning that the defining glomerular lesions may not be samples in renal needle core biopsy due to their focal nature. Hematoxylin and eosin-stained section shows sclerosis of glomerulus. Detachment of epithelial cells from glomerular basement membrane (GBM) is hallmark of disease (arrows) (400X).

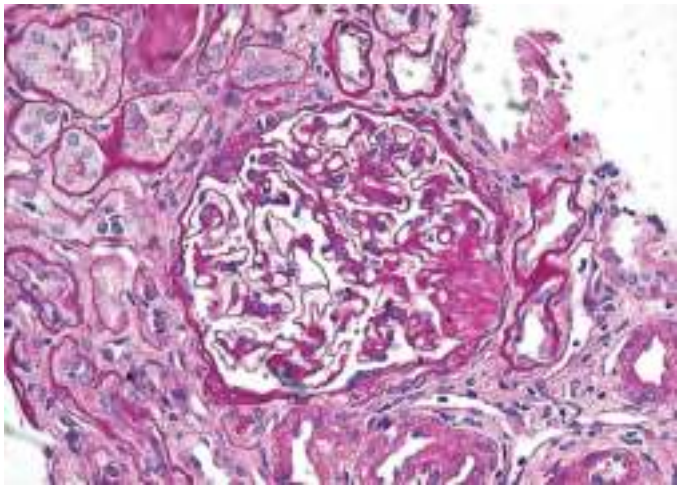


Fig. 22.33: Histology of focal segmental glomerulosclerosis (FSGS). FSGS is a histologic pattern of glomerulosclerosis affecting some glomeruli and only part of glomerular capillary tuft involving more than 50% of glomeruli and associated with heavy proteinuria ≥3.5 g/24 hours and progressive renal failure. Hematoxylin and eosin-stained renal tissue section shows focal and segmental glomerulosclerosis in lobules that appear to adhere to Bowman's capsule, which begins in the corticomedullary region. Focal tubular atrophy with interstitial fibrosis and hyalinization of afferent arterioles are present. It is worth mentioning that defining glomerular lesions may not be sampled in needle core biopsy due to focal nature of the disease (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

Treatment

Glucocorticoid therapy is the first line of treatment in children and adults with focal segmental glomerulosclerosis (FSGS) for a period of 3–6 months. Patients, resistant or intolerant to corticosteroids, are treated with immunosuppressive therapy with calcineurin inhibitors such as mycophenolate mofetil, or rituximab and tacrolimus (6–12 months).

MEMBRANOUS GLOMERULONEPHRITIS

Membranous glomerulonephritis (MGN) is the most common cause of insidious onset of nephrotic syndrome in adults (30–50 years) and in children. Its etiology can be primary or secondary.

- Membranous glomerulonephritis (MGN) occurs due to deposition of immune complex (electron dense) in glomerular basement membrane but not in the mesangial region accompanying diffuse thickening of glomerular basement membrane (5–10 times of normal GBM), without mesangial sclerosis, inflammatory cells and proliferation of resident glomerular cells. Proximal convoluted tubules contain hyaline droplets reflecting protein reabsorption. Progression of the disease results in glomerular basement membrane thickening, narrow capillary lumina, mesangial sclerosis and glomerulosclerosis.
- MGN is a slowly progressive disorder affecting males than females, which shows a little response to corticosteroid therapy. Prognosis is highly variable depending on its etiology.

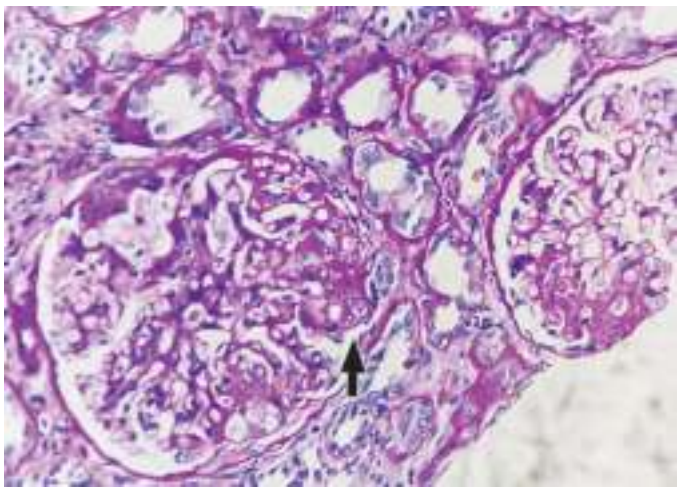


Fig. 22.34: Histology of tip lesion variant of focal segmental glomerulosclerosis (FSGS). Focal segmental glomerulosclerosis is histologically characterized by segmental sclerosis that involves a part of the glomerulus and only affects one but not all glomeruli renal biopsies. Patient with FSGS can present with the clinical manifestations of nephrotic syndrome or with hematuria, hypertension or renal insufficiency. Histologically, FSGS is classified into five variants: perihilar, tip, cellular, collapsing and not otherwise specified (NOS). Hematoxylin and eosin-stained renal tissue section examination in FSGS tip lesion shows segmental glomerulosclerosis (segmental obliteration of glomerular capillaries involving the tubular pole of glomerulus, hyalinosis, adhesions formation FSGS with tip lesion variant manifests with lower baseline serum creatinine associated with excellent response to treatment and lowest rate of progression) (400X).

- Histologic variants of membranous glomerulonephritis include MGN with crescents, MGN with antitubular basement membrane nephritis, transplant associated MGN and MGN with superimposed renal vein thrombosis.
- Renal biopsy is done to confirm the diagnosis of MGN. Immunosuppressive therapy plays a major role in the treatment of this disease.
- Jones methenamine silver-stained renal biopsy section shows characteristic black material in glomerular basement membrane that appears **'spikes' projections** around the capillary loops and '**domes**' due to immune complex deposits. Electron microscopy reveals effacement of podocytes.
- Antiphospholipase A2 receptor (PLA2R) antibodies are highly specific for the diagnosis of primary membranous glomerulonephritis.

Clinical Pearls: Membranous Glomerulonephritis

- Membranous glomerulonephritis is the most important cause of nephrotic syndrome in adults.
- Diffuse thickening of glomerular basement membrane occurs (5–10 times of normal GBM) due to *in situ* immune complexes (electron dense) in glomerular basement membrane but not in mesangial region.
- Most cases of primary membranous glomerulonephritis have PLA2R antibody positive deposits.
- Extensive subepithelial/intramembranous deposits are demonstrated by electron microscopy.
- Prognosis of MGN is highly variable depending on etiology of the disease.
- About 25% of MGN patients undergo spontaneous remission without treatment.
- Approximately 50% of patients have persistent proteinuria in nephrotic range and hematuria.
- Loss of antithrombin III and antiplasmin in urine may result in renal vein thrombosis.
- About 25% of patients develop chronic renal failure.
- Renal functions are markedly impaired.

Pathophysiology

Membranous glomerulonephritis may be primary or secondary. Membranous glomerulonephritis (MGN) may develop in the renal allograft either *de novo* or as a recurrence. These two forms of MGN may have different pathogenesis mechanisms, with different IgG subclass profiles in the immune deposits. Membranous glomerulonephritis is shown in Fig. 22.35. Etiology of membranous glomerulonephritis is given in Table 22.26.

- **Primary or idiopathic MGN:** Most cases of primary membranous glomerulonephritis are idiopathic (75% of adults and 20% of children) and are linked to HLA and caused by antibodies to renal autoantigen. Disease is considered the human model of Heymann nephritis, which in rat is produced by antibody to megalin antigen complex GP-330 on basal surface of visceral epithelial cells (megalin is homologous to LDL receptor), and brush border of epithelium of proximal convoluted tubules. Incidence of MGN is highest in teenagers and young adults.
- **Secondary MGN:** Secondary membranous glomerulonephritis is associated with immune complex deposits in glomerular basement membrane in diffuse granular fashion (usually referred to as lumpy bumpy), in the subepithelial region of glomerular basement membrane (GBM) in the setting of cancers (breast, lung, colon, melanoma and lymphomas), infectious agents (malaria, leprosy, syphilis, hepatitis B virus, filariasis, schistosomiasis), drugs (captopril, penicillamine, gold, mercury, trimethadione), and autoimmune disorders such as systemic lupus erythematosus, Hashimoto's thyroiditis (thyroglobulin), type 1 diabetes mellitus, angiofollicular lymph node hyperplasia and sarcoidosis or rarely rheumatoid arthritis. Proteinuria may occur due to glomerular injury induced by C5b–C9 membrane attack complex (MAC) in these cases.

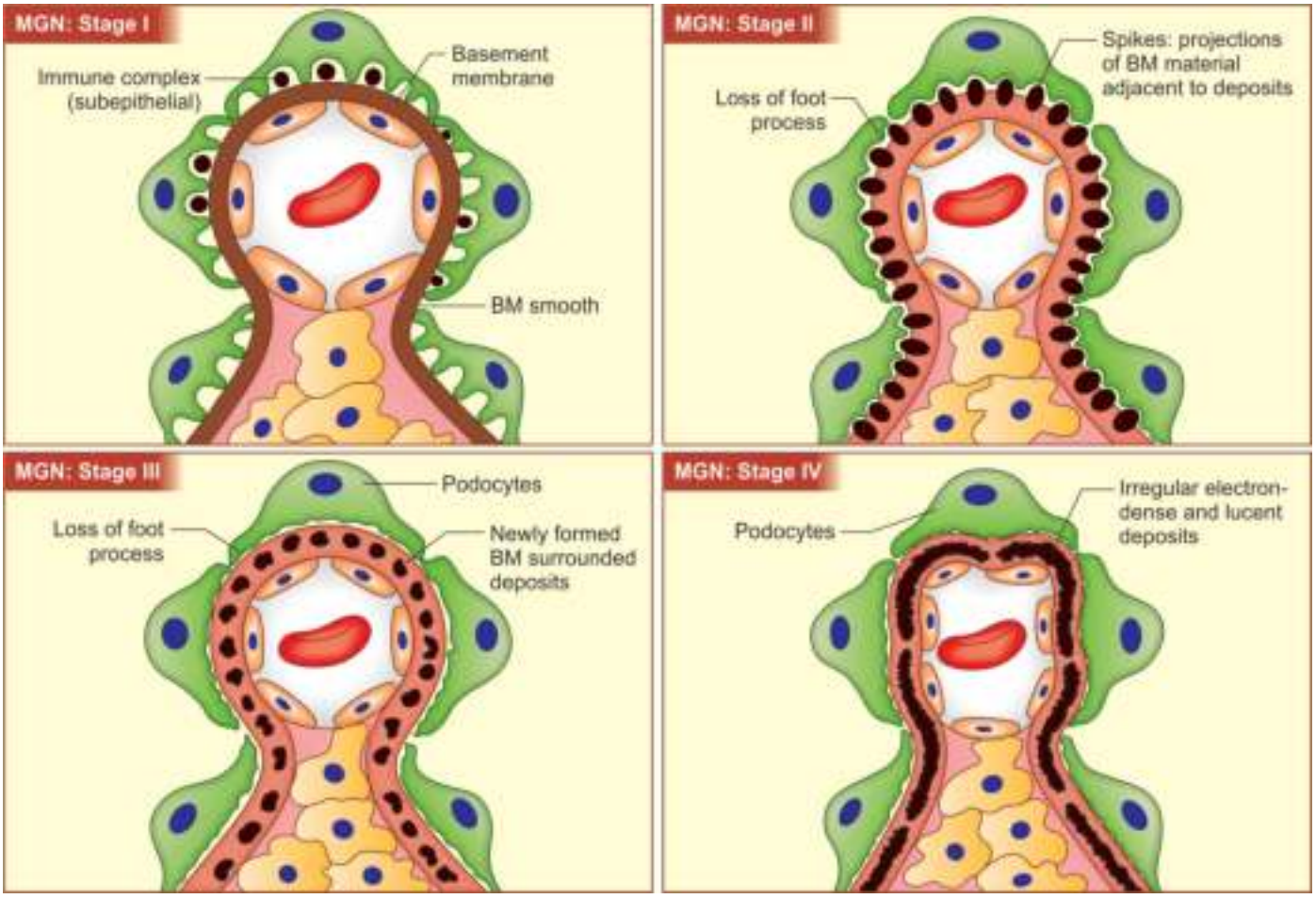


Fig. 22.35: Schematic diagram of membranous glomerulonephritis (MGN). Membranous glomerulonephritis is important cause of nephrotic syndrome. Its etiology can be primary or secondary. Disease occurs due to deposition of immune complex in subepithelial regions accompanying changes in the glomerular basement membrane. Renal biopsy is done to confirm the diagnosis. Immunosuppressive therapy plays a major role in the treatment of this disease. Electron microscopy reveals effacement of podocytes.

Table 22.26 Etiology of membranous glomerulonephritis

Categories	Examples		
Primary/idiopathic membranous glomerulonephritis (85%)			
In situ immune complex-mediated glomerulonephritis	Antibodies directed against GP-330 antigen present on brush border of epithelium of proximal convoluted tubules and visceral epithelium		
Secondary membranous glomerulonephritis (15%)			
Infectious agents	▪ Malarial parasite ▪ Mycobacterium leprae bacilli	▪ *Treponema pallidum* ▪ *Wuchereria bancrofti*	▪ *Schistosoma haematobium* ▪ Hepatitis B and C
Malignant tumor antigens	▪ Breast carcinoma ▪ Lung carcinoma	▪ Colon carcinoma ▪ Lymphoma	▪ Malignant melanoma
Drugs	▪ Gold ▪ Mercury	▪ Penicillamine ▪ Captopril	▪ Trimethadione
Autoimmune disorders	▪ Systemic lupus erythematosus (renal involvement giving pattern of membranous glomerulonephritis in 10% of cases)	▪ Hashimoto's thyroiditis ▪ Diabetes mellitus type 1	▪ Sarcoidosis ▪ Rheumatoid arthritis

Pathology Pearls: Ehrenreich and Churg Classification of Membranous Glomerulonephritis (MGN)

According to Ehrenreich and Churg classification of MGN is based on the appearance of electron-dense deposits.

- **MGN Stage 1:** MGN stage 1 shows small, sparse, electron-dense deposits on the visceral epithelial side of glomerular basement membrane (GBM).
- **MGN Stage 2:** MGN stage 2 shows larger electron-dense deposits causing glomerular basement membrane thickening along with effacement of foot processes giving characteristic '**spiles and domes**' appearance.
- **MGN Stage 3:** MGN stage 3 shows feature of stage 2 plus intramembranous coarse granular electron-dense deposits with formation of new glomerular basement membrane.
- **MGN Stage 4:** MGN stage 4 shows irregular electron-dense deposits, dissolution of electron-dense deposits. disrupted and sclerosed glomerular basement membrane.

Clinical Features

Membranous glomerulonephritis is the most common cause of nephrotic syndrome. Patient presents with insidious onset of nephrotic syndrome and occasionally hematuria and hypertension. One must rule out and treat secondary causes of MGN.

- Prognosis is variable and related to etiology such as spontaneous remission without treatment (33%). One-third of patients are stabilized with treatment.
- About 33% of patients develop persistent proteinuria, hypertension (50%), renal vein thrombosis (due to loss of antithrombin III and antiplasmin in urine) and hematuria, impairment of renal function (50%), and chronic renal failure (25%).
- Long-term prognosis of the disease may be improved by treating patients with high-dose corticosteroids and alkalinizing agents, e.g. cyclophosphamide, although associated with side effects.

Pathology Pearls: Membranous Glomerulonephritis

Light Microscopy

- Examination of hematoxylin and eosin-stained renal biopsy section reveals uniform diffuse thickening of glomerular wall without hypercellularity, without mesangial sclerosis and without inflammatory cells.
- Proximal convoluted tubules contain hyaline droplets, reflecting protein reabsorption.
- Progression of the disease leads to thickening of glomerular basement membrane, narrow capillary lumina, mesangial sclerosis and glomerulosclerosis. Histology of membranous glomerulonephritis (MGN) is shown in Figs 22.36 to 22.38. Comparison of stages of membranous glomerulonephritis by light, immunofluorescence and electron microscopy is given in Table 22.27.

Histochemistry

Jones methenamine silver stain highlights the proteinaceous basement membranes as black. It shows duplication of glomerular basement membrane with **'spikes and domes' appearance**. It results from the extension of basement membrane between and around the immune complex deposits. The spikes are basement membrane material, and the domes denoting immune complex deposits. Jones methenamine silver stain in membranous glomerulonephritis (MGN) is shown in Fig. 22.39.

Immunofluorescence Microscopy

Immunofluorescence microscopy of renal biopsy shows granular diffuse peripheral deposits of IgG or C3 also C5b–C9 or IgA or C4d outlining the glomerular capillary loops. It is a feature of immune complex-mediated glomerulonephritis.

Treatment

Clinical course of membranous glomerulonephritis makes it difficult to evaluate therapy. Patients are treated by corticosteroids, chlorambucil or cyclophosphamide chlorambucil or cyclophosphamide for six months. In case, remission is not achieved, patients may be treated by cyclosporine, adrenocorticotropic hormone, mycophenolate mofetil and rituximab. Treatment is effective on post-transplant recurrences of disease.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Membranoproliferative glomerulonephritis (MPGN) is also known as hypocomplementemic C3, lobular or mesangiocapillary glomerulonephritis.

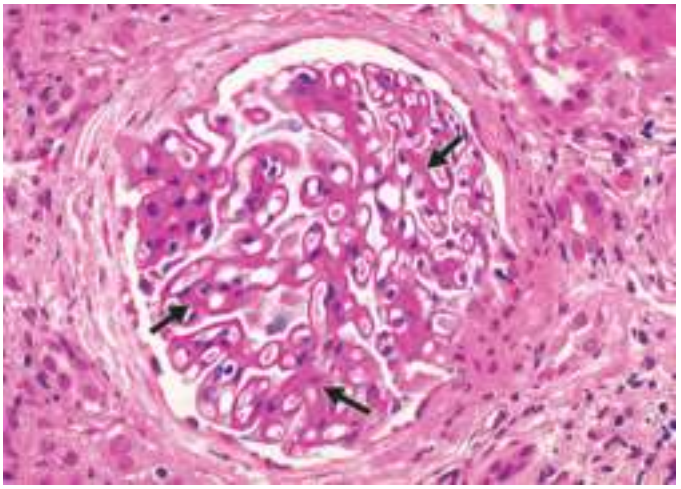


Fig. 22.36: Histology of membranous glomerulonephritis (MGN). Hematoxylin and eosin-stained renal biopsy section reveals uniform diffuse thickening of glomerular capillary walls without sclerosis and without inflammatory cells. Proximal convoluted tubules contain hyaline droplets, reflecting protein reabsorption. With progression of disease, glomerular basement membrane thickening, narrow glomerular capillary lumina, mesangial sclerosis and glomerulosclerosis (arrows) (400X).

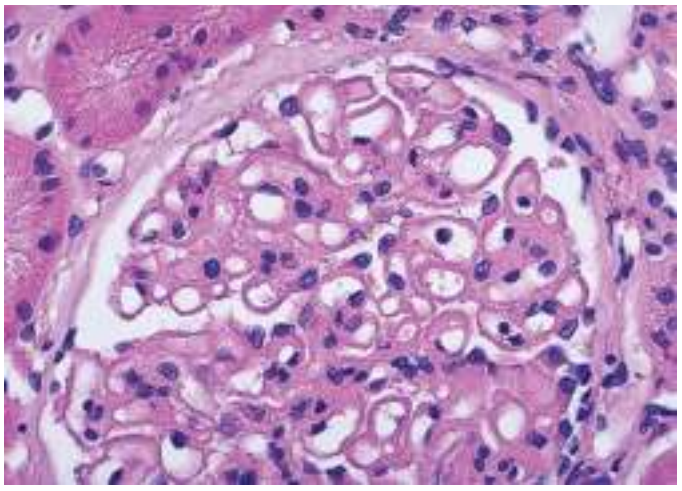


Fig. 22.37: Histology of membranous glomerulonephritis (MGN). Hematoxylin and eosin-stained renal tissue section shows uniform diffuse thickening of the glomerular capillary walls without hypercellularity and without mesangial sclerosis and without inflammatory cells. Proximal convoluted tubules contain hyaline droplets reflecting protein reabsorption. Progression of the disease results in glomerular basement thickening, narrow capillary lumina, mesangial sclerosis and glomerulosclerosis (400X).

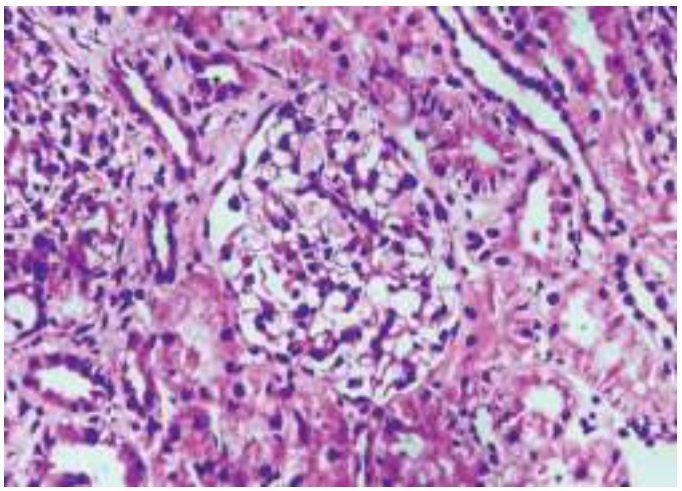


Fig. 22.38: Histology of membranous glomerulonephritis (MGN). Hematoxylin and eosin-stained renal tissue section shows diffuse thickening of the glomerular capillary walls due to *in situ* immune complexes (electron dense) in glomerular basement membrane but not in the mesangium, and without inflammatory cells. Progression of the disease results in thickening of GBM, narrow glomerular capillary lumina, mesangial sclerosis and glomerulosclerosis (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

Table 22.27 Comparison of stages of membranous glomerulonephritis by light, immunofluorescence and electron microscopy

Stage of MGN	Light Microscopy	Immunofluorescence Microscopy	Electron Microscopy
Stage I	Normal or slightly thickened GBM with slight vacuolization	Fine granular IgG, C3 deposits	Scattered small subepithelial electron dense deposits, no foot process effacement or spikes
Stage II	Moderately thickened GBM with spikes and vacuolization	Moderate sized, granular IgG, C3 deposits	Diffuse effacement of foot process
Stage III	Markedly thickened GBM, residual spikes and vacuoles, chain-like appearance	Coarsely granular IgG, C3 deposits	Intramembranous deposits, spikes, new membrane formation and diffuse effacement of foot process
Stage IV	Markedly thickening of GBM with few spikes, vacuoles and glomerulosclerosis	Focal IgG, C3 deposits	Sclerotic GBM, few deposits and lacunae

- Pattern of glomerular injury in MPGN is characterized by alterations in glomerular basement membrane (thickening and duplication), proliferation of endocapillary and mesangial cells, subendothelial and mesangial deposition of immunoglobulins and possible increased mesangial matrix, variable degree of effacement of foot process, caused by persistent antigenemia or circulating immune complexes resulting in increase in mesangial matrix and exudation of inflammatory cells.
- MPGN causes nephrotic syndrome in children (rarely) and adults (5%).
- Traditional classification of MPGN is based on electron microscopy findings: (a) MPGN type 1 (subendothelial and mesangial deposits), (b) MPGN type 2 (intramembranous dense ribbon-like deposits) and (c) MPGN type 3 (subendothelial and subepithelial deposits).
- Hematoxylin and eosin-stained renal biopsy section examination shows hypercellular glomeruli that exhibit lobular appearance in type 1 and type 2 MPGN. Mesangial cells proliferation results in splitting and thickening of glomerular basement membrane, hence giving a 'double contour' of glomerular basement membrane (GBM) and/or the 'tram-track appearance', resulting from reduplication of glomerular basement membrane due to ingrowth of mesangial region demonstrated with Jones methenamine silver stain and periodic acid–Schiff (PAS) stain.

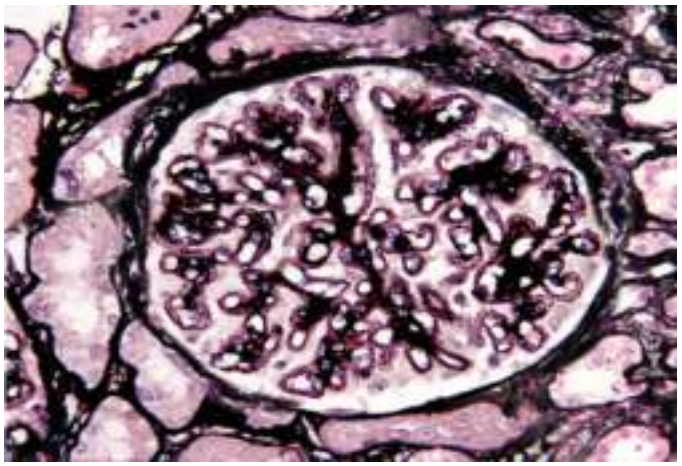


Fig. 22.39: Jones methenamine silver stain in membranous glomerulonephritis (MGN). Jones methenamine silver stained renal biopsy section shows characteristic black material in glomerular basement membrane that appears "spikes" projections around the capillary loops and 'domes' due to immune complex deposits (400X).

- C3-related membranoproliferative glomerulonephritis (MPGN)/dense deposit disease, also known as type 3 MPGN, is characterized by broad, linear, extremely electron dense deposits with C3 within glomerular basement membrane, mesangium, Bowman's capsule and tubular basement membrane, and low level of serum C3 are related to chronic activation of alternative complement system pathway resulting in the accumulation of complement components in the tissue, recruitment of leukocytes and inflammatory damage of glomeruli in hematoxylin and eosin-stained renal biopsy sections on light microscocpy.

Pathogenesis

Membranoproliferative glomerulonephritis may be caused by two mechanisms: (a) immunoglobulin mediated MPGN occurs in setting of chronic infections, autoimmune diseases, monoclonal gammopathy/dysproteinemias; (b) complement mediated MPGN occurs due to subendothelial and mesangial deposition of complement from dysregulation of alternative pathway; includes dense deposit disease (membranoproliferative glomerulonephritis type 2) and proliferative glomerulonephritis with C3 deposits; due to genetic mutations or development of autoantibodies to complement. MPGN is slowly progressive disorder with intermittent remission. It affects patients between 5 and 30 years of age and shows a little response to corticosteroid therapy.

Clinical Features

Patient initially develops nephritic syndrome slowly progressing to nephrotic syndrome and chronic renal failure; and later may develop renal vein thrombosis, hypertension, clinical manifestations of uremia and complex-mediated glomerulonephritis.

- Most patients have circulating antineutrophil cytoplasmic antibodies associated with vasculitis, i.e. Wegener granulomatosis, microscopic polyarteritis nodosa. MGPN disease occurs in three forms: type 1, type 2 and type 3.
- Comparison of types 1, 2 and 3 membranoproliferative glomerulonephritis (MPGN) is given in Table 22.28. Schematic representation of membranoproliferative glomerulonephritis type 1 and type 2 is shown in Fig. 22.40.

Pathology Pearls: Type 1 Membranoproliferative Glomerulonephritis (Type 1 MPGN)

- Type 1 MPGN constitutes 90% of cases. Patient presents with nephrotic syndrome and hypertension in 8–15 years of age.
- Disease occurs due to immune complex deposition and activation of classical and alternative complement pathways.
- About 66% of cases have persistent decrease in serum C3 due to its hypercatabolism; and reduction in total complement levels and borderline/reduced levels of C1q, C4 and properdin.
- Secondary MPGN occurs in the settings of systemic lupus erythematosus, hepatitis B, hepatitis C, HIV, malarial parasite, cytomegalovirus malarial parasite, cytomegalovirus, α_1-antitrypsin deficiency and malignancies.

Light Microscopy

- Examination of renal biopsy in type 1 classic MPGN shows marked proliferation of mesangial cells, large hypercellular glomeruli with accentuation of lobules exhibiting lobular appearance. Sometimes nodules are demonstrated in the mesangial region.
- Irregular thickening of the glomerular basement membrane occurs by interposition of mesangial cells between endothelium and glomerular basement membrane resulting in thickening of glomerular basement membrane due to ingrowth of mesangial cells..
- Neutrophils are often present. Crescents in glomeruli are demonstrated in 20% of cases. Proliferation of endothelial cells causes decrease in lumina of glomeruli resulting in decreased glomerular filtration rate. Histology of membranoproliferative glomerulonephritis (MPGN) is shown in Figs 22.41 and 22.42.

Histochemistry

Mesangial cell proliferation results in splitting and thickening of glomerular basement membrane, hence giving '**tram-track appearance**' or double contour appearance due to presence of complement, mesangial cells cytoplasm and matrix, and partly to the formation of new membrane matrix just beneath the endothelial cells demonstrated with PAS and Jones methenamine silver stain. Jones methenamine silver staining in type 1 membranoproliferative glomerulonephritis is shown in Figs 22.43 and 22.44.

Immunofluorescence Microscopy

Immunofluorescence microscopy reveals '**lumpy bumpy granular pattern**' (IgG, IgM, C3, C1q and C4) in mesangial and subendothelial regions in the new glomerular basement membrane formed. Immune complex deposits appear as spherical or ring-shaped deposits in mesangial region as well as subendothelial regions of glomeruli. Subepithelial immune deposits are composed of C3 and present in 15–20% of cases.

Electron Microscopy

Electron microscopy shows electron dense deposits in subendothelial and mesangial regions of glomeruli associated with increased mesangial matrix, mesangialization of glomerular capillary loops and fusion of foot processes.

Pathology Pearls: Type 2 Membranoproliferative Glomerulonephritis (Type 2 MPGN)

- Essential diagnostic feature of type 2 MPGN is not the membranoproliferative pattern but the presence of electron dense transformation of the glomerular basement membrane. Hence, it is also known as '**dense deposit disease**'. It accounts for 10% of cases.
- Disease may be familial or associated with partial lipodystrophy (loss of subcutaneous fat from fat and upper body).
- Disease is associated with uncontrolled activation of alternative complement system pathway. C3 nephritic factor, an autoantibody binds and stabilizes C3 convertase (C3bBb) and promotes C3 degradation resulting in very low serum C3 levels.
- There is only deposition of complement in the form of irregular ribbon-like or circular aggregates in lamina densa of glomerular basement membrane.
- Patients present with features of nephrotic syndrome than nephritic syndrome. Patients have poorer prognosis than type 1 MPGN. About 50% of patients develop renal failure over 10 years. About 80% of patients recur after renal transplant.

Light Microscopy

- Histologic features of type 2 MPGN exhibit less prominent cellular proliferation. Eosinophilic, refractile and ribbon-like thickening of glomerular basement membrane and basement membrane of Bowman capsule and tubules.
- Type 2 MPGN shows large hypercellular glomeruli with accentuation of lobules exhibiting '**lobular appearance**'. Irregular thickening of the glomerular basement membrane occurs by interposition of mesangial cells between endothelium and basement membrane.
- Mesangial cell proliferation results in splitting and thickening of glomerular basement membrane, hence giving '**tram-track appearance**' (double contour of GBM due to ingrowth of mesangial region). Histology of type 2 membranoproliferative glomerulonephritis is shown in Fig. 22.45.

Histochemistry

- Dense deposits are demonstrated by PAS and Masson's trichrome. Jones methenamine silver (JSM) stain demonstrates broad, linear, extremely electron dense deposits with C3 within basement membrane of glomeruli, tubules, Bowman's capsule, elastic lamina of blood vessels and sclerotic segments of glomeruli (glomerulosclerosis).
- Jones methenamine silver (JSM) stain in type 2 membranoproliferative glomerulonephritis (MPGN) complement related disease (C3 related glomerulopathy/dense deposit disease) is shown in Fig. 22.46.

Electron Microscopy

Electron microscopy shows electron dense deposits of immune complex within glomerular basement membrane, Bowman's capsule as long ribbon-like material.

Immunofluorescence Microscopy

Immunofluorescence microscopy shows linear or double contoured C3 and properdin (and absence of IgG, or C1q or C4) staining of glomerular basement membrane and brighter nodular or ring-like structures in mesangial region and not in dense deposits. C3 staining is also demonstrated in mesangial region, Bowman's capsule and tubular basement membranes.

Pathology Pearls: Type 3 Membranoproliferative Glomerulonephritis (Type 3 MPGN)

- Two rare morphologic subtypes of type 3 MPGN include: (a) Burkholder variant and (b) Anders variant. These are clinically similar to each other.
- Burkholder variant of type 3 MPGN has combined features of type 1 membranoproliferative glomerulonephritis and membranous glomerulonephritis. It is associated with hepatitis C and HIV coinfection.

Electron Microscopy

- Electron microscopy demonstrates subendothelial and subepithelial deposits and mesangial interposition associated with basement membrane spikes.
- Anders variant type 3 MPGN has features of hybrid of type 1 and 2 membranoproliferative glomerulonephritis.
- Electron microscopy demonstrates massive accumulation of deposits within basement membrane with membranous disruption, highlighted by Jones methenamine silver stain.

DIABETIC NEPHROPATHY

Diabetic nephropathy is a morphologic structural change in the glomeruli with mesangial matrix expansion encroaching on the glomerular capillary lumina that occurs due to long-standing hyperglycemia in diabetes mellitus. It is the most important cause of end stage renal disease in 55% of diabetic cases. Diagnostic criteria for diabetes mellitus include: fasting

Table 22.28 Comparison of types 1, 2 and 3 membranoproliferative glomerulonephritis (MPGN)

Parameter	MPGN Type 1	MPGN Type 2	MPGN Type 3
Frequency	90%	10%	Rare
Complement activation pathway	Classic pathway	Alternate pathway	Complement activation pathway absent
Immune complex deposits	IgG, IgM, C3, C1q, C4	C3 but lack IgG and C1q and C4	Nature of immune complex is unknown
Site of deposition of immune complex	Subendothelial region (most common), mesangial region and occasionally in subepithelial region of glomeruli	Electron dense deposits: C3 is deposited in the form of ribbon within lamina densa of basement membrane	Immune complex may be deposited in the subendothelial and subepithelial regions of glomeruli

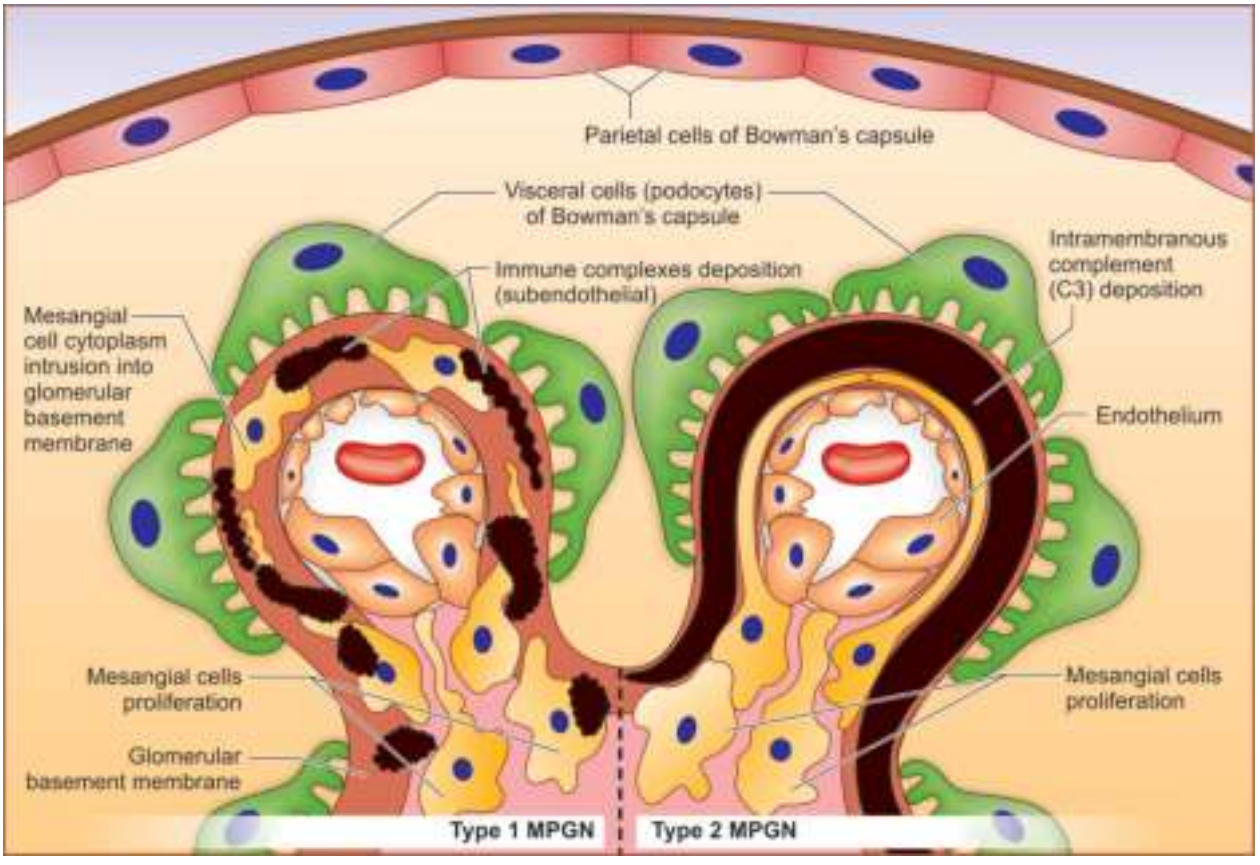


Fig. 22.40: Schematic diagram of membranoproliferative glomerulonephritis type 1 and type 2. Membranoproliferative glomerulonephritis (MPGN) is a morphologic pattern of glomerular injury, characterized by endocapillary and mesangial hypercellularity, mesangial and subendothelial deposits and duplication and thickening of glomerular basement membrane. MPGN accounts for nephrotic syndrome in children and adults. Traditional classification of MPGN 1 based on electron microscopy findings: MPGN type 1 (subendothelial and mesangial deposits), MPGN type 2 (intramembranous dense ribbon-like deposits) and MPGN type 3 (subendothelial and subepithelial deposits).

blood sugar >126 mg/dl at least on two different occasions or random blood sugar >200 mg/dl. Diffuse thickening of the glomerular basement membrane is the earliest morphologic change in diabetic patients.

- Diffuse glomerulosclerosis in diabetic glomerulopathy occurs due to accumulation of trapped proteins and synthesis of type IV collagen in extracellular matrix (ECM) encroaching on the capillary lumina. Reduced activity of mesangial matrix metalloproteinases (MMPs) is unable to degrade extracellular matrix proteins.
- Nodular glomerulosclerosis, also known as Kimmelstiel-Wilson disease, is characterized by sclerotic nodules in the peripheral region of intercapillary region of glomeruli formed due to accumulation of trapped proteins and synthesis

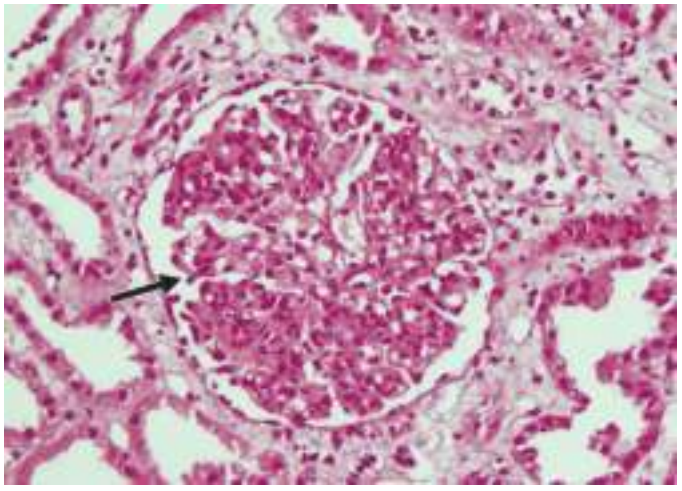


Fig. 22.41: Histology of membranoproliferative glomerulonephritis (MPGN). MPGN is a morphologic pattern of glomerular injury, characterized by endocapillary and mesangial hypercellularity, mesangial and subendothelial deposits and duplication and thickening of glomerular basement membrane. Hematoxylin and eosin-stained renal biopsy section examination shows hypercellular glomeruli that exhibit lobular appearance. Mesangial cells proliferation results in splitting and thickening of glomerular basement membrane, hence giving 'tram-track' appearance (double contour of GBM due to ingrowth of mesangial region) demonstrated with Jones methenamine silver stain (arrow) (400X).

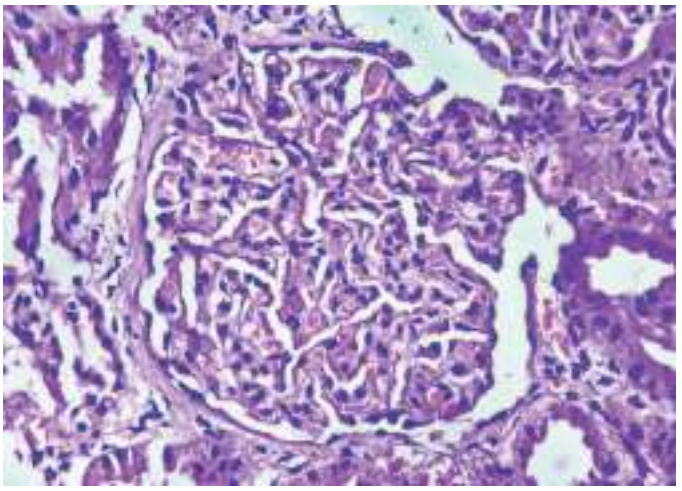


Fig. 22.42: Histology of membranoproliferative glomerulonephritis (MPGN). Hematoxylin and eosin-stained renal tissue section shows mesangial and endocapillary hypercellularity with lobular accentuation, irregular thickening of glomerular basement membrane by interposition of mesangial cells between glomerular capillary endothelium and basement membrane forming double contour/tram-track appearance as a result of immune complex deposit in subendothelial and mesangial region. Double contour of glomerular membrane/tram-track appearance can be appreciated by periodic acid–Schiff (PAS) or Jones methenamine silver stain (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

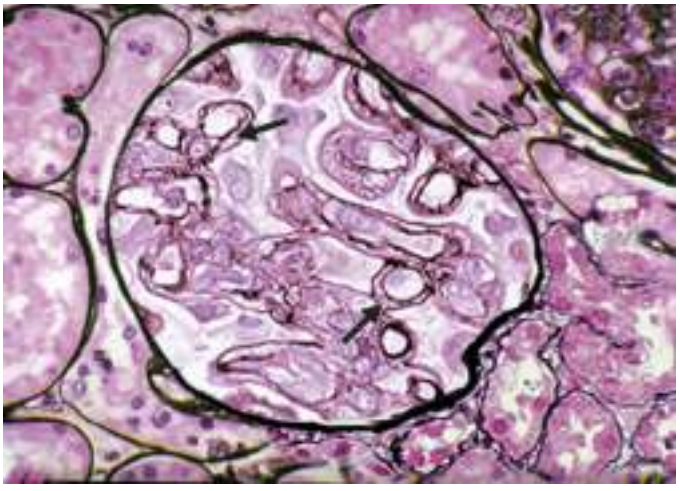


Fig. 22.43: Jones methenamine silver-stained renal biopsy section demonstrates a double contour to many glomerular basement membranes due to reduplication of glomerular basement membrane, and/or the 'tram-track appearance', resulting from reduplication of glomerular basement membrane (arrows) in type 1 membranoproliferative glomerulonephritis (MPGN) (400X). Patients present with nephrotic and/or nephrotic syndrome. Treatment of MPGN is directed at the underlying cause, when present. Idiopathic MPGN is treated by use of immunosuppressive agents.

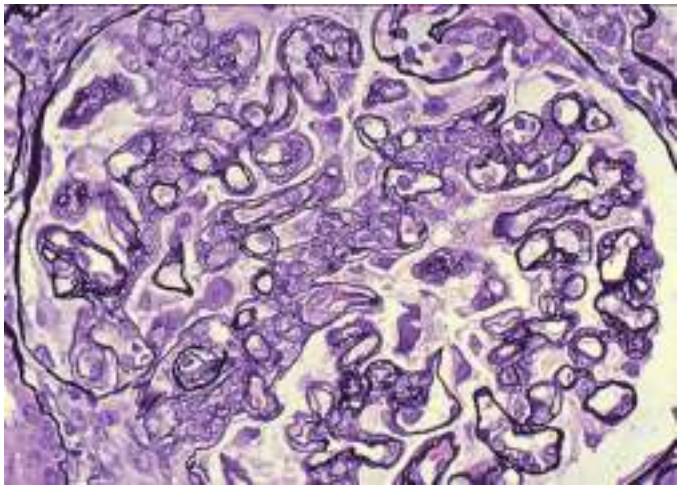


Fig. 22.44: Jones methenamine silver stain in type 1 MPGN. MPGN is characterized by subendothelial and mesangial electron dense deposits, mesangial interposition with splitting and duplication of glomerular basement membrane, possible increased mesangial matrix, variable degree of effacement of foot process. Double contour of glomerular membrane/tram-track appearance is observed by Jones methenamine silver stain (400X). (*Courtesy*: Dr. Neha Kumari and Dr. Tushar Kalonia)

of type IV collagen in mesangial matrix. Vascular hyalinosis is a common finding in nodular glomerulosclerosis. Nodules in diabetic glomerulosclerosis are periodic acid–Schiff (PAS) stain positive but negative Congo red stain.

Clinical Features

Diabetic nephropathy is a serious complication of uncontrolled diabetes mellitus that impairs renal normal functions of removing waste products and extra fluid from the body.

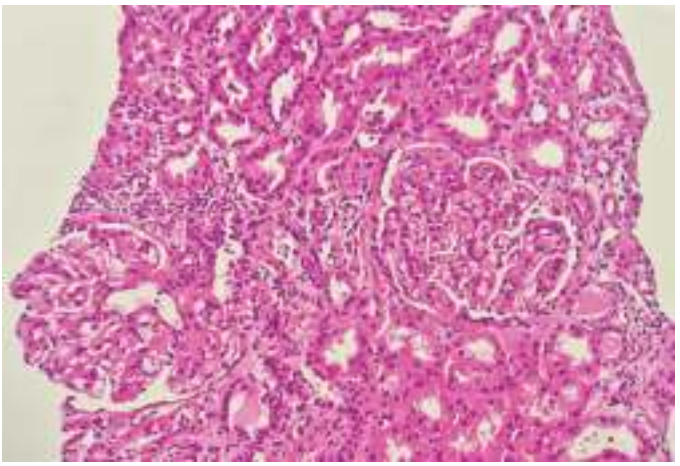


Fig. 22.45: Histology of type 2 MPGN. MPGN complement-related disease (C3-related glomerulopathy/dense deposit disease). C3-related glomerulopathy is characterized by broad, linear, extremely electron dense deposits with C3 within glomerular basement membrane, mesangium, Bowman's capsule and tubular basement membrane, and low level of serum C3-related to chronic activation of alternative complement pathway resulting in the accumulation of complement components in the tissue, recruitment of leukocytes and inflammatory damage of glomeruli. Hematoxylin and eosin-stained renal tissue section examination shows varied glomerular pathology: membranoproliferative glomerulonephritis (25–45%), mesangial proliferation (30–50%), acute exudative glomerulonephritis (10–20%) or crescentic glomerulonephritis (10–20%) (400X). (*Courtesy*: Dr. Neha Kumari and Dr. Tushar Kalonia.)

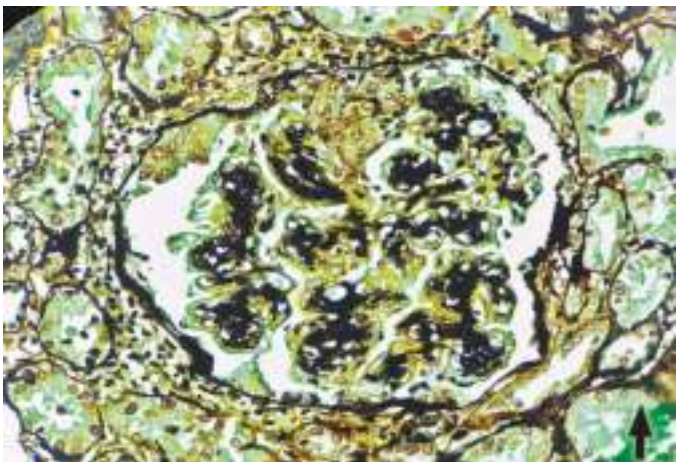


Fig. 22.46: Jones methenamine silver stain in type 2 MPGN complement-related disease (C3-related glomerulopathy/dense deposit disease). Jones methenamine silver stain highlights details of basement membrane of glomeruli, tubules, Bowman's capsule, elastic lamina of blood vessels and sclerotic segments of glomeruli (glomerulosclerosis). It delineates glomeruli within crescents and hence highlights the presence of extracapillary proliferation, also delineates mesangial regions of glomeruli which are argyrophilic (400X). (*Courtesy:* Dr. Neha Kumari and Dr.Tushar Kalonia.)

- Microalbuminuria is first sign of diabetic nephropathy; occurring after 10 years, and defined as excretion of urinary albumin >300 mg/day.
- Diabetic nephropathy manifests clinically as the nephrotic syndrome; however, it is compounded by chronic renal failure and hypertension. Retinopathy parallels nephropathy in diabetic patients.
- Diabetic nephropathy can be prevented by living healthy lifestyle and controlling diabetes mellitus and treating hypertension by captopril antagonist of angiotensin II.

Pathogenesis

Diabetic glomerulosclerosis occurs due to abnormal nonenzymatic glycosylation resulting from attachment of glucose to amino acids of serum proteins, mesangial matrix proteins and glomerular basement membrane. Mechanism of diabetic glomerulopathy is described as under.

- Glucose combines with proteins to form Schiff's base (reversible process), followed by formation of early glycosylated end products (reversible process) or (Amadori products); and then advanced glycosylated end products (irreversible process); which resist enzymatic degradation.
- Advanced glycosylated end (AGE) products induce binding of plasma proteins, i.e. immunoglobulins resulting in excessive mesangial matrix production. AGE products also bind to collagen fibers of glomerular basement membrane and tubular basement membrane, resulting in impairment of interaction.
- Other renal lesions in diabetic nephropathy are acute pyelonephritis, papillary necrosis, capsular drops

Table 22.29 Renal lesions in diabetic nephropathy

Renal Lesions	Comments
Glomerular lesions	
Diabetic glomerulosclerosis	Widening of mesangial areas occurs due to accumulation of basement-like stroma in the mesangial region, increased number of mesangial cells in 2–5 years after the onset of diabetes mellitus
Nodular glomerulosclerosis (also known as Kimmelstiel-Wilson disease)	Sclerotic nodules are formed in the peripheral region of intercapillary region of glomeruli demonstrated by PAS staining
Bowman capsule lesion	
Capsular drop	Accumulation of plasma proteins inside Bowman's capsule is known as capsular drop

Contd...

Table 22.29 Renal lesions in diabetic nephropathy (*Contd...*)

Renal Lesions	Comments
Glomerular subendothelial region lesion	
Fibrin cap	Fibrin cap is formed by plasma proteins deposition in subendothelial region
Papillary necrosis	Diabetic glomerulosclerosis leads to ischemia of papillae (papillary necrosis). Necrotic papillae may detach and pass in the urine as tissue fragments. Undetached papillae may resolve by fibrosis
Renal arterioles lesion	
Hyaline arteriosclerosis of renal arterioles	Hyaline arteriosclerosis of renal arterioles is the main cause of renal microangiopathy
Tubulointerstitial tissue lesions	
Armanni-Ebstein lesions	Deposition of glycogen in renal tubules is known as Armanni-Ebstein lesions in uncontrolled diabetes mellitus patients
Acute pyelonephritis	▪ Acute pyelonephritis occurs most frequently secondary to infection by bacteria or fungi and characterized by **pus in the tubule** and multiple grayish white areas of small microabscesses ▪ Microscopic urine analysis is the presence of white blood cell (WBC) casts

and fibrin cap. Renal lesions in diabetic nephropathy are given in Table 22.29.

Pathology Pearls: Diabetic Nephropathy

Light Microscopy

Diffuse and nodular glomerulosclerosis in diabetic nephropathy is shown in Fig. 22.47. Diffuse glomerulosclerosis in diabetes mellitus is shown in Fig. 22.48. Nodular glomerulosclerosis (Kimmelstiel-Wilson disease) in diabetes mellitus is shown in Figs 22.49 and 22.50. Benign nephrosclerosis in diabetes mellitus is shown in Fig. 22.51.

- **Diffuse glomerulosclerosis:** Diffuse glomerulonephritis parallels with widening of mesangial areas due to accumulation of basement-like stroma in the mesangial region, increased number of mesangial cells in 2–5 years after the onset of diabetes mellitus. Focal effacement of foot processes is common. Diffuse glomerulosclerosis associated with long-standing diabetes mellitus can be demonstrated by periodic acid–Schiff stain.
- **Nodular glomerulosclerosis:** Nodular glomerulosclerosis is also known as '**Kimmelstiel-Wilson disease**', which shows sclerotic nodules in the peripheral region of intercapillary region of glomeruli. Sclerotic nodules are formed in the mesangial region as a result of '**nonenzymatic glycation of proteins**' and accumulation of trapped proteins and synthesis of type IV collagen in mesangial matrix. The sclerotic nodules have a hypocellular matrix core which may be homogenous or exhibit lamellations with peripherally arranged mesangial nuclei. The sclerotic nodules vary in size that can be demonstrated by periodic acid–Schiff and silver methenamine stains. Nodular glomerulosclerosis leads to renal ischemia, tubular atrophy, interstitial fibrosis and ultimately resulting in small contracted kidneys. The remaining glomerular loops show signs of diffuse glomerulosclerosis.
- **Hyaline arteriolosclerosis:** The renal afferent and efferent arterioles may undergo hyalinization resulting in glomerular ischemia and hypertension in diabetic patients.
- **Acute pyelonephritis:** Glycosuria in diabetes mellitus predisposes to acute pyelonephritis by providing a rich medium or bacterial growth in the renal interstitial tissue. Patient presents with fever, urinary colic, and severe groin and flank pain.
- **Papillary necrosis:** Necrosis of the renal papillary tips may occur in severe cases. Renal papillary necrosis is serious complication of diabetic nephropathy. It can also be seen as a complication of chronic analgesic abuse, infectious pyelonephritis and sickle cell anemia. Necrotic papillae in the acute phases could be pale and soft or hemorrhagic. Renal papillae may become detached from cortical tissues and passed in the urine as tissue fragments. Necrotic papillae that are not detached may resolve as amorphous, fibrotic scarred tissue. Light microscopy reveals necrosis of the cellular elements of the papillae and hemorrhage and inflammatory reactions at the interface with viable areas.
- **Capsular drop:** Accumulation of plasma proteins inside Bowman's capsule is known as capsular drop.
- **Fibrin cap:** Accumulation of plasma proteins in the subendothelial region of glomeruli is known as fibrin cap.

Electron Microscopy

Electron microscopy reveals thickening of basement membrane of glomeruli and tubules, fusion of podocytes and accumulation of glycosylated basement membrane-like material in mesangial region.

RENAL AMYLOIDOSIS

Amyloidosis is characterized by deposition of abnormal extracellular fibrillar proteins in organs and tissues having an antiparallel, β-pleated sheet tertiary structure.

- Irrespective of molecular composition, amyloid always has the same characteristic histologic and ultrastructural appearance. Amyloid is resistant to enzymatic digestion, hence accumulates within tissue, interferes with function and destroys vital

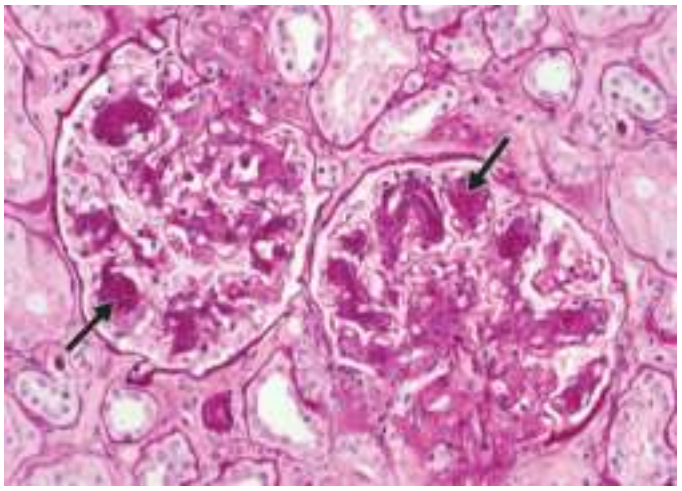


Fig. 22.47: Diffuse and nodular glomerulosclerosis in diabetic nephropathy. Hematoxylin and eosin-stained renal biopsy section shows diffuse thickening of glomerular basement membrane and tubules, expansion of mesangial matrix (diffuse/nodular or both) encroaching on the glomerular capillary lumina. In nodular glomerulosclerosis, nodules are formed due to accumulation of trapped proteins and synthesis of type 4 collagen (400X).

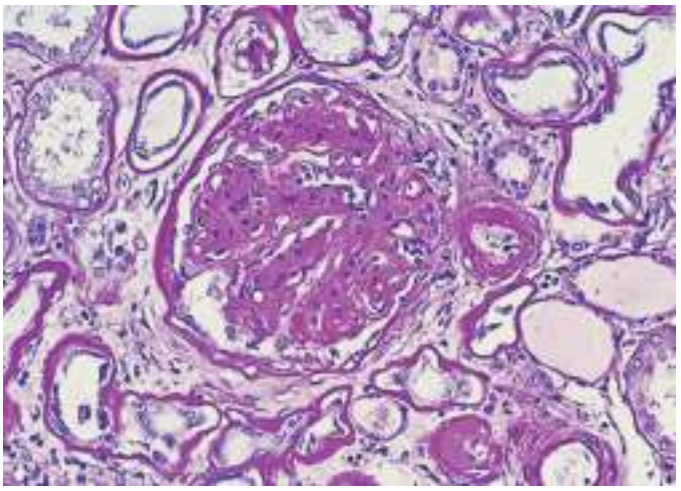


Fig. 22.48: Diffuse and nodular glomerulosclerosis in diabetes nephropathy. Hematoxylin and eosin-stained renal biopsy section shows diffuse thickening of glomerular basement membrane, increased accumulation of mesangial matrix proteins (collagen types 1, 2, and 4 and fibronectin) encroaching on the capillary lumina, and arteriolar hyalinosis. Other histologic findings can include fibrin caps, capsular drops and capillary microaneurysms (400X)

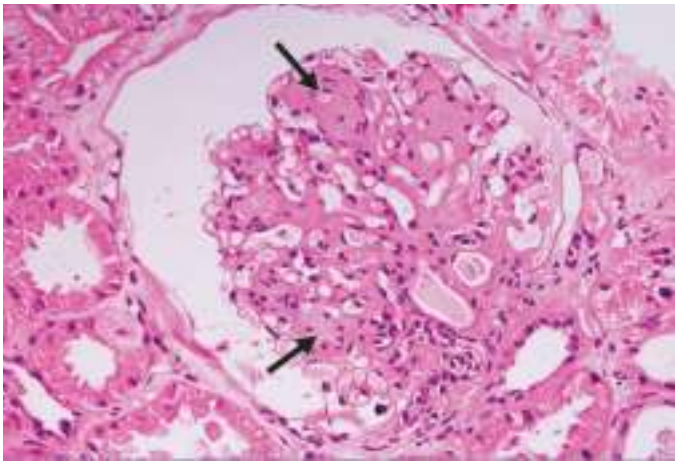


Fig. 22.49: Nodular glomerulosclerosis in diabetic nephropathy. Hematoxylin and eosin-stained renal biopsy section shows mesangial matrix expansion due to accumulation of collagen types 1, 2, and 4 and fibronectin encroaching on the capillary lumina, and arteriolar hyalinosis (400X).

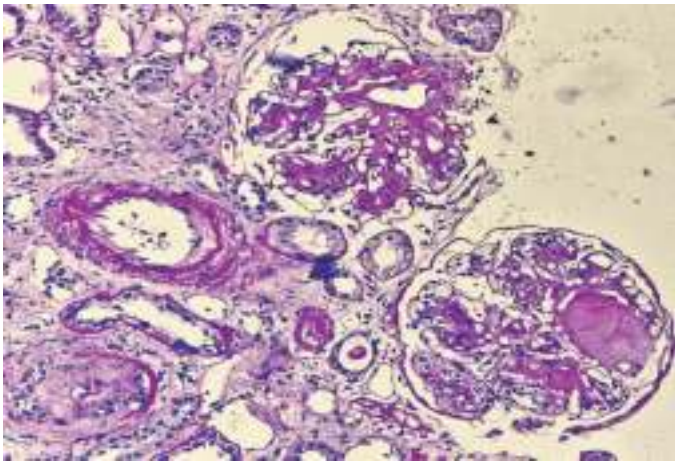


Fig. 22.50: Nodular glomerulosclerosis (Kimmelstiel-Wilson disease) in diabetes mellitus. Hematoxylin and eosin-stained renal biopsy section shows mesangial matrix expansion due to mesangial matrix proteins encroaching on the capillary lumina, and arteriolar hyalinosis (400X).

organs. Radioactive iodine labeled serum amyloid P (SAP) is used to assess amyloid deposition in nuclear medicine studies.

- Amyloid is composed of fibrillar proteins, and nonfibrillary glycoproteins (amyloid P component also called serum amyloid P [SAP], glycosaminoglycans and apolipoprotein E). Serum amyloid P component provides stability of amyloid deposits.
- Congo red stain apple-green birefringence under polarized microscopy is the most popular method for detection of amyloid material. Congo red fluorescence improves the diagnostic yield of conventional microscopy for both positive and negative cases. AA loses its Congo red-associated birefringence after oxidation in permanganate solution, whereas AL amyloid retains this feature.
- Kidneys are commonly involved in 80% cases of systemic amyloidosis. Most common clinical presentation of renal amyloidosis is nephrotic syndrome. Renal amyloidosis occurs due to deposition of amyloid material in primary (AL in multiple myeloma) and secondary amyloidosis (AA in chronic disorders). Patient develops chronic renal failure associated with fatal outcome.

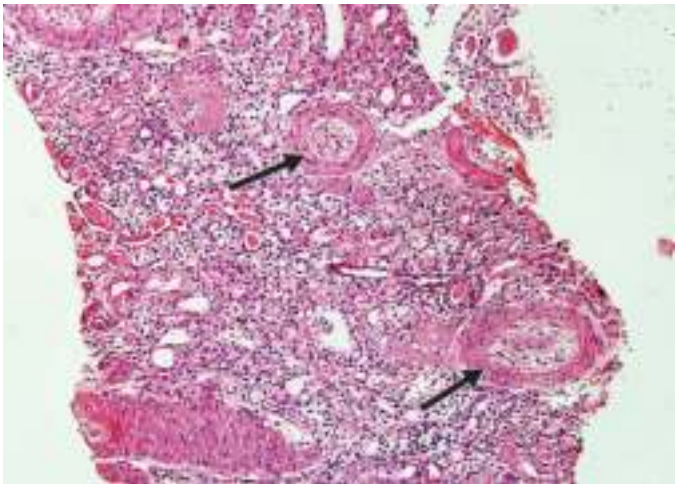


Fig. 22.51: Benign nephrosclerosis in diabetes mellitus. Benign nephrosclerosis refers to renal damage resulting from long-standing hypertension, diabetes mellitus and increasing age. Hematoxylin and eosin-stained renal biopsy section shows thickened and hyalinized arterioles and fibroelastic hyperplasia in lobular/arcuate arteries. There is presence of tubular atrophy, interstitial fibrosis, periglomerular fibrosis and glomerulosclerosis (arrows) (100X).

- In primary renal amyloidosis, κ or λ chains of immunoglobulins synthesized by clonal plasma cells are deposited initially in the mesangial matrix and then involving glomerular basement membrane (GBM). These immunoglobulins can be detected in serum or urine by electrophoresis.
- In secondary amyloidosis, amyloidosis is a well-known complication of chronic inflammatory disorder such as tuberculosis, bronchiectasis, rheumatoid arthritis, and osteomyelitis. These disorders stimulate the liver to synthesize amyloid-associated (AA) protein, an acute-phase reactant secreted by the liver. The kidneys (80%), liver, spleen, and adrenals are the most common organs involved in systemic amyloidosis. Schematic representation of renal amyloidosis is shown in Fig. 22.52.
- Hematoxylin and eosin-stained renal biopsy section in renal amyloidosis shows amorphous acellular amyloid deposit in the mesangium and extending along the inner surface of glomerular basement membrane (GBM) distorting glomerular lumina.
- Congo red stained renal biopsy section shows amyloid deposit in the mesangial region of glomeruli and blood vessels.

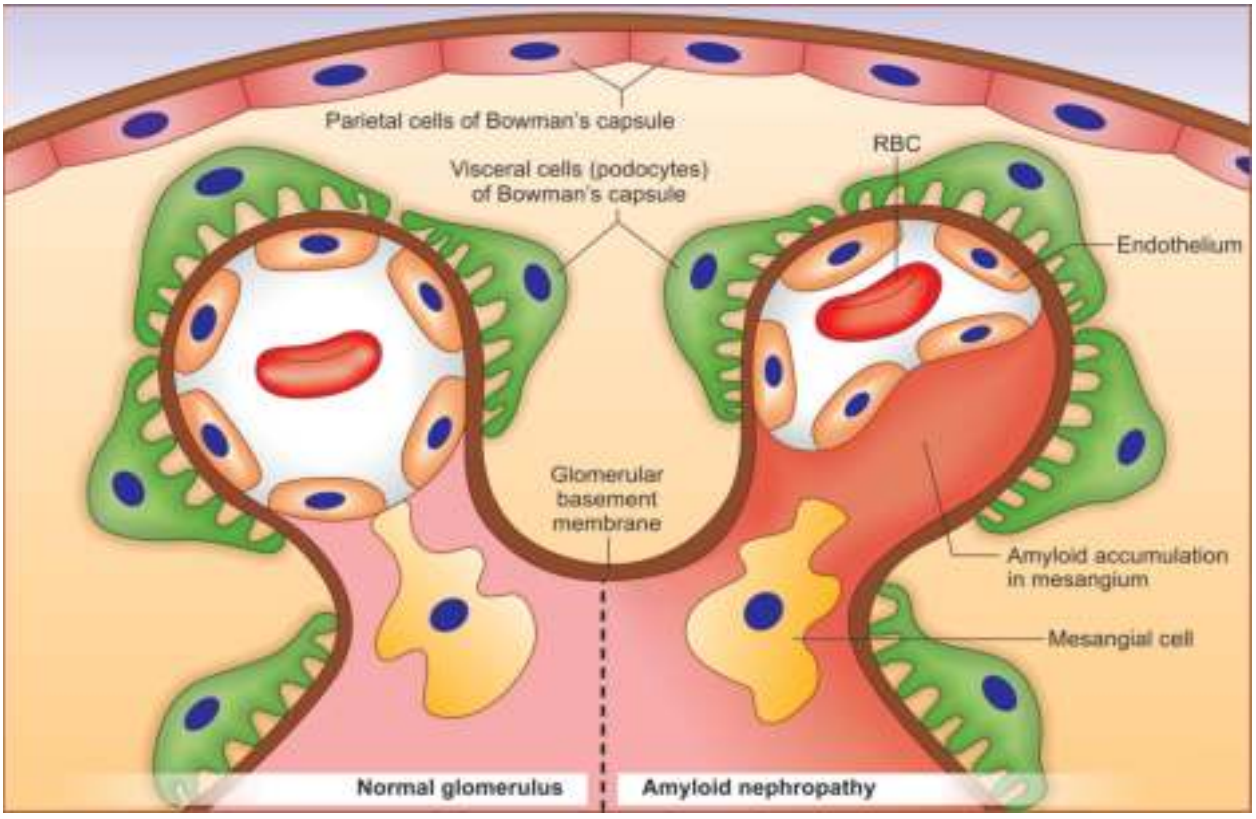


Fig. 22.52: Schematic representation of renal amyloidosis. Kidneys are most commonly affected by primary amyloidosis. Most common sign of renal amyloidosis is nephrotic syndrome. In amyloid light chain nephropathy (AL nephropathy), amyloid deposit in kidney first begins in the mesangium. Left half of schematic representation shows normal glomerulus. Right half of schematic representation shows amyloid deposit in the glomerular mesangial region.

Pathology Pearls: Renal Amyloidosis

- Amyloid refers to abnormal extracellular proteinaceous deposits in organs and tissues. Amyloid is insoluble and structurally dominated by β-pleated structure.
- Congo red stain 'apple-green birefringence' under polarized microscopy is the most popular method for detection of amyloid material.
- Congo red fluorescence improves the diagnostic yield of conventional microscopy for both positive and negative cases. Amyloid-associated (AA) loses its Congo red-associated birefringence after oxidation in permanganate solution, whereas amyloid-light chain (AL) retains this feature.

Clinical Features

Renal amyloidosis causes nephrotic syndrome in 5% of adult age group persons. Renal amyloidosis presents with nephrotic syndrome in middle-age adults, weight loss, organomegaly, monoclonal light chains in serum or urine.

- Duodenal biopsy is sensitive method for diagnosing amyloidosis in chronic kidney disease patients, and highly correlates with renal amyloidosis. About 50% of patients may have proteinuria in the nephritic range and hypertension.
- As the disease progresses, there will be renal insufficiency and chronic renal failure (small contracted kidneys) over 2–5 years.
- Renal manifestations caused by deposition of AA amyloid are clinically indistinguishable from that related to AL amyloid. Renal failure is a common cause of death in amyloidosis.

Pathology Pearls: Renal Amyloidosis

Gross Morphology

Kidney is enlarged, pale, and waxy with smooth surface with firm consistency. In advanced cases, the kidneys are contracted and shrunken due to vascular narrowing induced by deposition of amyloid. Cut section of kidneys reveals pale firm and waxy amyloid material in the renal cortex.

Light Microscopy

- Glomeruli show thickening of glomerular basement membrane. Amyloid material appears as amorphous, homogenous and eosinophilic in mesangial region, tubular basement membranes, renal vessels and interstitial tissue.
- Expansion of mesangial region obliterates the glomerular capillary loops rendering the glomerular filter leaky to plasma proteins leading to impaired renal functions.
- There is no cellular response to the amyloid deposits. The amount of amyloid in the glomeruli correlates roughly with the clinical manifestations of the disease. Histology of renal amyloidosis is shown in Fig. 22.53.

Histochemistry

- Amyloid material in glomerular mesangial matrix, glomerular and tubular basement membrane is demonstrated by Congo red staining under polarized microscopy, which reveals characteristic 'apple-green birefringence' (color of a Granny Smith apple).
- Amyloid material can also be demonstrated by various other stains, i.e. thioflavin T, thioflavin S, methyl violet, crystal violet.
- Pretreatment of tissue with potassium permanganate stained by Congo red demonstrates AL amyloid only but not AA amyloid.
- Congo red staining in renal amyloidosis is shown in Fig. 22.54. Periodic acid–Schiff (PAS): Jones methenamine silver stain in renal amyloidosis is shown in Fig. 22.55. Stains used to demonstrate amyloid material are given in Table 22.30.

Immunofluorescence Microscopy

Immunofluorescence microscopy of renal biopsy reveals non-specific trapping of proteins.

Immunohistochemistry Technique

Immunohistochemical staining for detecting SAA in secondary amyloidosis is shown in Fig. 22.56.

Electron Microscopy

Electron microscopy demonstrates fibrils 7.5–10 nm in diameter with randomly dispersed, nonbranching arrangement in mesangial and subendothelial regions of glomeruli.

MONOCLONAL IMMUNOGLOBULIN DEPOSITION DISEASE OF KIDNEY

Monoclonal immunoglobulin deposition disease is characterized by excessive production and deposition

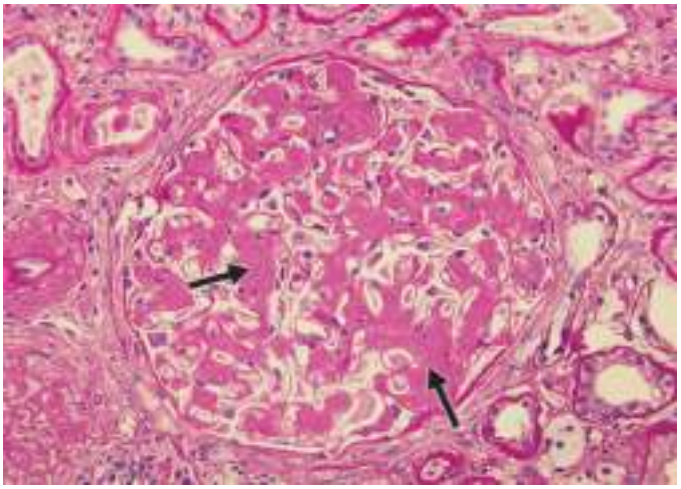


Fig. 22.53: Histology of renal amyloidosis. Hematoxylin and eosin-stained renal biopsy section shows amorphous acellular amyloid material deposit in the mesangium and extending along the inner surface of glomerular basement membrane distorting glomerular lumina (arrows) (400X).

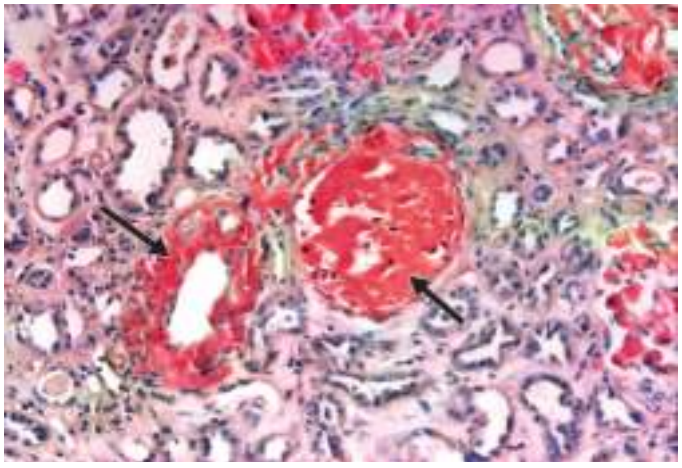


Fig. 22.54: Congo red staining in renal amyloidosis. Congo red stained renal biopsy section shows amyloid deposit in the mesangial region of glomeruli and blood vessels. It is worth mentioning that nodules in diabetic glomerulosclerosis are periodic acid–Schiff (PAS) positive but negative for Congo red stain (arrows) (400X).

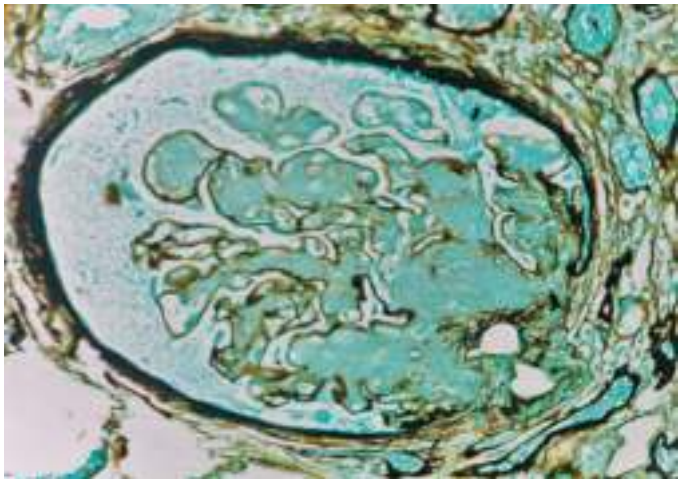


Fig. 22.55: Periodic acid–Schiff (PAS). Jones methenamine silver stain in renal amyloidosis. Jones methenamine silver stain delineates basement and connective tissue components. It is most commonly used in renal pathology to demonstrate abnormalities of the glomerular basement membrane. Glomerular basement membranes and oxidizable carbohydrates are stained black-argyrophilic. The mesangium of the glomerulus is expanded by amorphous, weakly periodic acid–Schiff positive material that produced an early nodular appearance (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

Table 22.30 Stains used to demonstrate amyloid material

Amyloid Stain	Color Imparted
Congo red with light microscopy	Rose-pink
Congo red with polarizing microscopy	Apple green birefringence similar to color of Granny Smith apple
Methyl violet (metachromatic stain)	Pink
Crystal violet (metachromatic stain)	Pink
Fluorescent stains (thioflavin T, thioflavin S)	Greenish
von Gieson stain	Khaki color
Periodic acid–Schiff (PAS) stain	Magenta color

Congo red stain is commonly used to demonstrate amyloid deposits in tissue sections.

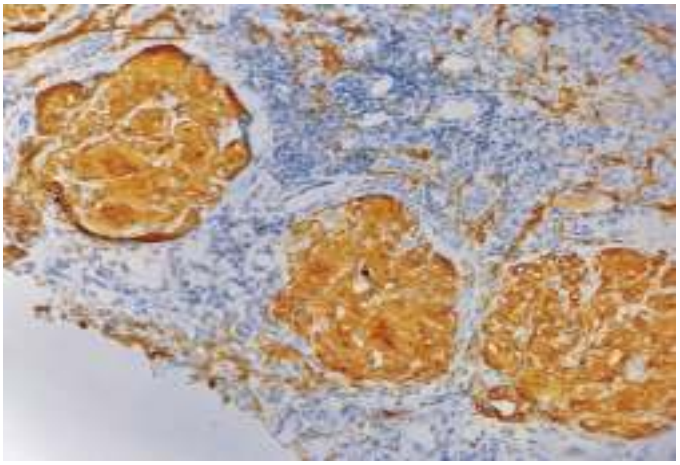


Fig. 22.56: Immunohistochemical staining for detecting SAA in secondary amyloidosis. Amyloidosis occurs when as abnormal protein, called amyloid, builds up in organs and tissues and interferes with normal function. Secondary amyloidosis (AA) is characterized by a protein called 'serum amyloid A'. This protein is produced by the body in response to chronic inflammation and infection the settings of tuberculosis and poorly controlled rheumatoid arthritis. Secondary amyloidosis (AA) commonly affects kidneys, liver and spleen. Heart involvement is extremely rare. Immunohistochemical staining is done by using antisera to 'serum AA 'on amyloid tissue in secondary amyloidosis (AA) (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

of nonamyloid immunoglobulin light chains in kidneys, liver, heart, nervous system and soft tissues in the setting of plasma cell dyscrasias.

- Monoclonal immunoglobulins are deposited in the glomerular basement membrane (GBM), tubular basement membrane, mesangium and interstitium. Periodic acid–Schiff (PAS) and Jones methenamine silver stain in renal monoclonal immunoglobulin deposition disease (MIDD) are shown in Fig. 22.57.
- Glomeruli are enlarged with (PAS) positive material in thickened capillary walls and mesangial nodules. Tubular basement is thickened and giving glassy appearance. Immunofluorescence stain shows granular κ (80%) or λ (20%) light chains along glomerular basement membrane, mesangial region, blood vessel walls and interstitium.
- MIDD subtypes include light chain (LCDD: 80% κ chain), light and heavy chain (LHCDD) and heavy chain deposition diseases (HCDD).
- Monoclonal immunoglobulin deposition disease is characterized by nodular sclerosing glomerulopathy, massive proteinuria in nephrotic range, and renal insufficiency. Some patients can develop hematuria and hypertension.

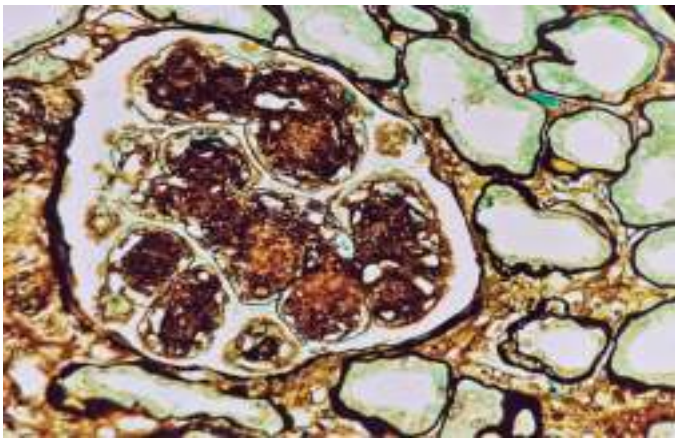


Fig. 22.57: Periodic acid–Schiff (PAS) and Jones methenamine silver stain in renal monoclonal immunoglobulin deposition disease (MIDD). Monoclonal immunoglobulins are produced by monoclonal plasma cells, which are found in the setting of plasma cell dyscrasias. Monoclonal immunoglobulins are deposited on the glomerular basement membrane, tubular basement membrane, mesangium and interstitium. The mesangial nodules are argyrophilic. The nonamyloidotic monoclonal immunoglobulin deposition disease is characterized by nodular sclerosing glomerulopathy, massive proteinuria in nephrotic range, and renal insufficiency, MIDD subtypes include light chain (LCDD: 80% κ chain), light and heavy chain (LHCDD) and heavy chain deposition diseases (HCDD) (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

LUPUS NEPHRITIS

Lupus nephritis is a common manifestation of systemic lupus erythematosus (SLE), that results in irreversible renal impairment in 60–70% cases. SLE is an autoimmune disease, that results in chronic inflammation and damage to multiple organs. SLE is diagnosed clinically and serologically with the presence of autoantibodies directed against DNA, RNA, and autologous proteins. Diagnostic criteria for systemic lupus erythematosus are given in Table 22.31.

- Many renal lesions encountered in lupus nephritis are also observed in other glomerulopathies. Hence, it is essential to provide a rational for harmonizing definitions for renal lesions irrespective of the glomerulopathy.
- In clinical practice, it is essential to obtain detailed information from the renal biopsy in paraffin-embedded sections stained by H&E stain and special histochemical stains and apply a basic format of the renal biopsy report. Renal biopsy must include approximately 10 glomeruli for evaluation.

Clinical Pearls: Diagnostic Criteria for Systemic Lupus Erythematosus

- Out of 11 diagnostic criteria for systemic lupus erythematosus (SLE), 4 are needed to confirm diagnosis.
- Eleven specific diagnostic criteria for SLE include: butterfly-shaped skin rash, raised rash patches on skin, sensitivity to light, ulcers in mouth or nose, arthritis in two or more joints plus swelling or tenderness, inflammation in the lining of heart and lungs, seizures or other neurological problems, and too much proteinuria.
- Fatigue, hair loss, antiphospholipid syndrome and elevated erythrocyte sedimentation rate are other findings in systemic lupus erythematosus, which are not part of specific diagnostic criteria.
- Hydralazine, procainamide and isoniazid drugs can produce (skin rash + joint pain + fatigue). Drug-induced lupus lesion never involves kidney and central nervous system.
- Drug-induced lupus gives anti-histone antibodies and always a positive antinuclear antibody. Complement level and anti-DS DNA are normal.
- Blood biochemistry findings include increased γ-globulin, decreased serum albumin, antinuclear test positive.
- LE cell phenomenon *in vitro* is positive (lupus cell preparation demonstrates LE cells).

Table 22.31 Diagnostic criteria for systemic lupus erythematosus

Organs	Comments
Skin manifestations and mucous membrane	Malar rash, photosensitivity rash, discoid rash and oral ulcers rash
Joints	Nonerosive arthralgia present (90%) and joint radiograph normal
Hematologic findings	Normocytic normochromic, leukopenia, thrombocytopenia, and hemolysis (any blood involvement counts as 1 criterion)
Cardiovascular system and blood findings	Libman-Sack's endocarditis (noninfective cardiac vegetations) and pericarditis
Renal manifestations	Lupus nephritis class I to class VI manifest with proteinuria in nephritic range to irreversible renal impairment in 60–70% cases
Cerebral manifestations	Behavioral change, cerebral stroke, seizures, meningitis
Serositis	Pericarditis, myocarditis, pleuritic chest pain, pneumonia pulmonary hypertension
Serology	▪ Antinuclear antibodies positive (95%) ▪ Anti-double-stranded (DS) DNA antibodies specific test (60–70%) ▪ Anti-Smith antibodies positive (10–20%) ▪ Each of serologic abnormalities counts as 1 criterion. Hence, if the person has joint pain and/or skin rash, and antinuclear antibodies and anti-double-stranded (DS) DNA antibodies, that the patient would have 4 criteria

Pathogenesis

Lupus nephritis occurs by two pathogenetic mechanisms: (a) formation of anti-DNA antibodies against planted DNA (*in situ* immune complex deposit) and trapped in the glomeruli, and (b) formation of immune complexes (DNA–anti-DNA) in circulation trapped in the glomeruli. In both mechanisms, immune complexes activate classical pathway of complement system resulting in injury to glomeruli, tubules and interstitial tissue.

- In lupus nephritis, autoantibodies are produced directed against DNA, RNA, and autologous proteins. Immune complexes are deposited in the glomeruli. Anti-double-stranded DNA antibodies may cross-react with glomerular basement membrane. Higher affinity autoantibodies may result in deposition in glomeruli.
- Cationic antibodies have a greater affinity for the anionic glomerular basement membrane. Immune complexes induce inflammatory response by the activation of the complement system and recruitment of inflammatory cells leading to injury to glomeruli, tubules and interstitial tissue.
- Glomerular thrombosis is another mechanism that plays a role of pathogenesis of lupus nephritis especially in patients with antiphospholipid syndrome and is believed to be the result of interaction between autoantibodies and negatively charged phospholipid proteins.
- Decreased serum C3 level correlates with SLE disease activity. In general, the more immune complex deposition and increased cellular proliferation, indicate poor prognosis. Renal changes are highly variable.

Clinical Features

Severity of lupus nephritis determines the clinical course. Patient presents with nephrotic syndrome but proteinuria may be in nephritic range. Approximately 90% of patients survive for 10 years. Patient develops irreversible renal impairment. Infection is the most common cause of death.

Pathology Pearls: Lupus Nephritis

Light Microscopy

Summary of pathological findings of lupus nephritis according to WHO criteria is given in Table 22.32. Activity and chronicity indices in lupus nephritis are given in Table 22.33.

- **Lupus nephritis class I:** Histologic examination shows mesangial immune complex deposits, with minimal mesangial cell hypercellularity or influx of leukocytes. Renal biopsy evaluation reveals four or more nuclei fully surrounded by mesangial matrix in the mesangial region not included the hilar region.
- **Lupus nephritis class II:** It is purely mesangial disease. Histologic examination reveals mesangial immune complex deposits and mesangial hypercellularity but without influx of leukocytes. Renal biopsy evaluation reveals four or more nuclei fully surrounded by mesangial matrix in the mesangial region not included the hilar region.
- **Lupus nephritis class III:** In lupus nephritis class III and class IV, the term endocapillary hypercellularity is used replacing endocapillary proliferation. Histologic examination shows glomeruli with segmental endocapillary hypercellularity involving <50% of glomeruli, mesangial hypercellularity, glomerular basement membrane thickening and early segmental necrosis demonstrated in Jones methenamine silver stained sections.
- **Lupus nephritis class IV:** In lupus nephritis class III and class IV, the term endocapillary hypercellularity is used replacing endocapillary proliferation.
  - Membranoproliferative glomerulonephritis is a form of endocapillary injury. Histologic examination shows subendothelial deposits, endocapillary and/or extracapillary proliferation in ≥50% of glomeruli giving '**wire-loop lesion**', and hyaline masses within glomerular capillary lumens caused by immune complex deposits (i.e. hyaline thrombi).
  - The term crescent refers to a renal lesion more than two cell layers, which consists of extracapillary hypercellularity and a variable mixture of cells. Fibrin and fibrous matrix may be demonstrated involving ≥10% of the circumference of Bowman's capsule.
  - Crescents can be cellular, fibrous and fibrocellular. Cellular crescents are composed of >75% cells and fibrin and <25% fibrous matrix. Fibrous crescents are composed of >75% fibrous matrix and <25% cells and fibrin, which are part of chronicity index. Fibrocellular crescents are composed of 25–75% cells, fibrin and the remainder fibrous matrix.
  - Fibrinoid necrosis is associated with glomerular basement membrane disruption and/or lysis of mesangial matrix, which lacks karyorrhexis. Active and chronic designations for class III/class IV lupus nephritis lesions are replaced by activity and chronicity indices in all cases.
  - Activity index includes fibrinoid necrosis as specific indicator. Segmental and global subdivisions of lupus nephritis IV have been eliminated.
- **Lupus nephritis class V:** Histologic examination demonstrates non-wire-loop lesion, membranous glomerulonephritis (thick glomerular loops), mesangial hypercellularity, influx of leukocytes, and subepithelial immune deposits in addition to subendothelial deposits.
- **Lupus nephritis class VI:** Lupus nephritis class VI lesion is rarely observed in renal biopsies. Class VI either needs to be eliminated or some fraction of globally advanced glomerulosclerosis, thickened glomerular capillary loops, tubular atrophy and interstitial fibrosis designated as the cut off between class IV and class VI.

Histochemistry

In general, the more immune complex deposition and the more cellular proliferation, the worse is the disease. Extensive immune complex deposition in the thickened glomerular loops gives so-called wire-loop appearance. Jones methenamine silver stain in wire-loop lesion in lupus nephritis is shown in Fig. 22.58.

Immunofluorescence Microscopy

Renal biopsy shows granular deposition of IgG, C3 and fibrin along glomerular basement membrane, in subendothelial and mesangial regions of glomeruli. It is indistinguishable from that of idiopathic membranous nephropathy. It is worth mentioning that subendothelial deposits are encountered in lupus nephritis and membranoproliferative glomerulonephritis.

Laboratory Findings

Decreased serum C3 level correlates with lupus nephritis activity index. Demonstration of anti-double-stranded DNA antibody is highly specific for renal disease correlating with disease activity index.

RAPIDLY PROGRESSIVE (CRESCENTIC) GLOMERULONEPHRITIS

Rapidly progressive (crescentic) glomerulonephritis (RPGN) is also known as crescentic glomerulonephritis and characterized by rapid progression to acute renal failure and end stage renal disease in 90% of cases within weeks or months. RPGN occurs due to severe glomerular injury resulting in exudation of inflammatory cells through the disrupted, segmentally necrotic glomerular basement membrane leads to the formation of crescents between the Bowman's capsule and the glomerular tuft involving >50% of glomeruli. Schematic representation of rapidly progressive (crescentic) glomerulonephritis is shown in Fig. 22.59.

Pathogenesis

Rapidly progressive (crescentic) glomerulonephritis (RPGN) is a clinical syndrome characterized by glomerular crescents involving >50% of glomeruli,

Table 22.32 Summary of pathological findings of lupus nephritis according to WHO criteria

Light Microscopy	Immunofluorescence Microscopy	Electron Microscopy	Clinical Syndromes and Prognosis
Lupus nephritis class I			
Minimal mesangial hypercellularity	Mesangial deposits	Mesangial deposits	Mild proteinuria and hematuria (asymptomatic) associated excellent prognosis
Lupus nephritis class II (25%)			
Mesangial hypercellularity increased mesangial matrix	Mesangial deposits	Mesangial deposits	Good prognosis
Lupus nephritis class III (20%)			
Focal glomerulonephritis (endocapillary hypercellularity and/or extracapillary hypercellularity in <50% of glomeruli)	Mesangial and glomerular loop deposits	Mesangial and subendothelial deposits	Mild proteinuria with or without hematuria, but normal renal functions associated with moderate prognosis
Lupus nephritis class IV (40%)			
Diffuse glomerulonephritis (endocapillary hypercellularity and/or extracapillary hypercellularity in ≥50% of glomeruli giving wire-loop lesion	Mesangial and glomerular loop deposits	Mesangial and subendothelial deposits	Nephritic syndrome, nephrotic syndrome associated with moderate to poor prognosis
Lupus nephritis class V (15%)			
Membranous glomerulonephritis (thick glomerular loops and mesangial cells hypercellularity)	Mesangial and glomerular loop deposits	Numerous subepithelial and scattered mesangial deposits	Nephrotic syndrome associated with moderate prognosis
Lupus nephritis class VI			
Advanced segmental glomerulosclerosis (>90% thickened glomerular loops, tubular atrophy and interstitial fibrosis)	Variable deposits	Variable deposits	Chronic renal failure associated with poor prognosis

Table 22.33 Activity and chronicity indices in lupus nephritis

Activity Index in Lupus Nephritis
▪ Endocapillary hypercellularity with or without leukocyte infiltration and reduction in lumen size ▪ Fibrinoid necrosis ▪ Cellular or fibrocellular crescents ▪ Subendothelial immune complex deposits identified as wire-loop lesions. Intraluminal immune aggregates (hyaline thrombi)
Chronicity Index in Lupus Nephritis
▪ Segmental/global glomerulosclerosis ▪ Fibrous adhesions ▪ Fibrous crescent

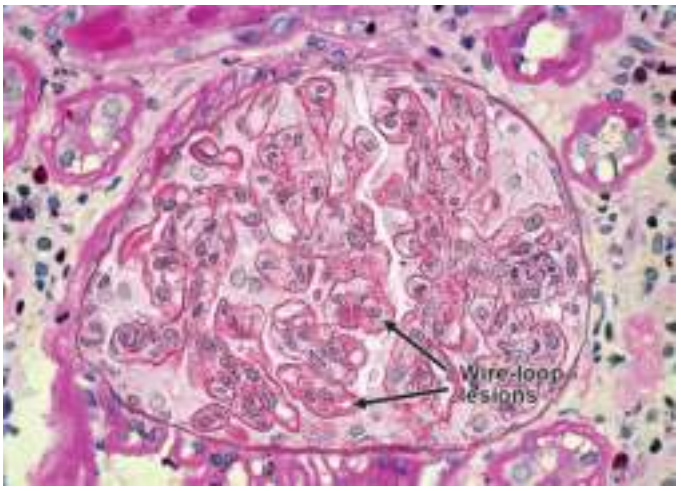


Fig. 22.58: Jones methenamine silver stain in wire-loop lesion in lupus nephritis. Jones methenamine silver stain in lupus nephritis delineates glomerular basement membrane and connective tissue components, which is most commonly used in renal biopsy to demonstrate abnormalities of the glomerular basement membrane. Glomerular basement membrane and oxidizable carbohydrates are stained black argyrophilic. Jones methenamine silver-stained renal biopsy thin about 2 μm section shows wire-loop lesion, which is one of the active lesions of lupus nephritis (arrows) (400X).

rapid decline in renal function, hematuria, proteinuria, oliguria or hypertension.

- RPGN occurs due to primary or secondary glomerulonephritis. RPGN is thought to be immune-mediated disorder, because most patients have antibodies to neutrophils (ANCA) in blood circulation.
- RPGN occurs in the settings of poststreptococcal glomerulonephritis, Goodpasture's syndrome,

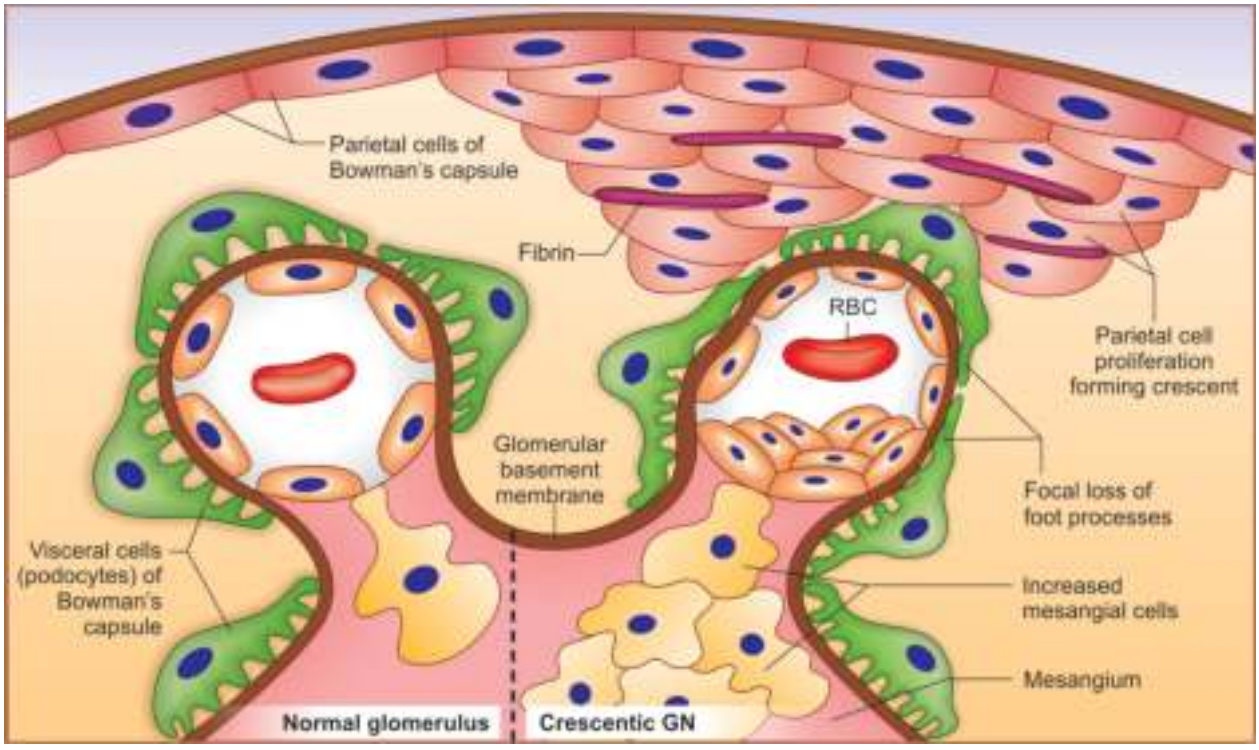


Fig. 22.59: Schematic representation of rapidly progressive (crescentic) glomerulonephritis. Rapidly progressive (crescentic) glomerulonephritis is a clinical syndrome characterized by rapid decline in renal function, hematuria, proteinuria, oliguria or hypertension. Glomerular crescents are observed in more than 50% of glomeruli. Left half of schematic representation shows normal glomerulus, whereas right half demonstrates crescent formation as a result of glomerular injury and escape of fibrin, which stimulates proliferation of parietal epithelial cells of Bowman's capsule. Crescents compress the glomerulus leading to impaired renal functions.

systemic lupus erythematosus, polyarteritis nodosa, Wegener's granulomatosis, IgA nephropathy, Henoch-Schönlein purpura and drug (penicillamine).

- Glomerular endothelial injury is a key driver of crescents formation, which causes damage and rupture of glomerular basement membrane, which triggers coagulation system cascade within Bowman's capsule space, ultimately leading to fibrin deposition. Fibrin stimulates parietal epithelial cells of Bowman's capsule to proliferate resulting in cellular crescents formation, which mount pressure and cause decline in glomerular filtration rate and thus impair renal function.
- Crescents are composed of parietal epithelial cells, macrophages and fibrin. Segmental fibrinoid necrosis of the glomerular tufts is present.

Pathology Pearls: Rapidly Progressive Glomerulonephritis (RPGN) Categories

Anti-GBM Antibody-mediated RPGN

Goodpasture's syndrome is characterized by IgG antibodies directed against noncollagenous domain of α-3 type 4 collagen in GBM and pulmonary capillaries. Patient presents with features of nephritic syndrome and hemoptysis. Immunofluorescence microscopy shows linear IgG, C3 and fibrin deposits.

Immune Complex-mediated RPGN

Immune complex-mediated RPGN may be caused by post-infectious glomerulonephritis, systemic lupus erythematosus, IgA nephropathy and Henoch-Schönlein purpura.

Pauci-immune Disease (ANCA Positive) RPGN

Most patients develop autoantibodies to neutrophils (ANCA), which activate neutrophils and cause them to adhere to endothelial cells. Neutrophils release oxygen-derived free radicals and hydrolytic enzymes resulting in glomerular endothelial cells injury.

Clinical Features

Goodpasture's syndrome begins with hemoptysis (70%) and nephritis. Rapidly progressive glomerulonephritis progresses to acute renal failure in a few weeks to months in 90% of cases. Anti-GBM antibodies are demonstrated in these patients. Plasmapheresis is useful in removing antibodies.

Surgical Pathology: Rapidly Progressive Glomerulonephritis (RPGN)

Gross Morphology

Progression of primary renal diseases and systemic disorders such as amyloidosis, diabetic nephropathy and multiple myeloma involving kidneys produce small contracted kidneys. Polyarteritis nodosa and healed infarct produce large scars in kidneys.There is loss of concentrating power of kidneys. Urine analysis shows fixed specific gravity: 1.010.

Light Microscopy

Histologic examination of renal biopsy shows glomeruli, tubules, blood vessels and interstitial tissue. Crescents are formed due to proliferation of parietal cells of Bowman's capsule, which compress glomeruli. Histology of rapidly progressive (crescentic) glomerulonephritis is shown in Figs 22.60 and 22.61.

Histochemical Stain

Jones methenamine silver (JSM) stain in rapidly progressive (crescentic) glomerulonephritis is shown in Fig. 22.62.

Immunofluorescence Microscopy

- **Linear immune complex pattern:** Linear immune complex pattern is observed in antiglomerular basement membrane disease and Goodpasture syndrome.
- **Granular immune complex pattern:** Granular immune complex pattern is demonstrated in immune complex-mediated glomerulonephritis.
- **Pauci-immune complex pattern:** Pauci-immune complex pattern is demonstrated in idiopathic crescentic glomerulonephritis.

CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is characterized by irreversible and progressive glomerulopathy and tubulointerstitial fibrosis, ultimately resulting in

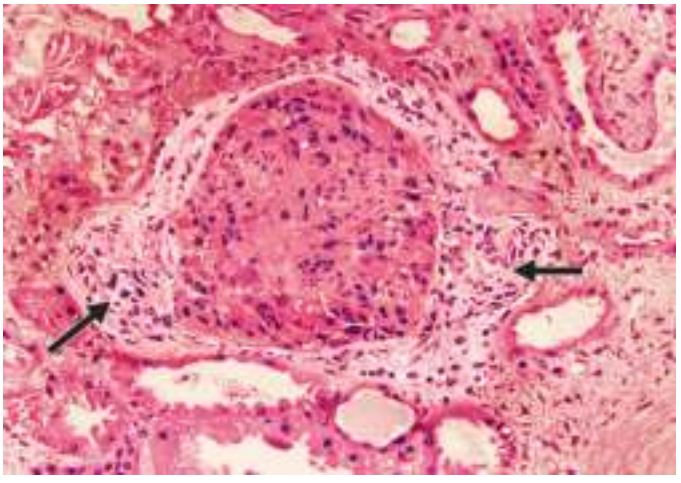


Fig. 22.60: Histology of rapidly progressive (crescentic) glomerulonephritis (RPGN). In hematoxylin and eosin-stained renal biopsy section examination, glomerular crescents are observed in more than 50% of glomeruli rapidly progressive (crescentic) glomerulonephritis. Endothelial injury is a key driver of crescents formation, which causes damage and rupture of glomerular basement membrane. This triggers coagulation cascade within Bowman's capsule space, ultimately leading to fibrin deposition. Fibrin stimulates parietal epithelial cells of Bowman's capsule to proliferate, resulting in formation of cellular crescents, which mount pressure and cause decline in glomerular filtration rate and thus impaired renal function. Arrows indicate cellular crescents (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

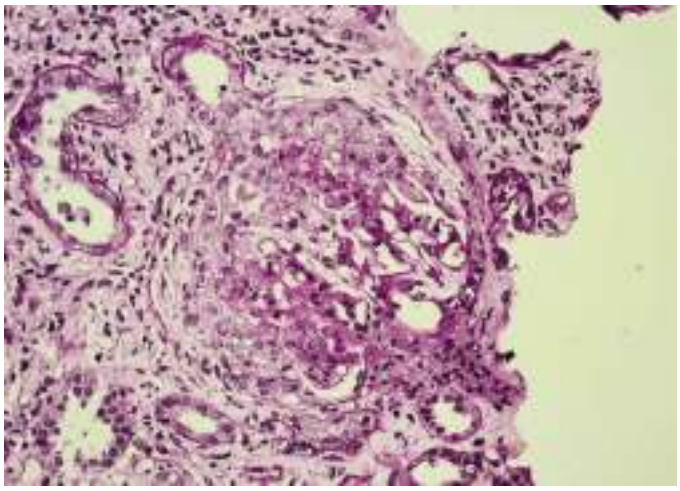


Fig. 22.61: Histology of rapidly progressive (crescentic) glomerulonephritis (RPGN). RPGN is a clinical syndrome characterized by glomerular crescents in more than 50% of glomeruli, rapid decline in renal function, hematuria, proteinuria, oliguria or hypertension. In hematoxylin and eosin-stained renal tissue section and extracapillary cellular profanation (crescents) are composed of parietal epithelial cells, macrophages and fibrin. Segmental fibrinoid necrosis of the glomerular tufts are present (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

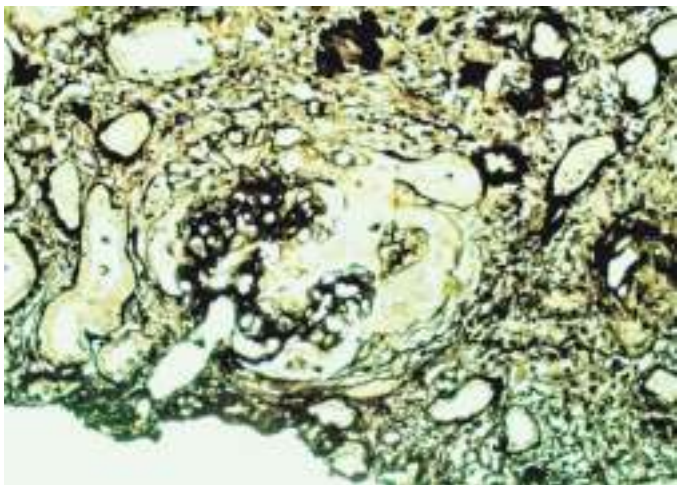


Fig. 22.62: Jones methenamine silver (JMS) stain in rapidly progressive (crescentic) glomerulonephritis (RPGN). JMS stain is a histochemical stain which highlights carbohydrates that can be oxidized to aldehydes. Aldehydes formed by oxidation of carbohydrates reduce a silver solution, causing the silver ions to bind to the carbohydrate and can be visualized. JMS stain highlights details of basement membrane of glomeruli, tubules, Bowman's capsule, elastic lamina of blood vessels and sclerotic segments of glomeruli (glomerulosclerosis). It delineates glomeruli within crescents and hence highlights the presence of extracapillary proliferation, also delineates mesangial regions of glomeruli which are argyrophilic (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

reduction in the glomerular filtration rate (GFR) and accumulation of uremic toxic products in the body.

- Progressive permanent destruction of nephrons in many kinds of kidney diseases eventually leads to chronic kidney disease (GFR <60 ml/minute per 1.73 square meters) persisting for more than three months. It is a state of progressive loss of renal functions ultimately resulting in the need for renal dialysis or renal transplantation.
- Chronic kidney disease is caused by rapidly progressive crescentic glomerulonephritis (80–90%), focal segmental glomerulosclerosis (50–80%), and membranoproliferative glomerulonephritis (50%), IgA nephropathy (30–50%), membranous glomerulonephritis (30–40%), diabetic nephropathy and rarely postinfectious glomerulonephritis (1–2%).
- Etiopathogenesis of chronic glomerulonephritis is shown in Fig. 22.63. Glomerular diseases progressing to chronic kidney disease are given in Table 22.34.

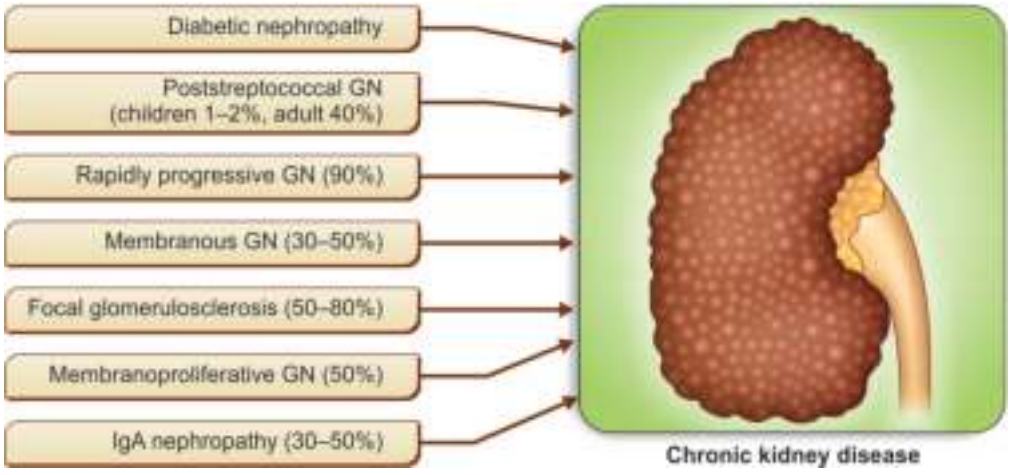


Fig. 22.63: Schematic representation of etiopathogenesis of chronic kidney disease. It is characterized by varying degrees of glomerular scarring that is always accompanied by cortical tubular atrophy, interstitial fibrosis, chronic inflammatory cells in the interstitium and arteriosclerosis. It leads to progressive renal failure. Kidneys are small contracted. External surface shows fine granularity known as 'grain leather' appearance.

Table 22.34 Glomerular diseases progressing to chronic kidney disease

Glomerular Diseases	Frequency (%)
Poststreptococcal glomerulonephritis	Children (1–2%) and adults (40%)
Rapidly progressive glomerulonephritis (RPGN)	90%
Membranous glomerulonephritis	30–50%
Focal glomerulosclerosis	50–80%
Membranoproliferative glomerulonephritis	50%
IgA nephropathy	30–50%

Glomerular filtration rate is <20% of normal in chronic glomerulonephritis.

Pathology Pearls: Chronic Kidney Disease

Gross Morphology

Bilaterally symmetrically small contracted kidneys occur due to long-standing injury to the glomeruli. External surface reveals diffuse fine granularity giving 'grain leather appearance'. Cut surface shows thinning of cortex. Peripelvic fat appears to be grossly increased, because of reduced renal parenchyma.

Light Microscopy

- **Glomeruli:** Hyaline obliteration of glomeruli transforms glomeruli into acellular eosinophilic masses (PAS+). Hyaline represents a combination of trapped plasma proteins, increased mesangial matrix, basement membrane-like material and collagen fibers.
- **Blood vessels:** Small- and medium-sized renal blood vessels are involved. Arteriosclerosis and arteriolosclerosis may be conspicuous as a result of accompanying hypertension.
- **Tubules:** These show dilation, atrophy, and loss of renal tubules. When the atrophic tubules contain eosinophilic proteinaceous casts, the resultant similarity in appearance to thyroid follicles is referred to as 'thyroidization' of tubules.
- **Interstitial tissue:** Kidneys show marked interstitial fibrosis with mononuclear cell infiltration.
- **Mesangial region:** There is increased mesangial matrix. Histology of chronic kidney disease is shown in Fig. 22.64.

CHRONIC KIDNEY DISEASE: LABORATORY FINDINGS AND CLINICAL MANIFESTATIONS

Most common laboratory findings in patients with chronic kidney disease include increased blood urea nitrogen and serum creatinine. There is disturbance of sodium, potassium, calcium, and phosphate and glucose metabolism. Acidosis is present. These patients need frequent hemodialysis.

- Urinalysis demonstrates isosthenuria fixed specific gravity due to loss of concentration and dilution functions of tubules, proteinuria, presence of waxy and broadcasts. Proteinuria diminishes due to obliteration of glomeruli.
- Azotemia refers to accumulation of waste products in blood, i.e. BUN, creatinine, urates, guanidino compounds, metabolic end products of nucleic acids, aliphatic amines, middle molecules (polypeptides) and parathormone. Parathormone is toxic to cells that augments contraction.
- Uremia is a set of clinical and laboratory findings encountered in patients with end stage kidney disease due to increased concentration of waste products in blood. Key laboratory features reflect inadequate excretion of metabolic products and minerals. Most patients are hypertensive and sometimes the dominant clinical manifestations are cerebral or cardiovascular accidents. Chronic renal disease is usually progressive.
- End stage renal disease has diminished glomerular filtration rate (GFR) <5% of normal range. The microscopic appearance of the end stage kidney is similar regardless of cause, that is why a renal biopsy in a patient with chronic renal failure yields a little useful information. The glomeruli are sclerosed. Tubules are often dilated and filled with pink casts and give an appearance of thyroidization. Blood vessels are thickened. There is presence of scattered chronic inflammatory cell infiltrates.
- Parameters in chronic kidney disease are given in Table 22.35. Etiology of chronic kidney disease is given in Table 22.36. Schematic representation of clinical manifestations of chronic kidney disease are

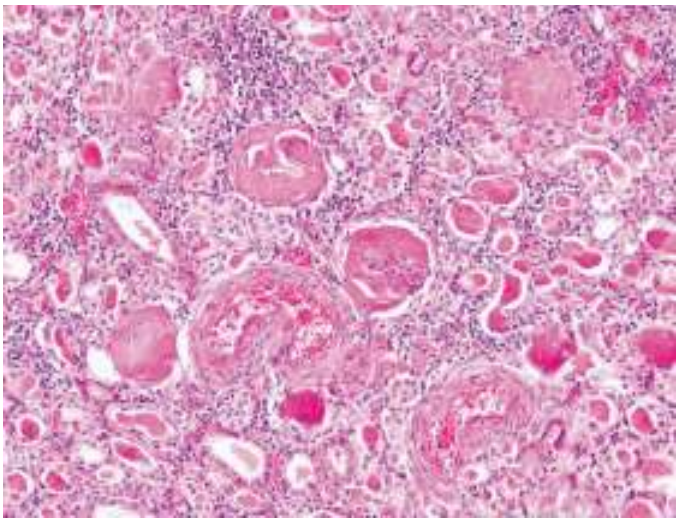


Fig. 22.64: Histology of chronic kidney disease. Hematoxylin and eosin-stained renal biopsy section shows glomerulosclerosis, tubular thyroidization (filled with colloid casts), tubular atrophy, hyalinization of blood vessels, intense diffuse lymphocytes and plasma cells in the interstitium and interstitial fibrosis (400X).

Table 22.35 Parameters in chronic kidney disease

Renal Reserve	Glomerular Filtration Rate (GFR)	Characteristics	
Diminished renal reserve	GFR (>50% of normal)	▪ Blood urea (normal range) ▪ Serum creatinine (normal range)	▪ Susceptible to uremia
Renal insufficiency	GFR (20–50% of normal)	▪ ↑Blood urea ▪ ↑Serum creatinine	▪ Sudden susceptible to uremia associated with hypertension, decreased concentrating ability of kidneys
Chronic renal failure	GFR (<20% of normal)	▪ ↑Blood urea ▪ ↑Serum creatinine	▪ Overt uremic manifestations (kidneys fail to regulate volume, solute composition and needing hemodialysis)
End stage renal disease	GFR (<5% of normal)	▪ ↑Blood urea ▪ ↑Serum creatinine	▪ Overt uremic manifestations with end stage kidney disease (kidneys fail to regulate volume, solute composition and needing hemodialysis)

Azotemia refers to accumulation of waste products in the body. Uremia refers to accumulation of waste products in the body with clinical manifestations.

Table 22.36 Etiology of chronic kidney disease

Etiology	Disorders	
Primary glomerular diseases	▪ Acute glomerulonephritis ▪ Antiglomerular basement membrane disease ▪ Chronic glomerulonephritis	▪ Goodpasture's syndrome ▪ Intercapillary glomerulosclerosis ▪ Rapidly progressive glomerulonephritis
Primary tubular diseases	▪ Chronic hypercalcemia ▪ Chronic potassium depletion	▪ Fanconi's syndrome ▪ Heavy metal poisoning (lead, cadmium)
Vascular diseases	▪ Ischemic renal diseases ▪ Long-standing nephrosclerosis	▪ Bilateral renal artery stenosis (congenital or acquired)
Infections	▪ Chronic pyelonephritis	▪ Tuberculosis
Obstructive disease	▪ Upper obstruction (calculi, neoplasms and retroperitoneal fibrosis)	▪ Lower obstruction (congenital anomalies of bladder or urethra, prostatic enlargement, urethral stricture)
Collagen diseases	▪ Diffuse systemic sclerosis (scleroderma) ▪ Systemic lupus erythematosus	▪ Polyarteritis nodosa
Metabolic diseases involving kidney	▪ Amyloidosis ▪ Chronic phenacetin over dosage ▪ Gout and hyperuricemic nephropathy	▪ Primary hyperparathyroidism ▪ Milk-alkali syndromes ▪ Sarcoidosis
Congenital anomalies	▪ Hypoplastic kidney ▪ Polycystic kidney disease	▪ Medullary cystic disease

shown in Fig. 22.65. Clinical manifestations in chronic kidney disease are given in Table 22.37.

Water and Electrolytes Imbalance

Electrolyte abnormalities in chronic kidney disease include either sodium loss (early) or sodium retention (late), hyperkalemia producing cardiac arrhythmias (potassium cannot be excreted, transcellular shift related to pH), and increased anion gap metabolic acidosis (retention of organic acids). Due to the diminished concentrating ability of kidney, patient develops polyuria leading to dehydration.

Acid–Base Equilibrium Imbalance

Under physiologic state, deamination of amino acids occurs in distal convoluted tubule (DCT) cells to form ammonia, which enters filtrate. Potassium and hydrogen ions also enter filtrate in exchange of sodium ions. Ammonia combines with hydrogen ions. In chronic glomerulonephritis, decreased formation of ammonia in the DCT cells, and decreased serum bicarbonate levels lead to decreased buffering action of hydrogen ions resulting in metabolic acidosis. Patient develops 'Kussmaul respiration' (sighing respiration).

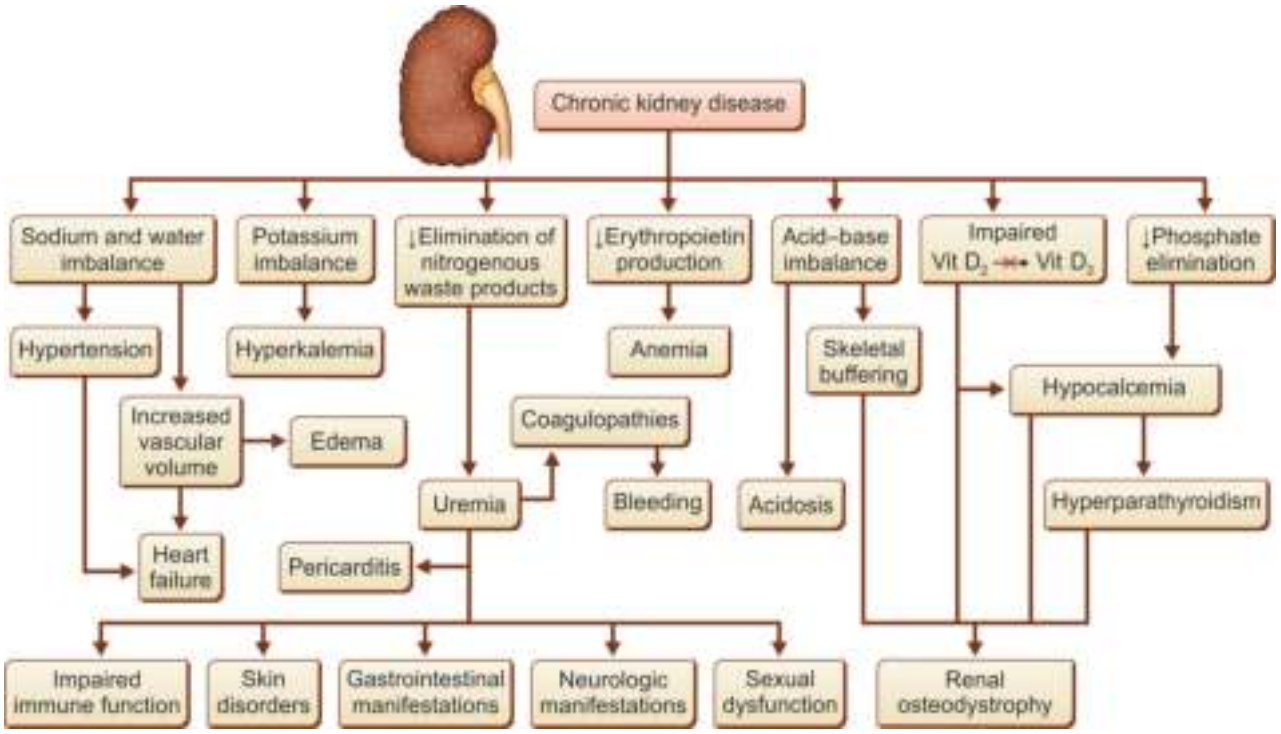


Fig. 22.65: Schematic representation of clinical manifestations of chronic kidney disease.

Table 22.37 Clinical manifestations in chronic kidney disease

System	Clinical Manifestations	
Cardiovascular system	▪ Hypertension present	▪ Serofibrinous pericarditis (occasional)
Respiratory system	▪ Hilar pneumonitis, X-ray shows 'batwing' opacity (uremic lung)	▪ Deep sighing respiration (Kussmaul respiration) ▪ Breath urine smell
Gastrointestinal tract	▪ Tongue is coated with ammoniacal or unpleasant taste, hiccup, anorexia, nausea, vomiting	▪ Diffuse or patchy ulcerations from mouth to anus results in gastrointestinal bleeding
Nervous system	▪ Headache, lassitude ▪ Drowsiness ▪ Weakness	▪ Muscle wasting ▪ Convulsions or coma
Ocular changes	▪ Eyelid swelling	▪ Arteriosclerotic retinopathy or hypertensive retinopathy
Dermal changes	▪ Skin grayish yellow due to urochrome deposit	▪ Pallor face
Hematological findings	▪ Normocytic normochromic picture with polychromasia	▪ Burr cells may appear
Biochemical abnormalities	▪ ↓ Glomerular filtration rate ▪ ↓ Creatinine clearance ▪ ↑ Blood urea ▪ ↑ Serum creatinine	▪ Urine concentrating ability decreased ▪ Disturbance of sodium, potassium, calcium, phosphate, and glucose metabolism ▪ Acidosis present
Urinary system	▪ Specific gravity fixed ▪ Urine analysis (proteinuria)	▪ Light microscopy reveals pyuria, hematuria, broadcasts

Cardiopulmonary Manifestations

In chronic kidney disease, patient develops hypertension, congestive heart failure and uremic pericarditis. Cardiopulmonary manifestations consist of accelerated atherosclerosis (increased VLDL from decreased catabolism), congestive heart failure with pulmonary edema (fine crepitations and dyspnea), hypertension (salt retention, volume overload, renin-angiotensin-aldosterone system), and uremic pericarditis (fibrinous pericarditis, often with hemorrhage), and uremic pneumonitis. Friction rub is heard on auscultation, which disappears, if the patient undergoes hemodialysis.

Gastrointestinal Tract Manifestations

Patients with chronic kidney disease develop gastrointestinal tract manifestations such as chronic esophagitis, hemorrhagic gastritis, peptic ulcer disease, enteritis, and intractable hiccups. Due to diffuse/patchy ulcerations in gastrointestinal tract, i.e. mouth to anus, patient presents with bleeding from gastrointestinal tract, nausea and vomiting. There is an increased susceptibility to infection (HBV, HCV, CMV, and HIV) owing to functional leukocyte abnormalities and the hazards of hemodialysis.

Hematologic Abnormalities

Normocytic anemias in chronic kidney disease most frequently occurs due to decreased synthesis of erythropoietin. Bleeding time is prolonged, because toxic metabolites cause functional defects of platelets, i.e. platelets aggregation and decreased platelet factor release. Patient has gastrointestinal tract bleeding.

Calcium and Phosphate Disturbances

Normally, vitamin D_2 to D_3 conversion takes place in kidney. Active metabolite D_3 maintains normal serum calcium level by enhancing calcium absorption at gastrointestinal tract level.

- In chronic kidney disease, vitamin D_3 active metabolite is not formed, hence leads to decreased absorption of calcium at gastrointestinal tract level. Hence, serum calcium is low.
- Hypocalcemia occurs owing to hypovitaminosis D due to loss of the 1α-hydroxylating enzyme and hyperphosphatemia as a result of decreased excretion, which drives calcium into tissue. Increased phosphate levels in blood reduce blood ionized calcium level, which stimulates parathyroid glands to produce parathormone. Hemodialysis fluid rich in aluminum inhibits calcium deposition in bones resulting in osteomalacia, osteoporosis and osteitis fibrosa cystica.

Pathology Pearls: Renal Osteodystrophy (Bone Changes in Chronic Renal Failure)

Renal osteodystrophy (skeletal abnormalities) occurs due to deranged calcium and phosphates metabolism. Bone alterations include osteomalacia (decreased bone mineralization), osteoporosis (decreased bone matrix; due to buffering action of metabolic acidosis), and osteitis fibrosa cystica from secondary hyperparathyroidism.

Osteomalacia

Osteomalacia refers to decreased mineralization of the organic bone matrix (osteoid). Hypovitaminosis D causes hypocalcemia leading to decreased bone mineralization. It produces fractures and bone pain.

Osteoporosis

Osteoporosis refers to decreased bone matrix due to metabolic acidosis. Loss of organic bone matrix and minerals causes an overall reduction in bone mass. It produces fractures and bone pain.

Osteitis Fibrosa Cystica

Hypovitaminosis D causes hypocalcemia, which stimulates production of parathormone resulting in secondary hyperparathyroidism, that increases bone resorption and causes cystic lesion in bone (jaw). Hemorrhage into the cystic lesions in jaw causes a brown discoloration. Histology of osteitis fibrosa cystica is shown in Fig. 22.66.

Dermatological Changes

Patient with chronic renal disease develops an array of cutaneous manifestations such as xerosis, pruritus, cutaneous pigmentation, sallow discoloration of skin due to accumulation of urochrome pigment in skin, skin infections, uremic frost and nail changes (e.g. half nails, koilonychia, splinter hemorrhages, onychomycosis, subungual hyperkeratosis, Mees lines, and Muehrcke's lines).

Neuromuscular Disturbances

Patient with chronic renal disease is most often afflicted with neurological complications, which can adversely affect the central and peripheral nerves. Common neurological complications in the patient include skeletal muscle weakness, functional peripheral nerve disease, encephalopathy, seizures, cerebral stroke and coma. Dietary potassium restriction may be useful to prevent peripheral neuropathy.

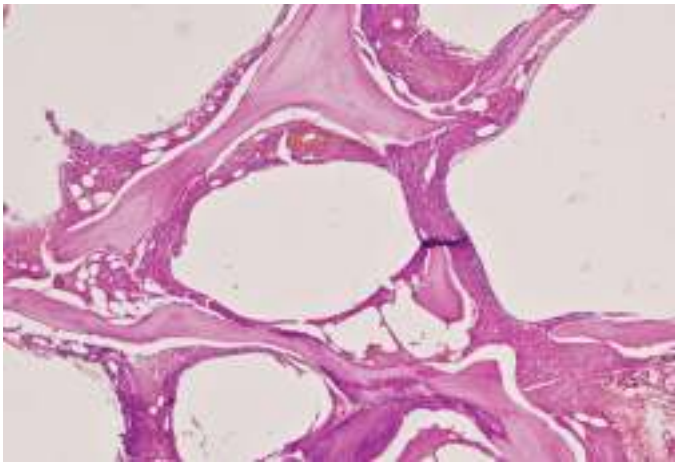


Fig. 22.66: Histology of osteitis fibrosa cystica. Renal osteodystrophy is the term used to describe different patterns of skeletal abnormalities that occur in patients with chronic renal disease and hyperparathyroidism. Osteitis fibrosa cystica is a manifestation of the effects of high levels of parathormone (PTH) on bone and is associated with a high bone turnover. Parathormone induces osteoclast activity which results in a bone breaks down. Hematoxylin and eosin-stained skeletal tissue section shows osteoclastic and osteoblastic activity with hemosiderin laden macrophages and cysts formation (400X).

CHRONIC KIDNEY DISEASE: MEDICAL/SURGICAL MANAGEMENT

Supportive care and treatment to manage symptoms of chronic kidney disease without performing dialysis or having a renal transplant, is called medical management.

Dialysis

Dialysis is the process of removing excess of waste metabolic waste products and toxins through selectively permeable membrane from the blood in patients whose kidneys no longer perform physiologic functions. Dialysis is performed by hemodialysis or peritoneal dialysis.

- In hemodialysis, the patient blood flows through a dialysis machine.
- In peritoneal dialysis, the dialysate is introduced into the peritoneal cavity; the peritoneum acts as the dialysis membrane.
- Dialysis machine itself can be thought of as an artificial kidney. Dialysate is the fluid that helps in removal of unwanted waste metabolic products from blood. In peritoneal dialysis, dialysate is introduced into the peritoneal cavity, in which peritoneum acts as the dialysis machine.

Renal Transplantation

Renal transplantation is the only surgical procedure of fully correcting the impaired renal functions for patient with end stage renal disease.

- **Indications:** All patients with chronic kidney disease stage 4 (GFR 15–29 ml/min) or end stage renal failure (GFR <15 ml/min) with progressive renal disease should be assessed for renal transplantation. It has been increasingly successful with renal graft survival in 80% of cases due to better ABO and HLA matching of the donor and improved post-transplant immunosuppression.
- **Contraindications:** Contraindications of renal transplantation include untreated malignancy, active infection, untreated HIV infection/AIDS, HBV or HCV infection and uncontrolled diabetes mellitus.
- **Complications:** Several complications can occur postoperatively, including delayed graft function, vascular and urethral complications. Patient with delayed renal graft function need dialysis in the first week after renal transplantation.
  - Early vascular complications includes renal artery and renal vein thrombosis, which can be recognized using a Doppler ultrasound. Aspirin and/or heparin should be administered postoperatively to reduce risk for renal vessels thrombosis.
  - Late vascular complications include renal artery stenosis several months after renal transplantation with uncontrolled hypertension. Renal angiography confirms the diagnosis and patients are treated by angioplasty.

RENAL BIOPSY EVALUATION AND INTERPRETATION

Evaluation of renal biopsy has a diagnostic role in renal disorders. Successful renal biopsy is a traumatic procedure; hence the patient's urine consists of RBCs after successful renal biopsy. Renal biopsy must consist of at least five glomeruli for diagnosing renal lesions. Kidney is composed of glomeruli, tubules, blood vessels and interstitial tissue. A renal biopsy specimen section is stained with H&E and examined by light microscopy, immunofluorescence microscopy, and electron microscopy (EM).

LIGHT MICROSCOPY

Routine and specialized stains help classify the glomerular disease as diffuse, focal, membranous, proliferative glomerulonephritis. In H&E stained tissue section, we should evaluate, overall cellularity, the symmetry and the thickness of the glomeruli.

- **Glomeruli:** With normal glomerular cellularity, cell nuclei are not clustered or overlapping. Clusters of cells in glomeruli, especially away from the hilum, indicate abnormal hypercellularity.
  - Glomerular cellularity is increased due to proliferation of glomerular cells (endothelial cells and mesangial cells), infiltration of leukocytes in glomeruli, and proliferation of extracapillary parietal cells of Bowman's capsule forming crescents.
  - Extracellular material is deposited in glomeruli such as amyloid deposit, fibrinoid necrosis in malignant hypertension, insudation of plasma proteins in benign hypertension (hyalinosis), and collagen matrix in glomerulosclerosis.
- **Tubules:** Normally tubules are arranged back-to-back. Inflammatory process of kidney widens intertubular space.
- **Blood vessels:** Blood vessels show nephrosclerotic changes in hypertension. There is increased extracellular material in glomeruli due to (a) immune complex deposits, (b) thickening or replication of glomerular basement membrane (GBM), (c) increase in collagen matrix (sclerosis), (d) insudation of plasma proteins (hyalinosis), (e) fibrinoid necrosis, and (f) amyloid deposit.
- **Interstitial tissue:** The renal interstitium is defined as the intertubular, extraglomerular, extravascular space of the kidney. It is bounded on all sides by tubular and vascular basement membranes, and diseased kidney.

Schematic representation of glomerular hypercellularity in various types of glomerulonephritis is shown in Fig. 22.67. Patterns of glomerular injury observed under light microscopy and some but not all diseases that cause patterns of glomerular injury are given in Table 22.38. Major glomerular patterns and differential diagnosis on light microscopy are given in Table 22.39. Histochemical stains used to evaluate renal biopsy are given in Table 22.40. Staining characteristics of normal and abnormal renal structures by selected histochemical stains are given in Table 22.41.

Pathology Pearls: Jones Methenamine Silver Stain used in Evaluation of Renal Biopsy

- Jones methenamine silver (JMS) stain is a histochemical stain, which highlights carbohydrates that can be oxidized to aldehydes. Aldehydes formed by oxidation of carbohydrates reduce silver solution, causing the silver ions to bind to the carbohydrate and can be visualized under light microscope.
- JMS stain highlights details of basement membrane of glomeruli, tubules, Bowman's capsule, elastic lamina of blood vessels and sclerotic segments of glomeruli (glomerulosclerosis).
- JMS stain delineates glomeruli within crescents and hence highlights the presence of extracapillary proliferation, also delineates mesangial regions of glomeruli, which are argyrophilic.

IMMUNOFLUORESCENCE MICROSCOPY

Immunofluorescence (IF) microscopy is a powerful technique that utilizes fluorescent labeled antibodies to detect specific target antigens. Fluorescein dye emits greenish fluorescence under UV light, which can be tagged to immunoglobulin molecules. Immunofluorescence microscopy identifies immune complex deposits, patterns of deposition of IgG, IgA, IgM, complement (C1q, C3), fibrin, light chains, and albumin.

Location of Immune Complex Deposits

Highly anionic macromolecules are excluded by glomerular basement membrane, which get trapped in subendothelial region of glomeruli. Cationic molecules bind to anionic sites, DNA nucleosome and other nuclear proteins. Highly cationic immunogens tend to cross subepithelial region of glomeruli. Neutral charged molecules are trapped in mesangial region of glomeruli.

- **Subendothelial immune complex deposits:** Immune complexes are deposited in subendothelial region in patient with systemic lupus erythematosus.
- **Subepithelial immune complex deposits:** Lumpy-bumpy (large size) immune complex deposits are demonstrated in poststreptococcal glomerulonephritis and membranous glomerulonephritis.

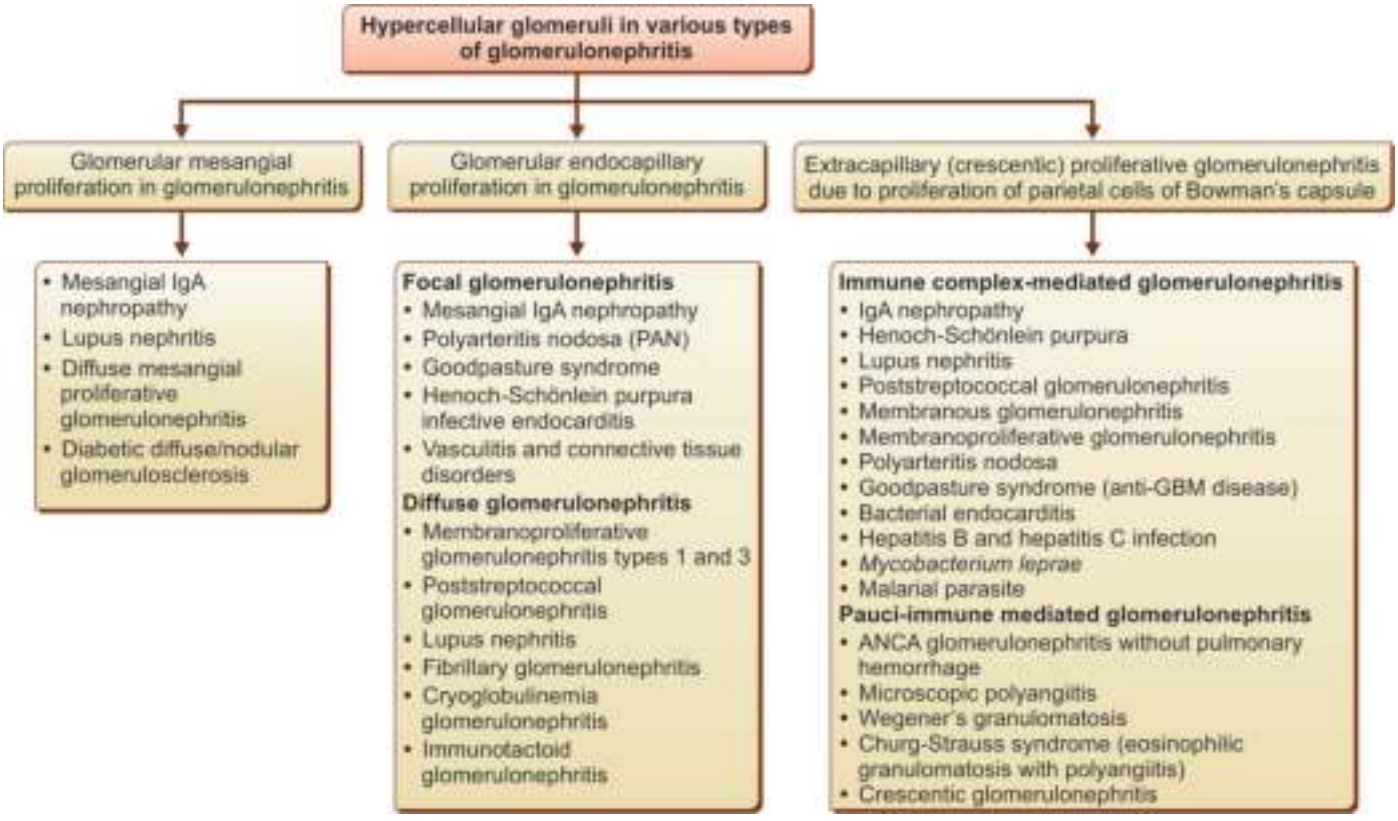


Fig. 22.67: Schematic representation of glomerular hypercellularity in various types of glomerulonephritis. It shows etiology of proliferation of mesangial cells, endocapillary and exocapillary hypercellularity.

Table 22.38 Patterns of glomerular injury observed under light microscopy and some but not all diseases that cause patterns of glomerular injury

No Glomerular Abnormality by Light Microscopy

- No glomerular disease
- Glomerular disease without light microscopic changes
  - Minimal change disease of glomeruli
  - Thin membrane disease of glomeruli
- Mild or early glomerular disease
  - Class 1 lupus nephritis
  - IgA nephropathy
  - C1q nephropathy
  - Membranous glomerulonephritis
  - Renal amyloidosis
  - Alport syndrome

Glomeruli with Thick Walls and Mesangial Expansion but Little or without Hypercellularity

- Diabetic glomerulosclerosis with diffuse glomerulosclerosis rather than nodular mesangial glomerulosclerosis (Kimmelstiel-Wilson disease)
- Secondary membranous glomerulopathy with mesangial immune deposits
- Dense deposit disease (type 2 membranoproliferative glomerulonephritis)
- Renal amyloidosis
- Monoclonal immunoglobulin deposition disease
- Fibrillary glomerulonephritis

Focal Segmental Glomerulosclerosis without Glomerular Hypercellularity

- Focal segmental glomerulosclerosis (primary or secondary)
- Chronic sclerotic phase of focal glomerulonephritis
- Alport syndrome (hereditary nephritis)

Mesangial or Endocapillary Glomerular Hypercellularity

- Focal or diffuse mesangioproliferative glomerulonephritis
- Acute (exudative) diffuse proliferative postinfectious glomerulonephritis
- Focal or diffuse endocapillary proliferative glomerulonephritis
- Membranoproliferative glomerulonephritis (type 1, type 2/dense deposit disease or type 3)

Extracapillary Glomerular Hypercellularity

- Antiglomerular basement membrane (anti-GBM) glomerulonephritis (granular immunoglobulin pattern by immunofluorescence microscopy)
- Immune complex crescentic glomerulonephritis (granular immunoglobulin deposit by immunofluorescence microscopy)
- ANCA crescentic glomerulonephritis (paucity of immunoglobulin deposit by immunofluorescence microscopy)
- Collapsing variant of focal segment glomerulosclerosis (including HIV nephropathy)

Membranoproliferative, Glomerular Lobular and Nodular Pattern

- Diabetic glomerulosclerosis with nodular mesangial glomerulosclerosis (Kimmelstiel-Wilson disease)
- Membranoproliferative glomerulonephritis (type 1, type 2/dense deposit disease or type 3B/3S)
- Monoclonal immunoglobulin deposition disease with nodular glomerulosclerosis
- Idiopathic (tobacco smoking associated) nodular glomerulosclerosis
- Thrombotic microangiopathy
- Fibrillary glomerulonephritis
- Fibrillary glomerulonephritis
- Immunotactoid glomerulopathy

Advanced Diffuse Global Glomerulosclerosis

- End stage glomerular disease
- End stage vascular disease
- End stage tubulointerstitial disease

Table 22.39 Major glomerular patterns and differential diagnosis on light microscopy

Parameters on Light Microscopy	Examples	
Minimal glomerular changes	▪ Minimal change disease (MCD) ▪ Thin glomerular membrane disease ▪ Early lupus nephritis	▪ Early mild IgA nephropathy ▪ Early diabetic nephropathy
Proliferative glomerulonephritis	▪ Post-infectious glomerulonephritis ▪ Lupus nephritis	▪ Henoch-Schönlein purpura
Focal segmental glomerulosclerosis	▪ Primary glomerulonephritis, NOS	▪ Secondary glomerulonephritis, NOS
Mesangial cell proliferation (hypercellular mesangial region)	▪ IgA nephropathy	▪ Lupus nephritis
Thick capillary loops	▪ Membranous glomerulonephritis ▪ Diabetic nephropathy	▪ Alport's syndrome ▪ Amyloidosis nephropathy (early stage)

Contd...

Table 22.39 Major glomerular patterns and differential diagnosis on light microscopy (*Contd...*)

Parameters on Light Microscopy	Examples	
Tram-track appearance	▪ Membranoproliferative glomerulonephritis ▪ Henoch-Schönlein purpura	▪ Lupus nephritis type 4
Nodular pattern	▪ Diabetic nephropathy ▪ Amyloid nephropathy	▪ Monoclonal immunoglobulin deposition disease (MIDD)
Crescents formation	▪ Goodpasture's syndrome ▪ Acute diffuse proliferative glomerulonephritis (occasional) ▪ Diffuse mesangioproliferative glomerulonephritis	▪ Henoch-Schönlein purpura ▪ Microscopic polyarteritis ▪ Wegener's granulomatosis ▪ Acute scleroderma involving kidney ▪ Segmental lupus nephritis with tuft necrosis

Table 22.40 Histochemical stains used to evaluate renal biopsy

Stains	Used to Demonstrate	
Hematoxylin and eosin stain	▪ Glomerular cellularity, symmetry and thickness ▪ Tubules	▪ Blood vessels ▪ Interstitial tissue
Periodic acid–Schiff (PAS) stain	▪ Glomerular basement membrane ▪ Tubular basement membrane	▪ Mesangial matrix
Jones methenamine silver stain	▪ Glomerular basement membrane	▪ Tubular basement membrane
Congo red stain	Amyloid deposit in glomeruli and blood vessels	
Masson's trichrome stain	Collagen deposition (focal segmental glomerulosclerosis)	
Fat stains (Sudan III, IV, black B, oil red O)	Fat microemboli in glomeruli	

Table 22.41 Staining characteristics of normal and abnormal renal structures by selected histochemical stains

Renal Tissue Components	PAS Stain	Masson's Trichrome Stain	Jones Methenamine Silver Stain
Normal renal structures			
Glomerular basement membrane	Red staining	Deep blue staining	Black staining
Tubular basement membrane	Red staining	Deep blue staining	Black staining
Mesangial matrix	Red staining	Deep blue staining	Black staining
Interstitial collagen fibers	No stained	Pale blue staining	No stained
Cell cytoplasm	No stained	Rust/orange granular	No stained
Abnormal renal findings			
Immune complex	Negative/positive	Bright red-orange homogenous	No stained
Fibrin	Weakly positive	Bright red-orange fibrillar	No stained
Amyloid	–	Light blue-orange	No stained
Tubular casts (Tamm-Horsfall protein)	Red	Light blue	Gray black
Insudative lesions	Negative/positive	Bright red-orange homogenous	No stained

- **Intramembranous immune-complex deposits:** Immune complex deposits are seen within glomerular basement membrane in systemic lupus erythematosus and membranoproliferative glomerulonephritis.
- **Mesangial deposits:** Immune complex comprising of IgA1 and complement are deposited in mesangial region of glomeruli in patient with IgA nephropathy.

Patterns of Immune Complex Deposits

Immune complexes deposited in glomeruli may show diffuse linear staining or lumpy bumpy or irregular (fluffy) patterns. Immunofluorescence microscopy is shown in Fig. 22.68A and B. Patterns of immune complex deposits by immunofluorescence microscopy in glomerulopathy are given in Table 22.42. Immunofluorescence microscopy is used to demonstrate

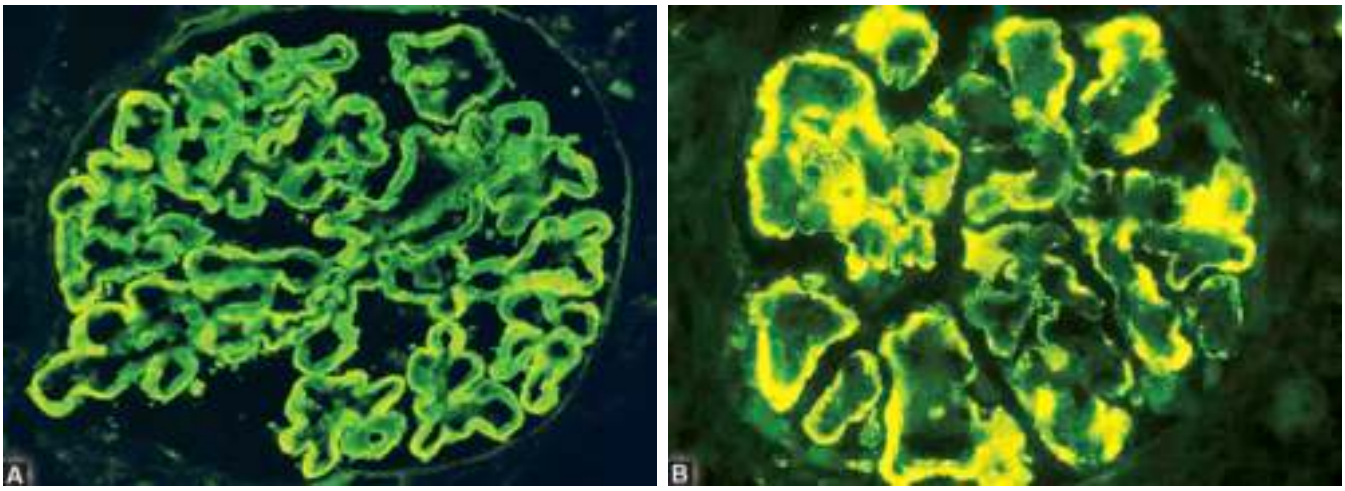


Fig. 22.68: Immunofluorescence microscopy in glomerulonephritis. Immunofluorescence and light microscopy is required for accurately diagnosing glomerulonephritis. (A) Linear deposition of endogenous immunoglobulin (Ig) along the glomerular basement membrane, (B) in contrast to a linear deposition of immunoglobulin, renal diseases associated with immune complex deposition has a granular 'lumpy-bumpy appearance.

Table 22.42 Patterns of immune complex deposits by immunofluorescence microscopy in glomerulopathy

Diffuse Linear Staining for IgG
Goodpasture's syndrome
Granular Lumpy-bumpy Immune Complex Deposits
▪ Poststreptococcal glomerulonephritis (subepithelial region) ▪ Membranous glomerulonephritis (intramembranous and subepithelial regions) ▪ IgA nephropathy (mesangial region) ▪ Lupus glomerulonephritis (subendothelial region)
Irregular (Fluffy) Immune Complex Deposits
Monoclonal light chains (AL amyloidosis), AA protein (AA amyloidosis)

immune deposits in glomerular diseases as given in Table 22.43.

- **Diffuse linear staining of GBM:** Diffuse linear staining for IgG is seen along the glomerular basement membrane in Goodpasture's syndrome.
- **Granular lumpy-bumpy immune complex deposits in glomeruli:** Granular lumpy-bumpy immune complex deposits are demonstrated in poststreptococcal glomerulonephritis (subepithelial region), membranous glomerulonephritis (intramembranous and subepithelial regions), IgA nephropathy (mesangial region) and lupus glomerulonephritis (subendothelial region). Their distribution is less uniform owing to differences in their charge, size, and solubility.
- **Irregular (fluffy) deposits in glomeruli:** Irregular (fluffy) staining is associated with monoclonal light chains (AL amyloidosis) and AA protein (AA amyloidosis).

Table 22.43 Immunofluorescence microscopy is used to demonstrate immune deposits in glomerular diseases

Subendothelial Immune Complex Deposits
Lupus nephritis (systemic lupus erythematosus)
Subepithelial Lumpy-bumpy Immune Complex Deposits
▪ Poststreptococcal glomerulonephritis ▪ Membranous glomerulonephritis
Intramembranous Immune Complex Deposits
▪ Membranoproliferative glomerulonephritis ▪ Lupus nephritis (systemic lupus erythematosus)
Mesangial Immune Complex Deposits
IgA nephropathy
Immune Complex Deposits along Glomerular Basement Membrane
Diffuse linear immunofluorescence for IgG and C3, fibrin in crescents is seen along the glomerular basement membrane in Goodpasture's syndrome.

ELECTRON MICROSCOPY

Electron microscopy (EM) is a useful diagnostic technique to supplement morphologic, immunohistochemical, cytogenetic and molecular analysis of tissues, although immunoperoxidase techniques have largely replaced EM for tumor diagnosis in surgical pathology.

- Electron microscopes are basically of two types: transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Renal biopsy is fixed in 2% glutaraldehyde embedded in epoxy resin. Ultrathin sections are obtained with microtome. Sections are placed on a specimen grid end of electron microscope. Digital image is obtained and analyzed.

- Renal biopsy examined under electron microscopy reveals site of immune complex deposits, thickening and replication of glomerular basement membrane, collagen matrix and fibrillary deposits.
- Electron dense immune complex deposits are demonstrated by electron microscopic study in membranous glomerulonephritis (intramembranous and subepithelial region), IgA nephropathy (mesangial region) and lupus glomerulonephritis (subendothelial region). Electron microscopy identifies structural abnormalities in minimal change disease affecting children responsible for nephrotic syndrome (e.g. effacement of foot process).
- Electron microscopy in glomerulopathy and immunofluorescence are given in Table 22.44. Immune complex deposits demonstrated by various immunofluorescence, light and electron microscopy in renal biopsy are given in Table 22.45.

Table 22.44 Electron microscopy in glomerulopathy and immunofluorescence

Electron Dense Immune Complex in Glomeruli
▪ Membranous glomerulonephritis (intramembranous and subepithelial region) ▪ IgA nephropathy (mesangial region) ▪ Lupus glomerulonephritis (subendothelial region) ▪ Minimal change disease (effacement of podocytes) ▪ Poststreptococcal glomerulonephritis (subepithelial region)
Glomerular Basement Membrane Thickening
Diabetic glomerulosclerosis
Glomerular Basement Membrane Replication
Membranoproliferative glomerulonephritis
Collagen Matrix Deposition
Focal segmental glomerulosclerosis
Fibrillary Deposit
Amyloid nephropathy (mesangial region)

Table 22.45 Immune complex deposits demonstrated by various immunofluorescence, light and electron microscopy in renal biopsy

Immunofluorescence Microscopy	Light Microscopy	Electron Microscopy
Diffuse linear immune complex deposits	Not visible	Not visible
Granular immune complex deposits	Bright red (Masson's trichrome stain)	Electron dense immune complex

TUBULAR DISEASES OF KIDNEY

Each of the convoluted tubules is transmitting urine from the glomeruli to the renal pelvis in the kidney. Water and salts are reabsorbed into the blood along their length. Once the glomerular filtrate passes through the proximal tubule in renal cortex, descending and ascending limb of loop of Henle. It enters the distal convoluted tubule in renal cortex.

- Proximal tubule absorbs essential metabolites by active process such as Na^+, K^+, Cl^-, HCO_3^-, amino acids, glucose and phosphates and water (80%) by passive process.
- Descending limb of loop of Henle is only permeable to water, hence absorbs water (10%) by passive process. Ascending limb of loop of Henle is impermeable to water, but permeable to salts, hence absorbs Na^+, chloride, K^+, Ca^+, HCO_3^- and Mg^+ by active process.
- Distal tubules absorb essential metabolites such as Na^+, Cl^- (absorption of Na^+, Cl^-, by aldosterone), Ca^+ and Mg^+ by active process; and water (5%) by passive process.
- Collecting ducts absorb water (5%) by the action of antidiuretic hormone (ADH). Hydrogen ions and organic acids secreted by proximal tubules enter peritubular network of blood vessels. Hydrogen ions are also secreted by distal tubule.

ACUTE TUBULAR NECROSIS

Tubular epithelial cells are particularly sensitive to anoxia and toxins, which cause tubular epithelial cell necrosis. Acute tubular necrosis (ATN) is a clinicopathologic entity with pathologic changes in the tubules when ischemic or nephrotoxic injuries adversely affect the kidneys. Ischemia-induced ATN represents about 60% of cases.

- ATN is a severe clinicopathologic entity, but potentially reversible, impairment of tubular epithelial cell function resulting in acute renal failure within 24 hours. Kidneys are not able to excrete waste metabolic products (urea and creatinine) and maintain normal fluid and electrolyte homeostasis.
- Complications of ATN include water and salt overload and metabolic acidosis. Prognosis of AT depends on the clinical stage. Treatment is usually supportive. Recovery most often occurs during the first month after acute renal failure, but can be seen even after one year.

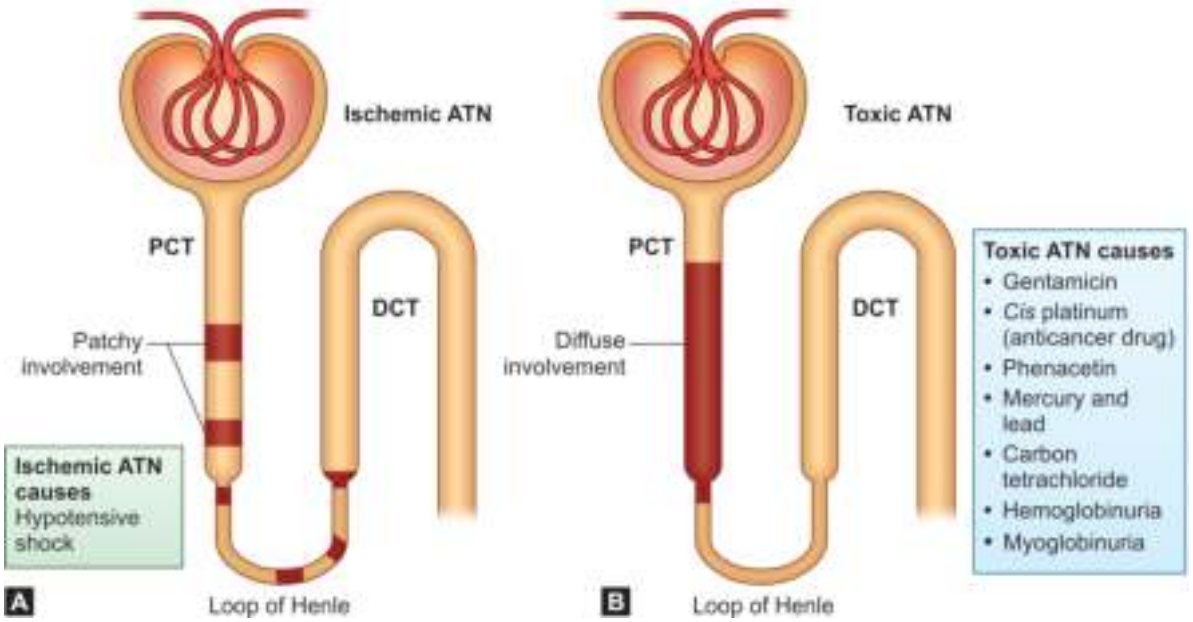


Fig. 22.69A and B: Schematic representation of acute tubular necrosis (ATN). ATN is classified as either ischemic or toxic types. Ischemic ATN occurs when the tubular epithelial cells do not receive enough oxygen that they are highly sensitive and susceptible to ischemia, due to their high metabolic activity. Toxic ATN occurs when the tubular cells are exposed to a nephrotoxic substance.

- Acute tubular necrosis is divided into ischemic and nephrotoxic. ATN is accompanied by oliguria (urine output <400 ml/24 hours) or anuria in most of cases. Some cases have polyuria (>800 ml/24 hours). Urine analysis shows hyaline granular or pigmented casts.
- Schematic representation of acute tubular necrosis (ATN) is shown in Fig. 22.69. Causes of acute tubular necrosis (ATN) are given in Table 22.46. Comparison between ischemic and nephrotoxic types of acute tubular necrosis (ATN) is given in Table 22.47.

ISCHEMIC ACUTE TUBULAR NECROSIS

Ischemic ATN results from reduced renal perfusion, usually associated with hypotension following massive hemorrhage, burns and septicemia including incompatible blood transfusion, acute pancreatitis and acute intestinal obstruction. Ischemia starts in outer cortex and extends inwards as blood pressure falls. Ischemic changes are marked in the cortical region (proximal tubule straight segment and thick ascending limb of loop of Henle), and medullary region (loop of Henle). Juxtamedullary nephrons usually escape ischemic injury, being closer to main renal arterial branches.

Pathogenesis

Tubular epithelial cells with their high rate of energy consuming metabolic activity and numerous organelles, are particularly sensitive to hypoxia and anoxia.

Table 22.46 Causes of acute tubular necrosis (ATN)

Ischemic Acute Tubular Necrosis
Major surgical procedures
Massive acute blood loss
Severe burns
Acute pancreatitis
Incompatible blood transfusion
Acute intestinal obstruction
Nephrotoxic Acute Tubular Necrosis
Endogenous products (e.g. hemoglobinuria, myoglobinuria)
Heavy metals (lead and mercury)
Antibiotics (e.g. gentamycin, penicillin, methicillin)
Anticancer drug (cisplatin)
Organic solvents (carbon tetrachloride, chloroform)
Nonsteroidal anti-inflammatory drugs (e.g. phenacetin)
Other toxic agents (ethylene, glycol, paraquat, poisonous fungi)
Radiographic contrast media

Acute tubular necrosis due to nephrotoxic agents is the most important cause of acute renal failure.

- Depletion of adenosine triphosphate (ATP) results in accumulation of intracellular calcium, which activates cellular hydrolytic enzymes.
- Phospholipase causes cell membrane injury. Protease causes rearrangement of cytoskeleton. Generation of reactive oxygen species amplify the tubular

Table 22.47 Comparison between ischemic and nephrotoxic types of acute tubular necrosis (ATN)

Ischemic Acute Tubular Necrosis (ATN)	Nephrotoxic Acute Tubular Necrosis (ATN)
Predisposing factors	
▪ Renal ischemia ▪ Major surgical procedures ▪ Massive acute blood loss ▪ Severe burns ▪ Acute pancreatitis ▪ Incompatible blood transfusion	▪ Hemoglobinuria ▪ Myoglobinuria ▪ Metals (lead, mercury) ▪ Drugs (gentamycin, penicillin, methicillin, phenacetin, cisplatin) ▪ Acute intestinal obstruction ▪ Chemical agents (carbon tetrachloride, chloroform, ethylene, glycol, paraquat poison) ▪ Poisonous fungi ▪ Radiographic contrast media
Proximal tubules and loop of Henle lesion patten	
Patchy lesions with detachment of tubular basement membrane	Diffuse lesions without detachment of tubular basement membrane
Regeneration of tubular epithelial cells	
Good regeneration on immediate restoration of blood supply	Poor regeneration
24 hours urinary output	
Oliguria (<400%)	Normal or increased urinary output

damage. Release of cytokines leads to recruitment of leukocytes. Influx of neutrophils leads to more tubular injury by liberating hydrolytic enzymes.

- Focal denudation of the tubular basement membrane occurs due to necrosis of individual tubular epithelial cells. Necrotic epithelial cells enter into some of tubular lumina. Ischemia causes patchy necrosis of proximal tubules and loop of Henle with intervening skip lesions.
- Tubular basement membranes are disrupted at ischemic sites, which prevent tubular cell regeneration. Ischemia and nephrotoxins cause necrosis of tubular cells as well as endothelial cells. Schematic representation of pathogenesis of acute tubular necrosis (ATN) is shown in Fig. 22.70.

Pathology Pearls: Pathogenesis of Acute Tubular Necrosis

- Necrosed tubular epithelial cells exfoliate and enter into distal tubular lumen, whih cells combine with Tamm-Horsfall proteins and form pigmented renal tubular cell casts in distal tubules. Tubular casts obstruct urinary outflow and increase intratubular pressure, which pushes fluid into the interstitium resulting in decreased glomerular filtration rate (oliguria).
- Vasoconstriction of renal afferent arterioles plays an important role in oliguria due to decreased glomerular filtration rate (GFR).
- GFR occurs as a result of decreased synthesis of vasodilators by injured endothelial cells such as nitric oxide and PGI_2; and increased synthesis of vasoconstrictor angiotensin II.
- Net result is decreased glomerular filtration rate. All these morphologic changes lead to reduced production of urine and cause oliguria. Initially, the changes induced by ischemia or toxins are reversible, but later become irreversible.

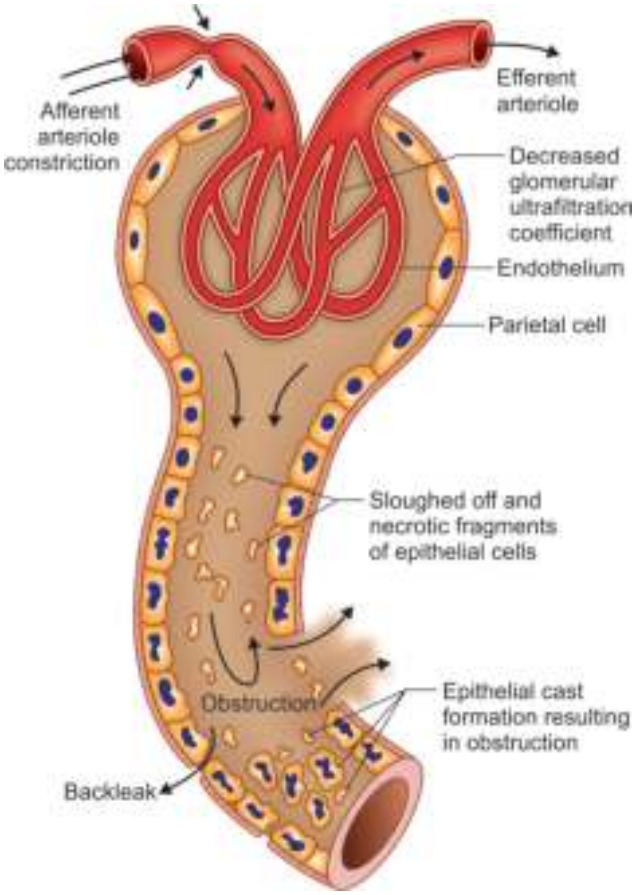


Fig. 22.70: Schematic representation of pathogenesis of acute tubular necrosis (ATN). Sloughing and necrosis of tubular epithelial cells lead to formation of casts in the tubules. Tubular casts cause obstruction and increase intraluminal pressure resulting in reduction of glomerular filtration rate. Tubular injury and increased intraluminal pressure are responsible for leakage of fluid from tubular lumen into the interstitium.

Clinical Features

Within 0–36 hours of ischemic tubular necrosis, patient presents oliguria (urinary output <400 ml/24 hours), salt and water overload, hyperkalemia, metabolic acidosis, uremia (elevated blood urea nitrogen and creatinine), BUN and creatinine ratio (<15) and urinary sediment showing tubular epithelial cells and granular casts. Patient needs dialysis to treat impaired renal functions. Mortality occurs in 5% of untreated cases if no damage to other organs occurs.

Pathology Pearls: Ischemic Acute Tubular Necrosis

Gross Morphology

The kidneys are usually enlarged. Cut surface reveals pale cortex and a congested medulla. No pathologic changes are seen in the glomeruli or blood vessels. The corticomedullary junction may be accentuated with deep red medullary regions.

Histologic and Ultrastructural Changes

There is a spectrum of morphologic changes in the renal tubules in acute tubular necrosis.

- **Early changes in tubules:** Initial ultrastructural changes in affected tubules vary from cell swelling, tubule dilated tubule, loss of periodic acid–Schiff (PAS) positive brush border of proximal tubules, blebbing of apical cell membrane, hyaline, granular and pigmented casts particularly in distal and collecting ducts, detachment of tubular epithelial cells forming eosinophilic tubular hyaline casts of Tamm-Horsfall protein (urinary glycoprotein normally secreted by these cells); white blood cells in dilated vasa recta, interstitial edema. Thinning of tubular epithelium occurs with enlargement of the lumen of the tubules. Changes in ischemic cases are usually milder and more focal than toxic ATN.
- **Late changes in tubules:** Early changes in tubules are followed by epithelial cell regeneration (flattened epithelium, dilated tubular lumina, large atypical nuclei with prominent nucleoli and mitotic figures and basophilic cytoplasm). If blood supply is not restored, irreversible changes in tubules occur such as detachment of tubular cell membrane. Nucleus undergoes changes such as pyknosis, karyorrhexis and karyolysis. Pigmented casts often represent hemoglobin or myoglobin in tubule lumen.

Electron Microscopy

Electron microscopic findings in acute tubular necrosis include simplification of tubular epithelium, cytoplasmic vacuolation, loss of brush borders, and small gaps in the lining of the tubular epithelium.

NEPHROTOXIC ACUTE TUBULAR NECROSIS

Nephrotoxic substances induced tubular epithelial injury is the most common cause of acute renal failure. Tubular epithelial cells being metabolically active absorb and concentrate these nephrotoxic agents. That is the reason that tubular cells are vulnerable to injury due to nephrotoxic substances.

- Nephrotoxic agents include antibiotics (penicillin, methicillin, cephalosporins, polymyxin), anticancer drugs (cisplatin), nonsteroidal anti-inflammatory drugs (NSAIDs, e.g. phenacetin), ethylene glycol, radiographic contrast media, heavy metals (mercury, lead) and organic solvents (carbon tetrachloride, chloroform). Prolonged intake of analgesic phenacetin causes renal papillary necrosis by inhibiting prostaglandin synthesis (vasodilator) resulting in ischemia of the renal medulla.
- Ethylene glycol also causes intratubular deposition of oxalate crystals visualized by polarized microscopy.
- Hemoglobinuria and myoglobinuria have nephrotoxic effect and cause acute tubular necrosis. Tubules show widespread necrosis of proximal tubular epithelial cells with sparing of distal and collecting tubules. Basement membrane of proximal tubules is intact and revealing vacuolation of cytoplasm. Nephrotoxic acute tubular necrosis is nonoliguric type of acute tubular necrosis.
- Patient has either normal urinary output or polyuria associated with more benign clinical course. Tubular findings in nephrotoxic acute tubular necrosis (ATN) are given in Table 22.48.

ACUTE KIDNEY DISEASE

Acute kidney disease (AKD), formerly known as acute renal failure is the sudden interruption of renal function as measured by glomerular filtration rate (GFR). AKD can lead to accumulation of water, sodium, and other metabolic products and cause electrolyte disturbances especially in hospitalized patients.

- According to KDIGO (kidney disease improving global outcomes), diagnostic criteria of acute kidney disease is the presence of any one of the parameters: (a) increase in serum creatinine by 0.3 mg/dl within 48 hours, (b) increase in serum creatinine 1.5 times of baseline within the prior seven days, and (c) urine output <0.5 ml/kg/hour for at least 6 hours. It is critical illness and treated promptly to restore renal functions.
- Acute kidney disease can also be caused by poor blood supply to kidney (prerenal etiology), or kidney disease (renal etiology) or obstruction to urine outflow (post-renal etiology). AKD is potentially reversible, however, if left untreated, permanent renal damage can lead to chronic renal failure.
- Hemolytic uremic syndrome (HUS) occurs due to ingestion of Shiga toxin-producing strains of *Escherichia coli* results in injury to glomerular

Table 22.48 Tubular findings in nephrotoxic acute tubular necrosis (ATN)

Nephrotoxic Agent	Extensive Necrosis of Tubular Cells along Proximal Tubule
Carbon tetrachloride	Neutral lipid accumulation/fatty change in injured cells, followed by tubular cell necrosis
Ethylene glycol	Ballooning and hydropic changes of proximal tubules, calcium oxalate crystals in tubular lumina
Hemoglobin/myoglobin	Numerous deeply pigmented, red-brown casts in distal and collecting ducts
Indinavir	Intraluminal clear crystals with mononuclear cell infiltration in tubules
Lead	Dark intranuclear inclusions and necrosis
Mercury	Large acidophilic inclusions
Tenofovir	Distinctive proximal tubular eosinophilic inclusions representing giant mitochondria
Vancomycin	Acute interstitial nephritis with lymphocytic and eosinophilic infiltrate and ATN

endothelial cells. It is not associated with angiopathy outside kidney. Patient presents with hemorrhagic diarrhea, microangiopathic hemolytic anemia and rapidly progressive renal failure.

ETIOPATHOGENESIS

Acute kidney disease (AKD) is defined as an abrupt impairment of renal function occurring over seven days or less. Chronic kidney disease refers to persistence of kidney disease for a period of >90 days. Untreated patients with AKD develop cardiovascular manifestations, end stage renal disease associated with diminished quality of life and mortality. Early identification of persistent acute kidney disease is important in order to initiate an extended evaluation and management protocol to prevent further kidney damage and associated mortality. Acute kidney disease occurs due to prerenal, renal and postrenal causes.

Prerenal Acute Kidney Disease

Prerenal acute kidney disease implies a reduction in the glomerular filtration rate (GFR) due to hypovolemia in the presence of normal renal function. This can be a part of systemic hypoperfusion resulting from hypovolemia or hypotension or selective hypoperfusion of kidney resulting from renal artery stenosis and aortic dissection.

- Hypovolemia may occur due to gastrointestinal fluid loss in settings of diarrhea, vomiting, massive hemorrhage and severe burns.
- Hypotension may result from deceased cardiac output in the settings of cardiogenic shock, massive pulmonary embolism, acute coronary syndrome, cardiac arrhythmias, cardiogenic shock, disseminated intravascular coagulation, arterial thrombi, eclampsia and malignant hypertension. Hypotension may result from systemic vasodilation in the settings of septic shock (gram-negative sepsis), anaphylaxis, anesthesia administration and hepatorenal syndrome.
- Renal blood flow may be decreased due to renal vasoconstriction caused by NSAIDs, radiographic iodinated contrast medium, amphotericin B and hepatorenal syndrome. If uncorrected, decreased renal blood flow most frequently progresses to ischemic acute tubular necrosis (ATN), which is the most common cause of acute kidney disease.

Intrarenal Acute Kidney Disease

Intrinsic renal diseases can target the glomeruli (e.g. acute glomerulonephritis, rapidly progressive crescentic glomerulonephritis), tubules (e.g. acute tubular necrosis due to ischemia, toxins), interstitial tissue (e.g. acute pyelonephritis and drug-induced acute interstitial nephritis), or blood vessels (e.g. malignant hypertension, polyarteritis nodosa), sickle cell disease, systemic lupus erythematosus, transfusion reaction, myeloma nephropathy, crush injuries and papillary necrosis. The tubular reabsorption of essential metabolites is greatly impaired leading to acute kidney disease.

Postrenal Acute Kidney Disease

Ureteral or urethral mechanical obstruction to urine outflow due to renal calculi, prostatic hyperplasia and invasive cervical carcinoma may result in acute renal failure. Renal causes of acute kidney disease other than acute tubular necrosis are given in Table 22.49.

CLINICAL FEATURES

Patient with acute kidney disease initially develops oliguria (urine output <400 ml/24 hours), azotemia (excess levels of urea in blood), and rarely, anuria (failure to secrete urine), which progresses to electrolyte imbalance, metabolic acidosis, uremic manifestations; renal dysfunction and disruption of systemic body systems. Patient develops headache, drowsiness, pulmonary edema. Kussmaul respiration, cardiac

Table 22.49 Renal causes of acute kidney disease other than acute tubular necrosis

Renal Disorder	Anatomical Renal Component Affected
Various glomerulopathies especially crescentic glomerulonephritis	Glomeruli
Crystal deposition (e.g. oxalates and sulphonamide)	Tubules lumen
Occlusion of renal artery, persistent vasoconstriction of intrarenal vessels, thrombotic thrombocytopenic purpura, malignant nephrosclerosis and bilateral cortical necrosis associated with separation of the placenta	Blood vessels
Acute interstitial nephritis of various types	Interstitial tissue of renal parenchyma

arrhythmias, heart failure, deranged blood pressure, systemic edema, dry mucous membrane and skin.

Urine analysis in acute kidney disease is given in Table 22.50. Laboratory diagnosis of acute kidney disease is given in Table 22.51.

- **Oliguric phase:** Patient with AKD develops oliguria (<400 ml/24 hours) due to poor renal perfusion (prerenal etiology). It results in impairment of kidney's ability to conserve sodium. There is increased blood urea nitrogen (BUN) and creatinine levels and decreased ratio of BUN to creatinine (from normal levels of 20:1 to abnormal decrease of 10:1).
- **Diuretic phase:** Patient with AKD develops slow rise in BUN and creatinine levels. Serum electrolytes (potassium, sodium) are decreased. Body water depletion occurs. Blood pressure falls. If untreated, patient has fatal outcome.
- **Recovery phase:** Well treated patient with AKD recovers with restoration of normal BUN and creatinine levels and urine output between 1 and 2 L/day.

Table 22.50 Urine analysis in acute kidney disease

Etiology of Acute Kidney Disease	Urinary Sediment Analysis
Acute tubular necrosis (ATN)	Dirty brown casts and exfoliated tubular epithelial cells
Acute tubulointerstitial nephritis	White blood cell casts and numerous pus cells
Acute poststreptococcal glomerulonephritis	Red blood cell casts, dysmorphic RBCs and proteinuria in nephritic range <2 g/24 hours

Table 22.51 Laboratory diagnosis of acute kidney disease

Laboratory Test	Prerenal Acute Kidney Disease	Intrinsic Acute Kidney Disease
FENa (fraction excretion of sodium; blood urea nitrogen and creatinine)	<1%	>1%
Blood urea nitrogen/ creatinine ratio	>20:1	<20:1
Urine sodium	<20 mEq/L	>20 mEq/L
Urine osmolality	>500 mOsm/kg	<500 mOsm/kg
Specific gravity of urine	>1.020	<1.010

TUBULOINTERSTITIAL DISEASES OF KIDNEY

Tubulointerstitial nephritis is inflammation of tubules and interstitial tissue, which occurs in the settings of infection, toxins, drugs, heavy metals (lead poisoning), irradiation, hypercalcemia, multiple myeloma and autoimmune disorders such as systemic lupus erythematosus, Sjögren's syndrome. Morphologic features of tubulointerstitial diseases are given in Table 22.52. Schematic representation of tubulointerstitial lesions in various renal disorders is shown in Fig. 22.71.

PYELONEPHRITIS

Pyelonephritis refers to bacterial infection of the renal parenchyma and/or renal pelvis. Depending on the course of disease, it is categorized: acute pyelonephritis or chronic pyelonephritis. Vesicoureteral reflux (VUR) with ascending bacterial urinary tract infections play an important role in the pathogenesis of pyelonephritis.

Table 22.52 Morphologic features of tubulointerstitial diseases

Nephrotoxic Agents
▪ Acute tubulointerstitial diseases ▪ Chronic tubulointerstitial diseases
Urate Nephropathy
Acute tubulointerstitial diseases in the setting of hyperuricemia
Hypercalcemia and Nephrocalcinosis
Deposition of calcium in renal parenchyma and tubular damage
Multiple Myeloma
Monoclonal light chains (Bence-Jones proteins) synthesized by plasma cells are directly toxic to the tubules resulting in formation of casts in tubules and thus obstruction to urine outflow and renal failure
Granulomatous Tubulointerstitial Diseases
▪ Tuberculous pyelonephritis ▪ Xanthogranulomatous pyelonephritis ▪ Wegener granulomatosis ▪ Sarcoidosis ▪ Fungal disease ▪ Malakoplakia

- **Acute pyelonephritis** produces severe symptoms as the kidneys are actively and aggressively attacked by bacteria. In some cases, bacteria may invade glomerular capillaries resulting in potentially life-threatening sepsis. Kidneys show radiating yellowish gray streaks in pyramids and abscess in cortex. There is moderate hydronephrosis and blunting of renal calyces.
- **Chronic pyelonephritis** results from incompletely treated acute pyelonephritis. The organisms continue to multiply and destroy the renal parenchyma. The process is slow and painless. The net result is permanent damage to kidneys with impaired renal function. Kidneys show wedge-shaped subcapsular scars, thinning of renal parenchyma, blunting of corticomedullary junctions and dilation and fibrosis of renal calyces in majority of cases.

ACUTE PYELONEPHRITIS

Acute pyelonephritis (APN) is inflammation of the renal parenchyma, calyces and pelvis, which often leads to scarring of the kidneys. Vesicoureteral reflux (VUR) with ascending infection is most common cause of acute pyelonephritis.

- Vesicoureteral reflux (VUR) carries infected urine up to the ureters into the renal pelvis and calyces resulting in entry of the bacteria through the papillae into the renal parenchyma. Bacteriuria is a typical finding in patients with acute pyelonephritis. Urinary tract infection is common due to shorter length of the female urethra especially during pregnancy.
- *Escherichia coli* from the feces causes distal colonization in the urethra especially in women due to short urethra.
- Ascending urinary tract infection by *Escherichia coli* is the most common cause of acute pyelonephritis in 85% of cases.

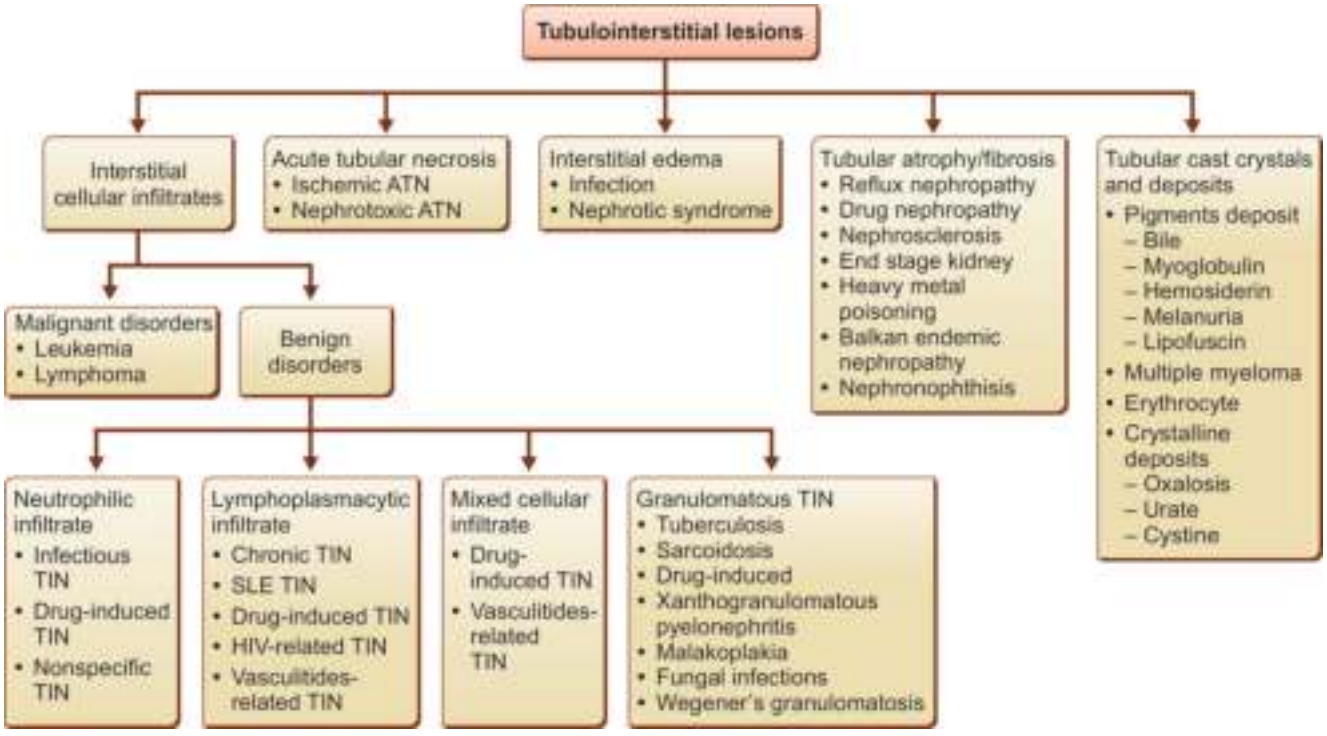


Fig. 22.71: Schematic representation of tubulointerstitial lesions in various renal disorders.

- Other gram-negative organisms include *Proteus vulgaris, Klebsiella pneumoniae*, enterococci, *Streptococcus faecalis*, Serratia, gram-positive pyogenic bacteria may cause acute pyelonephritis through hematogenous dissemination (uncommon cause) in the settings of repeated bacterial infections or hospital-acquired infection. Bacterial infection of the urinary bladder and urethra often precedes acute pyelonephritis.
- Acute pyelonephritis most often occurs in women than men, because women have short urethra injured during sexual intercourse resulting to urethritis and cystitis.

Predisposing Factors

Predisposing factors for acute pyelonephritis include urinary tract obstruction (e.g. calculi, prostatic hypertrophy), renal cystic diseases, diabetes mellitus, medullary sponge of kidney, vesicoureteral reflux (VUR), previous instrumentation of the urinary tract, pregnancy, sickle cell disease, immunosuppression, immunodeficiency, and neurogenic urinary bladder. Diabetic patients with glycosuria are at higher risk for developing acute pyelonephritis.

- **Vesicoureteral reflux (VUR):** Intravesical portion of the ureter is normally compressed during micturition, which prevents reflux of urine into the ureters. In vesicoureteral reflux, the intravesical portion of the ureter is not compressed during micturition, as a result urine refluxes into the ureters during micturition. If vesicoureteral reflux persists, infected urine ascends to the renal pelvis and renal parenchyma resulting in acute pyelonephritis and then progressing to chronic pyelonephritis.
- **Increased residual urine volume:** There is increased incidence of acute pyelonephritis in patients with atonic urinary bladder or flaccid urinary bladder caused by progesterone therapy; in which residual volume of urine is increased (normal residual volume 2–3 ml). Obstruction to urinary outflow due to nodular hyperplasia of prostate is also important cause of acute pyelonephritis.

Clinical Features

Patient with mild acute pyelonephritis may present with acute onset low-grade fever with or without flank pain in the lower back or costovertebral angle, frequent micturition, and voiding small quantity of urine at frequent intervals.

- In severe acute pyelonephritis, patient presents with sudden onset of spiking fever, flank pain, without gross hematuria, chills, nausea, vomiting, increased frequency of urination and painful urination (dysuria) if associated with lower urinary tract infection.
- Patient develops renal insufficiency in patients with bilateral and severe renal involvement with papillary necrosis.

Surgical Pathology: Acute Pyelonephritis

Gross Morphology

- Acute pyelonephritis is often a focal disease, and much of the kidney often appears normal. The kidneys develop grayish white areas of miliary pattern of small cortical microabscesses via hematogenous route in tubular lumens and interstitial tissue of cortex as well as medulla on the subcapsular surface.
- Accumulation of pus in the calyces and pelvis causes obstruction to urine outflow resulting in perinephric abscess in severe cases.
- Extension of suppuration through the renal capsule leads to formation of perinephric abscess.
- The urothelium of the renal pelvis and calyces may be hyperemic and covered by a purulent exudate. Gross morphology of acute pyelonephritis is shown in Fig. 22.72.

Light Microscopy

- Histologic examination shows an extensive neutrophil aggregate in the collecting tubules, collecting ducts lumina in the initial phase. Spillover of suppurative inflammation occurs into the interstitial tissue.
- Acute inflammation in calyceal and pelvic urothelium with occasional mucosal ulceration is present. Renal parenchymal destruction and abscess formation occur in severe cases. The glomeruli and blood vessels are spared initially. More severe inflammation occurs in renal papillae. Renal abscesses are more numerous in the cortex than medulla.
- Emphysematous pyelonephritis is characterized by empty spaces lacking epithelial cell lining distorting parenchyma, vascular thrombosis, ischemic necrosis, suppurative inflammation in adjacent parenchyma, widespread abscesses with papillary necrosis and cortical infarcts.
- Molding of neutrophils forms WBC casts in the tubules, which are passed in the urine along with free neutrophils and bacteria (pyuria). Histology of acute pyelonephritis is shown in Fig. 22.73.

Complications

Patients with acute pyelonephritis develop various complications, i.e. chronic pyelonephritis, pyonephrosis (due to obstruction to urine outflow), perinephric abscess, renal papillary necrosis and septicemia (endotoxic shock).

Laboratory Findings

Microscopic examination of urine reveals WBC casts, pus cells, hematuria and bacteriuria usually *E. coli*. Urine culture in suspected acute pyelonephritis cases is considered as significant, if excess of $(10)^5$ culture

forming units/milliliter is present, which excludes cases of extraneous bacterial contamination. Blood culture is positive in these cases.

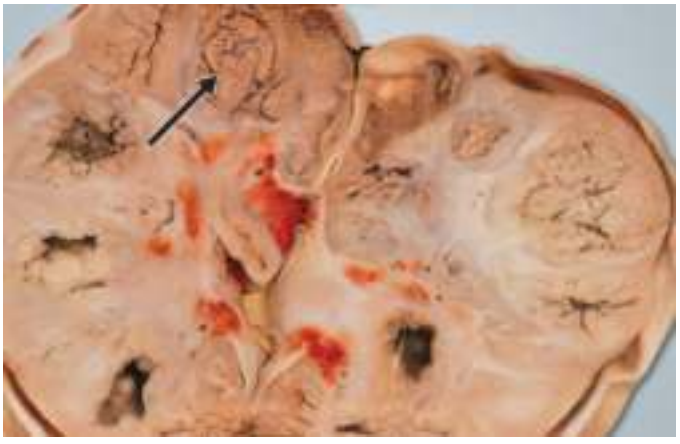


Fig. 22.72: Gross morphology of acute pyelonephritis. Acute pyelonephritis is a common bacterial pus forming infection of the collecting system and renal parenchyma most often occurs in young adult women, diabetics and immunocompromised individuals. *Escherichia coli* is the most common pathogen isolated from urine cultures or pus cultures in almost 70% of cases. Infection usually occurs via ascending route; however, it may also occur via hematogenous route. Signs and symptoms include sudden onset of pain in costovertebral angle, fever, pyuria or white blood cell casts in urine. Cut surface of kidney shows focal gray white, multiple abscesses or wedge-shaped areas of suppuration in the renal parenchyma (arrow). (*Courtesy:* Dr. Sujata Kanetkar, Senior Professor and Head of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

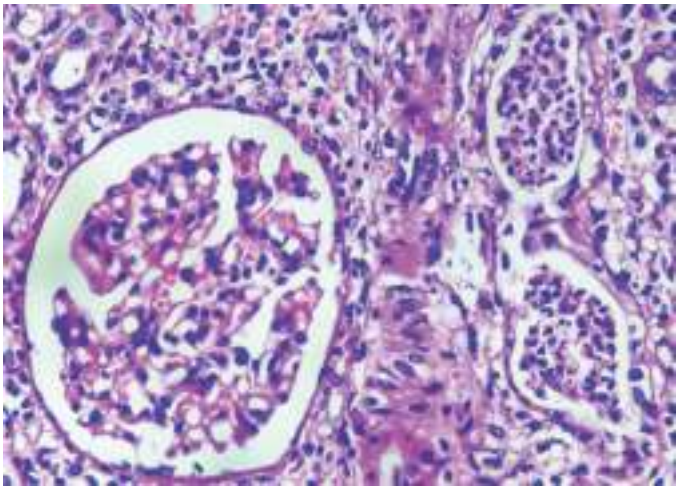


Fig. 22.73: Histology of acute pyelonephritis. Acute pyelonephritis is a common gram-negative bacterial infection of the renal pelvis and kidney in infants, children, women of childbearing age. Causative gram-negative organisms include *Escherichia coli*, Proteus, Klebsiella and Enterobacter from fecal flora. Patient presents with fever, chills, pain in groin region, nausea, vomiting, cloudy foul-smelling urine and frequent painful urination. Hematoxylin and eosin-stained renal tissue section shows patchy suppurative inflammation, primarily involving cortical region with edema, neutrophils in interstitium and tubular lumina, and also cortical abscess and necrosis (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

CHRONIC PYELONEPHRITIS

In chronic pyelonephritis, pathologic changes in the kidney occur as a result of recurrent and persistent ascending bacterial infection from urethritis or cystitis especially in young girls, secondary to vesicoureteral reflux (VUR), intrarenal reflux and urinary tract obstruction, which is termed reflux nephropathy. Inflammation usually affects one kidney causing small asymmetric contraction with scarred surface. The patient is usually asymptomatic.

Pathophysiology

Vesicoureteral reflux and urinary tract obstruction play key role in the pathogenesis of chronic pyelonephritis.

Vesicoureteral Reflux

Micturition is the process of emptying urine from urinary bladder. VUR is a condition in which urine flows backward from the urinary bladder to one or both ureters and sometimes to kidneys. Persons with VUR, the intravesical portion of the ureter is not compressed during micturition, as a result urine refluxes into the ureter, renal pelvis and renal parenchyma resulting in acute pyelonephritis and then progressing to chronic nonobstructive pyelonephritis. VUR targets children under 5 years of age due to an incompetent uretero-vesical junction.

Urinary Tract Obstruction

Obstruction may occur anywhere in the urinary system (e.g. renal tubules, renal pelvis, ureters, urethra, prostate, and bladder). Obstructive causes of obstructive uropathy in men are urinary bladder carcinoma, benign nodular hyperplasia of prostate, neurogenic bladder (e.g. spinal cord damage), and urethral stricture as a consequence of chronic urethritis. Pathologic changes in kidney occurs secondary to chronic obstruction to the urinary outflow leading to hydronephrosis.

- **Age group:** In children, the condition is most often congenital (e.g. posterior urethral valves and urethral strictures, ureteropelvic obstruction) and congenital ureteric abnormality (ureterocele). In adults, the condition is most often acquired as a result of urethral and ureteral strictures (instrumentation, radiation and inflammation), urinary tract tumors. In elderly persons, urinary tract obstruction occurs due to prostatic hyperplasia, prostatic cancer and nephrolithiasis.
- **Causes of obstruction:** Renal causes of obstructive uropathy include staghorn calculus, transitional cell carcinoma of renal pelvis and atrophic scarred cortex. Ureteric causes of obstructive uropathy include ureteral stone, transitional cell carcinoma, retroperitoneal fibrosis, surgical scars, and necrotic debris. Extrinsic obstructive causes of urinary tract

include pregnancy, endometriosis, and cervical carcinoma in women.

- **Pathologic renal changes:** Obstruction to urinary flow is associated with inflammation and dilatation of the ureter and renal pelvis, calyces especially polar compound papillae containing straight ducts with absence of valve-like action. Increased pressure in the urinary tract, reflux of urine with leakage into the interstitium, and ischemia of the inner medulla are key factors promoting renal atrophy, and diffuse U-shaped cortical fibrosis and scarring in renal polar areas.
- **Imaging technique:** Pathologic changes such as generalized atrophy of the kidney and dilatation of all portions of renal pelvis are visible with an intravenous pyelography (IVP) in chronic obstructive pyelonephritis. Treatment and prognosis depend on degree and chronicity of the obstruction.
- **Complications:** Complications of obstructive uropathy with urolithiasis are hydronephrosis, renal colic and superadded infection. Hydronephrosis leads to dilatation of the renal pelvis, collecting ducts, proximal and distal convoluted tubules. Calyces undergo atrophic changes and flattening with variable degrees of compression atrophy of the cortex and medulla. Loss of nephrons is accompanied by tubulointerstitial fibrosis.

Schematic representation of obstructive nephropathy is shown in Fig. 22.74. Causes of obstructive uropathy is given in Table 22.53. Schematic representation

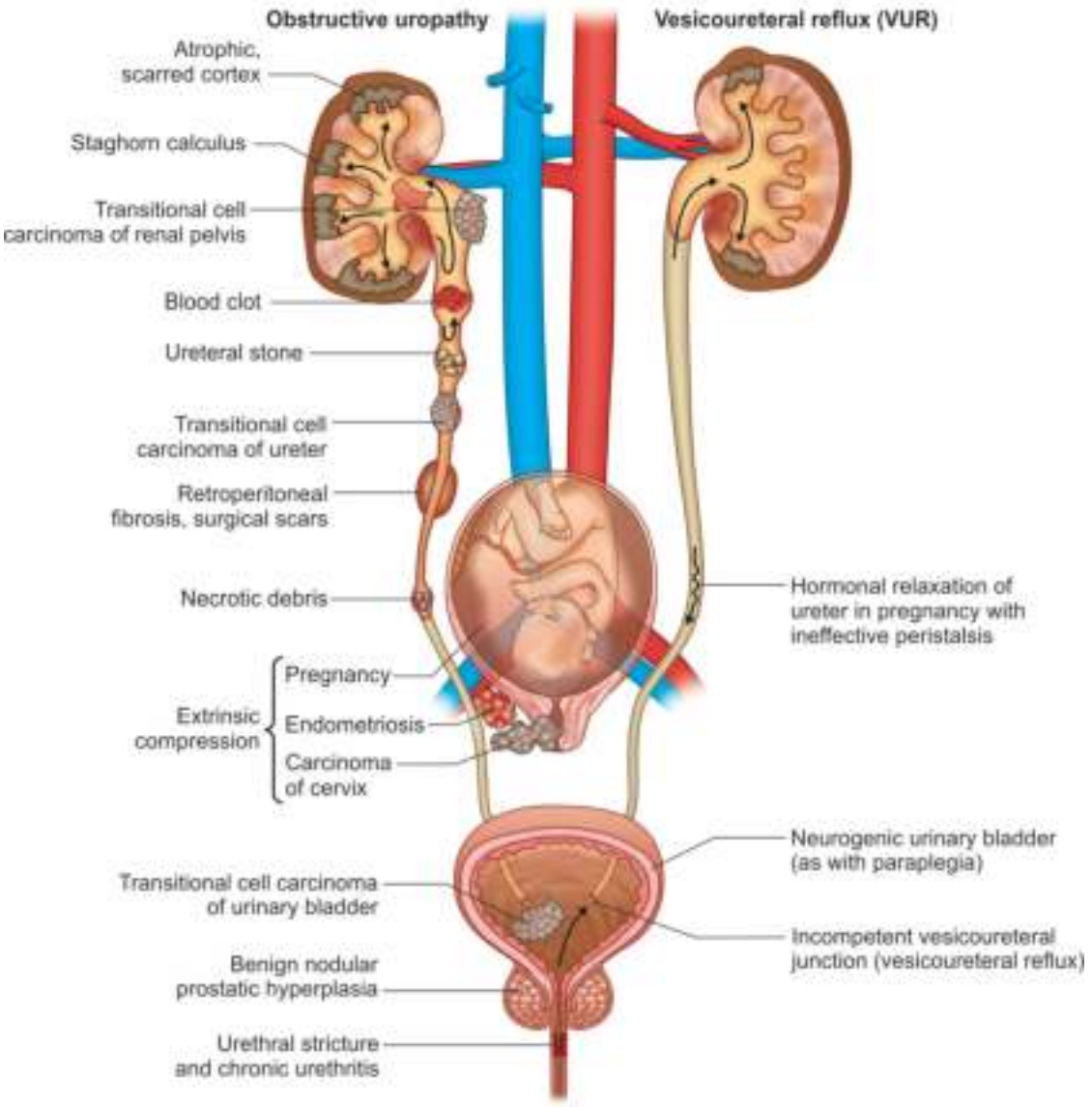


Fig. 22.74: Schematic representation of obstructive nephropathy. Obstructive uropathy is a disorder of the urinary tract that occurs due to obstruction in urinary flow and can be structural or functional. The back flow of urine into the unilateral or bilateral kidneys, depending on the location of the obstruction leads to hydronephrosis.

Table 22.53 Causes of obstructive uropathy

Renal Pelvis Obstruction
▪ Renal calculi
▪ Papillary necrosis
Ureter Obstruction
▪ Renal calculi
▪ Pregnancy
▪ Tumors that compress the ureter (ureteral stricture)
▪ Congenital disorders of the ureterovesical junction and ureteropelvic junction strictures
Urinary Bladder Obstruction
▪ Urinary bladder cancer
▪ Neurogenic urinary bladder
▪ Urinary bladder calculi
▪ Prostatic hyperplasia or prostate cancer
Urethra Obstruction
▪ Urethral strictures
▪ Congenital urethral defects

of chronic nonobstructive pyelonephritis is shown in Fig. 22.75. Schematic representation of vesicoureteral reflux (VUR) and role of chronic pyelonephritis is shown in Fig. 22.76. Comparison between chronic pyelonephritis and chronic glomerulonephritis is given in Table 22.54.

Clinical Features

Clinical presentation of chronic pyelonephritis is dependent on age and presence of prior diagnosis of urinary tract infections and vesicoureteral reflux.

- Patient presents with episodic manifestations of urinary tract infection or acute pyelonephritis, such as recurrent fever and flank pain.
- Bilateral involvement of kidneys leads to gradual loss of renal functions. Children with reflux nephropathy develop flank pain and other vague signs of inflammation, with or without hypertension and proteinuria resulting in chronic renal insufficiency.
- Radiologic findings show scars at the upper and lower poles of the kidney and dilatation of the renal pelvis. Prevention of recurrent urinary tract infections and surgical correction of severe vesicoureteral reflux in children can decrease the incidence of renal scarring.

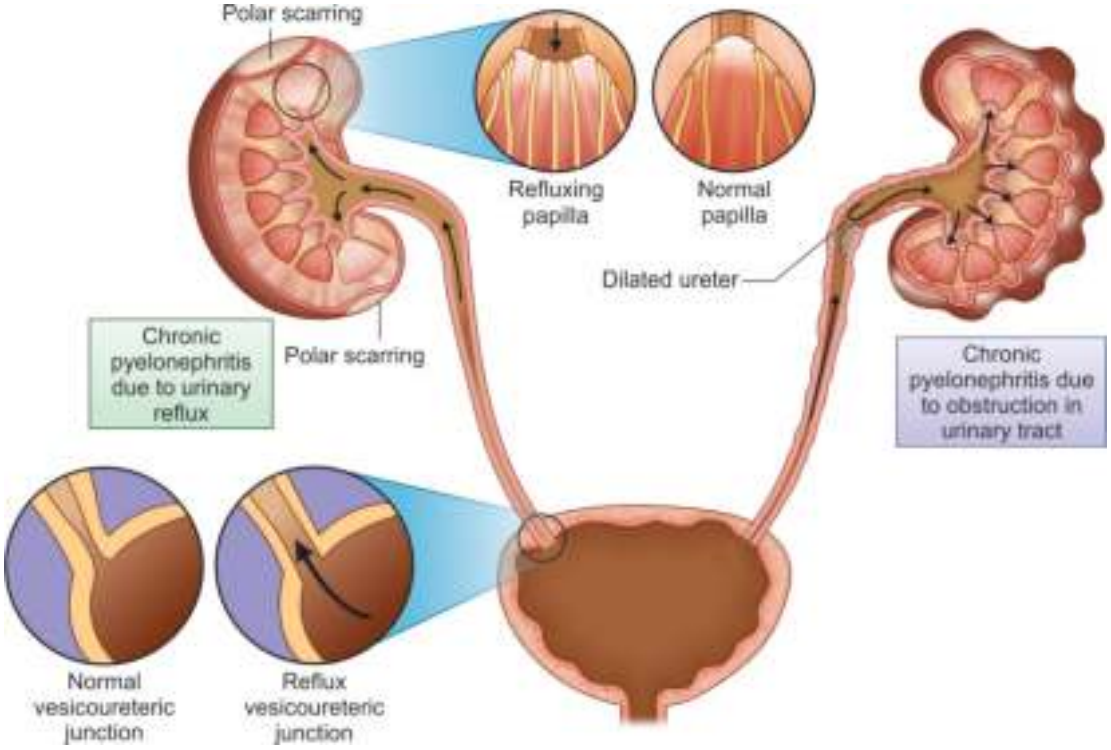


Fig. 22.75: Schematic representation of chronic nonobstructive pyelonephritis. Chronic pyelonephritis is characterized by renal inflammation and marked destruction of renal parenchyma due to recurrent or persistent infection of renal parenchyma, vesicoureteral reflux (VUR), anatomical anomalies or other causes of urinary tract obstruction such as strictures and calculi. Obstruction of the urinary tract leads to high pressure backflow of urine leading to destruction of all papillae in renal parenchyma. It leads to diffuse scarring at the poles of the kidney and thinning of overlying cortex.

Surgical Pathology: Chronic Pyelonephritis

Gross Morphology

- Renal parenchyma in chronic pyelonephritis is firm due to extensive fibrosis. External surface reveals broad asymmetric U-shaped scars involving cortex and medulla especially in upper pole well demarcated from the normal parenchyma.
- In chronic obstructive pyelonephritis, there is diffuse dilatation of the collecting system with occasional stones in the pelvis and calyces. Coarse scars are demonstrated on the cortical surface.
- Cut surface shows severe renal parenchymal thinning, deformity of the renal pelvis and calyces beneath the scars. Gross morphology of chronic obstructive pyelonephritis and hydronephrosis is shown in Fig. 22.77.

Light Microscopy

- Histologic examination of nephrectomy specimen shows scars consisting of a demarcated zone of diffuse atrophy of the renal parenchyma and loss of tubules, interstitial fibrosis, diffuse glomerulosclerosis, and interstitial inflammation as a result of lymphocytes and plasma cells infiltrate. Inflammation with lymphoid follicles beneath the urothelial lining of the renal pelvis suggests chronic inflammation. Scars are deep and involving renal medullary region in the upper and lower poles.
- Atrophic tubules contain eosinophilic proteinaceous casts, the resultant similarity in appearance to thyroid follicles is referred to as thyroidization. 'Thyroidization of the renal tubules' is characteristic finding but not pathognomonic.
- Thyroidization of tubules is less developed in chronic obstructive pyelonephritis. Inflammation with lymphoid follicles and neutrophils are present beneath the renal pelvis and urothelial lining in chronic obstructive pyelonephritis.
- Arteriosclerosis and hyaline arteriosclerosis are frequent when hypertension is present. Secondary focal segmental glomerulosclerosis and segmental renal dysplasia can be observed. Arteries can show endarteritis with narrowing of the lumen and fibrosis of media. Histology of chronic pyelonephritis is shown in Figs 22.78 to 22.80.

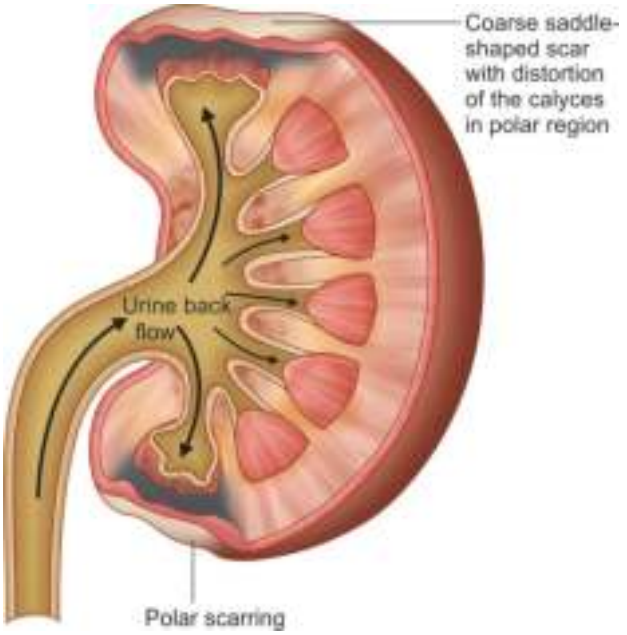


Fig. 22.76: Schematic representation of vesicoureteral reflux (VUR) and role of chronic pyelonephritis. Chronic pyelonephritis is characterized by renal inflammation with marked destruction of renal parenchyma and dilatation of the calyces resulting from previous infection induced by recurrent or persistent infection of renal parenchyma, vesicoureteral reflux, anatomical anomalies and urinary tract obstruction. VUR is a condition in which urine flows background from the urinary bladder to one or both ureters and sometimes to the kidneys leading to cause infection in the compound papillae located on the poles and then undergoing fibrosis. Note the relationship of the coarse saddle-shaped scars with distortion of the calyces.

Table 22.54 Comparison between chronic pyelonephritis and chronic glomerulonephritis

Parameters	Chronic Pyelonephritis	Chronic Glomerulonephritis
Gross morphologic features		
Kidney involvement	Unilateral involvement with coarse scars at poles	Bilateral involvement with diffuse granularity
Kidney contraction	Marked contraction	Moderate contraction
Histologic features		
Glomeruli involved	Patchy involvement	Diffuse involvement
Glomerular tuft proliferation	Absent	Present
Glomerular tuft thickening	Rare	Present
Changes in the Bowman's space	Relatively greater	Not usually present
Tubular loss relation to sclerosed glomeruli	Tubular loss not only confined to glomeruli	Tubular loss confined to glomeruli
Tubulointerstitial fibrosis	Coarse	Fine
Chronic inflammatory infiltrate	Marked	Less
Renal pelvis inflammation	Usually inflamed	Seldom inflamed
Vascular changes	Marked	Moderate
Necrotizing papillitis	Present	Absent

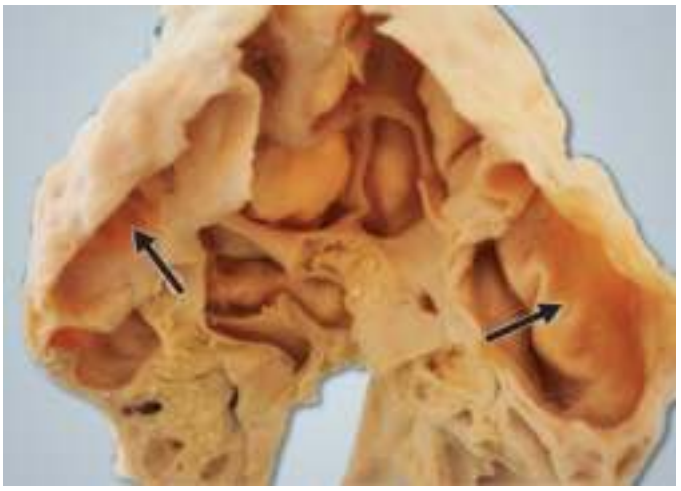


Fig. 22.77: Gross morphology of chronic obstructive pyelonephritis and hydronephrosis. Chronic pyelonephritis is defined as the marked reduction in kidney size, with marked destruction of renal parenchyma and dilatation of calyces forming cysts from previous persistent or recurrent infection. Cut surface shows marked dilatation of calyces caused by inflammatory destruction of papillae, with atrophy and scarring of the overlying cortex (arrows). (*Courtesy:* Dr. Sujata Kanetkar, Senior Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

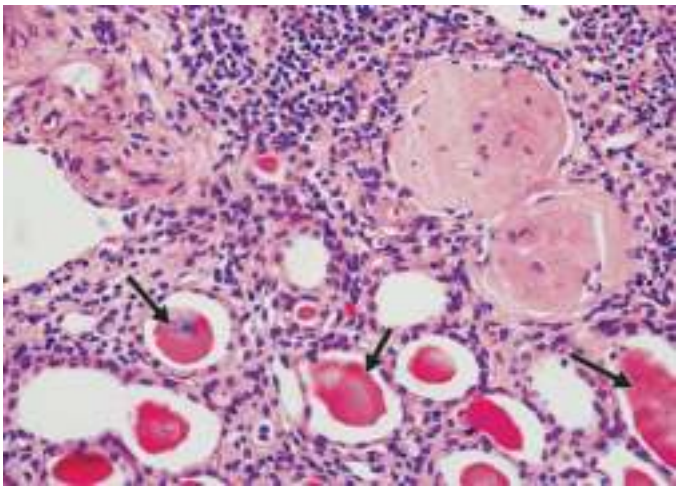


Fig. 22.78: Chronic pyelonephritis. Chronic pyelonephritis is characterized by tubulointerstitial nephritis, renal scarring with severe involvement of calyces and pelvis due to recurrent bouts of acute pyelonephritis. Hematoxylin and eosin-stained renal tissue section shows tubular thyroidization (filled with colloid casts), tubular atrophy, interstitial fibrosis and lymphocytes and plasma cells infiltrate in the interstitium, obliterative endarteritis of renal blood vessels and glomerulosclerosis (arrows) (400X).

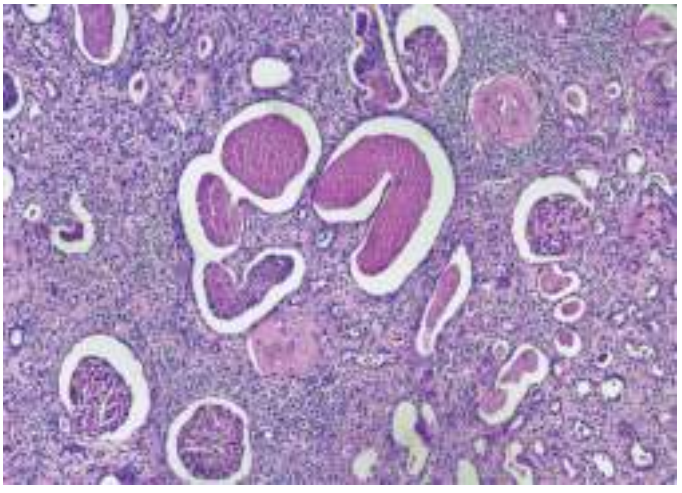


Fig. 22.79: Histology of chronic pyelonephritis. Chronic pyelonephritis is tubulointerstitial inflammation and renal scarring with severe involvement of calyces and pelvis. Patients may be asymptomatic, however can present with fever, malaise and flank pain. Diagnosis is with urine analysis, culture and imaging techniques. Hematoxylin and eosin-stained renal tissue section examination shows tubular thyroidization (filled with colloid casts), tubular atrophy, interstitial fibrosis and intense dense inflammation (numerous lymphocytes and plasma cells), obliterative endarteritis of blood vessels and interstitial Tamm-Horsfall protein. Normal glomeruli are observed in early disease course (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

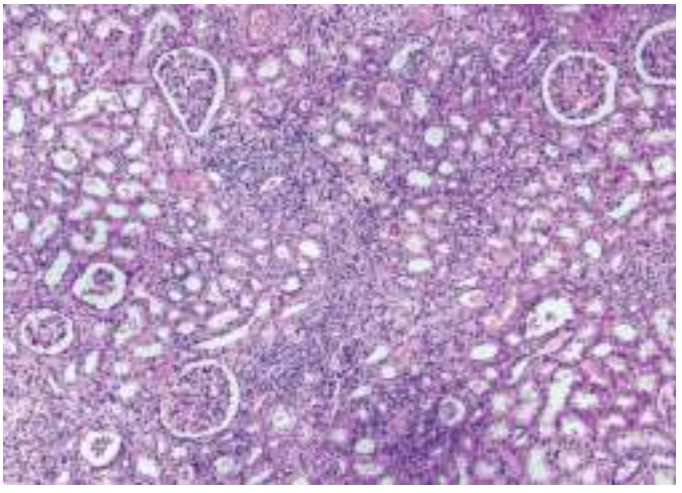


Fig. 22.80: Histology of chronic pyelonephritis. Chronic pyelonephritis is characterized by renal inflammation and scarring induced by recurrent or persistent renal infection in the settings of vesicoureteral reflux, urinary obstructions and major anatomic anomalies. It is more common in children than in adults. Hematoxylin and eosin-stained renal tissue section examination shows tubular thyroidization (filled with colloid casts), tubular atrophy, interstitial fibrosis and intense dense inflammation (numerous lymphocytes and plasma cells), obliterative endarteritis of blood vessels and interstitial Tamm-Horsfall protein. Normal glomeruli are observed in early disease course (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

XANTHOGRANULOMATOUS PYELONEPHRITIS

Xanthogranulomatous pyelonephritis is a destructive granulomatous inflammatory process of renal parenchyma resulting from long-standing urinary tract obstruction usually associated with staghorn calculus together with chronic infection with *Proteus mirabilis* and *Escherichia coli*.

- Sometimes long-standing infection may be localized by forming a destructive mass-like renal lesion. It

can be grossly and microscopically mistaken as clear cell variant of renal cell carcinoma.

- Xanthogranulomatous pyelonephritis usually affects 70% of middle-aged females. Urine cultures most frequently show *Escherichia coli* and *Proteus mirabilis*.
- Patient presents with pain and mass in the abdomen, lower urinary tract symptoms with gross hematuria and pyuria. Nephrectomy is curative for xanthogranulomatous pyelonephritis.

Surgical Pathology: Xanthogranulomatous Pyelonephritis

Gross Morphology

The kidney is enlarged with hydronephrosis. Renal pelvis contains calculi with characteristic 'staghorn' appearance, or some other evidence of obstruction. Cut surface reveals single or multiple yellow to orange nodules, which may mimic tumor nodules.

Light Microscopy

- In xanthogranulomatous pyelonephritis, the xanthogranulomatous inflammation is made up of mixed histiocytes with clear or foamy cytoplasm typically admixed with lymphocytes, plasma cells and multinucleated giant cells.
- Xanthogranulomatous nodules can have a zonal pattern with a central zone of neutrophils and necrosis rimmed by foamy histiocytes and fibroblastic response at the periphery. The pathologic changes include variable degree of renal tubular atrophy, tubular dilatation, squamous metaplasia of the urothelium, microabscesses and lymphoid follicle formation.
- The vascular stroma characteristic of clear cell variant of renal cell carcinoma is missing in xanthogranulomatous pyelonephritis.

Differential Diagnosis

- Xanthogranulomatous pyelonephritis should be differentiated from clear cell renal cell carcinoma for lipid-laden xanthomatous cells, sarcomatoid renal cell carcinoma and malakoplakia.
- Clear cell renal cell carcinoma has water clear cytoplasm, and is positive for cytokeratin and vimentin but negative for CD68.
- Sarcomatoid renal cell carcinoma is composed of prominent spindle cell proliferation or significant cytologic atypia in spindle cells. Epithelial component may be present.
- Malakoplakia is composed of eosinophilic histiocytes with Michaelis Guttmann bodies. Histology of xanthogranulomatous pyelonephritis is shown in Fig. 22.81.

TUBERCULAR PYELONEPHRITIS

Urogenital tuberculosis represent 25% extrapulmonary cases. Renal involvement in tuberculosis can be part of disseminated infection or localized genitourinary infection, which can evolve to pyonephrosis. Tubercular infection generally disseminates from the ureter to the urinary bladder causing granulomatous inflammation and fibrosis. Most patients have sterile pyuria which can be accompanied by microscopic hematuria. Urine analysis reveals acidic pH, proteinuria and pus cells.

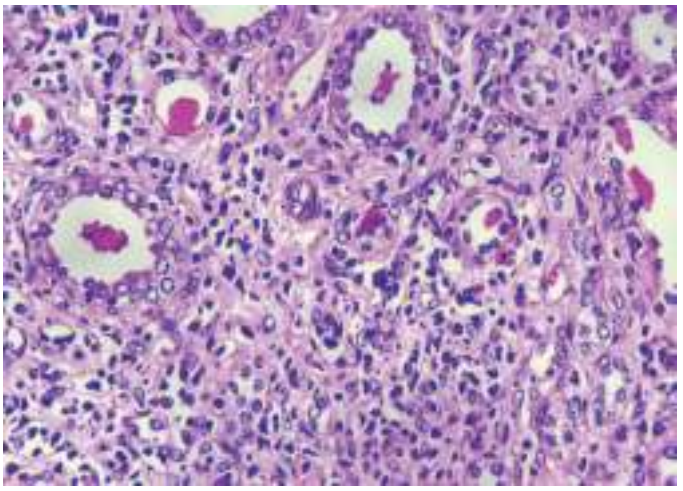


Fig. 22.81: Histology of xanthogranulomatous pyelonephritis. Xanthogranulomatous pyelonephritis is destructive inflammatory process that may complicate chronic pyelonephritis due to *Escherichia coli* and Proteus or nephrolithiasis, but resembling renal cell carcinoma. Xanthogranulomatous pyelonephritis is associated with pelvicalyceal obstruction and ulceration. Multiple yellow nodules are formed around calyces. Hematoxylin and eosin-stained renal tissue section shows replacement of renal parenchyma with CD68+ foamy histiocytes, occasional multinucleated giant cells and inflammatory cells (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)



Fig. 22.82: Gross morphology of tubercular pyelonephritis. Fibrous-walled abscess cavity filled with caseous material is shown on cut surface. This patient had a previous well-established history of pulmonary tuberculosis. Section from the patient's nephrectomy specimen shows a tuberculous granuloma, with central caseating necrosis and peripheral scarring (arrows). (*Courtesy:* Department of Pathology, Dr. DY Patil Medical College, Pune, Maharashtra.)

Surgical Pathology: Tubercular Pyelonephritis

Gross Morphology

Kidneys are enlarged. Cut surface reveals caseous material filling the renal pelvis. Gross morphology of tubercular pyelonephritis is shown in Fig. 22.82.

Light Microscopy

Tubercular pyelonephritis is characterized by a granulomatous reaction with epithelioid cell granulomas surrounding caseous necrosis material and Langhans' giant cells. Fibrosis and interstitial inflammation with lymphocytes and plasma cells are also observed around foci of caseous necrosis.

URATE NEPHROPATHY

Any condition associated with elevated levels of uric acid in the blood results in deposition of urate crystals in the tubules and interstitium, which is known as urate nephropathy.

- **Acute urate nephropathy:** Acute urate nephropathy occurs due to massive release of nucleic acid purines (uric acid) following aggressive treatment with allopurinol of disseminated hematolymphoid neoplasms (e.g. leukemia and lymphoma) resulting in increased serum uric acid level. Urate crystals are deposited in the renal tubules and interstitial tissue.
- **Chronic urate nephropathy:** Chronic urate nephropathy is characterized by deposition of crystalline monosodium urate in renal tubules and interstitial tissue in the medulla, forming pale yellowish '**tophi**' with surrounding foreign body inflammation, mononuclear cell infiltrates, and fibrosis. Patients with hyperuricemia may also develop nephrolithiasis with uric acid stones.

RENAL PAPILLARY NECROSIS

Renal papillary necrosis is characterized by coagulative necrosis involving papillae of renal medulla, classically associated with many underlying conditions, all of which predispose the patient to renal medullary ischemia.

- Majority of cases have one or more underlying predisposing factors such as chronic analgesic ingestion (acetaminophen, phenacetin, COX2 inhibitors), sickle cell anemia, diabetes mellitus, urinary tract obstruction/infection, vasculitis, tuberculosis, renal allograft and hepatocellular failure.
- Patient develops polyuria owing to an inability to concentrate urine, hematuria, renal colic resulting from renal papillae sloughing and urinary tract infection. Renal failure can rarely occur. Clinical course varies depending on underlying condition and number of renal papillae involvement.

Surgical Pathology: Renal Papillary Necrosis

Gross Morphology

Cut surface of kidney reveals necrotic medullary pyramids. Features of obstruction and fragments of sloughed papillae and calyces are possible present.

Light Microscopy

- Histologic examination in renal papillary necrosis reveals papillae showing geographic coagulative necrosis with ghosts of tubules visible. It shows overlying cortical atrophy, interstitial fibrosis and chronic inflammation. Distribution and duration of renal papillary necrosis vary due to underlying cause.
- In analgesic abuse induced renal papillary necrosis in various stages of progression, cut surface shows variable-sized foci of geographic necrosis, dystrophic calcification and sloughing of renal papillae.
- In diabetes mellitus induced renal papillary necrosis, all the renal papillae are at the same stage of acute coagulative necrosis, but dystrophic calcification is rare.
- In sickle cell disease or trait, only a few renal papillae may be focally affected, but dystrophic calcification is rare. In cases with urinary tract infection and obstruction, acute inflammation may indicate an underlying infection.

ACUTE INTERSTITIAL NEPHRITIS

Acute interstitial nephritis (AIN) is a clinicopathologic entity characterized by inflammation in the renal interstitium with various etiologies, often secondary to cell-mediated immune response to a drug acting as hapten. AIN is most common type of drug-induced kidney injury.

- AIN is characterized histologically by infiltrates of activated lymphocytes (T cells) and eosinophils in the renal interstitium, a pattern that indicates a type 4 cell-mediated immune reaction. Presence of eosinophilia and eosinophiluria indicates a type 1 hypersensitivity response. Urine analysis shows mild to moderate proteinuria, hematuria, and eosinophiluria (eosinophils in the urine). Withdrawal of the drug causes reversal of the disease.
- The most frequently implicated drugs in the pathogenesis of AIN include penicillin (particularly methicillin), rifampicin, sulfonamides, nonsteroid anti-inflammatory drugs (NSAIDs), allopurinol, and diuretics (thiazides, furosemide). AIN occurs two weeks after beginning a drug. Withdrawal of the drug usually results in reversal of the disease associated with excellent prognosis.
- Patient presents with classic triad: abrupt onset of fever, skin rash and eosinophilia with rapidly progressive acute renal failure, approximately 2 weeks following exposure to the drug administration. Eosinophilia and eosinophiluria are highly predictive. Renal functions are impaired with increased blood urea and serum creatinine. Hematuria, mild proteinuria and sterile leukocyturia are usually present.

Pathology Pearls: Analgesic Interstitial Nephritis

- Analgesic nephropathy is a common cause of chronic drug-induced interstitial nephritis. It is more common in women than men associated with chronic pain.
- The combination of acetaminophen (a metabolite of phenacetin) and aspirin in the setting of a patient (usually a woman) with chronic headache or pain is responsible for most cases.
- A cumulative analgesic ingestion of 3 kg or daily consumption of 1 g/day (>2 kg) over a span of 3 years is sufficient to produce analgesic interstitial nephritis.
- Complications of analgesic interstitial nephritis include renal papillary necrosis, hypertension, renal stones, hematuria, accelerated atherosclerosis, urothelial bladder transitional cell carcinomas (very high risk), tubulointerstitial fibrosis, and end stage renal disease are potential complications.

Pathogenesis

- Acetaminophen forms oxygen-derived free radicals, which bind with the renal tubular cells and cause oxidative cellular damage of renal tubules in the medulla.
- While aspirin inhibits prostaglandin synthesis, hence eliminating the vasodilator effects of prostaglandins.
- Vasoconstriction of renal vessels causes ischemic injury (coagulation necrosis) in the renal medullary papillae and tubulointerstitial fibrosis.
- Patient develops papillary necrosis of some or all papillae, which appears pale white infarcts. Sloughing of the necrosed renal papillae (renal papillary necrosis) is associated with gross hematuria, proteinuria, and colicky flank pain. An intravenous pyelogram reveals a ring defect where one or more renal papillae used to reside.
- Papillary necrosis may also occur in sickle cell anemia, diabetes mellitus, acute pyelonephritis, and obstructive uropathy.

Light Microscopy

Renal interstitial tissue is infiltrated by lymphocytes, plasma cells, macrophages, eosinophils and neutrophils. Eosinophils are frequently observed in drug-induced allergic acute interstitial nephritis, but represent a minor component of the inflammatory infiltrate. Tubulitis is common finding during active phase. Some degrees of tubular injury (tubular necrosis) can be found. Granulomatous inflammation is sometimes predominant.

LIGHT CHAIN CAST NEPHROPATHY IN MULTIPLE MYELOMA

Light chain cast nephropathy is the most common cause of death in multiple myeloma. Bence Jones proteins are the light chain of immunoglobulin filtered through the glomeruli, which are toxic to the tubules.

- At acidic pH of urine, the light chains of Bence Jones protein bind to Tamm-Horsfall glycoproteins synthesized by distal tubular epithelial cells and form casts in tubules. These casts in the tubules cause obstruction to urinary flow in the tubules. In some instances, the deposits of light chains do not become fibrillar and transform into AL amyloid.
- Nevertheless, these light chain deposits damage the glomerular basement membrane (GBM) and tubular basement membranes and cause renal failure. Hypercalcemia in multiple myeloma patient leads to nephrocalcinosis and urolithiasis.
- Light chain cast nephropathy may manifest either acute or chronic renal failure. Proteinuria is most often present in variable range (nephritic or nephrotic range).
- If patient presents with proteinuria in nephrotic range, AL amyloidosis or light chain deposition disease is more likely than light chain cast nephropathy.

Pathology Pearls: Light Chain Cast Nephropathy in Multiple Myeloma

Light Microscopy

- In light-chain cast nephropathy, tubular lesions show numerous dense hyaline casts in the distal tubules and collecting ducts in light chain cast nephropathy in multiple myeloma. These casts are brightly eosinophilic with angulated borders, which induce foreign body reaction, characterized by multinucleated giant cells and macrophages.
- Interstitial tissue is infiltrated by chronic inflammatory cells. More chronic lesions show tubular atrophy and interstitial fibrosis. Focal deposition of calcium is demonstrated in interstitial tissue.

Immunohistochemistry Technique

Immunohistochemical staining reveals that the casts containing light chains and Tamm-Horsfall glycoproteins.

RENAL TUBULAR FUNCTIONAL DISORDERS

FANCONI SYNDROME

Fanconi syndrome refers to generalized dysfunction of the proximal renal tubules, which may be hereditary or acquired. Patient has impaired absorption of glucose, amino acids, bicarbonate and phosphates. Patient presents with glycosuria, increased excretion of phosphates with decreased blood level of phosphates, amino acids and systemic acidosis.

CYSTINURIA

Cystinuria is an autosomal recessive disorder (few cases of autosomal dominant disorder) characterized by an inborn error of amino acid metabolism leading to excessive excretion of cystine due to defective reabsorption in renal proximal tubules.

- Cystinuria occurs due to mutations in the genes that encode the two subunits (neutral and basic amino

acid transport protein rBAT and b (0,+) amino acid transporter 1) of the amino acid transport system. Error in amino acid metabolism results in excess of cysteine in the urine associated with cysteine stones in urinary tract. The clinical symptoms of cystinuria are caused by the loss of poorly soluble cystine, which precipitates to form cystine stones in urinary tract. Most kidney stones are completely silent.

- Patient with large renal stones presents with an abrupt onset of severe, colicky pain in the flank radiating to the groin; and associated with either microscopic or macroscopic hematuria.

HARTNUP DISEASE

Hartnup disease is an autosomal disorder caused by the defective transport of neutral amino acids due to mutation in the neurotransmitter SLC6A19 in the small intestine and the renal proximal tubules. The abnormal urinary loss of tryptophan, a precursor of niacin leads to nicotinamide deficiency. Patient presents with pellagra-like skin eruptions, cerebellar ataxia, emotional instability and gross aminoaciduria.

NEPHROCALCINOSIS

Hypercalcemia may cause nephrocalcinosis or nephrolithiasis or urolithiasis. Nephrocalcinosis is a disorder in which there is too much calcium deposition in renal parenchyma and tubules with or without hypercalcemia. The disorders that can cause nephrocalcinosis include Alport syndrome, Bartter syndrome, medullary sponge renal disease, primary hyperoxaluria, renal tubular acidosis, renal transplant rejection and familial hypomagnesemia.

- Nephrocalcinosis is common in premature babies. In patients with nephrocalcinosis, extent of calcium deposition in renal parenchyma and tubules varies from microscopic to grossly visible demonstrated on abdominal radiographs.
- Majority of cases remain asymptomatic. Some cases have impaired concentrating ability of kidneys, however patients do not progress to end stage severe renal disease.
- In hypercalcemia, deposition of calcium in renal parenchyma is an example of metastatic calcification, in contrast to dystrophic calcification demonstrated at the site of renal parenchyma injury due to infarcts or cortical necrosis.
- Hypercalcemia due to primary hyperparathyroidism results in calcium deposition in renal parenchyma, which shows wedge-shaped scars interspersed with relatively normal renal tissue. These renal scars are composed of parenchymal atrophy and interstitial fibrosis. Etiology of hypercalcemia leading to nephrocalcinosis is given in Table 22.55.

Pathology Pearls: Nephrocalcinosis

Light Microscopy

- Basement membrane of renal tubules especially proximal tubules shows deposition of calcium. Calcium deposition also occurs in the cytoplasm of tubules and interstitial tissue.
- Tubular epithelial cells laden with calcium slough into the lumina in the form of aggregates as calcified casts. Scattered glomeruli show calcification in Bowman's capsule.
- Intrarenal arteries may also show calcification. Calcium deposits appear deeply basophilic in hematoxylin and eosin sections and black with von Kossa stain.

Electron Microscopy

Mitochondria of tubular epithelial cells contain abundant calcium deposits.

Table 22.55 Etiology of hypercalcemia leading to nephrocalcinosis

Increased Calcium Level due to Bone Resorption
▪ Primary hyperparathyroidism
▪ Renal osteodystrophy
▪ Neoplasms synthesizing parathormone or parathormone-like protein and metastases
Increased Intestinal Absorption of Calcium
▪ Milk-alkali syndrome
▪ Vitamin D excess
▪ Idiopathic hypercalcemia
▪ Sarcoidosis

UROLITHIASIS

Urolithiasis refers to the presence of stones in the kidneys, renal pelvis and calyces affecting both men and women. Ureterolithiasis refers to the presence of stones in the ureter. Urolithiasis refers to stones originating anywhere in the urinary system such as renal calyces, renal pelvis and urinary bladder in 80% of cases.

- Stone growth in urinary system starts with the formation of crystals of supersaturated urine which then adhere to the urothelium, thus creating the nidus

for subsequent stone growth in the setting of low urine volume and deficiency of urolithiasis inhibitors in urine such as pyrophosphate, diphosphonate, citrate, glycosaminoglycans and nephrocalcin, which prevent urolithiasis. Hypercalciuria promotes calcium oxalate/phosphate renal stones. Excessive intake of dairy products rich in oxalates and phosphates causes stone formation. Hydration prevents supersaturation of urinary solutes (e.g. calcium, oxalate, uric acid). Citrate chelates calcium constituent in urine, which binds calcium ions to form soluble complexes and thus decrease the free ion activity of unbound citrate and calcium.

- Recurrent urinary tract infection caused by urea splitting organisms such as *Proteus vulgaris*, *Klebsiella* species and Staphylococcus make the urine alkaline resulting in precipitation of ammonium magnesium phosphate stones known as staghorn stones filling entire renal pelvis and calyceal system.
- Renal stone requires a nidus to form, which comprises mucoprotein matrix (3–5%) and aggregated material derived from precipitation of urinary solutes, which constitutes 95–98% of the stone mass. Mucoprotein matrix of renal stones is composed of lipids, glycosaminoglycans (GAGs) and proteins (e.g. Tamm-Horsfall proteins, nephrocalcin, osteopontin, urinary prothrombin fragment 1, inter-alpha inhibitor, calgranulin, renal lithostathine, albumin, human urinary trefoil factor 1, hyaluronic acid, annexin II, CD44, matrix G1a protein, and monocyte chemoattractant protein 1).
- In most cases, the presence of a urinary stone is associated with an increased blood level and urinary excretion of its principal component. More than 90% of stones are radiodense on KUB radiographs.
- Complications of urolithiasis include acute kidney disease secondary to obstruction, anuria, urinary tract infection with renal obstruction and sepsis.
- Patient with renal stone presents with excruciating pain caused by acute distension of the ureter, usually by the transit of a small stone. Superadded infection is localized proximal to the site of obstruction, which may lead to infection of the renal parenchyma. Various cellular and extracellular molecular events during nephrolithiasis are given in Fig. 22.83.

Pathology Pearls: Risk Factors for Urolithiasis

- Anatomic anomalies of urinary tract (e.g. horseshoe kidney, ureteral structure)
- Family history of urinary tract stones
- Gout
- Hyperparathyroidism
- Relative dehydration, low urine volume, and deficiency of citrate in urine
- Metabolic disorders (e.g. hypercalciuria, hyperuricosuria, chronic metabolic acidosis)
- Cystinuria (an autosomal recessive inherited disorder)
- Drugs (e.g. diuretics and calcium/vitamin D supplements)
- Diet rich in oxalate (dairy products), urate, sodium and animal protein
- Obesity related nephrolithiasis

Pathology Pearls: Promoters and Inhibitors of Nephrolithiasis

Nephrolithiasis Promoters	Nephrolithiasis Inhibitors
▪ Calcium oxalate ▪ Calcium phosphate ▪ Sodium (promotes calcium absorption) ▪ Urate ▪ Low-urine volume ▪ Low-urine pH <5	▪ Citrate ▪ Magnesium ▪ Pyrophosphate ▪ Glycosaminoglycans ▪ Osteopontin ▪ Nephrocalcin ▪ High urine volume

Calcium phosphate and calcium oxalate aggregation are glycosaminoglycans, pyrophosphate and citrate.

MOLECULAR PATHOGENESIS OF UROLITHIASIS

Supersaturation of stone forming salts/solutes in urine is essential and excess of these salts by itself will not promote stone formation. The pathogenesis of calcium oxalate stone formation in the urinary tract is a multistep process and essentially includes nucleation, crystal growth, crystal aggregation and crystal retention.

- Promoters facilitate the stone formation while inhibitors prevent it. Besides low urine volume and low urine pH, high calcium oxalate, calcium phosphate, sodium, urate and cysteine also known to promote stone formation.
- High urine volume and many inorganic and organic substances such as citrate, magnesium pyrophosphate, diphosphonate, glycosaminoglycans, osteopontin, nephrocalcin are known to inhibit stone formation. Hydration prevents supersaturation of urinary solutes (e.g. calcium, oxalates, uric acid). Citrate chelates calcium constituent in urine. We shall discuss mechanism of stone formation and the role of promoters/inhibitors in crystallization.
- Upregulation of urolithiasis promoters and downregulation of urolithiasis inhibitors leads to stone formation within collecting system of kidney.
- Concentration of urinary urolithiasis promoters and inhibitors is influenced and regulated by both intestinal and renal transporters (gastrointestinal tract–renal axis). SLC26A1 gene encodes anion exchanger protein. SLC26A1 gene mutation causes

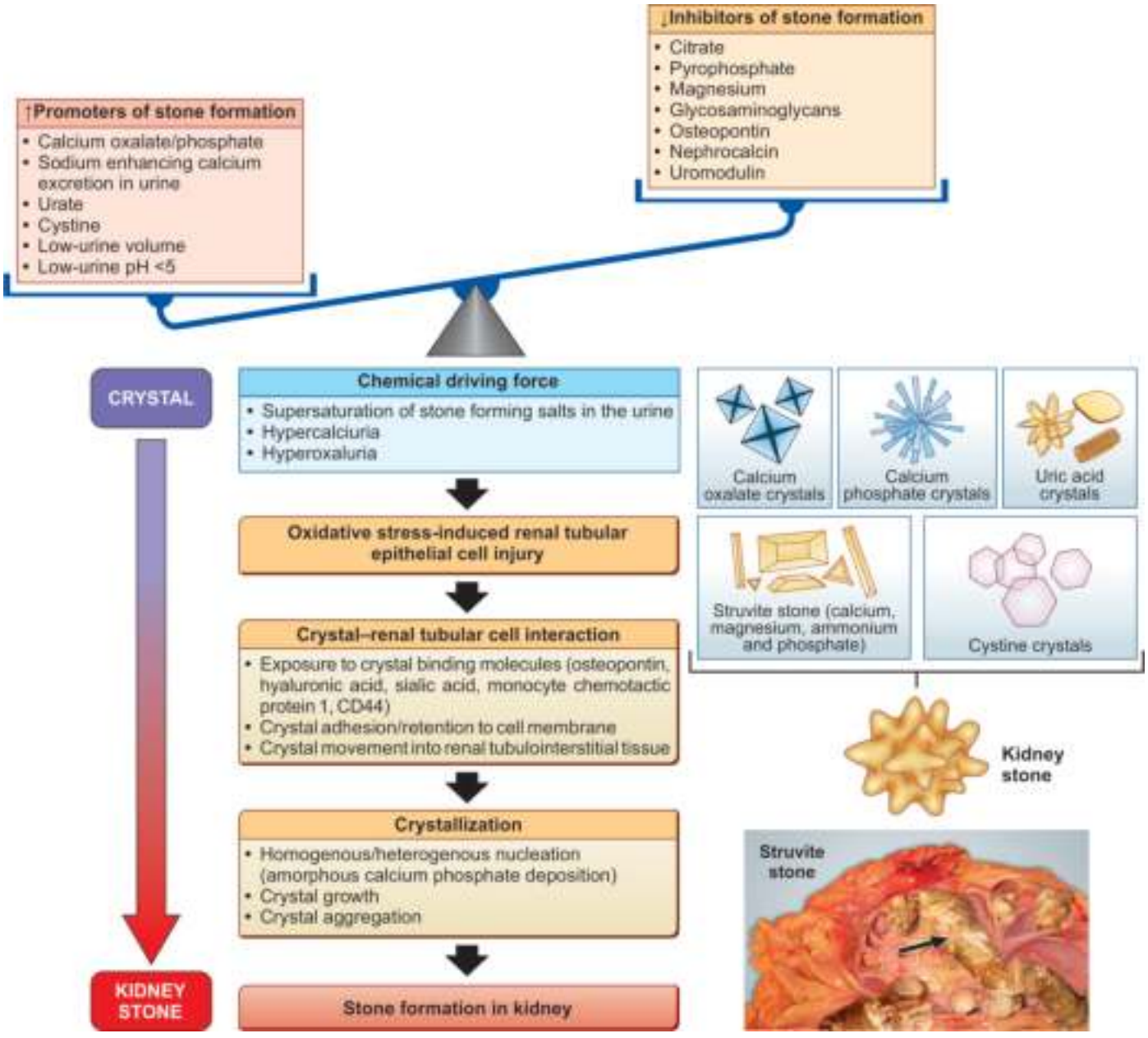


Fig. 22.83: Various cellular and extracellular molecular events during nephrolithiasis.

a recessive Mendelian form of nephrolithiasis. The transporters and ion exchangers are responsible for secretion and absorption of oxalates. Malabsorption of oxalates leads to urinary oxalate levels exceeding supersaturation and thereby promoting crystallization and consecutive stone formation.

- Depending on presence of salt constituents, urinary stones are of five types: (a) calcium oxalate/calcium phosphate (80–85%), (b) calcium, magnesium ammonium phosphate, also known as triple stones or struvite stones, forming staghorn calculus (5–10%), (c) uric acid/urate stones (5%), (d) xanthine stones, and (e) cystine stones (1–2%). With multifactor etiology and high recurrence rate, urinary tract stone disease provides a medical challenge. Composition, contributory factors and treatment of urinary stones are given in Table 22.56.

CALCIUM OXALATE AND PHOSPHATE STONES

Most renal stones contain calcium complexed with oxalate or phosphate, or a mixture of both anions. Calcium stones constitute 80–85% of urolithiasis, which are formed due to hypercalciuria (idiopathic 50%), increased absorption of calcium in the intestine and hypercalcemia.

- Hypercalcemia occurs in the settings of increased reabsorption of calcium by intestine, hyperparathyroidism (10% cases), ectopic production of

Table 22.56 Composition, contributory factors and treatment of urinary stones

Type of Stone	Frequency	Radiology	Conditions	Management
Calcium stones (calcium oxalate or calcium phosphate)	80–85%	Radiopaque	▪ Hypercalcemia and hypercalciuria ▪ Hyperparathyroidism ▪ Vitamin D intoxication ▪ Milk alkali syndrome ▪ Renal tubular acidosis ▪ Intestinal bypass surgery ▪ Hyperoxaluria	▪ Treatment underlying cause ▪ Increased fluid intake ▪ Diuretics ▪ Dietary restriction of food-rich in oxalates
Ammonium and magnesium phosphate or struvite stones (staghorn stones)	5–10%	Radiolucent	Infection (Proteus, *Klebsiella pneumoniae* and Pseudomonas) urine alkaline	▪ Increased fluid intake ▪ Treatment of UTI ▪ Acidification of urine
Uric acid stones (pH of approximately 5.5)	5–10%	Radiolucent	Hyperuricemia	▪ Increased fluid intake ▪ Uricosuric drug (allopurinol) ▪ Alkalization of urine
Cystine stones	1–2%	Radiolucent	Hereditary cystinuria	▪ Increased fluid intake ▪ Alkalization of urine

Calcium oxalate stones most often occur in adults and constitute 75–80% of cases.
Calcium phosphate stones are most common type of stones in children (10–20%).

parathormone in squamous cell lung carcinoma, osteolytic lesions, vitamin D intoxication, milk-alkali syndrome, sarcoidosis, and rarely primary hyperoxaluria. Excessive intake of dairy products rich in oxalates and phosphates causes stone formation.

- Increased serum calcium results in deposition of calcium in the basement membrane of renal tubules (nephrocalcinosis) and renal parenchyma. Calcium deposition is accompanied by and accumulation of macrophages and foreign body giant cells. Oxalate crystals are highlighted by polarized microscopy.
  - **Calcium oxalate stones:** Calcium oxalate stones most often occur in adults (75–80% of cases), which stones are radiopaque, which occur due to increased absorption of calcium oxalate from the bowel mucosa in Crohn's disease; and intake of certain foods (e.g. spinach, chocolate). The surface of calcium oxalate stone is rough, brown color probably due to old blood pigment.
  - **Calcium phosphate stones:** Calcium phosphate stones are most common type of stones in 10–20% of children. These stones are formed due to consumption of dairy products with recurrent urinary tract infections or distal renal tubular acidosis. Consistency of stones is hard/soft.

Pathogenesis of Calcium Oxalate or Phosphate Stones

Supersaturation of Urinary Salts

When the concentration of calcium oxalate or phosphate salt components increases beyond the saturation level, a state of supersaturation exists in the urine, which is thermodynamically unstable. Nephrolithiasis is a consequence of increased supersaturation of urinary salts in low urine volume and subsequent formation of crystalline particles. Renal stones are crystalline structures composed of calcium oxalate salts.

- The natural progression of the urine chemistry resulting in nephrolithiasis is urine saturation, urine supersaturation, crystal nucleation, crystal growth, crystal aggregation, crystal–cell interaction, endocytosis of calcium oxalate crystal by renal tubular epithelial cells, tubular cell injury, crystals retention by the urothelium and the continued growth of the renal stone on the retained crystals within kidneys.
- Nephrolithiasis occurs when concentration of ions such as calcium, oxalate, hydrogen, sodium and uric acid in the glomerular filtrate is higher than normal amounts. This condition is known as supersaturation and the supersaturated ions are more likely to come out of glomerular filtrate and crystalline. Risk factors for supersaturation of crystalline ions in glomerular filtrate include dehydration, high fat diet, animal protein, high salt intake and obesity.

Homogenous/Heterogenous Crystal Nucleation

The process of renal stone formation involves the formation of crystals that dissociate from the urine and accumulate on the lining surfaces of the urinary tract. Transformation from a liquid to a solid phase in a supersaturated solution is called crystal nucleation. Formation of renal stone occurs through homogenous or heterogenous nucleation.

- Homogenous nucleation occurs when calcium and oxalate ions complex to form small crystals that grow to larger pure calcium oxalate stones.

- Heterogenous nucleation occurs when calcium and phosphate ions complex to initially form small crystals of calcium phosphate. These small crystals of calcium phosphate form the substrate upon which calcium, oxalate subsequently deposits. This interaction results in a mixed calcium oxalate and calcium phosphate stone.
- Initially, homogenous crystal nucleation starts which progresses to heterogenous nucleation causing calcium oxalate crystal formation. Typically, the crystals form in the renal distal tubule, loop of Henle and/or collecting system. Most crystals are simply excreted unnoticed into the urine. However, sometimes, the crystals adhere to the epithelium of the tubules, particularly in the collecting system and form seed crystals that result in renal stone formation.

Crystal Growth

Crystal aggregation appears to be the major mechanisms for crystal growth. Crystal growth is one of the prerequisites for particle formation and thus stone formation. It has been postulated that after crystal nucleation within the renal tubule lumen, a nascent crystal either aggregates with other crystals to form a mass large enough to occlude the nephron or adheres to the urothelium.

Crystal Aggregation

Crystal aggregation has been recognized as an initial most crucial mechanism for renal stone pathogenesis. Microcrystals of calcium, oxalate and other salts can be found in concentrated normal urine or renal tubular fluid.

- Nevertheless, the individual urinary microcrystals are too small and are normally excreted by urinary or tubular fluid flow. In addition to crystal growth and adhesion of crystals to renal tubular epithelial cells, crystal aggregation causes the crystals to retain inside renal tubules by their rapidly enlarged size as a result of combination of several individual crystals into agglomerate.
- Many studies have revealed that deficiencies of urolithiasis inhibitors are associated with large aggregated crystals in the urine of renal stone cases. In addition, Tamm-Horsfall protein and various bacteria have been demonstrated to promote crystal aggregation.
- Quantitative analysis of crystal aggregation depends only on number of agglomerates and their size. Calcium oxalate crystal aggregate is analyzed by spectroscopy.

Crystal–Cell Interaction

Crystal–cell interactions are crucial in physiologic crystal retention and the early stage of calcium oxalate renal stone formation. Thus, crystals of calcium oxalate may be retained in the renal tubules to form stones by binding to the apical surface of tubular cells and subsequently undergoing endocytosis.

- Crystals of calcium oxalate are injurious to tubular epithelial cells due to oxidative stress and inflammatory response.
- The damaged tubular epithelial cells are released into the urine, which are capable of promoting crystal nucleation at much lower supersaturations of crystals.
- Damaged cell membrane tubules also provide sites for crystal attachment and eventual retention within the kidneys.
- Renal tubular epithelial damage cell may assist in movement of crystals from the intratubular lumen to the interstitial tissue leading to formation of apatitic Randall's plaques and ulceration to the renal papillary surface.

Endocytosis of Calcium Oxalate Crystals by Renal Tubular Epithelial Cells

Crystals are frequently precipitated in the renal tubular fluid of stone-forming as well as nonstone-forming persons. Following crystal precipitation in the nephron, these are rapidly excreted, which facilitates their excretion. This elimination process is thwarted, (prevented), when the crystals adhere to tubular epithelial lining.

- Many studies revealed endocytosis of calcium oxalate crystals occurs in renal tubular epithelial cells in a patient with primary hyperoxaluria, who received renal transplantation.
- Tamm-Horsfall proteins inhibit uptake of calcium oxalate crystals by renal tubular epithelial cells in distal tubular fluid.
- Nephrocalcin prevents attachment of calcium oxalate crystals to the plasma membrane and phagocytosis.

Relationship between Crystal–Cell Interaction and Renal Tubular Cell Injury

Both calcium oxalate crystals and oxalate ions induce an epithelial–mesenchymal transition (EMT) in the proximal tubular epithelial cells and contribute to renal tubular epithelial cell injury. TGF-β1 is the main cytokine synthesized by macrophages, which mediates of EMT.

- Macrophages exposed to calcium oxalate crystals stimulate TGF-β1 production and induce EMT in distal tubular epithelial cells. EMT plays a crucial role in both physiologic and pathologic states.
- The EMT is a process by which epithelial cells lose their polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells.

- Hyperoxaluria induces crystalluria, tubular crystalline deposits of calcium oxalate, interstitial fibrosis, tubulointerstitial inflammation and progressive renal failure.

MAGNESIUM, AMMONIUM, CALCIUM PHOSPHATE STONES (STRUVITE STONES)

Magnesium, ammonium, calcium phosphate stones, or staghorn stones also known as struvite stones or triple phosphate stones are the second most common form of renal stones. These are formed in patients with recurrent urinary tract infections by urea-splitting bacteria such as *Proteus vulgaris*, *Klebsiella* species and Staphylococcus. These constitute 15% of renal stones.

- Urease produced by these organisms convert urea into NH_3, thus causes alkaline pH of urine, resulting in precipitation of magnesium ammonium phosphate (struvite) and calcium phosphate (apatite). Staghorn calculi fill the entire pelvis and calyces.
- The struvite stone has flaking surface and gray white color. Because of large size staghorn stone filling entire renal pelvis and calyceal system, surgical removal is done. Antibiotics are given to eliminate urease producing bacteria. Because of large size staghorn stone filling entire renal pelvis and calyceal system, surgical removal is done. Antibiotics are given to eliminate urease producing bacteria. Struvite stone or staghorn stone (triple phosphate stones, calcium, magnesium ammonium phosphate) in kidney is shown in Fig. 22.84.

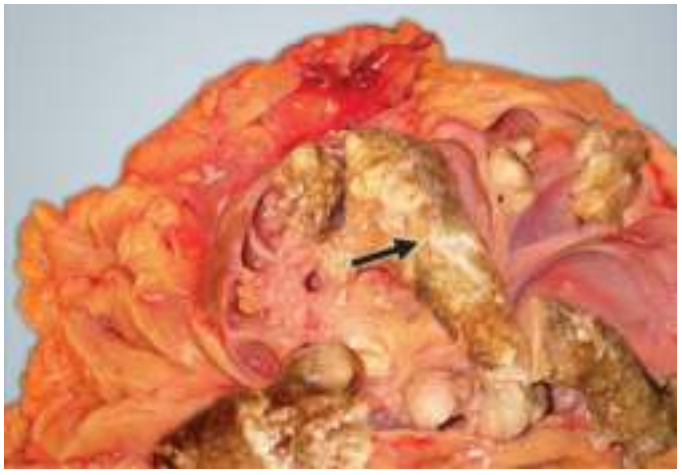


Fig. 22.84: Struvite stone or staghorn stone (triple phosphate stones, calcium, magnesium ammonium phosphate) in kidney. Staghorn stone, also sometimes called coral calculi that obtain its characteristic shape by forming a cast of the renal pelvis and calyces, thus resembling the horns of a stag. It can be complete or partial depending on the level of occupancy of the collecting system. It refers to struvite calculus involving the renal pelvis and extending into at least two calyces. Large staghorn stone is demonstrated (arrow).

URIC ACID STONES

Uric acid stones account for 6% of urolithiasis, which are formed due to increased serum uric acid in gout (hyperuricemia), chemotherapy for leukemia, uricosuric drugs or excess dietary proteins (meat).

- About 50% of patients lack elevated uric acid in blood or urine due to acidic pH. Long-standing diarrhea leads to small volume of very acidic urine responsible for uric acid stones formation.
- Uric acid stones are smooth light yellow-brown color, hard consistency and radiolucent. Initially uric acid stone is <2 cm in diameter, and may become staghorn calculi if located in renal pelvis.
- Uric acid crystals in collecting tubules are elongated and rectangular, or doubly refractile crystals in the interstitium with giant cell reaction. Allopurinol increases urinary pH and makes uric acid soluble in urine.

CYSTINE STONES

Cystine is a sulfur-containing amino acid obtained by the oxidation of two cystine molecules which are then linked via a disulphide bond. As the kidneys filter blood to create urine, cystine is normally reabsorbed back into the blood circulation. Persons with cystinuria cannot properly reabsorb cystine into their blood stream, so the amino acid cystine accumulated in their urine.

- As urine becomes more concentrated in the kidneys, excess cystine forms crystals. Aggregation of crystals become stones that may lodge in the urinary tract. Sometimes cystine crystals combine with calcium molecules in the kidneys to form large stones. These crystals and stones can block the urinary tract and reduce the ability of the kidney to eliminate waste metabolic products through urine. The cystine stones also provide sites for bacterial growth, where bacteria may cause urinary tract infections.
- Cystinuria is an autosomal recessive disorder due to mutations in the SLC3A1 (2p16.3) encoding heavy subunit (rBAT) or SLC7A9 (18Q13.1) genes that encode the two subunits leading to excess of cystine in the urine due to defective reabsorption in renal proximal tubules.
- Cystine stones are yellow-brown, round with smooth surface and radiopaque stones formed at low urinary pH. Cystine crystals are flat hexagons in urine. Most kidney stones are completely silent. Patient with large renal stones presents with an abrupt onset of severe, colicky pain in the flank radiating to the groin; and associated with either microscopic or macroscopic hematuria.

XANTHINE STONES

Xanthinuria is an autosomal recessive disorder, due to deficient xanthine oxidase, causing excessive xanthine levels and stones in one-third of patients. Xanthine stones are <2 cm, smooth, light yellow color, radiolucent; stones may or may not be visualized with plain radiograph.

STONE GRANULOMA

Endoscopic management of urolithiasis is one of the commonest urological procedures. A stone granuloma is a rare cause of ureteral obstruction following ureteroscopic stone fragmentation and removal. Stone granuloma can be caused secondary to recurrent ureteral obstruction and strictures. Delayed stone granuloma can also occur following pneumatic lithotripsy during ureteroscopy.

COMPLICATIONS OF UROLITHIASIS

Many complications may arise from urolithiasis especially if there is delay in diagnosis or inadequate treatment. The complications include acute or chronic kidney disease secondary to obstruction and uremia, anuria, urinary tract obstruction, chronic pyelonephritis, xanthogranulomatous pyelonephritis, pyonephrosis, renal calyceal rupture and urine extravasation, perinephric abscess and hypertension.

HYDRONEPHROSIS

Hydronephrosis describes urine-filled dilation of the renal pelvis and/or calyceal system as a result of obstruction. It is associated with progressive renal atrophy. The signs and symptoms of hydronephrosis depend upon whether the obstruction is acute (renal stone) or chronic, partial or complete, unilateral or bilateral. Renal stone produces acute hydronephrosis.

- Patient presents with sudden onset of intense pain in the flank region. Blood tests may demonstrate increased blood urea and serum creatinine or electrolyte imbalances. Urine analysis may indicate as elevated pH due to secondary obstruction of nephrons within the affected kidney. Clinical examination may detect a palpable abdominal or flank mass caused by the enlarged kidney due to hydronephrosis. Imaging studies, such as renal ultrasonography, intravenous pyelography, CT or MRI scan are important diagnostic tools in determining the presence and/or cause of hydronephrosis.
- Mild hydronephrosis appears to be relatively benign in nature, and in most cases, calyceal dilatation improves with time, and thus surgical intervention is not required. On the other hand, moderate or severe hydronephrosis often results in a significantly poor outcome and requires surgical intervention, and therefore, requires closer follow-up. Hydronephrosis due to nephrolithiasis is shown in Fig. 22.85.



Fig. 22.85: Nephrolithiasis inducing chronic pyelonephritis and hydronephrosis. Nephrolithiasis correlates with increased risk of chronic pyelonephritis with concurrent hydronephrosis. Cut surface of kidney shows a large calculus inducing hydronephrosis and chronic pyelonephritis (arrow). (*Courtesy:* Department of Pathology, Dr. DY Patil Medical College, Pune, Maharashtra.)

URINARY TRACT INFECTION

The most common complication of urolithiasis is the development of a urinary tract infection (UTI). When the urinary tract infection is associated with high fever, acute pyelonephritis is suspected. This is caused by bacteria spreading from urinary bladder to the kidney and invading the renal parenchyma. A urine culture is a test that can detect bacteria in urine. Urine analysis can find and identify the organisms that cause a urinary tract infection.

PYONEPHROSIS

Pyonephrosis is a disease causing suppurative destruction of the renal parenchyma. Hydronephrosis due to urolithiasis can be a cause of pyonephrosis, which is a urological emergency.

- Obstruction in the renal pelvis or upper part of ureter leads to superadded infection by *Escherichia coli*, Klebsiella, Proteus, enterococci, etc. and suppuration of the contents of the calyceal system known as pyonephrosis. The accumulation of purulent exudate in the hydronephrotic collecting system and abscess formation constitute the pathophysiology of pyonephrosis.



Fig. 22.86: Gross morphology of pyonephrosis. Pyonephrosis is characterized by accumulation of purulent debris and sediment in the renal pelvis and urinary collecting system resulting from urinary tract obstruction in the presence of pyelonephritis. The diagnosis of pyonephrosis is suspected when the clinical symptoms of fever and flank pain are combined with radiologic evidence of obstruction to the urinary tract (arrow). (*Courtesy:* Dr. Sujata Kanetkar, Senior Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

- The suppurative exudate fails to drain out due to obstruction. The surface of calyces becomes shaggy due to accumulation of pus. Extension of suppurative inflammation through the renal capsule leads to perinephric abscess. Gross morphology of pyonephrosis is shown in Fig. 22.86.

LABORATORY DIAGNOSIS OF UROLITHIASIS

Urine analysis is performed to demonstrate hematuria and crystals. It is also performed to study the composition of the stone (urine must be strained), since treatment is often tailored to the composition of the urinary stone.

- Approximately >50% of cases pass stones in urine within 48 hours. Recurrence of stone formation is seen in 50% of cases. X-rays kidney-ureter-bladder (KUB) may reveal radiopaque stones in >80% of patients.
- Enhanced spiral (helical) CT scan and ultrasonography are done to demonstrate urinary stones. Intravenous urography is helpful to locate urinary stone. Serum calcium and parathormone levels are increased in hyperparathyroidism.

MANAGEMENT OF UROLITHIASIS

Nephrolithotomy refers to removal of renal stones by making a small tract from the skin to the kidney. Extracorporeal shock wave lithotripsy (**ESWL**) is used to remove stones in the kidney or upper ureter. Shock waves are directed by a probe placed on the tummy or back. This shatters the stone into small fragments which then can be passed spontaneously. If ESWL does not work surgery can be used to remove staghorn stone. Urinary bladder stones are removed using a cystoscope, a special instrument which is passed through the urethra into the urinary bladder.

VASCULAR DISEASES OF KIDNEY

DIFFUSE RENAL CORTICAL NECROSIS SECONDARY TO SHOCK

Diffuse renal cortical necrosis refers to acute wide-spread ischemic necrosis limited to the large portions of the renal cortex of both kidneys; however the medulla is spared. Bilateral pale infarcts are seen in renal cortex. Massive tubular necrosis leads to fatal outcome. Renal cortical infarction may be patchy in some cases and compatible with survival. Vasa recta arising from juxtamedullary efferent arterioles supply arterial blood to the medulla. Thus, occlusion of the outer renal cortical vessels leads to cortical necrosis sparing medulla.

- Diffuse renal cortical necrosis occurs in the settings of hypovolemia and endotoxic shock. It most often occurs in pregnant women with pre-eclampsia, eclampsia, or abruptio placentae. Pregnant woman develops acute kidney disease. It is thought that the cause is a combination of end organ vasospasm and disseminated intravascular coagulation (DIC).
- Due to disseminated intravascular coagulation (DIC), necrosis is limited to renal cortex. Patient with severe renal cortical necrosis presents with acute renal failure. It is initially indistinguishable from that produced by acute tubular necrosis. Schematic representation of vascular lesions and histological patterns and their underlying causes is shown in Fig. 22.87.

Pathology Pearls: Diffuse Renal Cortical Necrosis

Gross Morphology

The extent of renal cortical necrosis varies from patchy to confluent. Complete occlusion of the arterial supply to renal cortex produces pale/while infarcts.

Light Microscopy

Kidneys show fibrin/platelets clots in arterioles and glomerular capillaries. Glomeruli, proximal and distal tubules are severely affected. Cellular outlines are maintained with loss of structural details. Dystrophic calcification occurs in areas of renal cortical necrosis.

RENAL INFARCTION

Renal infarction refers to ischemic necrosis due to obstruction of interlobar or larger branches of the renal artery. It most often occurs secondary to thromboembolic from the left heart (90%), in the settings of cardiac vegetations or complicated atherosclerotic plaque, nephrosclerosis (benign and malignant), vasculitis (e.g. polyarteritis nodosa), and sickle cell nephropathy. Gross morphology of renal infarct due to occlusion of arterial segment is given in Table 22.57.

SICKLE CELL NEPHROPATHY

Sickle cell nephropathy is the most common organ manifestation of sickle cell disease or sickle cell trait. Sickling of red blood cell may occur in peritubular capillaries in the medulla due to the low O_2 tension in the medulla.

- The rigidity of sickled erythrocytes results in obstruction of the microcirculation, with subsequent hypoxia and ischemic injury in many organs. The interstitial tissue in which the vasa recta course has a low oxygen tension. As a result, in patients with sickle cell disease, erythrocytes in the vasa recta tend to sickle and occlude the lumina. Infarcts in the medulla and papillae ensue, sometimes severe enough to cause renal papillary necrosis.
- The glomeruli are conspicuously congested with sickle cells. Patient develops renal papillary necrosis and pyelonephritis. Patient presents with asymptomatic hematuria (most common) due to infarction in the medulla. Kidneys lose concentrating ability of urine. Causes of renal papillary necrosis are given in Table 22.58.

NEPHROSCLEROSIS

Hypertension is defined as intermittent or sustained elevation of systolic blood pressure >139 mm Hg or diastolic blood pressure >89 mm Hg. Vascular changes in kidneys due to hypertension are known as benign or malignant nephrosclerosis. Features of benign and malignant nephrosclerosis in long-standing hypertension are given in Table 22.59.

- Approximately 15% of patients with mild to moderate hypertensive cause renal vascular changes known as benign nephrosclerosis. On gross morphologic examination, kidneys involvement shows fine granularity giving '**grain leather appearance**'.
- Renal vascular changes in severe hypertension are termed as malignant nephrosclerosis. Malignant

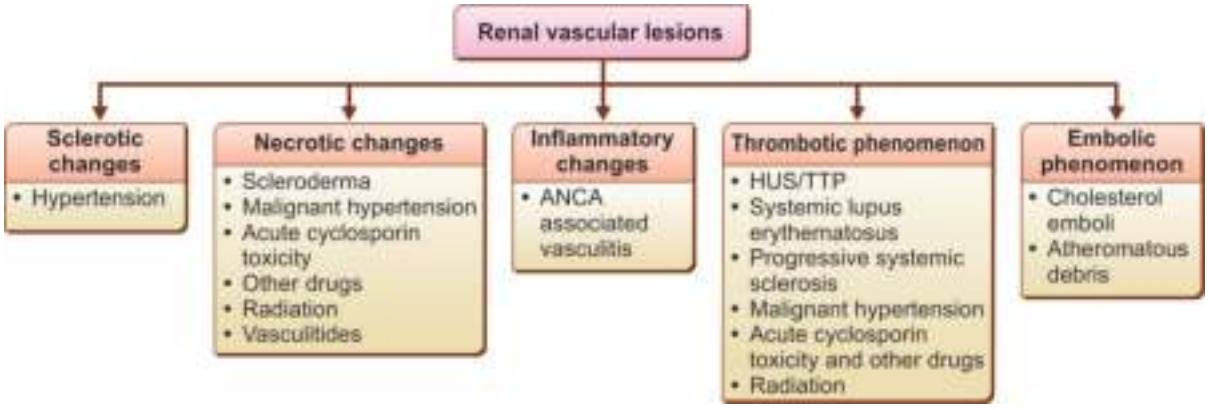


Fig. 22.87: Schematic representation of renal vascular lesions and histological patterns and their underlying causes.

Table 22.57 Gross morphology of renal infarct due to occlusion of arterial segment

Branch of Renal Artery	Size of Renal Infarct	Relationship to the Lobes
Segmental artery	4–5 cm	Entire lobe and portion of adjacent lobe
Interlobar artery	2–3 cm	Columns of Bertin and portion of adjacent lobe
Arcuate artery	0.5–1.0	One sixth to one eighth of a lobe extending to the middle of the lobe
Intralobular artery	0.1–0.2	Small portion of a lobe

Table 22.58 Causes of renal papillary necrosis

Disorder	Time Course of Papillary Necrosis	Number of Renal Papillae Affected	Infection	Calcification
Diabetic nephropathy with or without infection	10 years	Several renal papillae affected at same stage	80%	Rare calcification
Analgesic abuse most often associated with phenacetin	7 years	All renal papillae affected at different stages	25%	Frequent calcification
Sickle cell disease	Variable	Few renal papillae	–/+	Rare calcification
Lower urinary tract obstruction with or without infection	Variable	90% of renal papillae	90%	Frequent calcification

Table 22.59 Features of benign and malignant nephrosclerosis in long-standing hypertension

Parameters	Benign Nephrosclerosis	Malignant Nephrosclerosis
Incidence	Very common	Rarely occurs
Age group	Begins in <45 years of age and progresses	Young adults of 25–35 years
Sex distribution	Females > males	Males : females (1:1)
Etiology	Majority of cases suffer from primary/essential hypertension	Majority of cases develop malignant hypertension due to renal disease, however a few cases develop in long-standing primary/essential hypertension
Hypertension progression	Very slow progression over many years	Rapid progression within months to one or two years
Blood pressure range	Very slow rise of blood pressure with diastolic blood pressure ranging 90–120 mm Hg	Rapid rise of blood pressure with diastolic blood pressure ≥120 mm Hg
Injury to blood vessels	Mild to moderate injury to blood vessels	Severe injury to blood vessels
Large elastic and medium size arteries involvement	Potentiates slow formation of atheromatous plaque and accelerates arteriosclerosis	Potentiates formation of atheromatous plaque and accelerates arteriosclerosis
Arteriolar changes	Hyaline arteriolar thickening with narrowing of arteriolar lumen and reduplication of elastic lamina	Fibrinoid necrosis of vascular endothelium with occlusion of lumen by thrombus in vessels of kidney and other abdominal viscera
Pathogenesis of arteriolar changes	Benign hypertension increases endothelial permeability of arterioles resulting in deposition of plasma proteins beneath vascular intima and thus ischemia of tissue. These histologic changes are termed 'benign nephrosclerosis'.	Malignant hypertension causes severe damage to vascular endothelium of arterioles resulting in intravascular coagulation and fibrinoid necrosis with fixed vascular lumen. These histologic changes are termed 'malignant nephrosclerosis'.
Gross morphologic changes in kidneys	▪ Kidney size is decreased due to ischemia ▪ External surface shows grain leather appearance with thinned out cortex ▪ Tubular atrophy and increased interstitial fibrosis	▪ Small contracted kidneys due to marked diffuse glomerular sclerosis ▪ External surface shows flea-bitten kidney due to rupture of renal small vessels especially capillaries ▪ Tubular loss in relation to sclerosed glomeruli present
Clinical manifestations	Uremic manifestations are uncommon	Uremic manifestations are common due to chronic renal failure

hypertension causing necrosis of glomerular endothelial cells results in hemorrhage on the surface of kidney is known as 'flea-bitten kidney'. Other causes of flea-bitten kidneys are emboli from infective endocarditis, rapidly progressive glomerulonephritis, systemic lupus erythematosus, Goodpasture's syndrome and Henoch-Schönlein purpura. Causes of flea-bitten kidneys are given in Table 22.60.

Table 22.60 Causes of flea-bitten kidneys

Malignant hypertension
Emboli from infective endocarditis
Rapidly progressive glomerulonephritis
Systemic lupus erythematosus
Goodpasture's syndrome
Henoch-Schönlein purpura

BENIGN NEPHROSCLEROSIS LEADS TO OBLITERATION OF GLOMERULI

Benign nephrosclerosis refers to pathologic changes in the kidney secondary to chronic mild to moderate hypertension. Vascular disease of the kidneys can be both a cause and a consequence of hypertension.

- Renal involvement in hypertension is more common in Blacks than in Whites. Benign hypertension is most often asymptomatic until development of complications of the kidney and other organs.
- Vascular changes occur in interlobar, arcuate arteries and afferent arterioles located within the cortex of both kidneys in approximately 15% of patients with benign hypertension. End stage renal disease occurs in 25–30% of patients with long-standing benign hypertension. Maintaining normal blood pressure is the only way to slow the progression of benign nephrosclerosis.

Histologic Types of Vascular Changes

There are two types of vascular changes, i.e. hyaline arteriosclerosis and reduplication of elastic lamina. Similar changes are also seen in elderly persons (normal and hypertensive) and diabetes mellitus. Due to benign hypertension, persons develop increased permeability of vessels resulting in deposition of plasma proteins in subintimal regions of blood vessels. Lumina of the blood vessels is decreased leading to ischemic changes in organs.

Surgical Pathology: Benign Nephrosclerosis

Gross Morphology

- In benign hypertension, kidneys are symmetrically reduced in size and weight due to ischemia.
- Capsular surface of the kidneys is most often finely granular giving 'grain leather-like appearance', with V-shaped scars representing renal infarcts caused by the occlusion of medium-sized renal vessels.
- On cut section, cortex is thinned out. Simple cysts may also be present.

Light Microscopy

- Renal vascular changes in benign hypertension are known as benign nephrosclerosis. Focal global glomerulosclerosis, tubular atrophy and interstitial fibrosis are invariably seen with some degrees of chronic inflammation in the glomerulosclerotic regions.
- Renal artery branches such as afferent arterioles, interlobar and arcuate arteries exhibit intimal fibrosis, reduplication of the internal elastic lamina, and smooth muscle hyperplasia in concentric fashion and reduction of lumen size leading to hyaline arteriolosclerosis (thickening of the wall) and thus ischemia. It results in tubular loss or atrophy, increased interstitial fibrosis replacing nephrons, associated with infiltrates of lymphocytes without plasma cells. The eosinophilic deposits within arteriolar wall represent insudation of plasma proteins in small blood vessels.

Histochemical Stain

In benign nephrosclerosis, periodic acid–Schiff (PAS) stain shows arterial intimal thickening and atrophy of the media in the branch of renal artery.

Immunology

Immunoglobulin M (IgM) and C3 is most often present within hyaline layers of arterioles.

Electron Microscopy

Electron microscopy may reveal thickening and wrinkling of the glomerular capillary basement membrane.

MALIGNANT NEPHROSCLEROSIS LEADS TO FATAL RENAL DISEASE

Malignant nephrosclerosis is a form of thrombotic microangiopathy secondary to malignant hypertension (systolic blood pressure >220 mm Hg and/or diastolic blood pressure >130 mm Hg).

- Severely elevated blood pressure results in rapidly progressive vascular disease, which adversely affects the brain (cerebral stroke), heart (left ventricular hypertrophy and failure), kidney (renal failure), and retina (focal retinal hemorrhages and papilledema).
- Malignant nephrosclerosis can be a complication of either essential (primary) or secondary hypertension. Tobacco smokers, black young African-American persons and patients with underlying chronic renal disease are at higher risk of developing malignant nephrosclerosis.

Clinical Features

Patient presents with severe headache, blurred vision, neurologic symptoms, papilledema, congestive heart failure, acute renal failure. Microangiopathic hemolytic anemia and thrombocytopenia can be observed. Physical examination reveals very high blood pressure, pounding heart beat and bulging optic disc papilledema. The therapeutic goal is a gradual reduction of mean arterial pressure with intravenous vasodilators.

Surgical Pathology: Malignant Nephrosclerosis

Gross Morphology

Renal changes in severe hypertension are known as malignant nephrosclerosis. Renal arterioles and glomeruli rupture, resulting in 'flea-bitten kidney', multiple pinpoint petechial hemorrhages on the capsular surface of kidney.

Light Microscopy

Interlobular arteries show intimal edematous thickening with erythrocytes in the intima. Their lumen is greatly decreased and may be occluded by fibrin thrombi. Severe hypertension causes necrotizing arteriolitis and glomerulitis with intensely eosinophilic material giving fibrinoid necrosis and hyperplastic arteriolosclerosis (onion skin appearance). Renal tubules show atrophic changes. Tubules contain eosinophilic material known as thyroidization of tubules. There is tubular loss in relation to sclerosed glomeruli present. Patients develop chronic renal failure with manifestations of uremia.

- **Fibrinoid necrosis:** Severe hypertension injures glomerular capillary endothelial cells and causes increased vascular permeability, which leads to insudation of plasma proteins into the vessel wall. Severe endothelial injury causes intravascular coagulation and rupture of blood vessels. Lumen becomes narrow and fixed resulting in renal ischemia. This morphologic change in renal arterioles is known as fibrinoid necrosis. Focal acute tubular necrosis and small cortical infarcts may be present. Histologic features in fibrinoid necrosis in malignant hypertension is shown in Fig. 22.88.
- **Hyperplastic arteriolosclerosis:** Acute vascular injury is followed by smooth muscle proliferation with an increase in concentric layers of smooth muscle cells that yield the so-called 'onion skin appearance'. Smooth muscle hyperplasia occurs as a result of growth factors derived from platelets and inflammatory cells at the site of vascular injury. It is accompanied by the replication of elastic laminae, the growth of new cells beneath intima. Histologic features in hyperplastic arteriosclerosis in severe hypertension are shown in Fig. 22.89.

Immunopathology

Staining for fibrinogen and IgM may be observed in arterioles, small arteries and glomerular capillaries.

Electron Microscopy

Electron microscopy exhibits thickening and wrinkling of the glomerular capillary basement membranes. Glomerular capillary endothelial cells are swollen and the subendothelial space is widened by electron-lucent fluffy material. Platelet and fibrin thrombi may be present.

Consequences

Poor perfusion of the kidneys in malignant nephrosclerosis stimulates the release of renin, which serves to elevate systemic blood pressure even further by activation of renin-angiotensin-aldosterone system (renovascular hypertension).

THROMBOTIC MICROANGIOPATHY

Thrombotic microangiopathy refers to extensive endothelial damage due to multiple causes such as infections, drugs (cisplatin chemotherapy), autoimmune diseases, severe hypertension, and pregnancy.

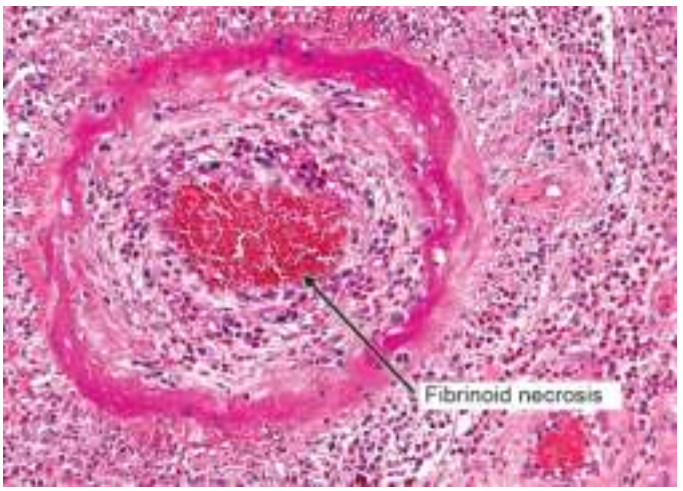


Fig. 22.88: Histologic features in fibrinoid necrosis in malignant hypertension. Malignant hypertension usually presents in 35–50 years of individuals and is characterized pathologically by fibrous endarteritis in the interlobular arteries and fibrinoid necrosis in the walls of a proportion of the efferent glomerular arterioles. The damage to the arteries leads to formation of pink fibrin, hence the term 'fibrinoid' (arrow) (400X).

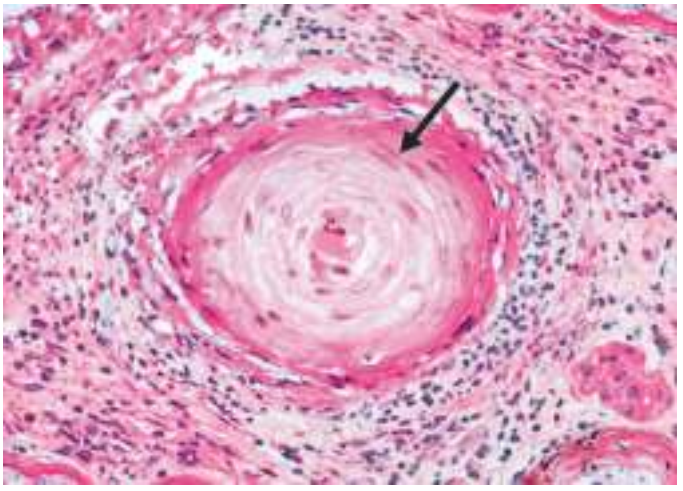


Fig. 22.89: Histologic features in hyperplastic arteriosclerosis in malignant hypertension. Pathologic changes in blood vessels observed in essential hypertension are similar to those seen in secondary hypertension due to renal disease or other diseases. Interlobular arteries exhibit intimal thickening and duplication of elastic lamina (elastosis) and there is hyaline change in the media of many arterioles (hyperplastic arteriosclerosis). Smooth muscle proliferation with an increase in concentric layers of smooth muscle cells that yield the so-called onion skin appearance (arrow) (400X).

- Alteration in blood flow leads to mechanical fragmentation of erythrocytes (schistocytes) and thrombocytopenia. This disorder is known as microangiopathic hemolytic anemia. Endothelial damage initiates a final common pathway of vascular changes such as thrombus formation resulting in focal ischemic necrosis. These vascular changes are to those seen in malignant nephrosclerosis.

- Histologic examination of affected kidneys exhibits arteriolar fibrinoid necrosis, arterial edematous intimal expansion, glomerular congestion, and vascular thrombosis.
- Patient presents with thrombocytopenia, hypertension, and renal failure. Causes of thrombotic microangiopathy are given in Table 22.61.

Table 22.61 Causes of thrombotic microangiopathy

Therapeutic Agents	
▪ Cisplatin	▪ Mitocin
▪ Cyclosporin	
Bacterial Infections	
▪ *Escherichia coli*	▪ Shigella
▪ Pseudomonas	
Autoimmune Disorders	
▪ Systemic lupus erythematosus	▪ Antiphospholipid antibody syndrome
▪ Scleroderma	
Other Disorders	
▪ Malignant hypertension	
▪ Pregnancy and postpartum period related factors	

TOXEMIA OF PREGNANCY INVOLVING KIDNEYS

Toxemia of pregnancy most often occurs during third trimester of first pregnancy. It most often affects kidneys, liver and brain (hemorrhages) in acute toxemia of pregnancy. Toxemia of pregnancy exists in two forms: pre-eclampsia and eclampsia.

- Pre-eclampsia refers to the triad of hypertension, proteinuria, and edema (fluid retention), during the third trimester of pregnancy. It usually begins insidiously after the 20th week of pregnancy.
- In eclampsia, pregnant women develop convulsion in addition to clinical manifestations observed in pre-eclampsia. Proteinuria is >3 g per day, and renal function declines. It is complicated by convulsions, and disseminated intravascular coagulation in eclampsia with fatal outcome.
  - Disseminated intravascular coagulation (DIC) is characterized by formation of fibrin thrombi in the liver, brain, and kidneys.

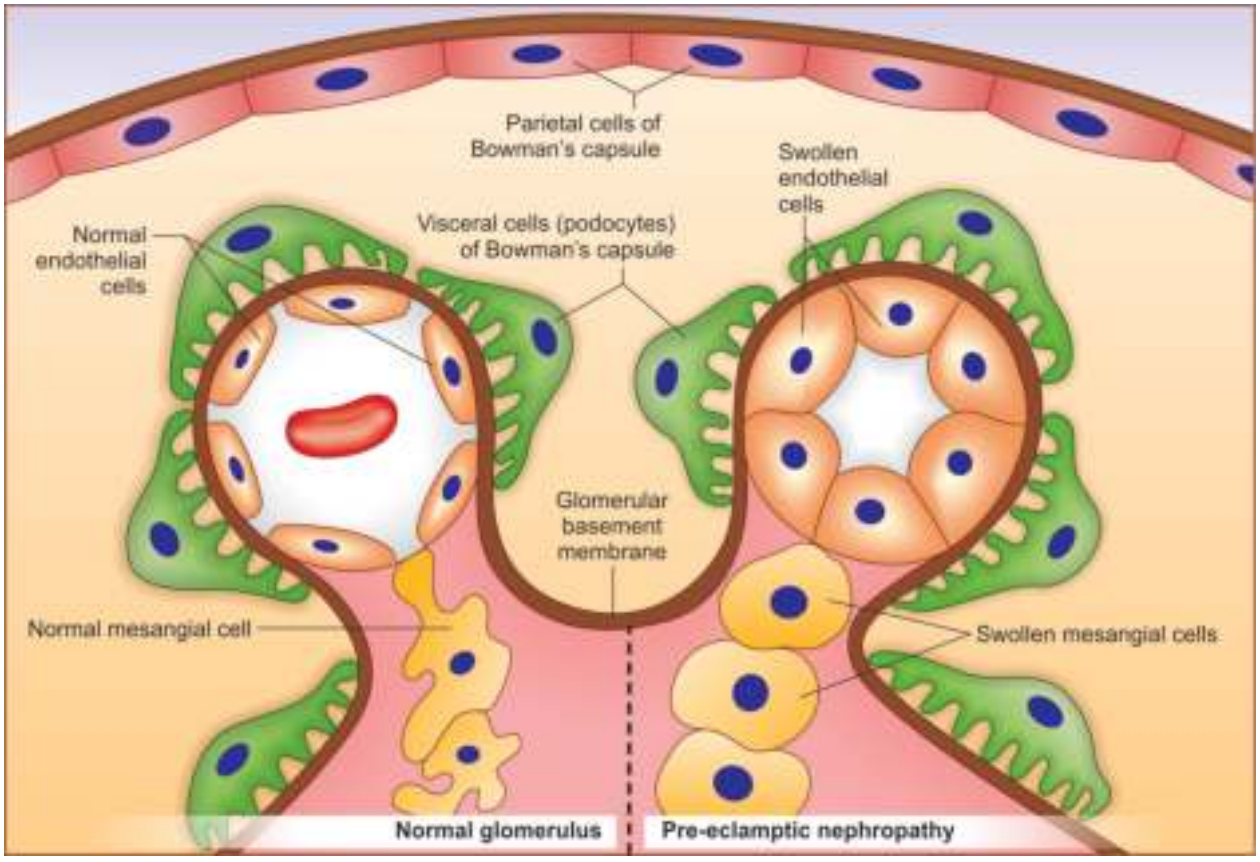


Fig. 22.90: Schematic representation of glomerulopathy in pre-eclampsia of pregnancy. Pre-eclampsia is a complication of pregnancy in the third trimester, usually primiparas, with maternal hypertension, proteinuria, periorbital edema, coagulation abnormalities, and disseminated intravascular coagulation in association with a characteristic glomerular lesion, endotheliosis. Glomerular endotheliosis represents a specific variant of thrombotic microangiopathy that is characterized by glomerular endothelial swelling with loss of endothelial fenestrae and occlusion of the capillary lumens.

- Patient develops puffiness on face, pitting edema and convulsions in true eclampsia. As the disease progresses the diastolic pressure persistently exceeds 110 mm Hg.

- Hematoxylin and eosin-stained section of kidney in pre-eclamptic nephropathy shows marked swelling of glomerular endothelial cells with marked narrowing of lumina. Mesangial cells and glomerular endothelial cells are enlarged and possess multiple vacuoles and vesicular structures. Schematic representation of glomerulopathy in pre-eclampsia of pregnancy is shown in Fig. 22.90.

VASCULITIS INVOLVING KIDNEYS

Renal vessels are involved in various types of vasculitis. Renal vasculitis (i.e. renal artery, interlobar arteries, arcuate arteries, efferent arterioles and glomeruli) may result in segmental/total infarction and renal parenchymal atrophy. Renal vasculitis tends to be severe and fulminant. Even with early diagnosis, about 33% of patients will progress to renal failure within five years. Vasculitis involving kidneys is given in Table 22.62.

Table 22.62 Vasculitis involving kidneys

Size of Vessel and Vascular Disorder	Renal Vessel Involved	Major Renal Manifestations
Small-sized vessel vasculitis		
▪ Immune complex-mediated vasculitis (Henoch-Schönlein purpura, cryoglobulinemia) ▪ Anti-GBM vasculitis (Goodpasture syndrome) ▪ ANCA vasculitis (Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome)	Glomeruli	Nephritis
Medium-sized vessel vasculitis		
▪ Polyarteritis nodosa ▪ Kawasaki disease	Interlobular arteries and arcuate arteries	Renal infarct and hemorrhage
Large-sized vessel vasculitis		
▪ Giant cell arteritis ▪ Takayasu's syndrome	▪ Main renal artery ▪ Main renal artery	▪ Renovascular hypertension ▪ Renovascular hypertension

CYSTIC DISEASES OF KIDNEY

The heterogenous group of renal cystic diseases comprises hereditary, developmental and acquired diseases. Each disease is distinguished by characteristic distribution of renal cysts. Accurate diagnosis of cystic renal diseases is important for two reasons: (a) appropriate management of delay onset of renal failure; and (b) appropriate genetic counseling in hereditary renal cystic diseases. Renal cystic diseases are given in Table 22.63.

ADULT POLYCYSTIC KIDNEY DISEASE

Adult polycystic kidney disease is autosomal dominant disorder. Though the genetic defect is present at birth, yet it most often manifests between 15 and 30 years of age. That is why this condition has also been called adult polycystic kidney disease. It is most often associated with berry aneurysm of the circle of Willis resulting in subarachnoid hemorrhage.

- Patient develops numerous bilateral cysts within the renal parenchyma that replace and ultimately destroy the renal parenchyma resulting in enlargement.
- The cysts originate from the tubules at any level, hence these cysts vary in size. Approximately 33% of patients develop hepatic cysts, whose lining resembles bile duct epithelium. PKD1, PKD2 and PKD3 gene mutations are associated with adult polycystic kidney disease. PKD1 gene encodes polycystin.
- Adult polycystic kidney disease manifests in the fourth decade or later life. Patient presents with sense of heaviness in the loins, bilateral flank and palpable large lobulated renal masses, hypertension, blood clots in the urine (hematuria), progression to end-stage renal disease after the age of 40 years. Secondary polycythemia may also occur. In women, adult polycystic kidney disease may be diagnosed at an earlier age during antenatal investigations, either as palpable masses or as impaired renal functions.

Table 22.63 Renal cystic diseases

Disorder	Inheritance	Pathology	Clinical Manifestations	Clinical Course
Adult polycystic kidney disease	Autosomal dominant	Kidneys and liver show large cysts. Berry aneurysm	Renal stones, hematuria, UTI and hypertension	Patient develops chronic renal failure at age of 40–60 years
Infantile polycystic kidney disease	Autosomal recessive	Patient presents with large cystic kidneys at birth	Liver fibrosis, splenomegaly, esophageal varices	Fatal during infancy and early childhood
Adult-onset medullary cystic disease	Autosomal dominant	Cysts at the junction of cortex and medulla	Salt wasting polyuria	Patient develops chronic renal failure at age of adulthood
Familial juvenile nephronophthisis	Autosomal recessive	Cysts at the junction of cortex and medulla	Anemia, growth retardation and salt losing polyuria	Patient develops chronic renal failure beginning at childhood
Medullary sponge kidney	None	Medullary cysts	Renal stones, hematuria and UTI	Patient has a benign course
Multicystic renal dysplasia	None	Kidneys are irregular with variable size cysts	It is associated with other renal anomalies	Surgery cures unilateral renal involvement. Patient with bilateral renal involvement develops chronic renal failure

- Diagnosis of adult polycystic kidney disease is established by ultrasonography, which demonstrates multiple bilateral renal cysts. Ultrasonography screening establishes diagnosis of adult polycystic kidney disease in 18 years of age in 95% of cases.
- Blood pressure control prevents development of chronic renal failure. Cardiac failure and urinary tract infection should be treated. Hemodialysis and renal transplantation are essential in management of these patients.

Surgical Pathology: Adult Polycystic Kidney Disease

Gross Morphology

Bilateral kidneys are enlarged up to 3 or 4 kg or more. The affected kidney contains large fluid-filled cysts up to 2 cm with areas of organized hemorrhage. Autosomal dominant adult polycystic kidney disease is shown in Fig. 22.91A and B.

Light Microscopy

- Adult polycystic kidney disease has saccular expansion of renal tubules and glomerular capsule that later dissociate and filled with fluid.
- Cysts are lined by cuboidal or flattened epithelium and possibly containing proteinaceous and serosanguineous fluid. Functional nephrons exist between the cysts with areas of global sclerosis, tubular atrophy, chronic inflammation and interstitial fibrosis.

INFANTILE POLYCYSTIC KIDNEY DISEASE

Infantile polycystic kidney disease is an autosomal recessive disorder. *In utero*, there is typically oligohydramnios because of poor renal functions and failure to form significant amounts of fetal urine. Cysts originate from the collecting ducts.

- Patient presents with multiple renal cysts evident at birth. The closed renal cysts are not in continuity with the collecting system. Infant suffering from polycystic kidney disease presents with bilateral flank masses, hypertension, oliguria and acute renal failure.
- Ultrasonography of kidneys shows cysts through cortex and medulla. Ultrasound of liver shows bile duct proliferation and hepatic fibrosis. Treatment is dialysis and renal transplantation.
- Ten-year survival rate is 80%. Death occurs shortly after birth in untreated cases. Infantile polycystic kidney disease is shown in Fig. 22.92.

Surgical Pathology: Infantile Polycystic Kidney Disease

Gross Morphology

- Both kidneys are markedly enlarged and tend to fill the retroperitoneum and displace abdominal contents.
- External surface of kidneys is smooth. The kidneys tend to be symmetrically enlarged. The cysts are quite small and uniform, perhaps 1–2 mm on average in cortex and medulla.

Light Microscopy

- The characteristic finding occurs in the later third trimester is renal cystic change with the cysts elongated and radially arranged. The cysts are lined by cuboidal epithelium.
- Cysts are smaller and present at right angles to the cortical surface.

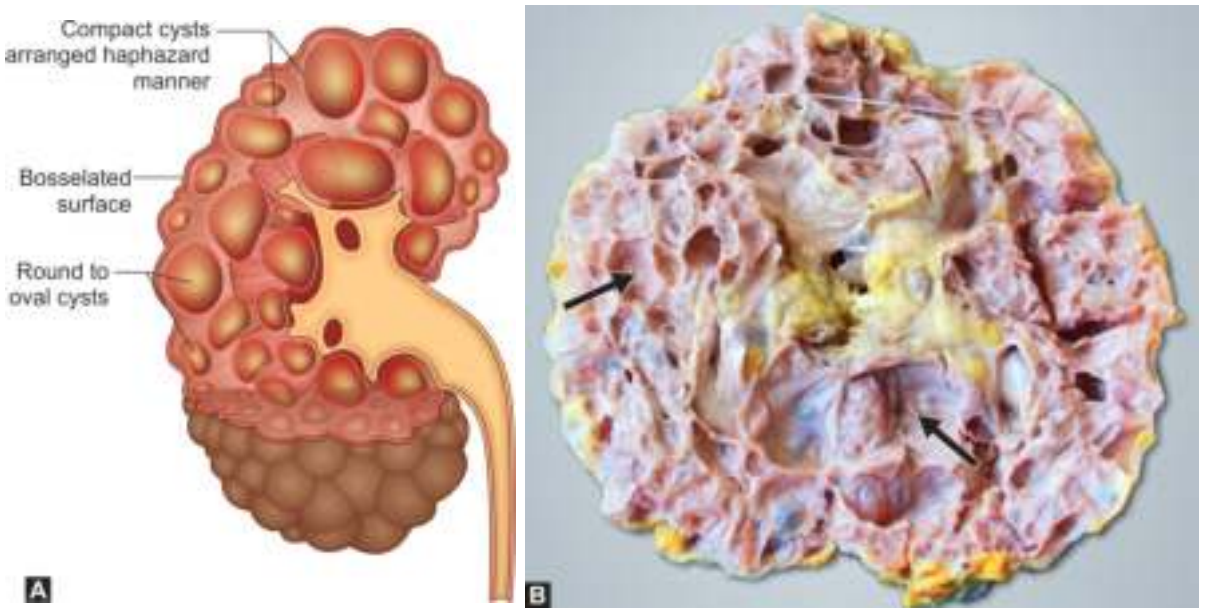


Fig. 22.91A and B: Autosomal dominant adult polycystic kidney disease. Autosomal dominant adult polycystic kidney disease occurs due to mutations in genes coding for polycystin 1 (PKD1 on chromosome 16p, most common) and polycystin 2 (PDK2, chromosome 4q). The renal parenchyma is almost completely replaced by cysts of varying size. The kidneys may be not much bigger than normal, but usually they are quite large (up to 8 kg). Cut surface shows subcapsular cysts up to 4 cm with very little normal renal tissue remaining. Cysts contain clear to brown fluid. Cut surface of kidney shows subcapsular multiple cysts (arrows).

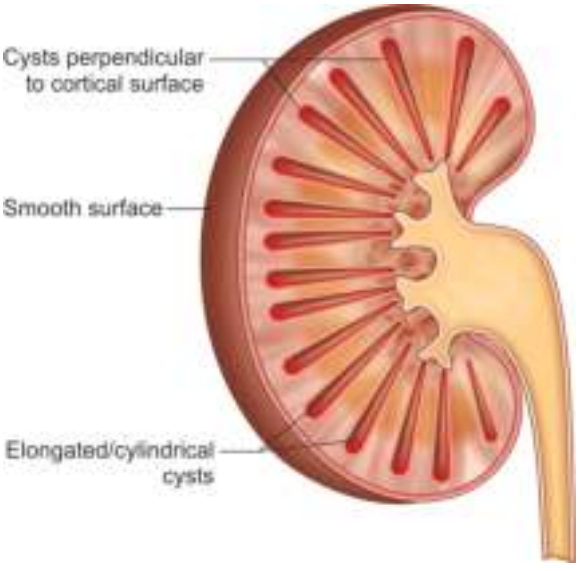


Fig. 22.92: Schematic representation of infantile polycystic kidney disease. Autosomal recessive polycystic kidney disease, also called infantile polycystic kidney disease usually presents with bilateral renal cystic disease at birth. Gross examination reveals large kidneys with smooth surface. Small cysts are present in the cortex and medulla. Dilated channels are perpendicular to cortical surface. Cysts are present in the medulla (collecting ducts). The disease has a poor prognosis.

SOLITARY RENAL CYST

Solitary renal cyst is comparatively rare. Renal cyst is solitary and well-defined and more common in women than men, which more often involves the lower pole of the kidney. Abdominal ultrasonography or computed tomography has led to the incidental detection of asymptomatic renal cyst. Complications of simple renal cyst include hemorrhage, infection, or rupture, which may result in development of complex cysts with calcification, and multiple lobules. Schematic representation of solitary renal cyst is shown in Fig. 22.93.

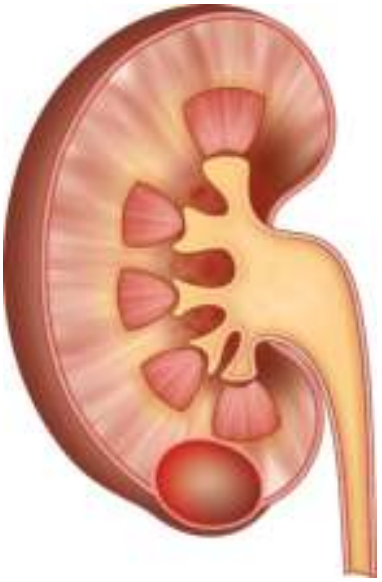


Fig. 22.93: Schematic representation of solitary renal cyst. Solitary renal cyst can occur in one or both kidneys. Simple cysts are usually round to oval in shape. These can range from the size of pea to the size of golf ball.

Surgical Pathology: Solitary Renal Cyst

Gross Morphology

- Solitary renal cyst is discrete lesion within kidney located in cortex. It extends outside the renal parenchyma and distorting the renal contour.
- Renal cyst is oval or circular in shape with well-defined outline. External surface is smooth, transparent, avascular, yellowish or bluish white in color. It is filled with homogenous clear or straw-colored fluid.

Light Microscopy

Light microscopy of solitary renal cyst is lined by a thin layer of fibrous tissue lined by a single layer of flattened or cuboidal epithelium.

MEDULLARY SPONGE KIDNEY

Medullary sponge kidney (also known as **Cacchi-Ricci disease**) is a congenital disorder of the kidneys and characterized by cystic dilatation of the collecting tubules in one or both fetus 'kidneys. In a normal fetus kidney, urine flows through collecting tubules, or tiny tubes as the kidney is being formed during a fetus' growth.

- Patient presents with renal colic (50–60%), urinary tract infection (20–35%), gross hematuria (10–20%) and hypercalcemia (30–50%). There is increased risk for development of kidney stones and urinary tract infection. Renal functions are impaired without development of renal failure.
- Radiologic findings are multiple small calculi within the renal papillae, dilated collecting ducts with the appearance of 'bristles on a brush'.
- Treatment focuses on complications (calculi and urinary tract infection) of medullary sponge kidney.

Surgical Pathology: Medullary Sponge Kidney

Gross Morphology

Kidney contains multiple small cysts (1–5 mm) involving the tip of renal papillae; one or multiple renal papillae possibly involved. Microcalculi are present in the dilated collecting ducts; possible lithiasis in the calyceal system. Medullary sponge kidney is shown in Fig. 22.94.

Light Microscopy

Dilated collecting ducts are lined by cuboidal or flattened epithelium, interstitium shows inflammation.



Fig. 22.94: Medullary sponge kidney. Medullary sponge disease, also known as Cacchi-Ricci disease, is a birth defect where changes occur in the tubules inside a fetus' kidneys. Patient presents with hematuria, calcium stone formation (nephrolithiasis) or signs of urinary tract infection such as polyuria and/or burning and pain during urination.

ACQUIRED RENAL CYSTIC DISEASE

Acquired renal cystic disease occurs in patients on long-term hemodialysis therapy in 10–20% of cases within the first three years of hemodialysis and 50% of cases within the first five years and 90% after ten years. Acquired cysts in kidney may form due to obstruction by oxalate crystals, hyperplasia or fibrosis. Multiple renal cysts, glomerular and tubular atrophy, and scarring are characteristic. The incidence of renal cell carcinoma is increased in 7% of patients. Atypical epithelial proliferations associated with gains of chromosome 7, 12, 17, 20 and Y, suggest these represent early neoplasms.

Surgical Pathology: Acquired Renal Cystic Disease

Gross Morphology

The kidneys are moderately enlarged weighing <800 g. Cut surface shows multiple cortical and medullary cysts containing clear fluid replacing >40% of renal parenchyma.

Light Microscopy

Renal cortical and medullary cysts are lined by flattened or cuboidal epithelium that may show focal pseudopapillae with nuclear enlargement and loss of polarity. These may contain oxalate crystals. Surrounding renal parenchyma shows global glomerulosclerosis, interstitial fibrosis and tubular atrophy.

CONGENITAL ANOMALIES OF KIDNEY

Congenital anomalies of kidney and urinary tract (CAKUT) can adversely affect functions of urinary tract, which occur from birth (congenital), although the abnormality may not become apparent until later in life.

RENAL AGENESIS

Renal agenesis is a condition in which a newborn is missing one or both kidneys. Unilateral renal agenesis (URA) is the absence of one kidney. Bilateral renal agenesis (BRA) is the absence of both kidneys, which is not compatible with life.

- Unilateral or bilateral renal agenesis is associated with other birth defects related to cardiovascular system, respiratory system, gastrointestinal system, skeletal muscles, bones, ears and eyes.
- Newborn babies with unilateral renal disease develop symptoms at birth or during childhood and not until later in life such as hypertension, proteinuria and swelling over face, hands and legs. Newborn babies with bilateral renal agenesis do not survive. Clinical examination reveals widely separated eyes with skin folds over eyes, low set eyes, flat broad nose, small chins, defects in arms and legs.
- Predisposing factors for renal agenesis and other defects include oligohydramnios, prepregnancy obesity, alcohol use and tobacco smoking. Potter syndrome in unborn infant is characterized by amniotic fluid, renal failure, duodenal atresia or with tracheoesophageal fistula.

MULTICYSTIC RENAL DYSPLASIA

Multicystic renal dysplasia results from the malformation of the kidney during fetal development. The kidney consists of irregular cysts of varying sizes leading to abdominal mass in infants. Multicystic renal dysplasia occurs in the setting of branchiootorenal (BOR) syndrome. BOR syndrome is characterized by malformations of the ears and kidneys. Mutations in three genes, EYA1 (located on 8q13, most common mutation in 40%), SIX1 and SIX5 have been observed in persons with BOR syndrome. Renal ultrasonography is the recommended initial imaging study.

Surgical Pathology: Multicystic Renal Dysplasia

Gross Morphology

Kidney is enlarged and distorted by variable-sized cysts involving one or both kidneys. Cut surface shows indistinct corticomedullary junction. Multicystic renal dysplasia is usually associated with ureteropelvic obstruction or ureteral atrophy/atresia.

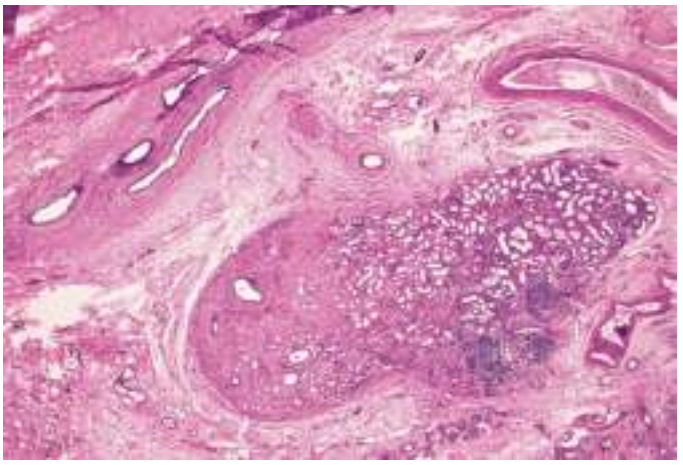


Fig. 22.95: Multicystic renal dysplasia. Multicystic renal dysplasia results from the malformation of the kidney during fetal development. The kidney consists of irregular cysts of variable sizes. Infants present with abdominal mass. Hematoxylin and eosin-stained renal tissue section shows disorganized renal parenchyma that is distorted by cysts of variable sizes, lined by flattened to cuboidal epithelium. It may contain nodular blastema (undifferentiated cells), islands of undifferentiated mesenchyme, cartilage (10–20%), immature collecting ducts and primitive glomerular structures (400X).

Light Microscopy

Multicystic renal dysplasia is composed of disorganized renal parenchyma due to multiple variable-sized renal cysts, which are lined by flattened to cuboidal epithelium. It may contain blastema component (undifferentiated cells), islands of mesenchyme, immature collecting ducts and primitive glomeruli. Histology of multicystic renal dysplasia is shown in Fig. 22.95.

SEVERE CONGENITAL ANOMALIES OF KIDNEYS AND URINARY TRACT

Severe congenital anomalies of kidneys and urinary tract (CAKUT) can result in life-threatening kidney failure and end-stage renal disease. Many different developmental abnormalities of urinary tract are classified as underdevelopment or absence of a kidney such as renal hypoplasia or renal agenesis; fluid-filled renal cysts, hydronephrosis, extra kidney or extra ureter; obstruction of ureter at ureteropelvic junction and posterior urethral valve. Genetic syndromes associated with severe congenital anomalies of kidneys and urinary tract are given in Table 22.64.

- CAKUT occurs due to combination of genetic and environmental factors. Role of genetic factors in pathogenesis of CAKUT is unknown in majority of cases.
- Many syndromes such as branchiootorenal syndrome, renal cysts and diabetes (RCAD) syndrome, renal

Table 22.64 Genetic syndrome associated congenital anomalies of kidney and urinary tract

Genetic Syndrome	Characteristics
Renal coloboma syndrome (also known as papillorenal syndrome)	Genetic disorder affects kidney and eye development, the kidneys are small and underdeveloped (hypoplastic), which can lead to end-stage renal disease (ESRD)
17q12 deletion syndrome	Severe malformation of kidney and urinary system, development of maturity-onset diabetes mellitus of young (MODY), intelligence instability (autism spectrum disorder, schizophrenia, bipolar disorder)
Renal cysts and diabetes (RCAD) syndrome	Maturity-onset diabetes mellitus of young (MODY); characterized by heterogeneous cystic renal disease and early-onset familial non-autoimmune diabetes mellitus
Fraser syndrome	Fused eyelids (cryptophthalmos), fusion of the skin between the fingers and toes (syndactyly), and abnormalities of genitalia and urinary tract
Townes-Brocks syndrome	Imperforate anus, abnormally shaped ears, and hand malformations that most often affect the thumb, and developmental anomalies of urinary system
Branchiootorenal syndrome (also called Melnick-Fraser syndrome)	Autosomal dominant disorder involving the kidneys, ears, and neck

coloboma syndrome, Fraser syndrome, and 17q12 deletion syndrome can cause urinary tract abnormalities.

- PAX2 genes encode proteins, which play important role in the embryogenesis of urinary tract. The genes most commonly involved in the pathogenesis of isolated cases of CAKUT include PAX2 associated with renal coloboma syndrome, HNF1B associated with 17q12 deletion syndrome, RCAD syndrome. Mutations in other genes are involved in the pathogenesis of isolated CAKUT. About 10–20% of CAKUT occur in families.
- In addition, environmental factors may play important role in the pathogenesis of isolated CAKUT.
- Risk for CAKUT is increased in those babies, whose mothers are suffering from diabetes mellitus, taking anti-epileptic drugs and diet deficient in vitamins and minerals such as folate and iron during pregnancy.
- Inherited CAKUT most commonly follows autosomal dominant pattern, which means one copy of altered gene in each cell is sufficient to cause CAKUT.
- In autosomal recessive pattern of CAKUT, both copies of the gene are mutated in each cell. Inheritance pattern is unknown in many cases of CAKUT. Congenital anomalies and malformations of the kidneys and urinary tract are given in Table 22.65.

Table 22.65 Congenital anomalies and malformations of the kidneys and urinary tract

Renal agenesis unilateral or bilateral (Potter disease)
Renal dysplasia
Renal hypoplasia
Renal fusion, ectopia, and duplication
Ureteropelvic junction obstruction
Vesicoureteral junction obstruction and vesicoureteral reflux
Ureteral duplication and ectopia
Ureterocele
Urethral atresia and posterior urethral valves
Urinary bladder exstrophy, persistent cloaca and rectovesical fistula

ECTOPIC KIDNEY

An ectopic kidney is a birth defect in which kidney does not reach the lumbar region and remains located in an abnormal position in pelvis or presacral region one in every 1,000 births.

- During fetal development, the baby's kidneys first appear as buds near the urinary bladder in the pelvic region.
- The fetal kidneys gradually move upward during fetal development. The fetal kidneys initially located in the lower abdomen move upward toward their permanent normal position in lumbar region.
- Sometimes, one of the kidneys fails to climb upwards and remains located in the pelvis or presacral region. Rarely, both the kidneys may remain in the pelvic region.
- Sometimes, ectopic kidney is observed as an incidental finding. Some patients may present with urinary problems.
- Vesicoureteral reflux (VUR) may cause urinary tract infection, urolithiasis and renal failure. Ectopic kidney can be detected by ultrasonography, nuclear scan, CT scan and MRI scan.

HORSESHOE KIDNEY

Fusion of both kidneys at their lower poles results in so-called horseshoe kidney in 90% of cases. It may

cause urinary tract obstruction because of impingement on the ureters.

- Predisposing factors for horseshoe kidney include abnormal migration of nephrogenic cells across the primitive streak, alterations in the intrauterine environment caused by intake of teratogenic drugs such as thalidomide and alcohol consumption during pregnancy. Horseshoe kidneys are often asymptomatic, and so are often identified incidentally.
- The kidneys are normally located in the retroperitoneal region between the transverse processes of T12 and L3. The left kidney is slightly higher than right kidney. The upper poles of the kidneys are normally positioned slightly medially and posteriorly relative to the lower poles. Horseshoe kidneys are different in three main ways: location, orientation, and vasculature.
- The horseshoe kidneys are located at L3 vertebra in the abdomen and pelvis, and exhibit great variation in the origin and number of renal arteries and veins.
- Normally, the renal ascent is coupled with a 90° medial rotation during six to eight weeks of development.
- Due to the isthmus, horseshoe kidneys experience malrotation, and consequently, the ureters need to either pass over the isthmus or down the anterior surface of the kidneys resulting in urinary drainage problems and stasis. Horseshoe kidney is detected by ultrasonography, CT scan and MRI.

URETER ANOMALIES

Ureter anomalies include congenital diverticulum, double ureters, ectopic ureter, hydroureter, megaureter, ureteral agenesis and ureteropelvic junction obstruction. Congenital diverticulum (all layers of ureter wall) occurs due to aberrant development of ureteric bud; and lined by urothelium, sometimes squamous metaplasia or urethritis glandularis. Patient may develop recurrent urinary tract infections.

- Double ureters are relatively incidental findings associated with duplication of renal pelvis in 2–3% of all persons. Ectopic ureter is more common in women than men, that may terminate into structures such as proximal urethra, seminal vesicle/ejaculatory duct, vas deferens, fallopian tube, uterus or vagina. Hydroureter may have neurogenic cause. Megaureter is often divided into obstructive type with smooth muscle defect and refluxing type with defective interstitial cell of Cajal-like cells.
- Ureteral agenesis occurs due to failure to develop mesonephric diverticulum, and is associated with failure of ipsilateral kidney to develop. Ureteropelvic junction obstruction occurs due to abnormal organization of smooth muscle bundles, which is the most common cause of hydronephrosis in children. Ureteropelvic junction obstruction is more common in males and on left side and bilateral in 20% of cases, which may be associated with agenesis of contralateral kidney.

NEOPLASTIC DISORDERS OF KIDNEY

Renal neoplasms are classified into two categories: primary (benign or malignant) and secondary tumors. Revised 2024 WHO classification of kidney neoplasms is given in Table 22.66. Common malignant neoplasms of the urinary tract are shown in Fig. 22.96. Classification of renal cell carcinomas has become challenging. According to the 2024 WHO classification, nomenclature of renal cell carcinomas is based on cytoplasmic appearance, architecture, combination of morphologic features, anatomic location, underlying disease, familial syndromes and specific genetic alterations. Panel of immunohistochemical markers applied in the renal neoplasms is given in Table 22.67.

Pathology Pearls: Metastases (Secondary Tumors) Involving Kidneys

- Metastatic (secondary) tumors to kidney include breast carcinoma, bronchus carcinoma, malignant melanoma and lymphomas.
- Metastases to kidneys are multiple involving both kidneys.
- Metastases exhibit central indentations, or umbilications, from necrosis.
- Clinical features in a patient with renal angiomyolipoma (hamartoma) initially are similar to renal cell carcinoma.

NEPHROBLASTIC AND CYSTIC TUMORS

According to 2024 WHO classification of renal tumors, nephroblastic and cystic tumors include nephrogenic rests, nephroblastoma (Wilms' tumor), cystic partially nephroblastoma and pediatric cystic nephroma.

WILMS' TUMOR (NEPHROBLASTOMA)

Wilms' tumor (nephroblastoma) is the most common pediatric solid malignant renal tumor, which can present a single nodule, as multifocal unilateral tumor or bilateral tumors. The tumor is often huge resulting

Table 22.66 Revised 2024 WHO classification of renal neoplasms

Renal Cell Neoplasms

- Clear cell renal neoplasms
  - Renal cell carcinoma clear cell variant
  - Multilocular cystic renal neoplasm of low malignant potential
- Papillary renal neoplasms
  - Papillary adenoma
  - Renal cell carcinoma papillary variant
- Oncocytic and chromophobe renal neoplasms
  - Renal oncocytoma
  - Renal cell carcinoma chromophobe variant
  - Other oncocytic neoplasms of kidney
- Collecting duct neoplasms
  - Collecting duct carcinoma
- Other renal tumors
  - Renal cell carcinoma papillary clear cell variant
  - Mucinous tubular and spindle cell carcinoma
  - Tubulocystic renal cell carcinoma
  - Acquired cystic disease-associated renal cell carcinoma
  - Eosinophilic solid and cystic renal cell carcinoma
  - Renal cell carcinoma, NOS
- Molecularly defined renal carcinomas
  - TFE_3-rearranged renal cell carcinoma
  - TFEB-altered renal cell carcinoma
  - ELOC (formerly TCEB1)-mutated renal cell carcinoma
  - Fumarate hydratase-deficient renal cell carcinoma
  - Hereditary leiomyomatosis and renal cell carcinoma
  - Syndrome-associated renal cell carcinoma
  - Succinate dehydrogenase-deficient renal cell carcinoma
  - ALK-rearranged renal cell carcinoma
  - Renal medullary carcinoma, NOS
  - $SMARCB_1$-deficient medullary-like renal cell carcinoma
  - $SMARCB_1$-deficient undifferentiated renal cell carcinoma, NOS
  - $SMARCB_1$-deficient dedifferentiated renal cell carcinoma

Contd...

Metanephric Tumors

- Metanephric adenoma
- Metanephric adenofibroma
- Metanephric stromal tumor

Mixed Epithelial and Stromal Neoplasms

- Mixed epithelial and stromal tumor
- Adult cystic nephroma
- Pediatric cystic nephroma

Renal Mesenchymal Neoplasms

- Adult renal mesenchymal renal neoplasms
- Angiomyolipoma
- Oncocytic angiomyolipoma with epithelial cysts
- Angiomyolipoma, epithelioid
- Hemangioblastoma
- Juxtaglomerular neoplasm
- Functioning juxtaglomerular cell neoplasm
- Renal medullary interstitial cell neoplasm
- Pediatric renal mesenchymal renal neoplasms
- Ossifying renal tumor of infancy
- Mesoblastic nephroma
- Classic congenital mesoblastic nephroma
- Cellular congenital mesoblastic nephroma
- Mixed congenital mesoblastic nephroma
- Malignant rhabdoid neoplasm of the kidney
- Clear cell sarcoma of kidney

Embryonal Neophroblastic Neoplasms of the Kidney

- Nephrogenic rests
- Perilobar nephrogenic rests
- Nephroblastomatosis
- Cystic partially differentiated nephroblastoma
- Nephroblastoma

Miscellaneous Germ cell Neoplasms

- Peripubertal-type teratoma
- Teratoma with carcinoid (neuroblastoma neoplasm)
- Renal hematopoietic neoplasms
- Yolk sac tumor, NOS
- Mixed teratoma yolk sac tumor

in abdominal distension. Patient usually presents with abdominal mass without symptoms or with abdominal pain, hematuria.

- Wilms' tumor is derived from embryonal mesonephric mesoderm. The tumor is composed of elements that resemble normal fetal tissue: (a) metanephric blastema component (abortive glomeruli, tubules), (b) immature mesenchymal stroma, and (c) immature epithelial elements. It occurs between 1–5 years of age and before 9 years of age in 90% of cases and rarely occurs after age of 9 years.
- In most instances, Wilms' tumor is sporadic (90%) and unilateral. However, in 5% of cases, tumor arises in the setting of several congenital syndromes in 10% of cases. It is diagnosed prior to six years of age in 90% of cases. Males and females are equally affected.
- WT1 tumor suppressor gene mutation is implicated in the development of Wilms' tumor. Molecular genetic signatures of Wilms' tumor, such as TP53 gene mutation and MYCN gene dysregulation may provide future additional prognostic and therapeutic information.
- Approximately 90% of Wilms' tumors have favorable histology. About 3–7% of tumors have anaplastic histology. Tumor resembles the fetal nephrogenic zone and attempts to form primitive glomeruli and tubules.
- The classic histologic pattern of Wilms' tumor is triphasic and composed of epithelial, blastemal,

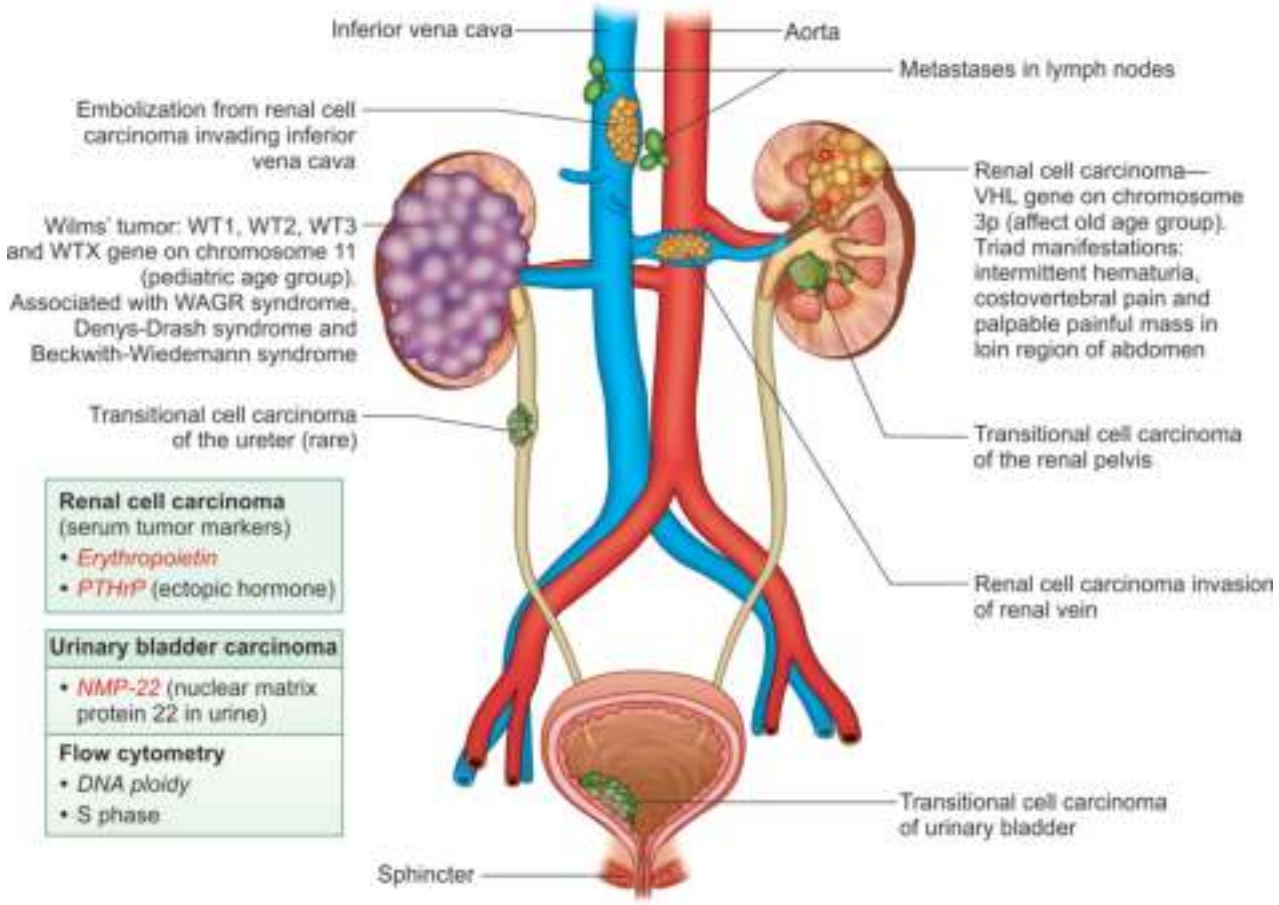


Fig. 22.96: Schematic representation of common malignant neoplasms of the upper and lower urinary tract.

Table 22.67 Panel of immunohistochemical markers applied in the renal neoplasms

Markers	Expression
Renal oncocytoma	
β-Defensin 1	Positive (100%)
Parvalbumin	Positive (100%)
Vimentin	Positive (100% to focal)
CD117 (proto-oncogene c-KIT is the gene encoding the receptor tyrosine-protein kinase, known as tyrosine-protein kinase, KIT, CD117)	Positive
CD7	Negative
GST-α	Negative
EpCAM	Negative
Clear cell renal cell carcinoma	
PAX2	Positive (best marker)
CAIX (carbonic anhydrase)	Positive (membranous diffuse staining), most sensitive and specific marker in inherited (100%) and sporadic cases (75%)
PAX8	Positive
RCC Ma	Positive
CD10	Positive
Vimentin	Positive
CK8	Positive
CK18	Positive
EMA	Positive
AE1/AE3	Positive
CAM5.2	Positive
α_1-Antitrypsin	Positive
S-100 protein	Positive
KS-cadherin	Negative/positive
CD117 (proto-oncogene c-KIT is the gene encoding the receptor tyrosine-protein kinase, KIT, CD117)	Negative/positive

Contd...

Contd...

Table 22.67 Panel of immunohistochemical markers applied in the renal neoplasms (*Contd...*)

Markers	Expression
Papillary (chromophil) renal cell carcinoma	
CK7	Positive
Recemase	Positive
PAX8	Positive
PAX2	Positive
AMACR	Positive
AE1/AE3 (low molecular cytokeratin)	Positive
EMA	Positive
MUC1	Positive
RCC Ma	Positive
CD10	Positive
CD117 (proto-oncogene c-KIT is the gene encoding the receptor tyrosine-protein kinase, KIT, CD117)	Positive
Vimentin	Positive/negative (variable staining)
CAIX (carbonic anhydrase)	Negative (except in areas of necrosis)
Chromophobe renal cell carcinoma	
Hale's colloidal iron (HCI)	Positive (blue cytoplasmic staining)
EpCAM (epithelial cell adhesion molecule)	Positive (new marker)
CD117 (proto-oncogene c-KIT is the gene encoding the receptor tyrosine-protein kinase, KIT, CD117)	Positive
TFE-3	Positive
Cathepsin K	Positive
PAX8	Positive
EMA	Positive
CK8/CK18	Positive
E-cadherin	Positive
Claudin 7	Positive
Ki-67	Positive (low expression)
N-cadherin	Negative
Vimentin	Negative
Renal cell carcinoma with Xp11.2 translocation and TFE3 fusions	
TFE-3 transcription factor	Positive (nuclear staining)
TFE-B transcription factor	Positive (nuclear staining)
CD10	Positive
AMACR	Positive
Vimentin	Positive
E-cadherin	Positive (66%)
CK19	Positive (30–50%)
EMA	Positive (30%)
Cathepsin K	Positive
Collecting duct carcinoma of Bellini	
PAX8	Positive
PAX2	Positive
Ulex europaeus lectins (Ulex-1)	Positive
Peanut agglutinin (PNA)	Positive
Mucin	Positive
Vimentin	Positive
CK8/18	Positive
CK19	Positive
EMA	Positive
S-100A1	Positive
Urothelial carcinoma of renal pelvis	
CK7	Positive (93%)
p63	Positive (92%)
CK903	Positive (92%)
CK20	Positive (70%)
Renal clear cell sarcoma	
Vimentin	Positive (hallmark is absence of any differentiation)
Wilms' tumor (nephroblastoma)	
Blastema component	
▪ WT1 ▪ Desmin	▪ Positive ▪ Positive
Epithelial component	
▪ WT1 ▪ Cytokeratin ▪ EMA ▪ CD57	▪ Positive ▪ Positive ▪ Positive ▪ Positive
Stromal component	
▪ Myogenin ▪ S-100 protein	▪ Positive (rhabdomyoblastic component) ▪ Positive (neural component)
Renal angiomyolipoma	
HMB-45	Positive
Melan A	Positive
Actin	Positive
Vimentin	Positive
TFE3 (transcription factor E3)	Negative/positive
Cathepsin K	Negative/positive

Contd...

and stromal elements. The proportion and degree of maturation of these components in tumor are observed in varying proportion in hematoxylin and eosin-stained renal tissue section.

- Biphasic and monophasic Wilms tumors are not infrequent. Epithelial and stromal elements may demonstrate different lines and degrees of differentiation, i.e. from poor to well differentiated.

Sporadic Wilms' Tumor

WT1 gene mutation has been demonstrated in stromal component of Wilms' tumor in some sporadic cases. It suggests that the stromal component of Wilms' tumor is neoplastic, which raises the possibility that undifferentiated blastema cells are precursors of the stromal and heterologous elements. It has been suggested that in addition to WT1; WT2 gene mutation plays an important role in the genesis of sporadic cases of Wilms' tumors.

Wilms' Tumor in Familial Syndromes

WT1 tumor suppressor gene is located on short arm of chromosome 11 (11p13). Wilms' tumor is caused by deletion or inactivation of WT1 gene. WT1 gene mutation also affects contiguous genes and causes other lesions in 10% of familial cases. Patient with familial syndromes may develop Wilms' tumor.

- **WAGR syndrome:** WAGR syndrome is characterized by Wilms' tumor (WT1 gene mutation), aniridia (absence of the iris) due to mutation of contiguous aniridia gene (PAX6 gene located on chromosome 11), genitourinary malformations and mental retardation.
- **Denys-Drash syndrome:** Denys-Drash syndrome (DDS) is characterized by Wilms' tumor (WT1 gene mutation), genitourinary malformations, intersexual disorders, tumors of the gonads (ovaries or testes) and glomerulopathy (mesangial sclerosis) resulting in chronic renal failure.
- **Beckwith-Wiedemann syndrome:** Beckwith-Wiedemann syndrome (BWS) is characterized by Wilms' tumor, unusual 'hemihypertrophy' (ranging from gigantism to asymmetric growth), tumors of the liver and adrenal glands, hepatosplenomegaly, macroglossia, malformations around the ear; and abdominal wall defects near the navel (omphalocele). This is thought to be caused by an overactive copy of an IGF_2 oncogene on chromosome 11, if becomes overactive; then it results in uncontrolled cell growth.

Clinical Features

Patient presents with large abdominal unilateral nontender mass, hematuria, hypertension (due to increased renin secretion), and pain abdomen due to pressure exerted by tumor on surrounding structures. Constitutional symptoms are anorexia, loss of appetite, generalized weakness and weight loss. Patient may present with traumatic rupture or lung metastases.

Mode of Spread

Wilms' tumor spreads into the perirenal soft tissue. Tumor may metastasize to regional lymph nodes via lymphatic route. Wilms' tumor may invade renal vein and spreads via hematogenous route to various organs in decreasing frequency such as lung (most common), liver, brain and peritoneum.

- Tumor rarely metastasizes to bones in contrast to neuroblastoma, where bone is the principal site.
- Tumor may metastasize to opposite kidneys (5–10%), adrenal glands, bowel, vertebrae and paraspinal region.
- Histologic examination establishes the diagnosis of Wilms' tumor. Fine needle aspiration cytology and frozen sections are unreliable.
- About 80–90% of patients are cured. However, small percentage of children develop second neoplasms.

Surgical Pathology: Wilms' Tumor (Nephroblastoma)

Gross Morphology

- The kidney is extensively replaced by large well-circumscribed bulging fleshy, pale white tumor demarcated from renal parenchyma.
- Cut surface reveals gray-white or pink-gray, lobulated, homogenous soft tumor enclosed in a rim of renal cortex and capsule. Cysts and lobular pattern are common. In pretreated cases, hemorrhage and necrosis are quite extensive. It is important that gross sampling must include adequate tumor from main tumor mass, renal sinus and its blood vessels and ureter.
- About 10% of tumors are bilateral or multicentric. Adequate sampling of tumor is essential. It should include at least one block per centimeter of largest dimension.
- Sections are required from renal pelvis, renal sinus, tissues surrounding renal artery and vein and the junction between normal kidney and tumor, tumor capsule and uninvolved kidney. Tissue is taken for molecular studies, Gross morphology of nephroblastoma (Wilms' tumor) is shown in Fig. 22.97.

Light Microscopy

Wilms' tumor resembles the fetal nephrogenic zone and attempts to form primitive glomerular and tubular structures. Tumor is composed of triphasic components. Presence of anaplastic features is associated with poor prognosis. Histology of nephroblastoma (Wilms' tumor) is shown in Figs 22.98 and 22.99.

- **Metanephric blastema:** It is composed of poorly developed abortive glomeruli and tubules with elongated /ovoid nuclei having molded/wedged shapes. Undifferentiated blastema is composed of primitive small blue cells with scanty cytoplasm, overlapping nuclei with finely dispersed chromatin arranged in diffuse, nodular, cord-like or basaloid pattern.

- **Immature mesenchymal tissue:** It is composed of striated muscle together with myxoid tissue, cartilage, bone and fat and thus creating a rather bizarre mixture.
- **Immature epithelial tissue:** It consists of immature squamous or mucinous epithelial tissue.

Electron Microscopy

Wilms' tumor resembles developing metanephros with well-developed cell junctions, microvilli and layer of thick flocculent coating around cell surface.

Immunohistochemistry Technique

Panel of markers is applied to formalin-fixed paraffin-embedded sections to demonstrate various components of Wilms' tumor such as blastema (WT1, desmin), epithelial component (WT1, cytokeratin, EMA) and stromal component (myogenin for rhabdomyoblastic component; S-100 protein for neural tissue).

Markers	Expression
Blastema component	
▪ WT1 ▪ Desmin	▪ Positive ▪ Positive
Epithelial component	
▪ WT1 ▪ Cytokeratin ▪ EMA (epithelial membrane antigen) ▪ CD57	▪ Positive ▪ Positive ▪ Positive ▪ Positive
Stromal component	
▪ Myogenin ▪ S-100 protein	▪ Positive (rhabdomyoblast component) ▪ Positive (neural component)

Molecular/Genetic Alterations

WT1 tumor suppressor gene is located on short arm of chromosome 11 (11p13). Wilms' tumor is caused by deletion or inactivation of WT1 gene. WT1 gene mutation also affects contiguous genes and causes other lesions in familial cases. Molecular genetic alterations in Wilms' tumor are given in Table 22.68.

Staging of Wilms' Tumor

A staging system is a standard way for the cancer care team to sum up the extent of the tumor. Staging system is used most often to describe the extent of spread of Wilms' tumors. This system divides Wilms' tumors into five stages using Roman numerals I through V. Staging of Wilms' tumor is given in Table 22.69.

Poor Prognostic Factors

Large size Wilms' tumor in children over two years of age is associated with poor prognosis. Diffuse anaplasia in stage II, III or IV tumors is also associated with poor outcome. Even small foci of anaplasia in the Wilms' tumor are associated with poor prognosis due to chemotherapy resistance. Presence of predominant epithelial component is demonstrated in stage I. Most blastema predominant tumors are stage III/IV.

Sporadic and inherited Wilms' tumor

Sporadic cases • Wilms' tumor 1 (WT1) gene mutation (85%) • WT1 gene mutation on 11p13 (15%)	**WAGR syndrome** • Wilms' tumor 1 (WT1) gene mutation (33%) • Aniridia and genital anomalies (100%)
Denys-Drash syndrome • Wilms' tumor 1 (WT1) gene mutation • Congenital nephropathy • Inter-sex disorders	**Beckwith-Wiedemann syndrome** • Wilms' tumor 2 (WT2) gene mutation on 11p15.5 • Hemihypertrophy • Increased risk of other tumor



Fig. 22.97: Gross morphology of nephroblastoma (Wilms' tumor). Patient usually presents with abdominal mass without symptoms or with abdominal pain, hematuria, hypertension and anemia. The kidney is extensively replaced by large solitary, spherical, bulging, fleshy, and pale white tumor demarcated from renal parenchyma. Cut surface reveals gray-white or pink-gray, lobulated soft tumor enclosed in a rim of renal cortex and capsule. In pretreated cases, hemorrhage and necrosis are quite extensive. It is important that gross sampling must include adequate sample tumor from main tumor mass, renal sinus and its vessels and ureter. (*Courtesy:* Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

Laboratory Diagnosis

Wilms' tumor is diagnosed by imaging techniques including abdominal ultrasonography, CT scan, and magnetic resonance imaging. Chest X-ray is done to rule out lung metastases. Kidney and liver function tests, FNAC and histologic examination are diagnostic tools.

RENAL ONCOCYTOMA

Renal oncocytoma is a benign epithelial tumor composed of uniform, round to polygonal cells with abundant, intensely mitochondria-rich eosinophilic

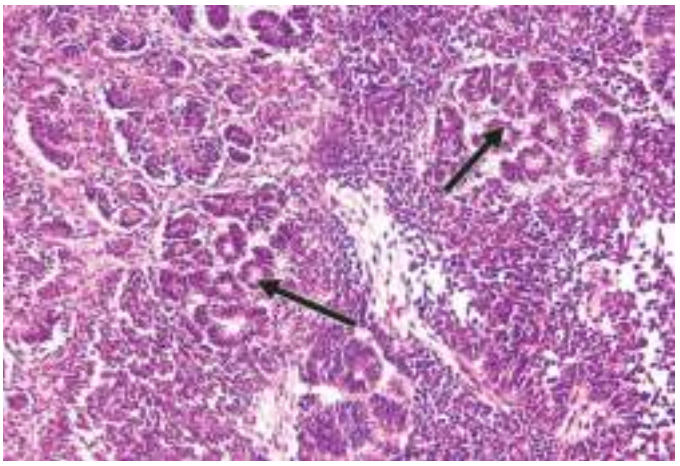


Fig. 22.98: Histology of nephroblastoma (Wilms' tumor). Nephroblastoma resembles the fetal nephrogenic zone and attempts to form primitive glomerular and tubular structures. Triphasic tumor consists of blastemal, epithelial and stromal components, which can be present in variable amount. Biphasic and monophasic tumors are not infrequent. Epithelial and stromal elements may demonstrate different lines and degrees of differentiation, i.e. from poor to well differentiated (arrows) (400X).

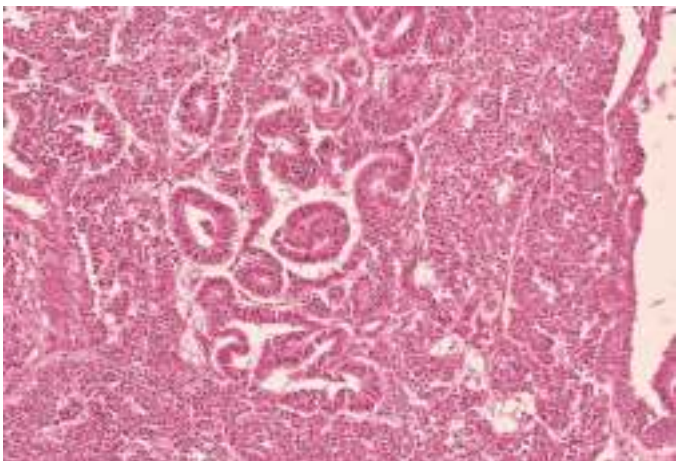


Fig. 22.99: Histology of nephroblastoma (Wilms' tumor). Wilms' tumor is most common pediatric renal tumor, which can present a single nodule, as multifocal unilateral lesions or a bilateral tumor. Approximately 90% of Wilms' tumors have favorable histology. About 3–7% of tumors have anaplastic histology. Molecular genetic signatures of Wilms' tumor, such as TP53 mutation and MYCN dysregulation may provide future additional prognostic and therapeutic information. The classic histologic pattern of Wilms' tumor is triphasic and composed of epithelial, blastemal, and stromal elements. The proportion and the degree of maturation of these components are present in varying proportion in hematoxylin and eosin-stained renal tissue section (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

Table 22.68 Molecular genetic alterations in Wilms' tumor

Cytogenetics of Wilms' tumor are deletion or mutations involving WT1 (11p13), WT2 (11p15), or WTX (Xp11.1)
Implicated genes are tumor suppressor genes such as WT1, WT2, WT3, WTX
Normal WT1, WT2, WT3, WTX encode zinc-finger transcription factor (DNA-binding tumor suppressor protein) expressed early in urogenital system development
Related assay(s) include IPOX for WT1
p53 abnormalities are demonstrated in anaplastic foci of Wilms' tumor
FWT1 (17q12–121) and FWT2 (19q13) mutations have been demonstrated in familial Wilms' tumor
Absence of t(12;15) of cellular mesoblastic nephroma
Absence of t(11;22) of Ewing's/PNET
Absence of 22q11.2 abnormalities of rhabdoid tumor
Absence of N-Myc of neuroblastoma

Table 22.69 Staging of Wilms' tumor

Stage	Wilms's Tumor involving Structures
Stage I	Tumor limited to the kidney with intact capsule and completely resected. There is no rupture of blood vessels
Stage II	Tumor has invaded renal capsule involving surrounding structures
Stage III	Tumor has spread beyond the kidney area involving abdominal or pelvic lymph nodes. Resection of tumor is not possible
Stage IV	The tumor has spread to distant structures such as the lungs, liver or brain. Metastases of lymph nodes outside abdomen and pelvis
Stage V	Bilateral renal involvement at diagnosis

and granular cytoplasm with uniform small, round and central nuclei with evenly dispersed chromatin. It is thought to arise from intercalated cells of collecting duct in the kidney. It accounts for 4–7% of adult renal epithelial tumors. It has wide range of age distribution in adult men above 50 years of age with a peak incidence in the seventh decade of life.

- About 66% of cases are detected as incidental finding on imaging techniques. It may coexist with renal cell carcinoma. Rarely, it is associated with chronic renal failure due to multiple tumors or large bilateral tumor. Patient may have renal oncocytoma as a component of Birt-Hogg-Dubé syndrome. Patient with this syndrome is at higher risk for renal cell carcinoma, lung cysts and spontaneous pneumothorax.
- Most patients are asymptomatic, however, some patients present with classic triad of hematuria, flank pain and flank mass as observed in cases of renal cell carcinoma. Imaging studies demonstrate solid homogenous mass in renal parenchyma and a central irregular scar, although not specific and not always present. Renal oncocytoma is treated by radical nephrectomy and associated with excellent prognosis. Comparison of gross and histologic features of some benign renal tumors is given in Table 22.70.

Table 22.70 Comparison of gross and histologic features of some benign renal tumors

Renal Papillary Cortical Adenoma	Renal Oncocytoma	Metanephric Adenoma
Gross morphology		
▪ Size <2 cm in subcapsular region ▪ Cut surface is pale yellow to gray	Well-circumscribed tumor arising from proximal and distal tubules in poles. Cut surface has a uniform mahogany, brown color due to mitochondrial lipochrome pigments	Small solid lesion in the renal cortex
Light microscopy		
Tumor is composed of small uniform cells with clear to eosinophilic cytoplasm forming branching papillary fronds	Tumor is composed of large epithelial cells called oncocytes, which have abundant eosinophilic homogeneous or granular cytoplasm (mitochondria) and uniform small nuclei	Tumor is composed of small round cells with bland nuclei and scanty cytoplasm. These are arranged in tubules and papillae with scant stroma. Areas of calcification may be seen

Pathology Pearls: Renal Oncocytoma

- Renal oncocytoma is a benign neoplasm. It is believed to arise from renal duct intercalated cells and are packed with defective mitochondria under electron microscopy.
- Tumor is well circumscribed mass renal cortex replacing renal parenchyma. Cut surface is mahogany brown to tan or yellow.
- Hemorrhage may be present but lacking necrosis. Large tumor can extend into and expand the perinephric fat, renal sinus even renal vein.
- Tumor is composed of large round eosinophilic cells (oncocyte) with dense granular cytoplasm with round irregular nuclei containing even chromatin and conspicuous nuclei arranged in small solid sheets in a loose connective tissue. Mitotic figures are rare or absent.
- Other architectures in renal oncocytoma include tubular, microcystic and macrocytic, packed nests projecting a solid appearance.

Surgical Pathology: Renal Oncocytoma

Gross Morphology

- Renal oncocytoma is well-circumscribed and unencapsulated in renal cortex measuring 3–6 cm. Central satellite scar in large tumor may be present in 30% of cases.
- Cut surface is solid, homogenous and mahogany or dark brownish yellow. Hemorrhage may be present in 20% of renal oncocytoma, but necrosis is rarely demonstrated.
- Bilateral and multiple renal oncocytomas in the kidney can be demonstrated invading renal capsule and renal vein in 5% of cases. Gross morphology of renal oncocytoma is shown in Fig. 22.100.

Light Microscopy

- Renal oncocytoma is composed of uniform round-to-polygonal cells with abundant, intensely eosinophilic owing to cytoplasmic mitochondria and granular cytoplasm, with small uniform, round and central nuclei with evenly dispersed chromatin and smooth contour of nuclear membrane.
- Tumor cells are arranged in nests, alveolar or tubular patterns. Stroma is myxoid or hyalinized in the central scar.
- Renal oncocytoma frequently demonstrate oncoblasts, and small tumor cells with minimal cytoplasm dense hyperchromatic nuclei. Tumor may show extensive papillary structure in occasional case. Tumor extension to perinephric fat without stromal response can occur in 20% of cases.
- Occasional renal oncocytoma tumor may demonstrate psammoma bodies, cystic change, invasion of capsule, perinephric fat and renal vein in 5% of cases. Renal cell carcinoma should always be ruled out first. Oncocytosis refers to numerous oncocytic lesions involving the kidney. Histology of renal oncocytoma is shown in Fig. 22.101.

Histochemistry

Hale's colloidal iron is showing luminal staining.

Immunohistochemistry Technique

Panel of markers applied on formalin-fixed paraffin-embedded sections of renal oncocytoma shows positivity for β-defensin 1, parvalbumin, CD117, vimentin.

Markers	Expression
▪ β-Defensin 1	▪ Positive (100%)
▪ Parvalbumin	▪ Positive (100%)
▪ CD117 (c-KIT)	▪ Positive
▪ Vimentin	▪ Negative (majority)/focal positivity in tumor cells within septa
▪ CK7	▪ Negative
▪ GST-α	▪ Negative
▪ EpCAM	▪ Negative

RENAL CELL CARCINOMA

Renal cell carcinoma (RCC) is most common cancer in men and women with male to female ratio 1:2 above 50 years of age.

- Risk factors for renal cell carcinoma include obesity, tobacco smoking, hypertension, acquired cystic kidney disease due to end stage renal disease, occupational exposure to trichloroethylene, and treated cases of neuroblastoma.
- Genetic susceptibilities estimated to account for 2–4%. Renal cell carcinoma may be sporadic or familial.



Fig. 22.100: Gross morphology of renal oncocytoma. Renal oncocytoma is a benign neoplasm. It is believed to arise from renal intercalated cells and are packed with defective mitochondria under electron microscopy. Tumor is well circumscribed mass in renal cortex replacing renal parenchyma. Cut surface is mahogany brown to tan or yellow. Areas of hemorrhage may be present but lacking necrosis. Large tumor can extend into and expand the perinephric fat, renal sinus even renal vein. (*Courtesy:* Dr. Neha Kumari.)

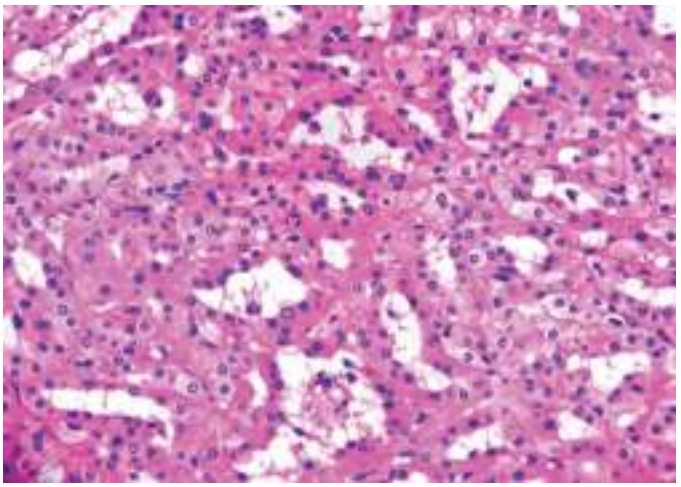


Fig. 22.101: Histology of renal oncocytoma. Renal oncocytoma is a benign neoplasm. It is believed to arise from renal intercalated cells and are packed with defective mitochondria under electron microscopy. Tumor is well circumscribed mass renal cortex replacing renal parenchyma. In hematoxylin and eosin-stained renal tumor tissue section, tumor is composed of large round eosinophilic cells (oncocyte) with dense granular cytoplasm with round irregular nuclei containing even chromatin and conspicuous nuclei arranged in small solid sheets in a loose connective tissue. Mitotic figures are rare or absent. Other architectures include tubular, microcystic and macrocytic, packed nests projecting a solid appearance (400X). (*Courtesy:* Dr. Neha Kumari.)

Pathology Pearls: Genetic Disorders Associated with Renal Cell Carcinoma

von Hippel-Lindau Disease

- von Hippel-Lindau (vHL) disease is an autosomal dominant disorder due to germline mutation of vHL gene mapped on 3p25. vHL is characterized by renal cysts and clear cell renal cell carcinoma. vHL is associated with bilateral and multifocal clear cell renal cell carcinomas.
- Other tumors in vHL disease include hemangioblastomas of cerebellum and retina, cysts of pancreas, liver, kidney, clear cell tumors at other sites, papillary cystadenoma of epididymis and pheochromocytoma.

Birt-Hogg-Dubé Syndrome

- Birt-Hogg-Dubé (BHD) syndrome is an autosomal dominant with incomplete penetration due to germline mutations in BHD gene mapped on chromosome 17p11.2, which codes for folliculin protein. This syndrome is characterized by renal oncocytoma, clear cell renal cell carcinoma and hybrid renal oncocytic/chromophobe renal cell carcinoma with areas of clear cells.
- Other lesions include lung cysts, spontaneous pneumothorax and skin lesions such as fibrofolliculomas, trichodiscomas, acrochordons.
- Renal tumors are detected mostly by imaging studies; and occasionally, patients present with flank pain, hematuria and palpable mass. It is essential for close follow-up of the renal tumors and minimal surgery with local resection of tumors to conserve renal function.
- Multiple tumor resections may be necessary in these patients. Hybrid renal oncocytoma tumor cells are positive for CD117 (c-KIT) and CK7 (patchy staining).

Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC) Syndrome

- Hereditary leiomyomatosis and renal cell carcinoma syndrome is an autosomal dominant disorder due to germline mutations of fumarate hydratase tumor suppressor gene mapped on 1q42.3–43 and contains 10 exons that encode fumarate hydratase protein, which is involved in tricarboxylic acid cycle. The fumarate hydratase gene biallelic inactivation is present in nearly all uterine leiomyomas and papillary (chromophil) renal cell carcinoma.
- Patient develops cutaneous or uterine leiomyomas and papillary (chromophil) renal cell carcinoma with characteristic large nucleus with a very prominent inclusion-like eosinophilic nucleolus.
- Cutaneous leiomyoma rarely progresses to leiomyosarcoma. Papillary (chromophil) renal cell carcinoma is aggressive neoplasm that grows quickly and metastasize early and the distant metastasis is most often detected at diagnosis.
- Majority of renal tumors are type 2 papillary (chromophil) renal cell carcinomas. Tumor is composed of large cells with abundant eosinophilic cytoplasm and large nucleus with prominent eosinophilic nucleoli with perinuclear halos.

Renal Tumors in Tuberous Sclerosis

Tuberous sclerosis (TSC) is the common genetic neurocutaneous disease due to mutations in TSC1 and TSC2 genes not involving vHL gene mutations and characterized by bilateral multifocal renal angiomyolipomas, clear cell renal cell carcinoma, renal oncocytoma, facial angiofibroma, periungual

fibroma, hypopigmented macules, giant astrocytoma, subependymal nodule, retinal hamartomas, cardiac rhabdomyomas, pulmonary lymphangioleiomyomatosis.

Constitutional Chromosome 3 Translocation Associated Renal Cell Carcinoma

Constitutional chromosome 3 balanced translocation has been linked to development of clear cell renal cell carcinoma, which is indistinguishable from that of sporadic clear cell renal cell carcinoma.

Pathology Pearls: Renal Cell Carcinoma—Histologic Variants

Histologic variants of renal cell carcinoma include clear cell variant, chromophil (papillary) variant, chromophobe variant, collecting duct carcinoma of Bellini in the medulla and Xp11 translocation pediatric renal cell carcinoma.

Clear Cell Renal Cell Carcinoma

- Clear cell renal cell carcinoma is most common variant and constituting 70–80% of cases, which arises from proximal tubular cells in cortex. von Hippel-Lindau (vHL) gene mutation on chromosome 3p has been demonstrated in sporadic and familial cases.
- Tumor is bright golden yellow tumor located in pole of kidney. On histologic examination, tumor is composed of round/polygonal cells with centrally placed round nuclei and abundant clear cytoplasm containing abundant glycogen and lipids; and arranged in solid cords, papillae, tubules or nests or alveolar pattern. Tumor cells express both cytokeratin and vimentin.

Papillary (Chromophil) Renal Cell Carcinoma

- Papillary (chromophil) renal cell carcinoma accounts for 10–15% of cases. It arises from distal tubular cells in cortex. It is never associated with vHL gene mutation located on chromosome 3.
- Trisomy or tetrasomy 7, 16 and 17 are common due to chromosomal gains. Loss of Y chromosome is seen in familial cases.
- PRCC gene on chromosome 1 fuses with TFE 3 gene on chromosome X. Fusion protein dysregulates mitotic checkpoints allowing abnormal segregation of chromosomes. It has worst prognosis. Tumor is composed of fibrovascular core lined by tumor cells arranged in papillary pattern. Psammoma bodies are observed in fibrovascular core.

Chromophobe Renal Cell Carcinoma

- Chromophobe renal cell carcinoma accounts for 5% of cases, which originates from intercalated cells of collecting ducts in cortex.
- Multiple chromosomal losses/hypoploidy occur in this variant. Tumor is composed of polyhedral cells with eosinophilic granular cells with prominent cell borders, eccentric nuclei, perinuclear halo and prominent cell borders resembling vegetable cells.
- Electron microscopy study reveals microvesicles 150–300 nm diameter. Hale's colloidal iron reacts with a substance present in the microvesicles. It has best prognosis.

Collecting Duct Carcinoma of Bellini

Collecting duct carcinoma of Bellini accounts for 2% of cases and arising from collecting ducts in medullary region. Tumor is circumscribed with central areas of necrosis. Reports on losses of chromosomes 1, 6, 14, 15, and 22 have been reported.

Pediatric Renal Cell Carcinoma with Xp11.2 Translocation and TFE3 Fusions

Pediatric renal cell carcinoma with TFE3 fusions occurs in children. Gene fusions are involving the TFE3 transcription factor. Gene is demonstrated on molecular and genetic analysis.

CLEAR CELL RENAL CELL CARCINOMA

Clear cell renal cell carcinoma is the most common histologic subtype of renal cell carcinoma. Essential features are neoplastic cells with clear or eosinophilic cytoplasm, characteristic delicate network of small blood vessels 'chicken wire vasculature', common molecular signature of vHL gene inactivation mapped on chromosome 3p12–3p26 (sporadic and familial cases) and upregulation of hypoxia-inducible factor (HIF).

- Clear cell renal cell carcinoma originates from proximal convoluted tubules in the renal cortex. Tumor accounts for 65–70% of adult renal carcinomas. It most often occurs in men than women, in the age group of 50–70 years.
- Tumor is bright golden yellow tumor located in upper (most common) or lower pole of kidney.
- Histologic examination reveals tumor cells with clear cytoplasm containing abundant glycogen and lipids. Tumor expresses both cytokeratin and vimentin on immunohistochemistry.
- Chromosome 3p alteration is present in almost all the cases. vHL gene alterations (loss of sequences or promoter hypermethylation) are present in 50–70% of sporadic clear cell renal cell carcinoma.

Pathology Pearls: von Hippel-Lindau Disease Associated Clear Cell Renal Cell Carcinoma

von Hippel-Lindau disease is an autosomal dominant disorder due to germline mutation of one allele of tumor suppressor in the von-Hippel-Lindau gene and characterized by a constellation of neoplasms, i.e. retinal hemangioma and cerebellar hemangioblastoma including renal cysts and clear cell renal cell carcinoma. Renal cysts are lined by clear cells. Renal tumors are bilateral and multifocal. Most renal tumors are asymptomatic and are detected in screening imaging studies.

- von Hippel-Lindau is a tumor suppressor gene located on 3p12 to 3p26. The vHL protein regulates hypoxia-inducible factor and hypoxia-induced genes. Loss of one allele of vHL

gene on 3p25–26 by loss of sequences or promoter hypermethylation has been demonstrated in sporadic (98%) and familial (2%) clear cell renal cell carcinomas.

- vHL gene mutation occurs by two mechanisms: (a) loss of sequences occurs on chromosome 3p either by deletion (3p12 to 3p26) or imbalanced translocation, i.e. (3;6, 3;8, 3;11), and (b) hypermethylation of vHL gene leads to inactivation of vHL gene in about 80% cases. When vHL gene is inactivated, the vHL protein complex cannot degrade HIF-1α, a transcription factor, that drives transcription of hypoxia associated genes including VEGF, PDGF and GLUT1.
- von Hippel-Lindau syndrome is an autosomal dominant disorder due to mutation of one allele of vHL tumor suppressor gene associated with tumors arising in multiple organs. Approximately 40% of all patients with vHL disease develop retinal hemangiomas, cerebellar hemangioblastomas and unilateral clear cell renal cell carcinoma.
- Metastatic renal cell carcinoma is the leading cause of mortality in patients with vHL disease. Early detection with nephron-sparing surgery is the basic aim for treatment of clear cell renal cell carcinoma. Patients with renal involvement in vHL disease have a better prognosis than do their sporadic counterparts.

Table 22.71 Risk factors for clear cell renal cell carcinoma

Sporadic Clear Cell Renal Cell Carcinoma
Prolonged tobacco smoking is major risk factor for sporadic clear cell renal cell carcinoma in 33% of cases
Obesity women are risk factor for sporadic clear cell renal cell carcinoma in obese women
Hemodialysis in chronic renal disease over five years induces acquired cystic disease of kidney leading to sporadic clear cell renal cell carcinoma
Loss of one allele from the short arm of chromosome 3 occurs in 90% of sporadic clear cell renal cell carcinoma and not inherited renal cell carcinoma
Inherited Clear Cell Renal Cell Carcinoma
Translocation t(3;8) or t(3;11) with breakpoint at the proximal end of 3p increases risk for inherited clear cell renal cell carcinoma occurs at 60 years in 85% of cases
vHL disease occurs due to mutation in vHL tumor suppressor gene close to oncogene raf-1 (chromosome 3p25). It is associated with inherited clear cell renal cell carcinoma in 28% of cases

Predisposing Factors

Tobacco smoking is most important risk factor for development of renal cell carcinoma in sporadic cases. Other risk factors are adult polycystic disease, patients on dialysis, obesity in women, hypertension, analgesic abuse, von Hippel-Lindau disease, tuberous sclerosis (but not via vHL mutations), occupation exposure to polycyclic hydrocarbons, lead and asbestos. Risk factors for clear cell renal cell carcinoma are given in Table 22.71.

Clinical Features

Clear cell renal cell carcinoma (CCRCC) is detected as incidental finding on imaging in 60–80% of cases. Hematuria is the single most common presenting sign. The tumor most often invades renal veins and can extend up the vena cava reaching right side of heart with early dissemination.

- Metastases via hematogenous route reach to lung, liver, bone, soft tissue, and adrenal glands. Hematuria is the most frequent presentation. Large tumor is palpable on clinical examination. Malignant cells are rarely detected in the urine in renal cell carcinoma.
- Renal oncocytoma and renal angiomyolipoma are often difficult to differentiate from renal cell carcinoma by imaging techniques.
- Patient with 10 cm size tumor presents with clinical triad: (a) grossly visible intermittent hematuria (50–60%), (b) costovertebral pain (45%) and (c) palpable mass with pain in loin region of abdomen (30%).
- Grossly visible painless hematuria should be considered a sign of urinary tract carcinoma until proven otherwise. Constitutional symptoms include fever, malaise, weakness, and weight loss.

Physical Examination

In clear cell renal cell carcinoma, edema in lower legs occurs due to extension of tumor in the inferior vena cava. Sudden onset of left-sided varicocele occurs in old persons due to obstruction of left testicular vein draining directly into left renal vein by the tumor. Metastases of clear cell renal cell carcinoma to pelvis bone and femur result to pathologic fracture. About 5% of patients develop manifestation related to metastases in lung, opposite kidney, adrenal gland, gingival, central nervous system, larynx, bronchi, nasal cavity, thyroid gland, skin and soft tissue.

Paraneoplastic Syndrome

In clear cell renal cell carcinoma tumor synthesizes ectopic hormones in 5% of cases resulting in clinical manifestations: hypertension (renin synthesis), polycythemia (erythropoietin synthesis), hypercalcemia (parathormone-like peptide synthesis) and feminization or masculinization (gonadotropins synthesis), Cushing's syndrome (ACTH synthesis). Patient may also develop hepatic dysfunction, acquired dysfibrinogenemia, and amyloidosis. Peripheral blood smear examination may reveal eosinophilia and leukemoid reactions in 5% of cases. Systemic manifestations of renal cell carcinoma are given in Table 22.72.

Table 22.72 Systemic manifestations of renal cell carcinoma

Substances Synthesis by Renal Cell Carcinoma	Systemic Manifestations
Prostaglandins synthesis	Fever
Parathyroid hormone-related peptide	Hypercalcemia
Erythropoietin synthesis	Increased RBCs count in 4% of cases
Prolactin	Galactorrhea
Renin synthesis	Hypertension
Exact mechanism unknown	Gynecomastia

Amyloidosis occurs in 3% of cases of renal cell carcinoma due to stimulation of serum amyloid (SAA) synthesis by liver.

Surgical Pathology: Clear Cell Renal Cell Carcinoma

Gross Morphology

- Clear cell renal cell carcinoma is unilateral solitary tumor in 90% of cases, bilateral involvement in 1% (usually with von Hippel-Lindau or tuberous sclerosis).
- Tumor can be unifocal (90%) or multifocal (10%). Tumor is well-circumscribed, partially encapsulated spherical renal cortical mass.
- Cut surface of tumor shows variegated appearance (solid and cystic areas), orange/golden yellow mass due to presence of lipids in tumor cells. Hemorrhage, necrosis and fibrosis are present. Soft fleshy areas may herald sarcomatous component.
- Frequent involvement of renal vein and renal sinus is present. Tumor may invade the perinephric fat, renal sinus (cavity within the kidney occupied by renal pelvis, renal calyces, blood vessels, nerves), adrenal gland, surrounding tissues and renal vein. Invasion of renal vein is a common mode of spread.
- During processing of the nephrectomy specimen, examination of renal vein determines metastatic potential of tumor.
- At least three blocks of tumor in renal sinus interface should be submitted. Uninvolved renal parenchyma should be sampled distant from tumor.
- Lymph nodes should be isolated in radical nephrectomy specimens performed in <10% of cases. Gross morphology of clear cell renal cell carcinoma is shown in Figs 22.102 and 22.103.

Light Microscopy

- Clear cell renal cell carcinoma is composed of large round/polygonal cells with distinct membrane, centrally placed round nuclei and abundant clear cytoplasm-rich in glycogen and fat washed during processing of tumor. In some cases, cytoplasm may be granular. Tumor cells form compact solid cords, papillae, tubules or nests or alveolar, microcystic (containing extravasated red blood cells or eosinophils) and occasional macrocytic architectural patterns.
- Tumor cells are arranged in nests and sheets and separated by a network of arborizing small, delicate, thin-walled blood vessels with dilated sinusoidal vascular spaces giving **'chicken wire' appearance**. Other minor patterns of arrangement of tumor cells include trabeculae, papillary, tubular and microcystic.
- The stroma may be reduced, but well vascularized. Some tumors may have pseudopapillary architecture due to high-grade changes and loss of cell cohesion. Cystic changes with serosanguineous fluid or blood are most often present.
- Renal vein margin is positive only when adherent tumor is visible microscopically at the actual margin.
- High-grade clear cell renal cell carcinoma shows rhabdoid or sarcoid differentiation with high-grade spindle cells containing homogenous eosinophilic cytoplasm and eccentric nucleus and globular eosinophilic inclusions. Heterologous elements may be present.
- Clear cell renal cell carcinoma, should be differentiated from chromophil (papillary) renal cell carcinoma, chromophobe renal cell carcinoma, renal oncocytomas, clear cell papillary (chromophil) renal cell carcinoma, and epithelioid angiomyolipoma. Histology of clear cell renal cell carcinoma is shown in Figs 22.104 and 22.105.

Histochemistry

- In clear cell renal cell carcinoma, tumor cells are rich in glycogen (PAS positive) and fat.
- Lipids are dissolved during processing of tissue in paraffin-embedded specimens. Fat can be demonstrated by frozen section technique by applying fat stains (Sudan III, Sudan IV, Sudan black or oil red O). There is absence of mucus in tumor cells.

Electron Microscopy

In clear cell renal cell carcinoma, tumor cells contain abundant glycogen, well defined long microvilli similar to brush border of normal proximal tubules, numerous cell junctions. These also demonstrate variable fat, scant organelles with scant microvesicles.

Immunohistochemistry Technique

- Clear cell renal cell carcinoma expresses both cytokeratins (8, 18) and vimentin. While other types express keratins 7 and 19.
- Other markers used include epithelial membrane antigen (EMA), α_1-antitrypsin, S-100 and angiotensinogen converting enzyme (ACE). Using immunostains potentially supporting primary or metastatic clear cell renal cell carcinoma include PAX2 or PAX8. PAX2 is the best marker with 50–80% sensitivity.

Markers	Expression
▪ PAX2	▪ Positive (best marker)
▪ CAIX (carbonic anhydrase)	▪ Positive (membranous diffuse staining), most sensitive and specific marker in inherited (100%) and sporadic cases (75%)
▪ PAX8	▪ Positive
▪ RCC-Ma	▪ Positive

▪ RCC-Ag	▪ Positive
▪ CD10	▪ Positive
▪ Vimentin	▪ Positive
▪ CK8	▪ Positive
▪ CK18	▪ Positive
▪ EMA	▪ Positive
▪ AE1/AE3	▪ Positive
▪ CAM5.2	▪ Positive
▪ α_1-Antitrypsin	▪ Positive
▪ S-100 protein	▪ Positive (rare case)
▪ CEA	▪ Positive (rare case)
▪ K903	▪ Negative
▪ KS-cadherin	▪ Negative/positive
▪ CD117 (proto-oncogene c-KIT gene encoding the receptor tyrosine-protein kinase, KIT, CD117)	▪ Negative/positive

Prognostic Factors

Prognostic factors for clear cell renal cell carcinoma are Fuhrman's nuclear grading and International Society of Urological Pathology (ISUP) nucleolar grading in clear cell renal cell carcinoma, histologic features and necrosis of tumor. Fuhrman's nuclear grading and ISUP nucleolar grading in clear cell renal cell carcinoma is given in Table 22.73.

Fuhrman Nuclear Grading System

Fuhrman classification of renal cell carcinoma is a 3-tiered system in which nuclear size, shape and nucleolar prominence are assessed simultaneously to assign a grade. An International Society of Urological Pathology (ISUP)/WHO Grading System based on nucleolar prominence appears to be a more powerful prognostic discriminator and has been recommended as a substitute.

- Grade 1: Nucleoli are inconspicuous or absent at objective magnification 400X

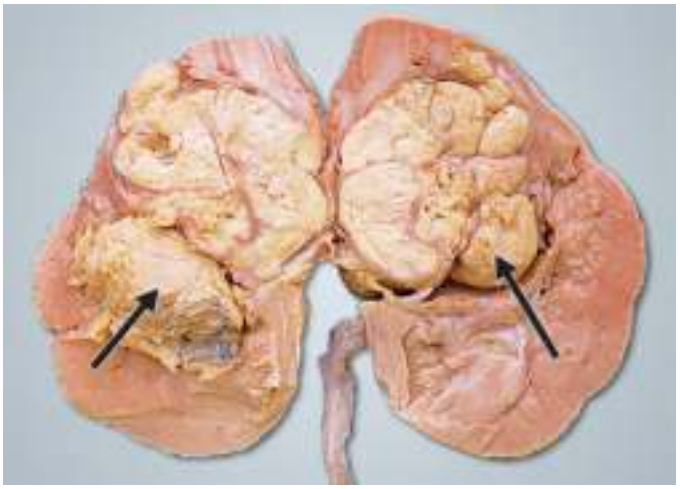


Fig. 22.102: Clear cell renal cell carcinoma. Tumor is well circumscribed, partially encapsulated spherical mass. Cut surface shows variegated appearance (solid and cystic areas). On cut surface, tumor is golden yellow mass due to presence of lipids in tumor cells. Areas of hemorrhage and necrosis are present (arrows) (400X). (*Courtesy:* Dr. Sujata Kanetkar, Senior Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

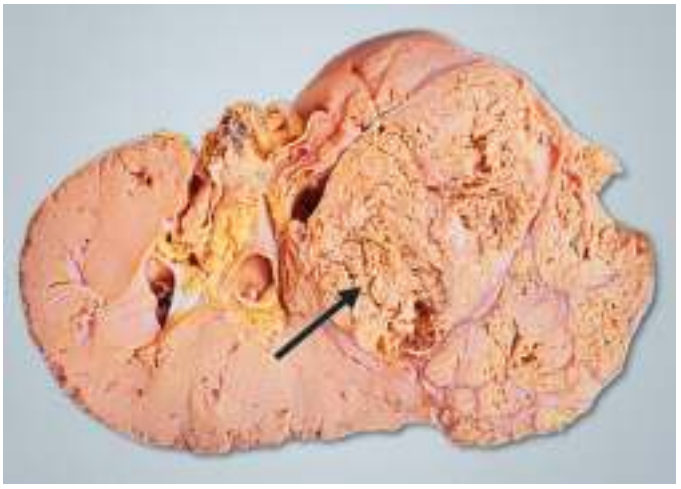


Fig. 22.103: Clear cell renal cell carcinoma. Tumor is well circumscribed, partially encapsulated spherical mass. Cut surface shows variegated appearance (solid and cystic areas). On cut surface, tumor is golden yellow mass due to presence of lipids in tumor cells. Areas of hemorrhage and necrosis are present (arrow). (*Courtesy:* Department of Pathology, Dr. DY Patil Medical College, Pune, Maharashtra.)

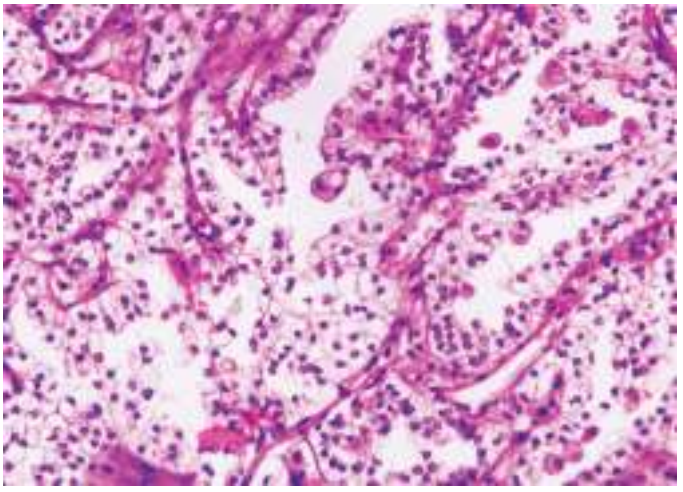


Fig. 22.104: Clear cell renal cell carcinoma. In hematoxylin and eosin-stained tumor section, tumor is composed of large round/polygonal cells with centrally placed round nuclei and abundant clear cytoplasm. The cytoplasm of tumor cells appears clear because it is rich in glycogen and fat, which are washed out during histologic processing of the tissue. Tumor cells are forming solid cords, papillae, tubules or nests or alveolar pattern. These cells are demarcated by a network of thin-walled blood vessels in the delicate supporting stroma. The stroma may be reduced, but well vascularized (400X).

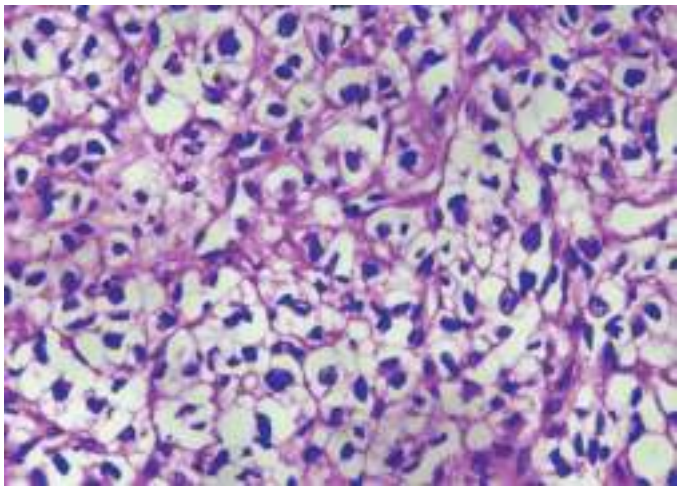


Fig. 22.105: Clear cell renal cell carcinoma, In hematoxylin and eosin-stained renal tumor tissue section examination, tumor is composed of tumor cells with clear cytoplasm and distinct membrane with clear to granular eosinophilic cytoplasm. Presence of network of arborizing small thin-walled blood vessels is important diagnostic feature for cases with granular eosinophilic cytoplasm. Tumor cells are arranged in solid nests and sheets, alveolar, microcystic (containing extravasated red blood cells or eosinophilic) and occasional macrocytic architectural patterns. High-grade clear cell renal cell carcinoma shows rhabdoid differentiation with high-grade tumor cells containing homogenous eosinophilic cytoplasm and eccentric nucleus and globular eosinophilic inclusions (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

Table 22.73 Fuhrman's nuclear grading and ISUP/WHO nucleolar grading in clear cell renal cell carcinoma

Grade	Nuclear Features
Grade 1	▪ Nuclei small round and uniform (10 μm) ▪ Nucleoli inconspicuous or absent at objective magnification 400X
Grade 2	▪ Nuclear outline irregular (15 μm) ▪ Nucleoli prominent at objective magnification 400X
Grade 3	▪ Nuclei irregular (20 μm) ▪ Nucleoli prominent at objective magnification 100X
Grade 4	▪ Nuclei (20 μm size, extreme pleomorphism, hyperchromatic, multinucleated giant cells, sarcomatoid and/or rhabdoid change) ▪ Nucleoli (single or multiple) at objective magnification 400X

- Grade 2: Nucleoli are prominent at objective magnification 400X
- Grade 3: Nucleoli are prominent at objective magnification 100X
- Grade 4: Presence of extreme nuclear pleomorphism, multinucleated giant cells, sarcomatoid and/or rhabdoid change at objective magnification 400X.

Histology of Tumor

Clear cell renal cell carcinoma with sarcomatoid and rhabdoid differentiation have higher rates of tumor recurrence and metastasis. Presence of sarcomatoid and rhabdoid differentiation should be mentioned in the report.

Necrosis in the Tumor

Coagulative tumor necrosis has an independent prognostic significance. It is essential to report the amount and percentage of necrosis present in the tumor.

Mode of Spread

Clear cell renal cell carcinoma metastasizes in 30% of cases. Tumor invades renal capsule and perinephric fat. Tumor metastasizes to para-aortic and other lymph nodes via lymphatic route. Tumor has tendency to invade through renal vein into inferior vena cava (IVC) and then extends intraluminally, with thrombus formation in 5–50% of cases. Tumor may extend up to right chambers of heart in 1% of cases. Tumor can disseminate via hematogenous route to lungs (50–70%), bones (33%), liver (18%), skin (8%) and brain (8%).

Staging System

Staging system is a way of describing the size of tumor and whether it has spread into nearby lymph nodes or other parts of the body via hematogenous route. The stage is one of the most important factors in deciding how to treat the cancer and determining how successful treatment might be. Anatomic staging of clear cell renal cell carcinoma is given in Table 22.74.

Laboratory Investigations

Complete work up of clear cell renal cell carcinoma includes hemogram, urine examination for malignant cells, blood biochemical tests (serum calcium, liver and renal functions) and imaging techniques. Ultrasound of the abdomen especially kidneys, intravenous

Table 22.74 Anatomic staging of clear cell renal cell carcinoma

Stage	Extent of Spread of Tumor
Stage I	Tumor confined within capsule of kidney (prognosis 10 years survival in 70% of cases)
Stage II	Tumor invading perinephric fat
Stage III	Tumor invading the renal vein or inferior vena cava (A), or regional lymph node involvement (B), or both (C)
Stage IV	Tumor invading adjacent viscera (excluding ipsilateral adrenal) or distant metastases

pyelography, renal arteriography, bone scan may show involvement of the bones due to metastases. Diagnosis of clear cell renal cell carcinoma is confirmed on histologic examination.

Treatment and Prognosis

Stage 1 of clear cell renal carcinoma is treated by radical/nephrectomy, radiotherapy and arterial embolization. Systemic chemotherapy is not effective against advanced disease. About 15–20% of patients with advanced disease responds to immunotherapy. Receptor tyrosine kinase inhibitors are administered in advanced stage disease. About 30% of patients with clinically localized disease develop local and distant recurrence after surgery. Approximately 50% of patients have fatal outcome.

MULTILOCULAR CYSTIC RENAL CELL CARCINOMA

Multilocular cystic renal cell carcinoma is a variant of clear cell renal cell carcinoma. Tumor is composed of multiple cysts lined with low-grade clear cells, and without solid or expansile nodules. Tumor accounts for 5% of all clear cell renal cell carcinomas and has lower stage than other renal cell carcinomas. Patients are treated by partial or radical nephrectomy, who have excellent prognosis without recurrence or metastasis reported after surgical excision. Multilocular cystic renal cell carcinoma demonstrates chromosome 3p deletion and vHL gene mutations.

Surgical Pathology: Multilocular Cystic Renal Cell Carcinoma

Gross Morphology

Multilocular cystic renal cell carcinoma is a solitary well circumscribed and entirely cystic mass without solid tumor mass and necrosis.

Light Microscopy

In multilocular cystic renal cell carcinoma, cysts are lined by a single layer or focally stratified clear cells. The clear cells contain small uniform and round or oval without prominent nucleoli. Small nests of clear cells are present in the fibrous septa. There is no solid or expansile clear cell nodules.

Immunohistochemistry Technique

Markers	Expression
▪ EMA	▪ Positive
▪ CD10e	▪ Positive
▪ AE1/AE3	▪ Positive

PAPILLARY (CHROMOPHIL) RENAL CELL CARCINOMA

Papillary (chromophil) renal cell carcinoma is a histologic subtype of renal cell carcinoma (RCC) with predominant tubulopapillary architecture and characteristic genetic alterations.

- Tumor may be sporadic or familial and never associated with vHL gene mutation. Rarely, tumor is a part of hereditary papillary renal cell carcinoma syndrome or hereditary leiomyomatosis and renal cell carcinoma syndrome.
- Tumor, is derived from distal tubular epithelium of cortex with predominantly papillary or tubulopapillary architecture, often well-circumscribed and encapsulated features.
- Papillary (chromophil) renal cell carcinoma is second most common encountered renal cell carcinoma accounting for 10–15% of cases. It most often occurs in elderly males and females. Tumor has two morphologic subtypes with distinct molecular pathways and clinical behavior.

Clinical Features

Patient presents similarly to other subtypes of renal cell carcinoma; typical triad of palpable mass, flank pain and hematuria (uncommon seen in 5–10% of cases). Tumor has tendency to present at an early stage.

- Tumor is detected as incidental finding on imaging in more than 15% of cases. In majority of patients, tumor is unilateral.
- However, patients may have bilateral and multifocal tumors compared to other subtypes of renal cell carcinomas.
- Tumor fungates into calyces, involves renal pelvis, and extends into ureter. It may also invade the renal vein, inferior vena cava and right atrium.

Radiologic Findings

Papillary (chromophil) renal cell carcinoma may appear hypovascular due to spontaneous tumor necrosis. Foci of calcification are demonstrated in 30% of cases. Radiology techniques may detect differences between clear cell renal cell carcinoma, and papillary (chromophil) renal cell carcinoma.

Surgical Pathology: Papillary (Chromophil) Renal Cell Carcinoma

Gross Morphology

- Papillary (chromophil) renal cell carcinoma, is most often well-circumscribed with prominent pseudocapsule and friable located in renal cortex pouring out of kidney. Tumor may be solitary or multifocal.
- Cut surface is tan-yellow to red-brown with granular papillary architecture. Large tumor may show cystic degeneration, fibrosis, hemorrhage and extensive necrosis. Majority of papillary (chromophil) renal cell carcinoma has familial predisposition.

- Tumor looks necrotic on gross examination than microscopically less necrotic than expected. Gross morphology of papillary (chromophil) renal cell carcinoma is shown in Fig. 22.106.

Light Microscopy

- Papillary (chromophil) renal cell carcinoma is most often well-circumscribed with prominent pseudocapsule.
- Tumor is composed of papillary fronds or tubulopapillary architectures varying proportions with central fibrovascular cores lined by a single layer of tumor cells with round uniform nuclei without nucleoli; which may contain foamy macrophages, psammoma bodies and hemosiderin.
- The stroma is usually scanty but highly vascularized. Papillae can coalescence to impart a solid growth pattern with glomeruloid structures. About 5% of tumors demonstrate sarcomatoid change.
- It is essential to assess nucleolar prominence under high power field with greatest nuclear pleomorphism. Grading is done according to International Society of Urological Pathology (ISUP) nucleolar grade.
- Papillary (chromophil) renal cell carcinoma is further classified into type 1 and type 2 based on following morphology. Type 1 papillary (chromophil) renal cell carcinoma prognosis is better than type 2 subtype.
  - **Type 1 papillary (chromophil) renal cell carcinoma:** Tumor is composed of papillae lined by a single layer of cells with scanty basophilic cytoplasm and low nuclear grade associated with better prognosis; with molecular/cytogenetic alterations (+7, +17 and other trisomies, MET mutations).
  - **Type 2 papillary (chromophil) renal cell carcinoma:** Tumor is composed of papillae lined by pseudostratified layers of cells with more abundant eosinophilic cytoplasm and higher nucleolar grade associated with worse prognosis; with multiple molecular/cytogenetic alterations. Tumors may be multifocal involving both kidneys.
- Histology of papillary (chromophil) renal cell carcinoma is shown in Figs 22.107 and 22.108.

Electron Microscopy

Papillary (chromophil) renal cell carcinoma shows variably sized microvilli, small amounts of cytoplasmic lipid, but no glycogen.

Immunohistochemistry Technique

Panel of markers applied on formalin-fixed paraffin-embedded sections from papillary (chromophil) renal cell carcinoma expresses positivity for CK7, AMACR, AE1/AE3, EMA, MUC1, RCC-Ma, CD10, PAX2, PAX8 and vimentin. Tumor cells are positive for CK7 in type 1 (80%) and type 2 (20%).

Markers	Expression
CK7	Positive
Recemase	Positive
PAX8	Positive
PAX2	Positive
AMACR	Positive
AE1/AE3 (low molecular cytokeratin)	Positive
EMA	Positive
MUC1	Positive
RCC-Ma	Positive
CD10	Positive
CD117 (proto-oncogene c-KIT is the gene encoding the receptor tyrosine-protein kinase, KIT, CD117)	Positive
Vimentin	Positive/negative (variable staining)
CAIX (carbonic anhydrase)	Negative except in areas of necrosis

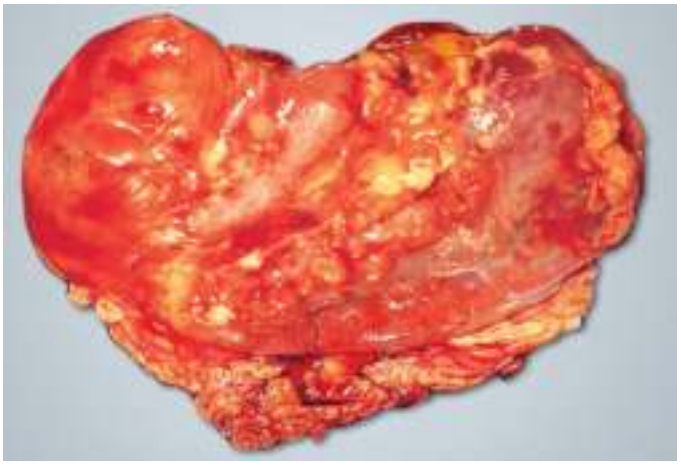


Fig. 22.106: Papillary (chromophil) renal cell carcinoma. Tumor is most often circumscribed with prominent pseudocapsule and friable located in renal cortex. Tumor may be multifocal. Cut surface shows tumor with papillary protrusions. Large tumor may contain cystic degeneration, fibrosis, hemorrhage and necrosis. The great majority of renal cell carcinoma, chromophil/papillary has familial predisposition.

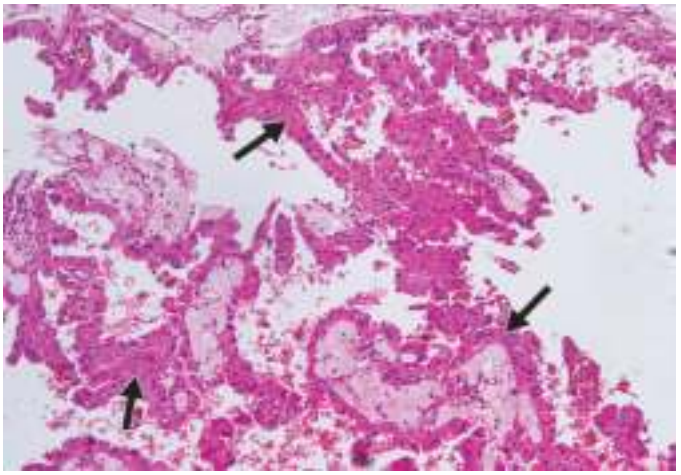


Fig. 22.107: Histology of papillary (chromophil) renal cell carcinoma. In hematoxylin and eosin-stained renal tumor section, tumor is composed of papillae or tubulopapillary architecture with fibrovascular cores covered by cuboidal or low-columnar cells with round, uniform nuclei without nucleoli. Fibrovascular cores may contain foamy macrophages, psammoma bodies and hemosiderin. The stroma is usually scanty but highly vascularized (arrows) (400X).

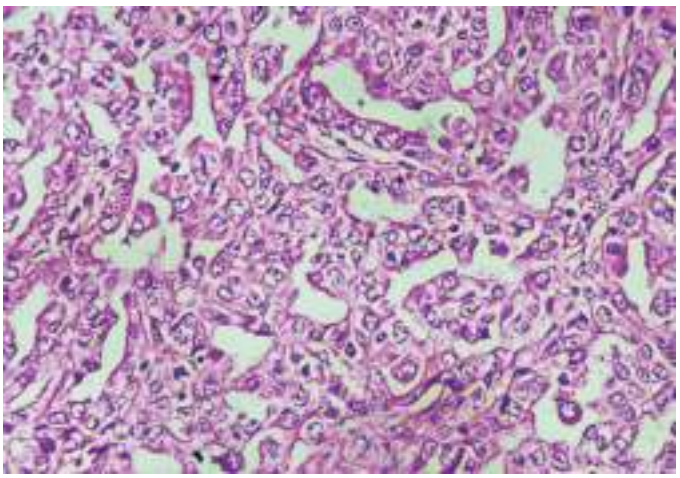


Fig. 22.108: Histology of papillary (chromophil) renal cell carcinoma (PRCC). Tumor has two histologic variants: type 1 and type 2 PRCC. Type 1 PRCC is composed or papillae lined by a single layer of tumor cells with scant basophilic cytoplasm and low-grade nuclear grade. Tumor has +7, 17+ and other trisomies, MET gene mutations and associated with better prognosis. Type 2 PRCC is composed of papillae lined by pseudostratified layer of tumor cells with more abundant eosinophilic cytoplasm and higher nuclear grade. Multiple genetic changes are present and associated with worse prognosis. Hematoxylin and eosin-stained renal tumor tissue section examination shows well circumscribed tumor with prominent pseudocapsule composed of papillae or tubulopapillary architecture with fibrovascular cores which may contain foamy macrophages, psammoma bodies and hemosiderin pigment (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

Molecular Genetic Alterations

Papillary (chromophil) renal cell carcinoma is never associated with vHL gene mutation located on chromosome 3. There is typical gain of chromosomes 7 and 17 (trisomy) in sporadic cases.

- Type 1, papillary (chromophil) renal cell carcinoma demonstrates +7 (75%), +17 (80%), +12, +16, +20, +3q, –Y, –X (p or q for all chromosomes).
- Type 2 papillary (chromophil) renal cell carcinoma, demonstrates heterogenous cytogenetic alterations such as CpG island methylation or loss of CDKN2A tumor suppressor gene and associated with poor prognosis.
- Familial papillary (chromophil) renal cell carcinoma may be seen in familial papillary renal cell carcinoma syndrome (type 1 PRCC) and in Hogg-Dubé syndrome. Loss of Y chromosome is seen in familial cases in men. PRCC gene on chromosome 1 fuses with TFE 3 gene on chromosome X. Fusion protein dysregulates mitotic checkpoints allowing abnormal segregation of chromosomes.
- Patient develops bilateral hereditary papillary renal cell carcinoma due to **germline mutation MET proto-oncogene** mapped on chromosome 7q31 in hereditary papillary renal cell carcinoma syndrome or fumarate hydratase gene mapped on chromosome 1q42, 3–43 in hereditary leiomyomatosis and renal cell carcinoma syndrome.

Treatment

Patient with papillary (chromophil) renal cell carcinoma is treated by resection and tyrosine kinase inhibitor therapy. About 80% of type tumors have an alteration in MET gene sequence or copy number and may be susceptible to inhibitors of MET pathway.

CHROMOPHOBE RENAL CELL CARCINOMA

Chromophobe renal cell carcinoma is a subtype of renal cell carcinoma comprising large pale and/or pleomorphic eosinophilic cells with prominent cell borders, finely reticular pale cytoplasm, perinuclear halos and wrinkled, angulated (raisinoid) nuclei. Tumor arises from distal convoluted tubules and cortical intercalated cells collecting ducts.

- Out of three histologic variants of renal cell carcinoma, prognosis of chromophobe renal cell carcinoma is excellent. Men and women are equally affected. Majority are sporadic and incidental without gender preference with mean age 55 years.
- Birt-Hogg-Dubé syndrome is an autosomal dominant disorder characterized by multiple tumors (bilateral chromophobe renal cell carcinomas, renal oncocytomas or hybrid oncocytic/chromophobe tumor), small dome-shaped popular fibrofolliculomas over face, neck, and upper trunk, lung cysts and spontaneous pneumothorax. Mutations in the folliculin gene (FLCN) at 17p11.2 leads to premature truncation and loss of function of the folliculin protein.

Clinical Features

Chromophobe renal cell carcinoma confined within renal capsule is associated with good prognosis. Patient presents with hematuria, flank mass and pain. Prognosis of chromophobe renal cell carcinoma is much better than clear cell renal cell carcinoma with morality less than 10% and five-year survival in more than 90% of cases. Sarcomatoid morphological features in chromophobe renal cell carcinoma and invasion in perinephric fat are associated with worse prognosis. Patients are usually treated by partial nephrectomy.

Molecular Genetic Alterations

Multiple chromosomal losses or extreme hypoploidy is seen on cytogenetic study in chromophobe renal cell carcinoma.

Poor Prognostic Factors

Chromophobe renal cell carcinoma showing sarcomatoid change, microscopic necrosis, small vessel invasion and distant metastasis is associated with poor prognosis.

Surgical Pathology: Chromophobe Renal Cell Carcinoma

Gross Morphology

- Chromophobe renal cell carcinoma is well-circumscribed but not encapsulated with tan to light brown. Size of the tumor ranges 2–22 cm (mean 8 cm). Tumor is solid and well-circumscribed but not encapsulated.
- Cut surface is homogenous, light brown to tan. Areas of hemorrhage and small cysts are demonstrated in 25–30% of cases. Tumor can be solitary (85%) and may be multifocal (≤15%). Calcification is observed in 48% of tumors.
- Central scarring is demonstrated in ≤15%. It is difficult to differentiate chromophobe cell carcinoma from renal oncocytoma. Hemorrhage and necrosis are rare.

Light Microscopy

Classic variant of chromophobe renal cell carcinoma

- Classic variant of chromophobe renal cell carcinoma is composed of large pleomorphic cells with pale and highly reticulated cytoplasm, and distinct cell borders eccentrically placed angulated (raisinoid) nuclei with a perinuclear halo.
- Tumor cells are arranged in solid growth, nests or broad alveoli/trabeculae. These cells are commonly mixed with smaller cells with granular cytoplasm.
- Tumor cells appear as 'vegetative cells' due to retraction of cytoplasm giving cobblestone appearance.
- Tumor is composed of three types of cells: (a) small cells with solid slightly granular eosinophilic cytoplasm, (b) cells have perinuclear halo or translucent zone in a background of pale, flocculent but not clear cytoplasm; and (c) large, polygonal cells with hard cell border, abundant cytoplasm with reticular pattern. Tumor cells are arranged in solid sheets with a concentration of the largest cells around blood vessels. Histology of chromophobe renal cell carcinoma is shown in Fig. 22.109.

Eosinophilic variant of chromophobe renal cell carcinoma

- Eosinophilic variant of chromophobe renal cell carcinoma is composed entirely of intensely eosinophilic cells.
- Tumor cells appear as 'vegetative cells' due to retraction of cytoplasm imparting a '**cobble stone appearance**'. Tumor cells have varying degrees of eosinophilic cytoplasm and granularity in the periphery adjacent to the cytoplasmic membrane, and irregular, wrinkled, and angulated nuclei (raisinoid) with perinuclear clearing (halos).
- Medium-sized blood vessels with eccentrically hyalinized walls are observed. It is worth mentioning that formalin fixation may obscure cytoplasmic features.

Histochemistry

Hale's colloidal iron stain reveals strong blue cytoplasmic staining in chromophobe variant of renal cell carcinoma. This staining pattern is absent in clear cell variant and chromophil variant of renal cell carcinoma.

Electron Microscopy

- Electron microscopy of chromophobe renal cell carcinoma reveals numerous oval cytoplasmic microvesicles (possibly from mitochondrial outpouchings) measuring from 150 to 300 nm in diameter.
- These oval cytoplasmic microvesicles are not found in any other renal tumor and that are considered diagnostic.
- It is quite likely that Hale's colloidal iron reacts with a substance present in the microvesicles. Eosinophilic variant is similar but with more mitochondria.

Immunohistochemistry Technique

Panel of markers applied on formalin-fixed paraffin-embedded sections of chromophobe renal cell carcinoma expresses positivity for EMA, CK7, E-cadherin, claudin 7, EpCAM and CD117.

Markers	Expression
EpCAM (epithelial cell adhesive molecule)	Positive (new marker)
CD117 (c-KIT)	Positive
CK7	Positive
TFE3	Positive
Cathepsin K	Positive
PAX8	Positive
EMA	Positive
CK8/CK18	Positive
E-cadherin	Positive
Claudin 7	Positive
Ki-67	Positive (low expression)
N-cadherin	Negative
Vimentin	Negative

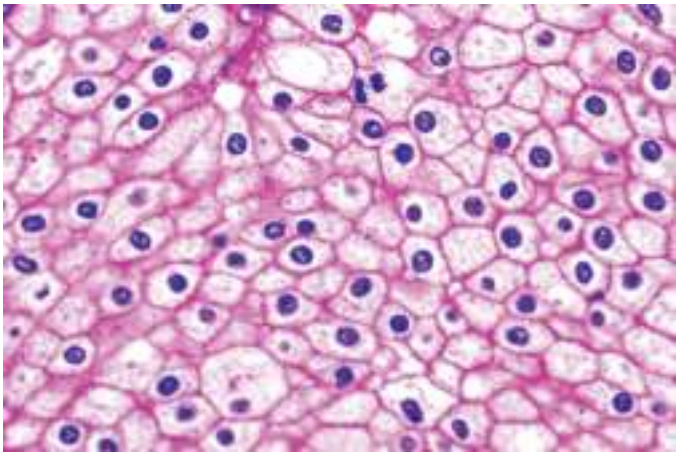


Fig. 22.109: Histology of chromophobe renal cell carcinoma. Hematoxylin and eosin-stained renal tumor tissue section examination shows confluent solid growth with nests, sheets or alveoli/trabeculae composed of two types of cells: (a) type 1 cells are pale polygonal cells with sharply defined plant-like cell borders (vegetative cells); abundant cytoplasm with eccentrically placed irregular, wrinkled and angulated nuclei with coarse chromatin and a perinuclear halo, and (b) type 2 cells are smaller cells with finely granular eosinophilic cytoplasm in predominant eosinophilic variant. Stroma is minimal and composed incomplete fibrovascular septae around solid sheets (400X).

Table 22.75 Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 fusions

Xp11 Chromosomal Translocation	Gene Fusion
t(X;17) (p11.2; q25)	ASPL-TFE3
t(X;1) (p11.2q21)	PRCC-TFE4
t(X;1) (p11.2q34)	PSF-TFE3
t(X;1) (p11.2q23)	CLTC-TFE3
inv(X) (p11.2; q23)	NONO-TFE3

Karyotyping Xp11 chromosomal translocation renal cell carcinoma is IPOX for TFE and cathepsin K. Alveolar soft part sarcoma shows der(17) t(X;17) (p11.2;q25) and gene fusion ASPL-TFE3.

PEDIATRIC RENAL CELL CARCINOMA WITH Xp11.2 TRANSLOCATION AND TFE3 GENE FUSION

Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 gene fusion is distinctive type of renal cell carcinoma characterized by chromosomal translocation involving TFE3 gene on chromosome Xp11.2 or TFEB gene on chromosome 11q12. It is also called MiT translocation subgroup renal cell carcinoma, which most often affects young patients. It represents 30–70% of pediatric renal cell carcinoma. Presence of dense calcifications in tumor resemble nephrolithiasis, and obstruction of the renal pelvis promotes extensive obscuring xanthogranulomatous pyelonephritis. Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 fusions is given in Table 22.75.

Clinical Features

Pediatric renal cell carcinoma with Xp translocation is associated with prior chemotherapy for other malignancies. Patient presents with high stage disease associated with poor prognosis. Majority of cases are reported in children and young adults. Clinical presentation is similar to renal cell carcinoma in adults. Patients are treated by nephrectomy. Patient has indolent clinical behavior in lymph node positive and metastasis negative. Clinical course is aggressive in metastasis positive children and adults.

Surgical Pathology: Pediatric Renal Cell Carcinoma with Xp11.2 Translocation and TFE3 Gene Fusion

Gross Morphology

- Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 gene fusion has a fibrous pseudocapsule ranging in size from 3 to 14 cm in diameter.
- Cut surface of tumor is yellow or gray white to tan yellow with frequent hemorrhage, necrosis and calcification. Sometimes, tumor imparts egg-shell consistency. Gross morphology of pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 fusion is shown in Fig. 22.110.

Light Microscopy

Pediatric renal cell carcinoma with different chromosomal translocations exhibits somewhat different morphological features.

- **ASPL-TFE3 gene fusion pediatric renal cell carcinoma:** Tumor is composed of cells with abundant partly clear or granular, eosinophilic cytoplasm with sharp, discrete borders and vesicular nuclei and prominent nucleoli. Tumor cells are arranged in nests and papillae or pseudopapillae surrounded by thin-walled capillary vessels. '**Psammomatous calcification**' and '**hyaline nodules**' are frequent. Sometimes central cells in nests are discohesive imparting an alveolar or bloody garland appearance. Mitoses are rare/absent. Stromal macrophages and psammoma bodies, if present are limited in number. Histology of pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 fusion is shown in Fig. 22.111.
- **PRCA-TFE3 gene fusion pediatric renal cell carcinoma:** Tumor is composed of cells arranged in more compact nested and less papillary pattern. Tumor cells have a less abundant cytoplasm, fewer psammomatous calcification and hyalinized fibrovascular cores.

Immunohistochemistry Technique

Panel of markers applied on formalin-fixed paraffin-embedded sections from tumor expresses positivity for TFE 3, TFEB, CD10, AMACR, vimentin, E-cadherin, CK19, EMA and cathepsin K.

Markers	Expression
TFE3 transcription factor	Positive (nuclear staining)
CD10	Positive
AMACR	Positive
Vimentin	Positive
E-cadherin	Positive (66%)
CK19	Positive (30–50%)
EMA	Positive (30%)
Cathepsin K	Positive

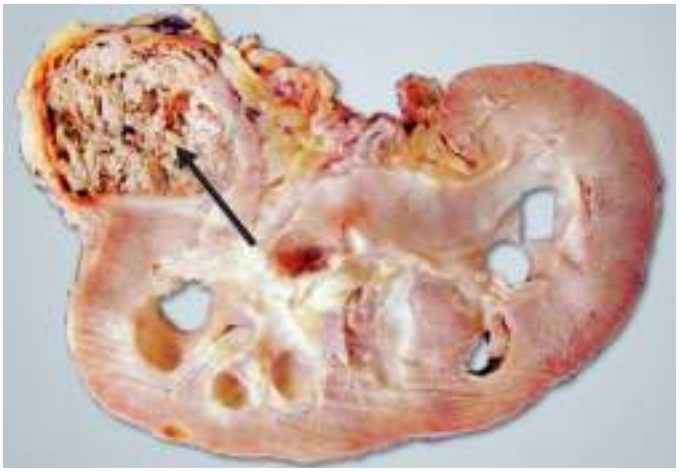


Fig. 22.110: Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 gene fusion. Tumor has a fibrous pseudocapsule. It is located at lower pole. Cut surface is tan yellow or gray white with varying degrees of hemorrhage, necrosis and calcification not distinct from other variants of renal cell carcinoma. It may sometimes, impart egg-shell consistency (arrow).

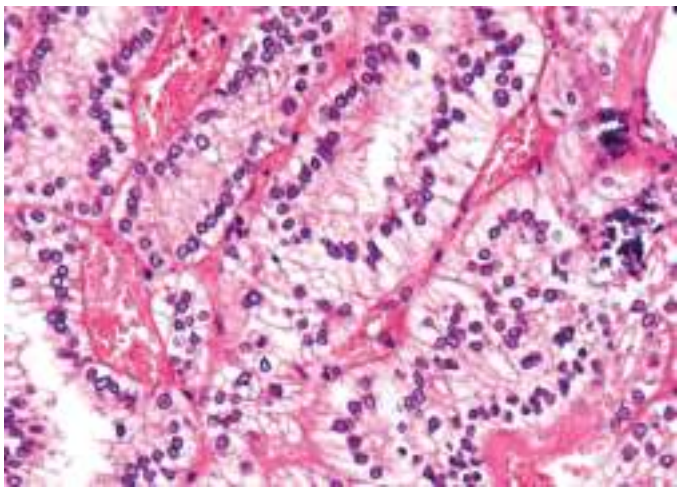


Fig. 22.111: Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 gene fusion. Tumor harbors gene fusions involving members of the MiT family of transcription factors including TFE3 and TFEB. MiT refers to microphthalmic associated transcription factors. In hematoxylin and eosin-stained renal tissue sections, tumors are histologically diverse, often has papillary, alveolar and nested growth pattern with clear eosinophilic cells, high-grade nuclei and psammoma bodies. Tumor cells are surrounded by thin-walled vessels. Tumor is more common in children and young adults (400X).

COLLECTING DUCT CARCINOMA OF BELLINI

Collecting duct carcinoma of Bellini is a rare highly aggressive renal cell carcinoma of renal medulla in adults accounting for 2% of cases, which arises from principal cells of distal collecting ducts of Bellini in the medulla and involving both medulla and cortex because of large size tumor. It affects elderly persons with mean age of 55 years and male predominance.

- Essential major diagnostic criteria of tumor are involvement of renal medulla, high-grade cuboidal cells or hobnail cells, predominant tubular architecture, infiltrative growth pattern, marked stromal desmoplasia.
- Minor diagnostic features of tumor are central location of tumor, tubulopapillary architecture, tumor cells with mucin, inflammatory polymorphonuclear cells in the stroma and extensive infiltration of tumor cells in the renal parenchyma, extrarenal tissue and blood vessels. Essential diagnostic features of collecting duct carcinoma of Bellini are shown in Table 22.76.

Clinical Features

Patients present with pain, gross hematuria, weight loss, flank mass and distant metastases in 66% of cases. Sometimes, tumor is detected incidentally on radiologic study.

- On CT scans, normal outer contours are usually maintained in affected kidneys; and the tumor mass involves medulla and protrudes into the renal sinuses. Cytology of urinary sediment shows malignant cells.
- Tumor usually invades surrounding tissue and metastasizes to regional lymph nodes via lymphatic route and liver, lungs, bones or contralateral kidney via hematogenous route. Majority of patients develop lymph node and widespread metastasis in liver, lungs, bone and adrenal glands at presentation.
- The tumor is highly aggressive associated with worst prognosis with fatal outcome within the first 2 years.
- Patients are treated by nephrectomy with adjuvant and immunotherapy.

Table 22.76 Essential diagnostic features of collecting duct carcinoma of Bellini

Major Diagnostic Criteria
Medullary involvement
Tubular architecture
Growth pattern infiltrative
Marked desmoplasia of stroma
Cytologically high-grade cuboidal or hobnail cells
No other renal cell or urothelial carcinoma
Minor Diagnostic Criteria
Centrally located large tumor
Tubulopapillary architecture
Inflammatory polymorphonuclear cells in the stroma
Extensive renal, extrarenal and vascular infiltration
Cytologically tumor cells with mucin positivity

Molecular Genetic Alterations

Molecular genetic information in collecting duct carcinoma of Bellini is still very limited in these tumors, but losses of chromosomes 1q32.1–32. 2, 6q, 8p, 14, 15, 21q; and 22 have been reported. It is not associated with loss of 3p, and trisomies 7 and 17. HER2/neu amplification has been demonstrated in 45% of cases. Other genomic alterations include NF2 (30%), SETD2 (25%), SMARCB1 (18%) and CDKN2A (12%). Loss of chromosome 3p is rare in these cases.

Radiologic Findings

CT imaging demonstrates good correlation with histologic findings, which shows solid mass with cystic component with infiltrative/expansile/exophytic growth in the renal medulla involving renal sinus, perinephric tissue, regional lymph nodes and distant metastases to liver, lungs, bones or contralateral kidney.

Surgical Pathology: Collecting Duct Carcinoma of Bellini

Gross Morphology

Collecting duct carcinoma of Bellini is predominantly located in the medulla and involving cortex. Tumor has irregular extensively infiltrating margins and infiltrative into renal sinus fat and perinephric tissue and renal vein. Cut surface of tumor is firm gray mass measuring 2.5–12 cm (mean 5 cm). Areas of cystic, hemorrhage and necrosis are common. Tumor may demonstrate satellite nodules and renal vein invasion.

Light Microscopy

- Collecting duct carcinoma of Bellini is complex, infiltrative and poorly circumscribed tumor. Tumor is composed of high-grade cuboidal cells or hobnail cells with large pleomorphic nuclei with prominent nucleoli and coarse chromatin arranged in cords, tubules, tubulopapillary or tubulocystic structures embedded into inflamed desmoplastic stroma. Intracytoplasmic and intraluminal mucin may be present. Numerous mitotic figures are present.
- Tumor may demonstrate microcystic change due to dilation of tubular structure. Marked stromal desmoplasia and inflammatory polymorphonuclear cells in the stroma are present.
- Tumor may exhibit sarcomatoid dedifferentiation. Adjacent to the tumor, tubular epithelium lining collecting ducts may appear dysplastic.

Electron Microscopy

- Collecting duct carcinoma of Bellini shows features of adenocarcinoma, including intracellular and extracellular lumina.
- Well-formed cell junctions, prominent basal lamina and short apical microvilli are demonstrated.

Immunohistochemistry Technique

Panel of markers applied on formalin-fixed paraffin-embedded sections from tumor expresses positivity for PAX8, PAX2, Ulex europaeus lectin 1 (Ulex-1), peanut lectin agglutinin (PNA), mucin, vimentin, CK8/18, CK19, EMA and S-100A1.

Markers	Expression
PAX8	Positive
PAX2	Positive
Ulex europaeus lectin 1 (Ulex-1)	Positive
Peanut lectin agglutinin (PNA)	Positive
Mucin	Positive
Vimentin	Positive
CK8/18	Positive
CK19	Positive
EMA	Positive
S-100A1	Positive
p63	Negative
Uroplakin II	Negative
CD10	Negative
AMACR	Negative
E-cadherin	Negative
CAIX	Negative
OCT3/4	Negative
CD117	Negative

UROTHELIAL CARCINOMA OF RENAL PELVIS

Renal pelvis, ureters and urinary bladder are lined by transitional epithelium. Urothelial carcinoma of renal pelvis is a morphologic variant of high-grade tumor similar to urothelial carcinoma of urinary bladder affecting adults. Tumor is composed of bland nested pattern, that could be difficult to differentiate from florid von Brunn's nests; but more irregular and most often invasive. It accounts for 5–10% of primary renal tumors. Clinical outcomes are similar to invasive high-grade urothelial carcinoma associated with poor prognosis.

Predisposing Factors

Cigarette smoking is the most common cause of renal pelvis carcinoma. There is increased risk of renal pelvis carcinoma in patients with intake of analgesic drugs (phenacetin), cyclophosphamide drug for longer period, and prolonged exposure of occupational workers in aniline dye and rubber industries.

- Patient presents with hematuria and flank pain. Risk factors of tumors of renal pelvis are similar to bladder cancer, suggesting a '**field effect**'.
- In aniline and rubber industries, prolonged exposure to β-naphthylamine absorbed by gastrointestinal tract reaches liver. In the liver, hydroxylation of β-naphthylamine forms 1-hydroxy-2-naphthylamine (active carcinogenic metabolite), that combines with glucuronic acid and excreted in the urinary tract.
- This molecule is deconjugated by urinary bladder mucosal enzyme glucuronidase, thus exposing the urothelium to the active carcinogen resulting in urothelial carcinoma of renal pelvis.

Clinical Features

Patient most frequently presents with hematuria and flank pain. Transitional epithelial cells lining papillae may detach and may be found in urine, important for diagnosis. Tumor fragmentation may block urinary outflow and cause flank pain and hydronephrotic changes. Even a small tumor may cause hematuria, renal colic, or urinary obstruction and can be diagnosed in early stages of development. Excision of the entire ureter is necessary because of the high frequency of concurrent and subsequent urothelial carcinoma of ureter.

Surgical Pathology: Urothelial Carcinoma of Renal Pelvis

Gross Morphology

Tumor is thickened, irregular with or without papillary component invading wall of renal pelvis. Tumor may invade renal vein and metastasize to various organs. It may result in fragments breaking off from the tips of fronds; and the tumor cells are detected in urine on cytologic examination.

Light Microscopy

- Urothelial carcinoma of renal pelvis is composed of anastomosing irregular-sized nests lacking cytoarchitectural orientation to overlying urothelium with more irregular projections into the stroma especially in the deep aspect. The overlying urothelium is most often unremarkable.
- Cytologically, tumor cells are bland with focal atypia. Central lumens of the nests contain necrotic cellular debris. Stroma of the tumor may be normal or desmoplastic.

Immunohistochemistry Technique

Panel of markers applied on formalin-fixed paraffin-embedded sections from tumor expresses positivity for CK7, p63, CK903, CK20, and p53.

Markers	Expression
CK7	Positive (93%)
p63	Positive (92%)
CK903	Positive (92%)
CK20	Positive (70%)
p53	Positive
BCL-2	Negative
p27	Negative

MESENCHYMAL TUMORS OCCURRING MAINLY IN CHILDREN

Mesenchymal tumors of kidney occurring in children include clear cell sarcoma, rhabdoid tumor, congenital mesoblastic nephroma and ossifying renal tumor of infancy.

CLEAR CELL SARCOMA OF KIDNEY

Clear cell sarcoma is a childhood kidney tumor most often diagnosed at 2–3 years of age. Tumor arises from renal medulla.

- There are many histologic variants of clear cell sarcoma: classic, myxoid, sclerosing, cellular, epithelioid (acinar or trabecular), palisading Verocay body, spindle cell, storiform and anaplastic.
- Classic clear cell sarcoma is composed of small plump tumor cells arranged in nests and cords and separated by networks of delicate fibrovascular stromal septa in only 20% of cases.
- BCOR gene mutation is implicated in the pathogenesis of clear cell sarcoma of kidney. Patients are treated by doxorubicin (adriamycin).

Clinical Features

Child presents with large well-circumscribed unilateral abdominal mass with mean size 11.5 cm. Bone metastases occur in 20–40% of patients more frequent than Wilms' tumor. About 5% of patients have metastatic disease at clinical presentation involving bone, lung, brain and liver.

Surgical Pathology: Clear Cell Sarcoma of Kidney

Gross Morphology

- Clear cell sarcoma of kidney is unilateral and unicentric renal mass with irregular but sharp margin in central region or medulla.
- Cut surface of tumor is homogenous tan/gray or gelatinous and firm with occasional cystic area.

Light Microscopy

- Classic pattern of clear cell sarcoma of kidney is composed of small and plump tumor cells arranged in nests and cords and separated by network of delicate fibrovascular septa (also called chicken wire appearance).
- Spindle shape cells are present near the vascular spaces, hence called septal cells, which contain smooth chromatin without prominent nucleolus, and chromatic appear washed out, similar to papillary carcinoma of thyroid gland.
- Clear cell sarcoma of kidney contains 20% clear cells as a result of intracytoplasmic vacuoles or an extracellular mucopolysaccharide matrix. Vascular invasion by tumor cells is common. Histology of clear cell sarcoma of the kidney (CCSK) is shown in Fig. 22.112.

Immunohistochemistry Technique

Markers	Expression
Vimentin	Positive
Cyclin D1	Positive
CD34 (highlights septal capillaries)	Positive
MIB1 (Ki-67)	Positive

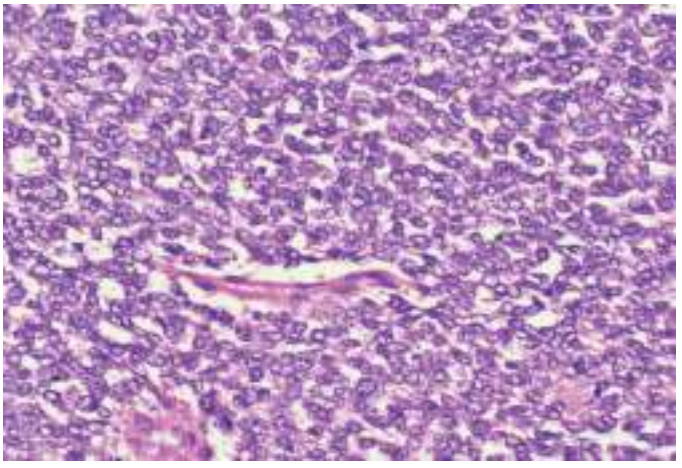


Fig. 22.112: Histology of clear cell sarcoma of the kidney (CCSK). CCSK is highly malignant neoplasm occurring in the same age group as Wilms' tumor. Originally, it was called bone metastasizing renal tumor of childhood. However, CCSK is resistant to conventional therapy, but often responsive to doxorubicin containing regimens, In H&E stained renal tissue section, tumor is highly cellular consisting of proliferating monotonous undifferentiated malignant cells of small to medium size with clear or faintly eosinophilic cytoplasm containing pleomorphic round to oval nuclei with inconspicuous nucleoli, 1–5 mitoses/high power field. Tumor shows delicate vascular stroma and extensive areas of necrosis. Tumor infiltration occurs beyond renal capsule with vascular embolization (400X). (*Courtesy:* Dr. Neha Kumari and Dr. Tushar Kalonia.)

TUMOR-LIKE LESIONS OF KIDNEY

RENAL ANGIOMYOLIPOMA (HAMARTOMA)

Renal angiomyolipoma is a hamartoma comprised of fat, smooth muscle and thick-walled blood vessels in varying proportion and haphazardly arranged, which may occur as sporadic or in association of tuberous sclerosis. It is often associated with tuberous sclerosis syndrome. It most often occurs in adults of 50 years of age. It rarely occurs in pediatric age group.

- **Sporadic renal angiomyolipoma:** Patient develops multifocal, small and bilateral renal asymptomatic or symptomatic (hematuria) lesions. Occasionally, lymph node involvement occurs but without fatal outcome.
- **Tuberous sclerosis associated renal angiomyolipoma:** It is caused by mutations in the TSC1 or TSC genes which encode the hamartin and tuberin proteins, respectively. Tuberous sclerosis is associated with multiple hamartomas within brain (as cortical tubers subependymal hamartomas), face, and kidneys (renal angiomyolipoma in 80% of cases).

Molecular Genetic Alterations

Renal angiomyolipoma in children exhibits molecular derangements such as 9q34, 16p13.3, 5q, TSC1, TSC2 and tumor suppressor genes.

Clinical Features

Patient may present with flank pain/loin mass and hematuria. Clinical picture mimics like renal cell carcinoma. Histopathologic examination confirms the diagnosis.

Surgical Pathology: Renal Angiomyolipoma

Gross Morphology

Renal angiomyolipoma reveals well circumscribed mass having variegated appearances replaces the upper pole of this kidney. In 25% of cases, the angiomyolipoma may be confused with malignancy because of extension outside the renal capsule. Gross morphology of renal angiomyolipoma is shown in Fig. 22.113.

Light Microscopy

Renal angiomyolipoma comprises mature adipose tissue, smooth muscle and thick-walled blood vessels in varying proportion and haphazardly arranged. Histology of renal angiomyolipoma is shown in Fig. 22.114.

Immunohistochemistry Technique

Panel of markers applied on formalin-fixed paraffin-embedded sections of renal angiomyolipoma expresses HMB-45, melan A, actin and vimentin.

Markers	Expression
▪ HMB-45	▪ Positive
▪ Melan A	▪ Positive
▪ Actin	▪ Positive
▪ Vimentin	▪ Positive
▪ TFE3 (transcription factor E3)	▪ Negative/positive
▪ Cathepsin K	▪ Negative/positive



Fig. 22.113: Gross morphology of renal angiomyolipoma. Tumor is usually unilateral and unifocal. Tumor ranges from 0.5 to 25 cm with a mean of 6 cm. Tumor is circumscribed, not capsulated with pushing border. Cut surface shows gray white (smooth muscle component) and yellow tissue (adipose tissue component) appearance. Tumor may involve the intrarenal venous system, renal vein or vena cava despite benign nature. Multiple or bilateral tumors suggest underlying tuberous sclerosis. Imaging often is sufficient to render the diagnosis. Radiologic findings depend on the amount of adipose tissue within the tumor. Definitive diagnosis of renal angiomyolipoma is done by histopathologic examination of tissue.

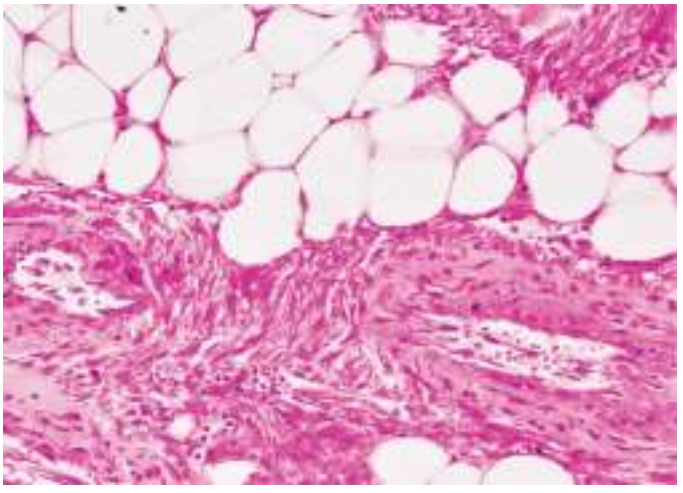


Fig. 22.114: Renal angiomyolipoma. Renal angiomyolipoma can occur sporadically or in younger patients with tuberous sclerosis. Sporadic angiomyolipoma cases are usually diagnosed in middle age adults. Renal angiomyolipoma, classic variant is a benign neoplasm composed of admixture of thick dysmorphic blood vessels, smooth muscle and adipose tissue in variable proportions and haphazardly arranged observed in hematoxylin and eosin-stained renal tissue sections (400X).

RENAL FUNCTION TESTS

The kidneys play a vital role in excretion of waste products (urea, creatinine, uric acid), reabsorption of essential metabolites, maintenance of acid–base equilibrium and calcium homeostasis, regulation of water and electrolytes; and synthesis of erythropoietin, renin and 1, 25-dihydroxyvitamin D.

- The functional unit of the kidney is the nephron which consists of the glomerulus, proximal and distal tubules, and collecting duct.
- Assessment of renal function is important in the management of patients with kidney diseases affecting various components of the kidneys.
- Generally, blood urea and serum creatinine are analyzed by taking random samples to assess renal functions. Other biochemical tests are performed such as blood sugar, serum proteins (total and albumin and globulin ratio), lipid profile and serum calcium.
- Clinically, the most practical tests to assess renal function is to get an estimate of the glomerular filtration rate (GFR) and to check for proteinuria (albuminuria).
- Azotemia refers to accumulation of nitrogenous products in the body. Uremia refers to clinical manifestations due to accumulation of nitrogenous metabolic products.
- Prerenal azotemia is caused by a decrease in cardiac output due to blood loss and congestive failure. Renal azotemia is caused by parenchymal damage to kidneys. Postrenal azotemia is caused by obstruction to urinary outflow below kidneys in the settings of prostatic hyperplasia, prostatic carcinoma and stones in ureters.
- Normal blood chemical values are given in Table 22.77. Laboratory investigations used in renal disease are given in Table 22.78.

Table 22.77 Normal blood chemical values

Substance	Normal Values
pH of blood	7.35–7.45
Blood urea nitrogen	8.0–20.0 mg/dl (2.9–7.1 mmol/L)
Creatinine	0.6–1.2 mg/dl (50–100 mmol/L)
Sodium	135–145 mEq/L
Potassium	3.5–5 mEq/L
Chloride	98–106 mEq/L
Carbon dioxide (CO_2 content)	24–29 mEq/L
Calcium	8.5–10.5 mg/dl (2.1–2.6 mmol/L)
Uric acid	▪ Male: 2.4–7.4 mg/dl (14–440 μmol/L) ▪ Female: 1.4–5.8 mg/dl (80–350 μmol/L)

Table 22.78 Laboratory investigations used in renal disease

Investigation	Reason to Evaluate	Comments
Serum potassium	▪ The kidneys are the main route of excretion of potassium ▪ Hyperkalemia occurs in acute renal failure. Levels may reach life-threatening state	Frequent monitoring of serum potassium levels, is therefore, essential
Serum sodium	Most renal diseases have a little impact on serum sodium levels. Serum sodium levels are vital in diabetes insipidus and syndrome of inappropriate ADH secretion (SIADH)	Close monitoring is vital if large volume of fluids are administered in acute renal failure and patient on hemodialysis
Blood urea	Urea is a nitrogenous waste product of proteins synthesized by the liver and excreted by kidneys	Its estimation provides a measure of the severity of the renal insufficiency in acute and chronic kidney disease
Serum creatinine	Creatinine is derived from muscle and normal levels exhibit muscle mass and racial variability	Serum creatinine levels are increased in acute and chronic kidney disease
Serum proteins	Serum proteins are decreased in nephrotic and nephritic syndrome	Proteins are lost in urine in variable proportion depending on severity of glomerular injury
Serum lipids	Serum lipids are increased in nephrotic syndrome	Liver compensates protein loss by synthesizing lipoproteins leading to increased serum lipid levels
Blood sugar	Blood sugar levels increased cause diabetic nephropathy	Blood sugar monitoring is essential in diabetes mellitus

Contd...

Table 22.78 Laboratory investigations used in renal disease (*Contd...*)

Investigation	Reason to Evaluate	Comments
Serum calcium and phosphate	Kidneys activate vitamin D_2 to D_3. This activation is impaired in chronic kidney disease	Hypocalcemia is accompanied by increased parathormone level as a consequence phosphate level are raised. Magnesium levels should be monitored
Serum complement concentration	Serum complement may be altered in systemic lupus erythematosus	Serum complement level is decreased in poststreptococcal glomerulonephritis
Arterial blood gases	Kidneys participate in maintaining acid–base equilibrium	Patient with acute renal failure develops metabolic acidosis
Serum autoantibodies	▪ Most relevance to nephrology is antineutrophilic cytoplasmic antibody (ANCA) in vasculitis ▪ Serum antinuclear antibodies (ANA) to double-stranded DNA for systemic lupus erythematosus	▪ Serum antineutrophilic cytoplasmic antibody (ANCA) is analyzed in renal vasculitis ▪ Serum antinuclear antibodies (ANA) to double-stranded DNA are analyzed in lupus nephritis
Serum immunoglobulins and electrophoresis	Serum immunoglobulins and electrophoresis are analyzed in suspected cases of myeloma causing renal impairment	Light chains form casts in tubules leading to renal impairment
Imaging techniques	▪ Plain abdominal radiograph is helpful to demonstrate calculi in urinary tract ▪ Ultrasonography evaluates obstruction, hydronephrosis and neoplasms	Nuclear medicine scans provide data of the overall excretory function of kidneys
Hemogram	Erythropoietin level is decreased in chronic renal failure. Patient develops anemia	Transferrin bound iron loss occurs in nephrotic syndrome resulting in iron deficiency anemia
Bleeding and coagulation studies	These studies are essential prior to renal biopsy	Kidney biopsy is contraindicated in bleeding and coagulation disorders. Hypercoagulable state may occur in nephrotic syndrome
Cystatin C (recent biomarker)	Cystatin C is encoded by CST3 gene. It belongs to type 2 cystine gene family. It is found in all tissues. It is excreted in urine	Increased cystatin C concentration in blood indicates impaired glomerular filtrate and renal functions

GLOMERULAR FILTRATION RATE ASSESSMENT

Glomerular filtration rate (**GFR**) represents the flow of plasma from the glomerulus into Bowman's space over a specified period and is the chief measure of kidney function. The normal GFR for an adult is 90–120 ml per minute.

- Glomerular filtrate should be freely filtered at the glomerulus. Glomerular filtrate rate depends upon: (a) renal blood flow, (b) hydrostatic pressure glomeruli (normal 60 mm Hg due to smaller lumen of efferent arterioles), (c) number of functional glomeruli, (d) blood colloidal pressure (normal 25 mm Hg), and (e) back pressure in tubules (normal 15 mm Hg).
- Filtration pressure is necessary to drive fluid across the glomerular basement membrane. It is equal to the difference between the blood pressure in the glomeruli (60 mm Hg) and sum of osmotic pressure exerted by plasma proteins (27 mm Hg) and intratubular pressure (15 mm Hg).
- Filtration pressure = Blood pressure in the glomeruli – (osmotic pressure 27 mm Hg in glomeruli + intratubular pressure 15 mm Hg).

EXOGENOUS MARKER OF GLOMERULAR FILTRATION RATE ASSESSMENT

Exogenous marker of glomerular filtration rate assessment includes infusion of inulin and then measurement of blood levels after a specified period to determine the rate of clearance of inulin. Its use is limited because purified inulin is expensive, difficulty in measuring glomerular filtration rate and time consuming for both patients and clinicians. Normal inulin clearance is 100–150 ml/minute.

- Other markers used for assessment of glomerular filtration rate are radioisotopes such as chromium-51 ethylenediaminetetra-acetic acid (**51 Cr-EDTA**), and technetium-99-labeled diethylenetriamine-pentaacetate (**^{99}Tc-DTPA**).

- The most promising exogenous marker used is the nonradioactive contrast agent such as **iohexol**, especially in children.

CREATININE CLEARANCE

Creatinine clearance is the most commonly used endogenous marker for assessment of glomerular function. Renal clearance of a substance is the amount of blood or plasma completely cleared off in a given time. The calculated clearance of creatinine is used to provide an indicator of glomerular filtrate rate. It involves the collection of urine specimen over a period of 24-hour preferably over an accurately timed period of 5–8 hours since 24-hour collected urine samples are notoriously unreliable. Creatinine clearance is then calculated using the equation: C = (U × V)/PC = clearance, U = urinary concentration, V = urinary flow rate (volume/time, i.e. ml/min), and P = plasma concentration.

- Normal creatinine clearance is 90–120 ml/minute. Creatinine is the by-product of creatine phosphate in skeletal muscle, and it is produced at a constant rate by the body. Creatinine clearance should be corrected for body surface area.
- Due to tubular secretion, creatinine overestimates glomerular filtration rate by around 10–20%. Creatinine clearance is decreased in elderly persons, acute and chronic kidney disease. Creatinine clearance is increased in normal pregnancy.
- The amount of creatinine produced per day depends on skeletal muscle bulk, and thus, there is a difference in creatinine ranges between males and females with lower creatinine values in children and those with decreased skeletal muscle bulk. Diet also influences creatinine clearance values.

TUBULAR REABSORPTION TESTS

The renal tubules play an important role in reabsorption of electrolytes, water, and maintaining acid–base balance. Electrolytes such as sodium, potassium, chloride, magnesium, phosphate can be analyzed in the urine as well as glucose. Urinary osmolality is analyzed to assess concentrating ability of urine tubules. A urinary osmolality greater than 750 mOsmol/kg H_2O indicates a normal concentrating ability of tubules.

- A water deprivation test can be performed to exclude nephrogenic diabetes insipidus as opposed to excessive intake of water. Nephrogenic diabetes insipidus (NDI) is an inability to concentrate urine due to impaired renal tubule response to vasopressin (ADH), which results in excretion of large amount of dilute urine. It can be inherited or occur secondary to conditions that impair renal concentrating ability.
- **Ammonium chloride test** can be performed to confirm diagnosis of distal renal tubular acidosis with failure to acidify the urine to a pH <5.3.
- Renal tubular acidosis is characterized by an hyperchloremic acidosis with a normal anion gap. Diagnosis is confirmed by alkaline urine pH >5 despite a metabolic acidosis (plasma bicarbonate <17.5 mmol/L) in the presence of a normal glomerular filtration rate.
- In ammonium chloride test, the ammonium chloride is converted to urea in the liver with the consumption of bicarbonate. The bicarbonate is replaced by renal synthesis with excretion of hydrogen ions. Ammonium chloride test stresses the ability of the kidneys to excrete acid and any failure to excrete acid will result in metabolic acidosis. Proximal renal tubular acidosis in Fanconi syndrome is characterized by aminoaciduria, glycosuria, and phosphaturia and bicarbonate wasting.

BLOOD BIOCHEMISTRY

BLOOD UREA NITROGEN

Urea or blood urea nitrogen (BUN) is a nitrogen-containing compound formed in the liver as the end product of protein metabolism (amino acid and pyrimidine) and urea cycle. About 85% of urea is eliminated via kidneys and rest is excreted via gastrointestinal tract. Urea is partially reabsorbed in the proximal tubules.

- Blood urea nitrogen levels depend on: (a) glomerular filtration rate, (b) protein content in diet, (c) proximal tubule reabsorption, and (d) functional status of the urea cycle. Normal serum blood urea nitrogen (BUN) is 7–18 mg/dl.
- Blood urea nitrogen is increased in prerenal diseases (gastrointestinal bleeding, dehydration, catabolic states, high protein diet, congestive heart failure), renal diseases (acute renal failure, chronic renal failure) and postrenal obstructive disorders (prostatic nodular hyperplasia, prostatic carcinoma, urolithiasis). Causes of increased blood urea nitrogen concentration are shown in Table 22.79.
- Blood urea concentration is decreased during pregnancy due to expansion of plasma volume. Blood urea concentration is also decreased in the settings of decreased synthesis of urea in cases of cirrhosis, fulminant hepatocellular failure; and dysfunctional urea cycle. Decreased amino acid degradation in the settings of kwashiorkor and starvation also results in decreased blood urea concentration.

Table 22.79 Causes of increased blood urea nitrogen concentration

Causes	Comments
Prerenal causes	▪ High protein diet, gastrointestinal hemorrhage ▪ Increased protein catabolism such as surgery, trauma, extreme starvation ▪ Impaired renal perfusion due to extracellular fluid loss, cardiac failure, hypoproteinemia
Renal causes	Reduced glomerular filtrate due to acute or chronic renal diseases
Postrenal causes	Obstruction to urine outflow due to benign prostatic hypertrophy, prostatic carcinoma, urethral stricture, obstruction and stone

SERUM CREATININE

Normal serum creatinine level is 0.6–1.2 mg/dl. Creatinine is metabolic end product of creatine in skeletal muscle. Creatine binds phosphate in skeletal muscle for ATP synthesis. Creatinine is filtered in the kidneys and not reabsorbed or secreted. It is excellent metabolic product for renal clearance testing. Serum creatinine concentration varies with age and skeletal muscle mass, i.e. increased with age and decreased in skeletal muscle wasting. Causes of increased and decreased serum creatinine are similar to those for serum blood urea nitrogen (BUN).

RATIO OF BLOOD UREA NITROGEN TO SERUM CREATININE

Ratio of blood urea nitrogen to serum creatinine is ≤15. Creatinine is filtered and is neither reabsorbed nor secreted in kidneys. Urea is filtered and partly reabsorbed in the proximal tubule. Ratio of blood urea nitrogen to serum creatinine depends etiology at the level of prerenal, renal and postrenal level. In prerenal disease the ratio is close to 20:1, while in intrinsic renal disease it is closer to 10:1.

SERUM CYSTATIN C

Cystatin C is an endogenous glomerular filtration rate marker encoded by CST3 gene. It is synthesized by all nucleated cells of body. It functions as protease inhibitor. Cystatin C is freely filtered at the glomerulus.

- Serum level of cystatin C is inversely correlated with the glomerular filtration rate (GFR). Increased serum cystatin C concentration indicates impaired glomerular filtration rate and renal functions. The renal handling of cystatin C differs from creatinine.
- While both cystatin C and creatinine are freely filtered by glomeruli, once cystatin C is filtered, it is reabsorbed and metabolized by proximal tubules, unlike creatinine.
- Unlike creatinine, cystatin C is not affected by age, skeletal muscle bulk, or diet. Hence, cystatin C is a more reliable marker of glomerular filtration rate than creatinine particularly in early stage of renal impairment. It is worth mentioning that cystatin C concentration may be affected due to malignant, thyroid disease, and tobacco smoking.

NOVEL SERUM, URINE AND TISSUE BIOMARKERS

Several novel serum, urine and tissue biomarkers have been reported to be useful to assess renal injury, which include serum biomarkers, urine biomarkers and tissue biopsy.

- Low-molecular proteins such as cystatin C, β_2-microglobulin, and retinol-binding protein present in the systemic circulation are filtered by glomeruli.
- Glomerulopathy leads to elevated concentration in the blood. Some proteins synthesized in response to cellular/tissue injury neutrophil gelatinase-associated lipocalin (NGAL).
- Novel biomarkers include kidney injury molecule 1 (KIM-1), L-type fatty acid-binding protein (L-FABP), and fibroblast growth factor 23 (FGF23). Their optimum clinical utility will be realized with ongoing studies.
- Serum neutrophil gelatinase-associated lipocalin (NGAL) is a novel biomarker for acute renal injury in critically ill children with septic shock. Kidney injury molecule (KIM-1), a transmembrane protein is a novel biomarker for human renal proximal tubular injury.
- L-type fatty acid-binding protein (L-FABP) is present in the cytoplasm of human renal proximal tubules. It is upregulated in renal proximal tubular injury.
- Fibroblast growth factor 23 (FGF23) is synthesized by bone, which acts on kidney indicating that FGF23 is systemic novel biomarker.
- Anti-phospholipase A2 receptor (PLA2R) antibodies are highly specific for the diagnosis of primary membranous glomerulopathy. Novel biomarkers for renal injury are given in Table 22.80.

URINE ANALYSIS

The best specimen for urine analysis is a freshly voided midstream urine. Midstream urine is utilized as it is less likely to be contaminated by commensal between bacteria and epithelial cells. Urine analysis involves 24 hours urine output, physical observation

Table 22.80 Novel biomarkers for renal injury

Markers	Role in Clinical Practice
Serum biomarkers	
Serum creatinine	Glomerular filtrate rate assessment
Serum cystatin C	Glomerular filtrate rate assessment (specific and accurate)
Serum neutrophil gelatinase-associated lipocalin (NGAL)	Marker of glomerular injury in children with septic shock (elevated serum NGAL in acute and chronic renal injury)
Serum kidney injury molecule 1 (KIM-1)	Unregulated in human renal proximal tubular injury
Serum L-type fatty acid-binding protein (L-FABP)	Upregulated in human renal proximal tubular injury
Serum asymmetric dimethylarginine (ADMA)	Analysis of endothelial function (inhibits nitric oxide synthase), elevated in disease progression and death
Serum PLA2R antibody	Specific marker for primary membranous glomerulonephritis
Urine biomarkers	
Urine creatinine	Analysis of renal clearance
Urine protein/albumin	Nonspecific marker of renal injury, degree of proteinuria correlates with progression of disease
Urine L-FABP	Correlates with proteinuria and predicts disease progression
Urine NGAL	Marker for tubular epithelial cell injury in acute and chronic renal disease
Urine IGFBP7 and TIMP-2	Marker of acute renal injury and cell cycle arrest
Urine uromodulin	Decreased excretion in diabetic nephropathy
Urine collage fragments	Decreased excretion in chronic renal disease
Urine MMP-7	Marker of Wnt/β-catenin signal pathway
Urine α_1-antitrypsin	Marker of glomerulopathy with nephrotic syndrome
Urine retinol binding protein (RBP)	Marker correlated with renal function
Urine β-trace protein	Marker correlated with renal function
Urine β_2-microglobulin	Marker correlated with renal function
Urine inulin	Exogenously administered marker to assess glomerular filtration rate (inulin better marker than creatinine)
Urine iothalamate	Exogenously administered marker to assess glomerular filtration rate
Urine iohexol	Exogenously administered marker to assess glomerular filtration rate
Renal DTPA scan	Exogenously administered marker to assess glomerular filtration rate by renal DTPA scan
Urine EDTA	Exogenously administered marker to assess glomerular filtration rate
Tissue biopsy	
α-SMA	Predicts renal disease progression marker for myofibroblasts
Tissue transglutamase	Positive immunohistochemical staining correlated with worsened fibrosis
MMP-1, MMP-9, IL-8, β_4-integrin, urokinase receptor	Gene subset that predicts disease progression from renal biopsy samples
PLA2R	Anti-PLA2R: a serological marker for primary membranous nephropathy

DTPA: Diethylenetriaminepentaacetic acid; EDTA: Ethylenediaminetetra-acetic acid; GFR: Glomerular filtration rate; L-FABP: Liver-type fatty acid-binding protein; MMP: Matrix metalloproteinase; NGAL: Neutrophil gelatinase-associated lipocalin; PLA2R: Phospholipase A2 receptor; RBP: Retinol-binding protein; SMA: Smooth muscle actin.

(color), pH, specific gravity, chemical and microscopic analysis by dipstick. Normal values for routine urinalysis are given in Table 22.81. Correlations of urine analysis and renal diseases are given in Table 22.82.

24 HOURS URINE OUTPUT

The normal range for 24-hour urine volume in adults is 800–2,000 ml/24 hours; with two liters fluid intake per day. Normal value ranges may vary slightly among different laboratories.

Table 22.81 Normal values for routine urinalysis

Parameters	Urinary Finding
General characteristics and measurements	
Total 24 hours urinary output	600–2500 ml (average 1200 ml)
Color	Yellow
pH	4.5–8.0 (average 5 or 6)
Specific gravity	1.005–1.025 with normal fluid intake
Chemical determinants	
Proteins	Nil
Sugar	Nil
Ketone bodies	Nil
Blood	Nil
Bilirubin	Nil
Leukocyte esterase	Nil
Nitrate for bacteria	Nil
Urobilinogen	0.5–4.0 mg/dl
Urinary sediment analysis	
Hyaline casts	Nil/occasional
Red blood cells	Nil
White blood cells	Nil
Epithelial cells	Present
Calcium oxalate crystals	Present

Contd...

Table 22.82 Correlations of urine analysis and renal diseases

Disorder	Urine Analysis	Comments
Glomerular disorders		
Acute glomerulonephritis	▪ Macroscopic hematuria (smoky colored urine) ▪ Proteinuria in nephritic range ▪ Red blood cell casts	▪ Poststreptococcal glomerulonephritis • Periorbital edema • Hypertension • Serum proteins and complement decreased • Blood urea, serum creatinine increased
Goodpasture syndrome	▪ Macroscopic hematuria ▪ Proteinuria ▪ Red blood cell casts	Antibodies directed against glomerular basement membrane
Wegener's granulomatosis	▪ Macroscopic hematuria ▪ Proteinuria ▪ Red blood cell casts	Antineutrophilic antibody
Henoch-Schönlein purpura	▪ Macroscopic hematuria ▪ Proteinuria ▪ Red blood cell casts	Following respiratory infections in children
Membranous glomerulonephritis	▪ Microscopic hematuria ▪ Proteinuria	▪ Autoimmune disorder of adults ▪ Generalized edema ▪ Serum proteins decreased ▪ Hypercholesterolemia
IgA nephropathy	▪ Hematuria ▪ Proteinuria	IgA immune complexes in mesangial region
Nephrotic syndrome	▪ Proteinuria in nephrotic range ▪ Renal tubular epithelial cells ▪ Oval fat bodies ▪ Fatty and waxy casts	Caused by many glomerulopathies (e.g. minimal change disease, membranous GN, membrano-proliferate GN, diabetic nephropathy, amyloid nephropathy)
Minimal change disease	▪ Proteinuria in nephrotic range ▪ Fat droplet	▪ Following allergic reactions in children ▪ Generalized edema ▪ Serum proteins decreased ▪ Hypercholesterolemia ▪ Prognosis excellent after steroid therapy

Contd...

Table 22.82 Correlations of urine analysis and renal diseases (*Contd...*)

Disorder	Urine Analysis	Comments
Focal segmental glomerulosclerosis	▪ Proteinuria ▪ Hematuria	▪ Drugs abuse ▪ HIV infection
Tubular disorders		
Acute tubular necrosis	▪ Renal tubular epithelial cells and casts ▪ Positive blood reagent strip	Induced by ischemia, hemoglobinuria, myoglobinuria and antibiotics
Fanconi's syndrome	▪ Proteinuria ▪ Glycosuria	General failure of tubular reabsorption
Renal glycosuria	Glycosuria	Failure of active transport of only glucose
Tubulointerstitial disorders		
Cystitis	▪ High pH ▪ Leukocyte esterase positive ▪ Nitrite positive ▪ WBCs ▪ Bacteria	Urinary bladder infection
Acute pyelonephritis	▪ Leukocyte esterase positive ▪ Nitrite positive ▪ WBC casts	Tubular infection indicated by presence of WBC casts
Chronic pyelonephritis	▪ High pH ▪ Leukocyte esterase positive ▪ Nitrite positive ▪ WBCs granular, waxy and broadcasts	Pyogenic infection due to major anatomic abnormalities may presents with fever, malaise and flank pain
Acute interstitial nephritis	▪ Hematuria ▪ Proteinuria ▪ WBC casts	▪ Induced by medications ▪ Eosinophils in urine ▪ Bacteria absent
Renal lithiasis (calculi)	Microscopic hematuria	Patient presents with severe renal colic

- Oliguria is defined as urine output <400 ml/24 hours. Urine output is decreased in dehydration, hypovolemic shock, acute and chronic kidney disease, multiple organ failure and urinary dysfunction retention.
- Polyuria is excessive or an abnormally large production or passage of urine (>2.5–3.0. L/24 hours in adults). Polyuria occurs in the settings of diuretics use, uncontrolled diabetes mellitus, central diabetes insipidus and nephrogenic diabetes insipidus.

PHYSICAL EXAMINATION

The normal color of urine is straw colored in the presence of dehydration urine is a darker color.

- Cloudy or milky urine usually accompanied by bad smell possibly indicates urinary tract infection.
- Red urine may indicate hematuria or porphyria or represent the dietary intake of food like beets.
- Pink, red or light brown urine is caused by hemolytic anemia, neoplasms located in kidney or urinary bladder and porphyria.
- Dark yellow or orange urine, indicates recent intake of vitamin B containing nutrient supplement, carotene, phenazopyridine, rifampicin, warfarin and laxative. Urine color in various conditions is given in Table 22.83.

pH AND SPECIFIC GRAVITY

Normal urine pH is 4.5–8.0 (average 5 or 6) and specific gravity as 1.005–1.025 with normal fluid intake. Urine specific gravity is a measure of the ratio of the density of urine to the density of water. Specific gravity is estimated by Vogal's urinometer.

- Increased urine specific gravity means increased concentration of solutes in the urine in the settings of dehydration, diarrhea, excessive sweating, glucosuria, renal artery stenosis decreased renal blood flow and hepatorenal syndrome.
- Specific gravity is decreased in the settings of renal failure, pyelonephritis, diabetes mellitus, acute tubular necrosis, interstitial nephritis and excessive intake of fluids.

Table 22.83 Urine color in various conditions

Color	Conditions
Normal urine color	Yellow amber colored
Dark yellow or orange color	▪ Concentrated urine ▪ Diet-rich in carotene and vitamin B complex ▪ Durgs—rifampicin, warfarin, laxative and phenazopyrine
Red color	▪ Hematuria ▪ Myoglobinuria ▪ Hemoglobinuria ▪ HbO_2
Cloudy or milky color	Leukocytes in urinary tract infection
Orange	Urates
Brown	Bilirubin
Brown black color	▪ Melanin ▪ Methemalbuminemia
Blue/green color	▪ Sorbitol therapy ▪ M-Dopa therapy
Yellow color	▪ Pus cells ▪ Tetracycline therapy

CHEMICAL EXAMINATION

Urine dipstick provides qualitative analysis of different analytes in urine using chemical analysis. Dipstick uses dry chemical methods to detect for the presence of protein, glucose, blood, ketones, bilirubin, urobilinogen, nitrite, and leukocyte esterase. The color changes following interaction of the urine with the chemical reagents impregnated on the paper of the dipstick are compared to the color chart guide to interpret the results. Analytes tested on urine dipstick-protein should not be detectable in normal urine specimens. Schematic representation of dipstick is shown in Fig. 22.115.

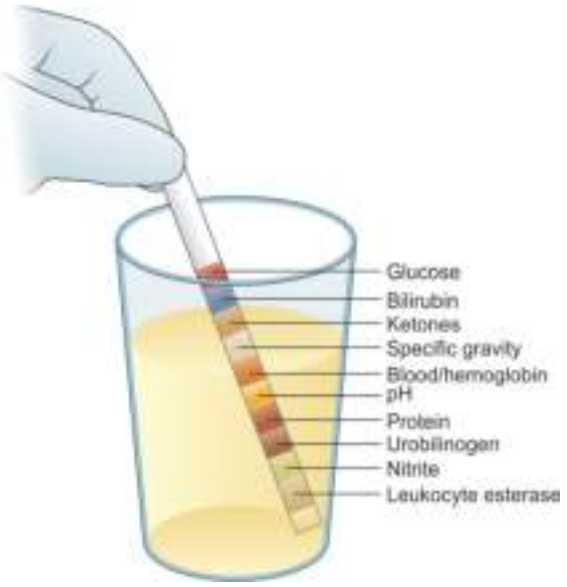


Fig. 22.115: Schematic representation of dipstick. Dipstick is a bedside qualitative estimation of specific gravity, leukocyte esterase and pH, urinary chemical constituents such as, protein, glucose, ketone bodies, bilirubin, urobilinogen, hemoglobin.

Albuminuria/Proteinuria

Normal persons excrete proteins 150 mg/24 hours in urine (30% albumin; 30% globulins; 40% Tamm-Horsfall protein).

- Microalbuminuria refers to the presence of albumin 30–300 mg per day, which is a marker of glomerular endothelial dysfunction and cardiovascular disease.
- Urine albumin may be analyzed in 24-hour urine sample in early morning/random specimens as an albumin/creatinine ratio. Presence of albuminuria on two occasions with the exclusion of a urinary infection indicates glomerular dysfunction.
- Functional proteinuria is observed in fever, congestive heart failure and orthostatic position.
- Frank proteinuria is defined >300 mg/24 hours. Selective proteinuria refers to presence of albumin in urine. Nonselective proteinuria refers to presence of both albumin and globulin in the urine.
- Increased amount of protein in urine may be due to injury to glomeruli (glomerulonephritis), incomplete tubular reabsorption of proteins (interstitial nephritis, Fanconi syndrome due to inability to absorb glucose, amino acids, phosphate, bicarbonate and uric acid; and Hartnup's syndrome due to defect in reabsorption of neutral amino acids (tryptophan) in the gastrointestinal tract and kidneys), overflow proteinuria (multiple myeloma—Bence Jones proteins, myoglobinuria) and urinary tract infection and tumors.
- Nephritic syndrome is characterized by proteinuria (<2 g/24 hours), oliguria (<400 ml/24 hours), passage of smoky colored urine, periorbital edema and hypertension.
- Nephrotic syndrome is characterized by massive proteinuria (≥3.5 g/24 hours), hypoproteinemia, hypercholesterolemia and generalized edema.

Glucosuria

Glucose is the main type of sugar in the bloodstream, which is the major source of energy for body's cells. Glucose is derived from foods and other substances. Several hormones including insulin and glucagon regulate glucose levels in the blood. Glucose is not detectable in urine samples of normal persons. Glucosuria may be present in diabetes mellitus and pregnancy. Renal glycosuria occurs when the renal threshold is decreased.

Ketone Bodies

Ketone bodies are produced by the liver and used as an energy source when glucose is not readily available. Acetoacetate and 3β-hydroxybutyrate exist in

abundance. While acetone is least abundant. Ketone bodies are normally present in the blood. Blood ketone bodies level is increased in the settings of fasting, vomiting, prolonged exercise and diabetic ketoacidosis. Therefore, these persons excrete abundant ketone bodies in the urine.

Bile Salts

Bile salts are composed of the four different types of free **bile acids** such as cholic acid, deoxycholic acid, chenodeoxycholic acid and lithocholic acid. Each of these bile acids may turn combine with glycine or taurine to form more complex acids and salts. Normal urine does not contain bile salts. Bile salts are detected in the urine in obstructive jaundice.

Bile Pigments

Bile pigment is the product of hemoglobin breakdown excreted in bile. There are two types of bile pigments: (a) bilirubin (yellow in color) and (b) biliverdin (green in color). Biliverdin is the oxidized form of bilirubin in the bile pigment excreted through urine. Normal urine does not contain bilirubin. The presence of bile pigments is an indication of liver dysfunction. Jaundice occurs due to liver dysfunction, which results in the detection of abnormally high levels of bilirubin in the bloodstream and urine, as the liver is unable to filter out excess bilirubin.

Urobilinogen

Urobilinogen is a by-product of bilirubin that is eventually eliminated through urine and stool. A urobilinogen in urine analysis measures the quantity of urobilinogen in a urine sample ranging from 0.1 to 1.8 mg/dl. Urobilinogen is formed from reduction of bilirubin. Bilirubin is a yellow-colored pigment produced by breakdown of red blood cells. Normal urine contains some quantity of urobilinogen. Urobilinogen is absent in urine in conjugated hyperbilirubinemia and increased in the settings of liver diseases such as hepatitis and cirrhosis.

MICROSCOPIC EXAMINATION

The microscopic urine analysis involves wet-preparation analysis of urine to assess in the presence of red blood cells, white blood cells, hyaline casts, and crystals as well as microorganisms. In normal urine red blood cells per high-power field is between 0 and 3 and white blood cells (WBCs) per high-power field is between 0 and 5.

Hematuria

Presence of red blood cells indicates renal injury. Causes of hematuria are glomerulonephritis, interstitial renal disease, renal papillary necrosis, renal cell carcinoma, transitional cell carcinoma of urothelium, urinary tract infection, urolithiasis, renal tuberculosis and exacerbation by anticoagulants. Causes of hematuria are given in Table 22.84.

Table 22.84 Causes of hematuria

Glomerulonephritis
Interstitial renal disease
Renal papillary necrosis
Renal cell carcinoma
Transitional cell carcinoma of urothelium
Urinary tract infection
Urolithiasis
Renal tuberculosis
Exacerbation by anticoagulants

Pus Cells in Urine

Pus cells are demonstrated in urinary tract infection. Presence of nitrites and leucocyte esterase in urine are indicators of urinary tract infection. Some bacteria, for example, Enterobacteriaceae, convert nitrates to nitrites.

Casts in Urine

Hyaline casts consist of proteins and occur in glomerular disease. Red blood cell casts usually denote glomerulonephritis. White blood cell casts are demonstrated in patients of pyelonephritis.

Crystals in Urine

Crystals may also be detected in urine. The type of crystals formed depends on urine pH. Calcium oxalate, uric acid, amorphous urate, leucine, tyrosine and cysteine crystals are present in acidic pH urine. Amorphous phosphate, triple phosphate, calcium carbonate and ammonium biurate crystals are present in alkaline pH urine.

- Calcium oxalate crystals are envelope-shaped and are present in ethylene glycol poisoning or primary and secondary hyperoxaluria.
- Uric acid crystals are needle-shaped and are associated with gout.
- Triple phosphate crystals are rectangular are demonstrated in pseudogout.
- Cysteine crystals are hexagonal and seen in cystinuria. Schematic representation of urinary sediment analysis is shown in Figs 22.116 and 22.117.

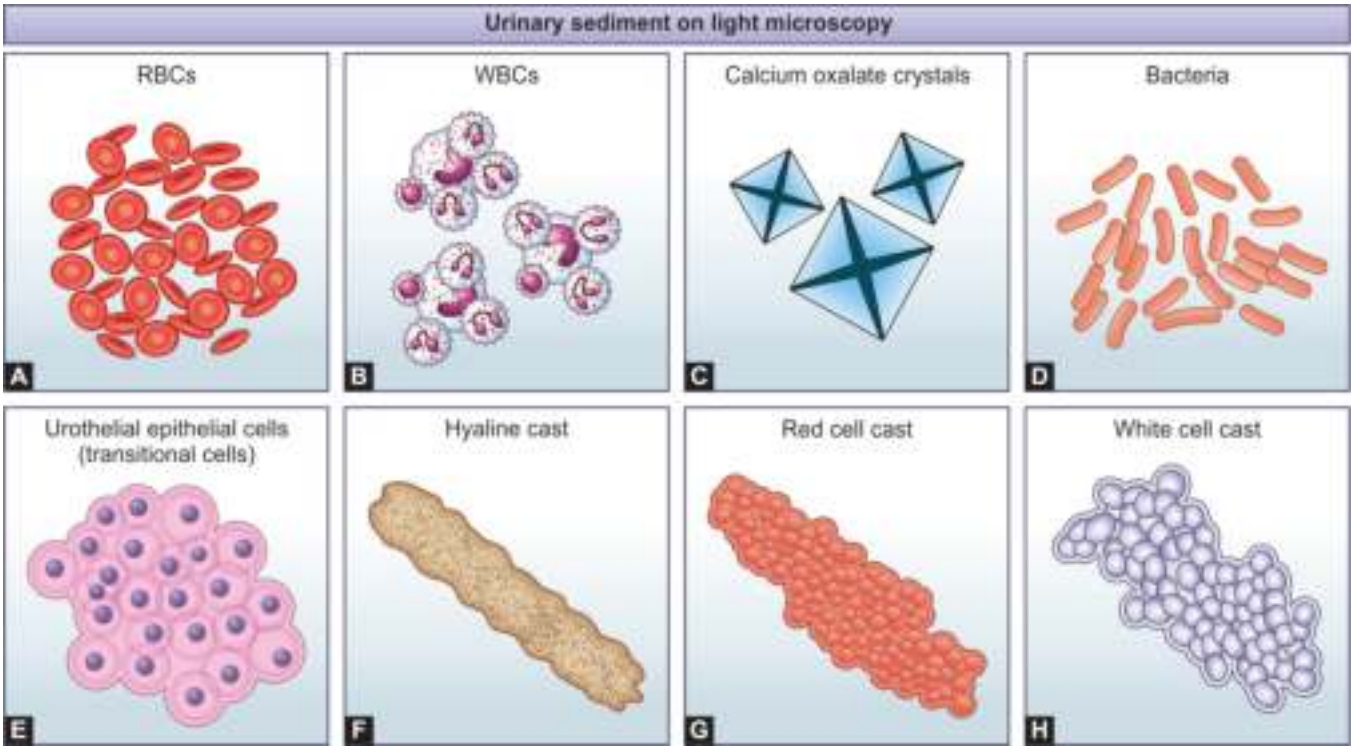


Fig. 22.116A to H: Schematic diagram of urinary sediment. Urinary sediment is examined under light microscopy to demonstrate RBCs, pus cells, crystals, bacteria, epithelial cast, hyaline cast and white cell cast in various urinary tract disorders.

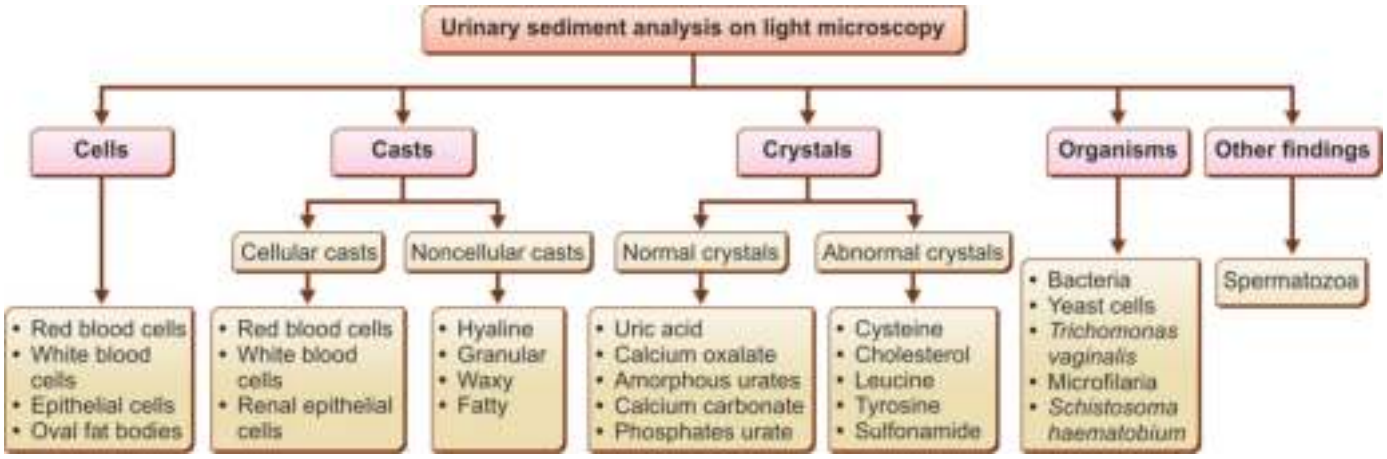


Fig. 22.117: Urinary sediment analysis by light microscopy.

URINE CULTURE AND SENSITIVITY TEST

Urine contains a low-level of bacterial community, known as a microbiota in healthy adults, who are free from a urinary tract infection (UTI). The female and male urinary tracts are relatively same except for the length of urethra. Female urethra is short, hence prone to urinary tract infection.

- Certain conditions can also increase the risk for urinary tract infection, including diabetes mellitus, nephrolithiasis, an enlarged prostate in men, pregnancy, as well as urinary tract surgery. However, in the process of urine specimen collection, some contamination occurs from skin microbiota.
- Patients with urinary tract infection present with pain or burning during urination. Urine may look cloudy or bloody, and it might give off a bad smell. UTI is diagnosed by taking a urine sample and checking for bacteria.
- Urine culture and sensitivity is used to diagnose UTI and to identify the bacteria or yeast causing urinary tract infection. If bacteria multiply, an antibiotic sensitivity or susceptibility test can identify the antibiotic most likely to eradicate those bacteria.

CHAPTER

23

Urinary Bladder and Male Reproductive System

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

DISORDERS OF URINARY BLADDER

- Anatomy and histology
- Benign non-neoplastic disorders
  - Acute cystitis
  - Chronic cystitis
  - Granulomatous cystitis
  - Interstitial cystitis
  - Hemorrhagic cystitis
  - Papillary and polypoidal (bullous) cystitis
  - Eosinophilic cystitis
  - Cystitis glandularis and/or cystitis cystica
  - Malakoplakia of urinary bladder
  - Exstrophy of urinary bladder
  - Urachal sinus
  - Urachal diverticulum
  - Urinary bladder diverticula
- Primary urothelial neoplasms
  - Urothelial carcinoma
    - Noninvasive papillary urothelial neoplasia
    - Endophytic/inverted urothelial neoplasia
    - Flat urothelial lesions with atypia
    - Invasive urothelial carcinoma
  - Primary squamous cell carcinoma
  - Primary adenocarcinoma
  - Botryoid rhabdomyosarcoma

DISORDERS OF TESTES

- Testes: overview
  - Anatomy
  - Histology
  - Spermatogenesis
  - Semen (seminal fluid)
    - Semen analysis
    - Cryopreservation of spermatozoa
- Inflammatory disorders of testis
  - Bacterial orchitis
  - Mumps orchitis
  - Tuberculous epididymo-orchitis
  - Granulomatous orchitis
  - Syphilitic orchitis
- Neoplastic disorders of testis
  - Germ cell tumors of testis
    - Seminoma of testis
    - Spermatocytic seminoma of testis
    - Embryonal carcinoma of testis
    - Yolk sac tumor
    - Teratoma of testis
    - Choriocarcinoma of testis
    - Mixed germ cell tumors of testis
    - Burnt-out germ cell tumors of testis
  - Sex cord/stromal tumors of testis
    - Sertoli cell tumor (androblastoma) of testis
    - Leydig cell tumor of testis
  - Other malignant tumors of testis
    - Testicular lymphoma
    - Testicular plasmacytoma
    - Leukemic infiltration and myeloid sarcoma of testis
    - Metastases in testis
- Neoplastic disorders of mesothelium
  - Adenomatoid tumor
- Non-neoplastic disorders of mesothelium
  - Testicular torsion
  - Hydrocele
  - Hematocele
  - Spermatocele
  - Varicocele
  - Male hypogonadism
  - Male impotence
  - Cryptorchidism
  - Testicular atrophy
  - Male pseudohermaphroditism

DISORDERS OF PROSTATE GLAND

- Anatomy and histology—prostate
- Inflammatory disorders—prostate
  - Acute bacterial prostatitis
  - Asymptomatic inflammatory prostatitis
  - Chronic prostatitis
  - Nonspecific granulomatous prostatitis
- Benign nodular hyperplasia of prostate gland
- Neoplastic disorders of prostate
  - Prostatic intraepithelial neoplasia
    - Genetic alterations and immunohisto-chemical markers
  - Intraductal and invasive prostatic adenocarcinoma
    - Molecular genetic alterations
    - Clinical features
    - Diagnostic modalities
    - Serum tumor markers in prostatic adenocarcinoma
    - Gleason grading system
    - Gleason staging system
    - Prognostic factors
    - Therapeutic correlation

DISORDERS OF PENIS

- Anatomy of penis
- Congenital anomalies of penis and miscellaneous disorders
- Inflammatory disorders of penis
- Neoplastic disorders of penis

DISORDERS OF URINARY BLADDER

ANATOMY AND HISTOLOGY

Urinary bladder is a hollow musculomembranous sac, which acts as a reservoir for the urine. It is the most anterior organ of the pelvic viscera. It has superior, right inferolateral, left inferolateral and posterior surfaces. It is subperitoneal organ and has parietal peritoneum only on its superior surface. Urine enters the urinary bladder via ureters and exits via the urethra.

- Body of the urinary bladder is lined by transitional epithelium, which can hold 400–1000 ml of urine.
- Trigone urinary bladder has the shape of inverted triangle found on the fundus formed by right and left ureteral orifices. Once the trigone is stretched to a certain degree, signal is transmitted to the brain that urinary bladder needs to be emptied.
- Ureteral orifices are slit-like openings through which ureters enter the urinary bladder on the posterolateral angles of the trigone of the urinary bladder.
- Neck is the lowest portion of urinary bladder through which the urethra arises. Internal urethral sphincter is composed of smooth muscle located at the junction of urethra and the urinary bladder innervated by S2–S4 nerves of the pelvic plexus, which prevents the retrograde ejaculation of semen into the urinary bladder.
- Detrusor muscle is composed of inner and outer longitudinal, and middle circular layer.
- Urinary bladder is supplied by parasympathetic pelvic nerve, pudendal somatic nerve and sympathetic hypogastric nerve.
- Urinary bladder is supplied by internal iliac arteries. Superior vesical arteries supply anterosuperior surface of the urinary bladder. In males, inferior vesical arteries supply fundus and neck of the urinary bladder. In females, vaginal arteries, obturator and inferior gluteal arteries supply the posteroinferior surface of the urinary bladder. The veins draining from the urinary bladder correspond to the arteries. Veins from the vesical venous plexus drain into internal iliac veins.
- Lymphatic vessels in both sexes leave the superior surface of the urinary bladder and drain into external iliac lymph nodes. Lymphatic vessels from the fundus drain into the internal iliac lymph nodes. Lymphatic vessels from the neck of the urinary bladder drain into the sacral and common iliac lymph nodes.
- Urinary bladder is lined by specialized stratified epithelium, the urothelium termed 'transitional epithelium'. Urothelium is present in ureter, urinary bladder and proximal urethra. Apical layer of urothelium is umbrella-shaped, intermediate layer consists of two to three layers of polygonal cells; and basal layer is composed of two to three layers of small cuboidal cells. Lamina propria is the suburothelial layer separating the urothelium by a basement membrane and underlying muscularis mucosa. Muscularis propria is composed of smooth muscle coat with the underlying tissue. Serosa is connective tissue covered with the mesothelial lining partially on the surfaces of the urinary bladder, that has a peritoneal covering, which also contains blood vessels of various sizes.

BENIGN NON-NEOPLASTIC DISORDERS

Benign non-neoplastic disorders of urinary bladder include cystitis (acute, chronic, granulomatous, interstitial, hemorrhagic, papillary polypoid, eosinophilic), cystitis glandularis, malakoplakia, exstrophy, persistence of the urachal sinus, vesicoumbilical fistula or urachal diverticulum and urinary bladder diverticulum. Benign non-neoplastic disorders of urinary bladder are given in Table 23.1.

Table 23.1 Benign non-neoplastic disorders of urinary bladder

Benign Non-neoplastic Disorders	Etiology
Acute infectious cystitis. Intermittent urinary obstruction in females	▪ *Escherichia coli* (85%) ▪ *Klebsiella pneumoniae* ▪ *Proteus vulgaris* ▪ Enterococci
Chronic infectious cystitis	Cystoscopy or indwelling catheters for prolonged periods and intermittent urinary obstruction
Granulomatous cystitis	Bacteria, fungi, parasites and intravesical immunotherapy
Chronic interstitial cystitis	Middle-aged and elderly women
Hemorrhagic cystitis	▪ Cyclophosphamide therapy ▪ Adenovirus infection in immunocompromised persons
Papillary and polypoid (bullous) cystitis	Prolonged indwelling catheter

Contd...

Table 23.1 Benign non-neoplastic disorders of urinary bladder (*Contd...*)

Benign Non-neoplastic Disorders	Etiology
Eosinophilic cystitis	▪ Lamina propria of urinary bladder is infiltrated by eosinophils due to allergic and parasitic disorders ▪ Eosinophils may be present in the adjoining areas of urothelial carcinomas
Cystitis glandularis	▪ Chronic bladder inflammation ▪ Urinary bladder exstrophy ▪ Chronically infected neurogenic bladder ▪ Urinary bladder stones ▪ Urinary bladder with indwelling catheter
Malakoplakia	Soft yellow mucosal plaque shows abundant epithelioid histiocytes (known as Hansemann histiocytes) containing basophilic intracytoplasmic granules known as Michaelis-Gutmann bodies containing iron (Perls' stain) and calcium (von Kossa)
Exstrophy of urinary bladder	Absence of the anterior part of the urinary bladder and abdominal wall
Persistence of the urachal sinus	Persistence of the urachal sinus results in drainage of urine from the umbilicus of a newborn
Vesicoumbilical fistula or urachal diverticulum	Occur due to failure of the urachus to involute
Urinary bladder diverticulum	Benign nodular hyperplasia prostate resulting in obstruction and recurrent infections

ACUTE CYSTITIS

Ascending infection from the urethra is the most common route of infection causing cystitis. It most often occurs in women because of short urethra, especially during pregnancy. In acute cystitis, urinary bladder stroma is edematous and hemorrhagic; infiltrated by neutrophils of variable intensity. Patient presents with dysuria, increased frequency of urination, a sense of urgency, and discomfort over the urinary bladder (Fig. 23.1).

Pathogenesis

Escherichia coli is the most common cause of acute cystitis in 85% of cases. *Proteus vulgaris*, *Klebsiella pneumoniae*, and Enterococci are also responsible for acute cystitis in some cases. *Staphylococcus saprophyticus*, a coagulase negative bacterium, is associated acute cystitis in 10–20% of young sexually active females.

- Predisposing factors for acute cystitis prior instrumentation or catheterization, radiation therapy and chemotherapy. Urine examination reveals inflammatory cells.
- Causative agent can be identified by urine culture. Most cases of acute cystitis respond well to treatment with antimicrobial agents.

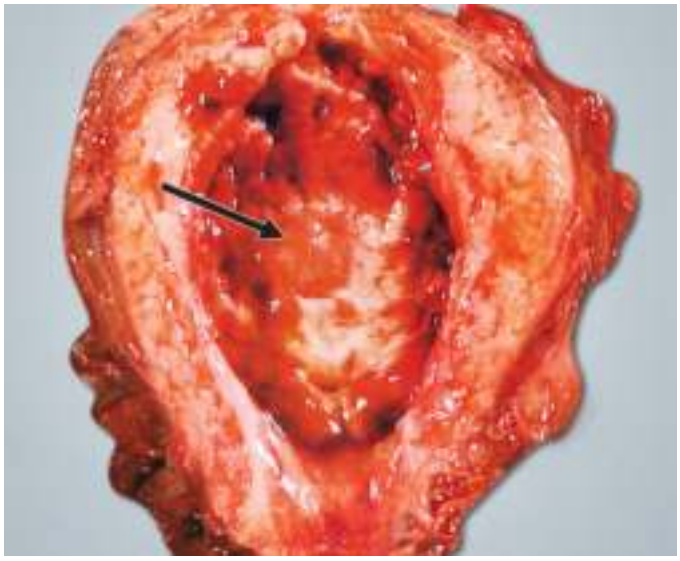


Fig. 23.1: Acute cystitis. The mucosal surface of urinary bladder is red and inflamed. The patient had an indwelling catheter for many weeks before death from other pathology (arrow) 400X.

CHRONIC CYSTITIS

Chronic cystitis is associated with lack of resolution of the inflammatory reaction in patients with acute cystitis. It occurs due to introduction of pathogens into the urinary bladder during cystoscopy or indwelling catheter remaining for prolonged periods. Urinary bladder stroma shows predominance of lymphocytes and fibrosis of the lamina propria.

GRANULOMATOUS CYSTITIS

Granulomatous cystitis is caused by bacteria, fungi, parasites and intravesical immunotherapy. Histologic features of urinary bladder biopsy specimen show granulomas and chronic inflammatory cells.

INTERSTITIAL CYSTITIS

Interstitial cystitis, also referred as Hunner's ulcer, which may be of autoimmune nature. The disorder is most commonly seen in middle-aged women with systemic lupus erythematosus or other autoimmune disorders. Mucosa and submucosa of urinary bladder show infiltration by chronic inflammatory cells with numerous mast cells. Patient presents with

suprapubic pain, frequency and urgency with or without hematuria.

HEMORRHAGIC CYSTITIS

Hemorrhagic cystitis is caused by cyclophosphamide therapy and adenovirus infection in immunocompromised persons.

PAPILLARY AND POLYPOID (BULLOUS) CYSTITIS

Papillary polypoid (bullous) cystitis occurs due to prolonged indwelling catheter. Lesion is difficult to differentiate from low-grade urothelial neoplasm.

EOSINOPHILIC CYSTITIS

Eosinophilic cystitis is characterized by presence of eosinophils in lamina propria of urinary bladder due to allergic conditions and parasitic infestations. Eosinophils may be present in the adjoining areas of urothelial carcinomas.

CYSTITIS GLANDULARIS AND/OR CYSTITIS CYSTICA

Cystitis glandularis occurs due to number of chronic stimuli such as recurrent cystitis, neurogenic urinary bladder, exstrophy of urinary bladder and urinary bladder chronically irritated by stones or an indwelling catheter. Nests of urothelium (Brunn nests) grow inward and undergo metaplastic changes (cystitis glandularis) and/or cystic dilatation (cystitis cystica). There is no evidence that cystitis glandularis/cystitis cystica predisposes to urinary bladder carcinoma.

Surgical Pathology: Cystitis Glandularis
Gross Morphology
Cystitis glandularis takes the form of focally or diffusely inflamed edematous or polypoid bladder mucosa.
Light Microscopy
Histologic examination of biopsy specimen reveals glandular structures and cystic spaces within the urinary bladder wall infiltration by chronic inflammatory cells and intestinal metaplasia (Fig. 23.2).

MALAKOPLAKIA OF URINARY BLADDER

Malakoplakia is granulomatous disorder of urinary bladder of unknown etiology, which involves skin and other organs such as kidneys, colon, lung and prostate gland. Predisposing factors for malakoplakia of urinary bladder are chronic infections, immunosuppressive state and cancers. Malakoplakia is characterized by formation of soft yellow-brown plaques on the mucosal surface of the urinary bladder.

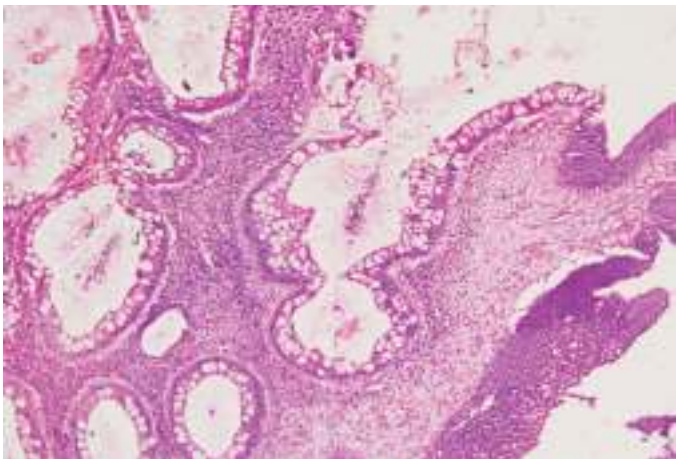


Fig. 23.2: Cystitis glandularis. Histologic examination of urinary bladder specimen reveals glandular structures and cystic spaces within the urinary bladder wall infiltration by chronic inflammatory cells and intestinal metaplasia (400X).

- Malakoplakia of urinary bladder probably occurs due to deficient phagocytic function of macrophages and recurrent infections by *Escherichia coli* and *Proteus vulgaris*.
- On cystoscopy, malakoplakia mimics neoplasia in patient with a long history of recurrent urinary infection, and recent hematuria.
- Soft yellow-brown plaques on the mucosal surface of urinary bladder represent expansion of mucosa by the presence of numerous histiocytes with lysosomal dysfunction, abundant eosinophilic cytoplasm containing PAS positive granules.
- Some of the macrophages show laminated, basophilic deposits of calcium termed **Michaelis-Gutmann bodies**. Histologic features of malakoplakia of urinary bladder are shown in Fig. 23.3.

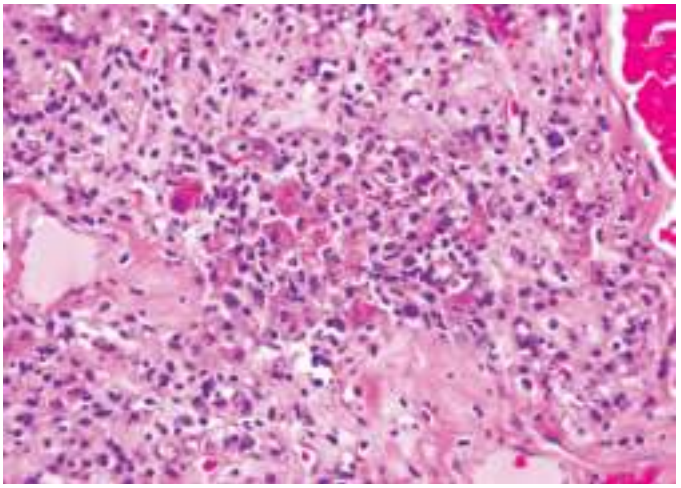


Fig. 23.3: Malakoplakia of urinary bladder. It shows intracellular and extracellular target-like structures termed Michaelis-Gutmann bodies formed due to deposition of calcium (400X).

EXSTROPHY OF URINARY BLADDER

Exstrophy of urinary bladder refers absence of the anterior surface of the urinary bladder and abdominal wall; that can be recognized at the time of birth. In some male infants, exstrophy of urinary bladder is associated with epispadias (incomplete formation of the penile urethra).

- In patients with exstrophy of urinary bladder, posterior wall of urinary bladder undergoes mechanical trauma and undergoes squamous or glandular metaplasia and recurrent infections.
- Despite surgical repair, there is an increased risk for squamous cell carcinoma or adenocarcinoma of urinary bladder in the age group of 50–60 years.

URACHAL SINUS

Urachal sinus is a rare type of vitellointestinal duct anomaly. Vitellointestinal duct or omphalomesenteric duct (OMD) is an embryonic structure, which connects the yolk sac to the midgut and failure of its involution results in various anomalies including Meckel's diverticulum, patent vitellointestinal duct, sinus tract, sinus cyst and umbilical polyp.

- Clinical presentation is persistent umbilical discharge such as urine and fecal matter in pediatric age group. Moreover, urachal sinus has different clinical course in adults than pediatric age group in which it undergoes involution and has a benign clinical course.
- The remnants of urachal sinus are prone to recurrent infection and development of malignancy.
- A proper diagnostic workup by clinical examination and imaging tool is required.
- Early excision of the urachal sinus at initial diagnosis is key for preventing further morbidities.

URACHAL DIVERTICULUM

Urachal diverticulum is the rarest type of congenital urachal anomaly, which occurs due to failure of the distal urachus to close its point of connectivity to the urinary bladder. The disorder is usually asymptomatic but can be associated with recurrent urinary tract infections and other complications such as vesicoumbilical fistula.

URINARY BLADDER DIVERTICULA

Urinary bladder diverticula secondary to benign nodular hyperplasia of prostate is a complication that can lead to obstruction to urinary flow, stone formation in urinary bladder, urinary tract infection (UTI) and development of tumors in the urinary bladder.

Cystoscopy can demonstrate solitary or multiple sac-like outpouchings in the urinary bladder wall.

PRIMARY UROTHELIAL NEOPLASMS

UROTHELIAL CARCINOMA

Urothelial carcinoma (historically known as transitional cell carcinoma) accounts for 90% of all primary tumors of the urinary bladder. Most cases of urothelial carcinoma *in situ* progress subsequently to invasive urothelial carcinoma by invading the lamina propria.

- Development of urothelial carcinoma seems to be dependent on a combination of genetic and environmental factors (tobacco smoking, industrial petrochemical exposure, aniline dyes particularly benzidine and β-naphthylamine).
- *Schistosoma haematobium* is pathogenetically related to both urothelial carcinoma and squamous cell carcinoma but not adenocarcinoma of urinary bladder.

Table 23.2 Features of urothelial lesions with atypia, noninvasive papillary urothelial neoplasms and invasive urothelial neoplasms

Urothelial Lesion	Morphological Features on Cystoscopy	Histologic Features	Malignant Potential and Recurrence after Surgery
Flat urothelial lesions with atypia			
Urothelial dysplasia	Flat lesion	▪ Dysplastic urothelial cells with loss of polarity ▪ Nuclei enlarged with coarse chromatin	▪ Malignant potential (15%) ▪ No recurrence after surgery
Urothelial carcinoma *in situ* (CIS)	Flat lesion	Dysplastic cells of entire urothelium from basal layer to surface	▪ Malignant tumor ▪ No recurrence after surgery
Papillary urothelial neoplasms			
Urothelial papilloma	Single papillary frond associated with painless hematuria	▪ Tumor cells with delicate papillary frond ▪ Minimal branching or fusion	▪ Malignant potential (2%) ▪ Recurrence after surgery (<80%)
Inverted papilloma	Sessile or polypoidal growth	▪ Tumor cells are forming anastomosing cords growing towards lamina propria ▪ Urothelial cells bland	▪ No malignant potential ▪ Rare recurrence after surgery

Contd...

Table 23.2 Features of urothelial lesions with atypia, noninvasive papillary urothelial neoplasms and invasive urothelial neoplasms (*Contd...*)

Urothelial Lesion	Morphological Features on Cystoscopy	Histologic Features	Malignant Potential and Recurrence after Surgery
Noninvasive papillary urothelial neoplasm of low malignant potential (PUNLMP)	Single papillary frond of 2–5 cm size	▪ Tumor cells are with large nuclei forming fused papillae ▪ Mitotic figures rare in basal cells	▪ Malignant potential tumor (4%) ▪ Recurrence after surgery (25–30%)
Noninvasive papillary urothelial carcinoma (low-grade)	Single or multiple tumors	▪ Tumor cells are pleomorphic forming frequent branching and fusion of papillae ▪ Mitoses occasional at any level	▪ Malignant potential tumor (2–20%) ▪ Recurrence after surgery (64–70%)
Noninvasive papillary urothelial carcinoma (high-grade)	Tumor invading urinary bladder wall	▪ Tumor cells are pleomorphic forming frequent branching and fusion of papillae ▪ Mitoses frequent	▪ Malignant potential high-grade tumor (18%) ▪ Recurrence after surgery (56–68%)
Invasive urothelial neoplasms			
Urothelial carcinoma with squamous differentiation or glandular differentiation and trophoblastic differentiation	Exophytic cauliflower-like or ulcerated growth	▪ Tumor cells contain hyperchromatic nuclei (well differentiated to poorly differentiated showing squamous, glandular or trophoblastic differentiation) ▪ Mitoses numerus	▪ Malignant potential high-grade tumor ▪ Recurrence after surgery (100%)
Squamous cell carcinoma	Large fungating solitary tumor with necrosis	Well-differentiated squamous cell carcinoma	▪ Malignant potential high-grade tumor ▪ Recurrence after surgery (100%)
Adenocarcinoma	Growth invading smooth muscle	Well-differentiated adenocarcinoma	▪ Malignant potential high-grade tumor ▪ Recurrence after surgery (100%)

- Most cases of urothelial carcinoma of urinary bladder affect persons over the age of 50 years. However, it may rarely affect young adults and children. Men are more affected than women. Features of urothelial lesions with atypia, noninvasive papillary urothelial neoplasms and invasive urothelial neoplasms are given in Table 23.2.

Noninvasive Papillary Urothelial Neoplasia

Recent 2016 WHO and the International Society of Urological Pathology (ISUP) classified noninvasive papillary neoplasms into flat lesions and papillary neoplasms: urothelial papilloma, papillary urothelial neoplasm of low-malignant potential (PUNLMP), low-grade papillary urothelial carcinoma; and high-grade papillary urothelial carcinoma. Histologic features used to classify urothelial papillary lesions according to recent 2016 WHO and International Society of Urological Pathology (ISUP) are given in Table 23.3.

- Noninvasive urothelial papilloma of exophytic type is composed of relatively simple branching papillary excrescences, histologically normal lining epithelium with orientation of the neoplastic cells, perpendicular to the basement membrane.
- Noninvasive urothelial neoplasm of low-malignant potential (PUNLMP) is similar, but epithelial lining appears hyperplastic compared with normal epithelium.
- Noninvasive low-grade papillary carcinoma has a more disorganized urothelium that loses polarity with mild atypia. The papillae may be more complex and confluent compared to PUNLMP.
- Noninvasive high-grade papillary urothelial carcinoma is composed of neoplastic cells of variable size and shape with hyperchromatic nuclei.

Endophytic/Inverted Urothelial Neoplasia

Endophytic/inverted urothelial neoplasia is a benign epithelial tumor, which usually affects adults and elderly males. It is usually a solitary tumor located in the trigone or neck regions of urinary bladder or prostatic urethra.

- Patient presents with hematuria and/or urinary obstruction.

Table 23.3 Histologic features used to classify urothelial papillary lesions according to recent 2016 WHO and International Society of Urological Pathology (ISUP)

Parameters	Noninvasive Papilloma	Noninvasive Papillary Neoplasms of Low Malignant Potential	Noninvasive Low-grade Papillary Carcinoma	Noninvasive High-grade Papillary Carcinoma
Neoplasm architecture				
Papillae	Delicate papillae	Delicate papillae, occasionally fused	Papillae delicate, fused, branching	Papillae delicate, fused, branching
Organization of cells	Organization of cells identical to normal	Polarity of cells identical to normal; any thickness; cohesive	▪ Predominantly ordered organization of cells ▪ Minimal cells crowding and loss of polarity ▪ Variable thickness ▪ Cells cohesive	▪ Predominantly disordered organization of cells ▪ Loss of polarity frequent ▪ Variable thickness ▪ Cells discohesive
Cytomorphological features				
Nuclear size	Identical to normal cells	Nuclear size may be uniformly enlarged	Nuclear size may be uniformly enlarged	Nuclear size enlarged with variation in size
Nuclear shape	Identical to normal cells	Cells elongated, round-oval with uniform size	Cells round-oval; slight variation in size and shape and contour	Moderate or marked pleomorphism
Nuclear chromatin	Fine chromatin	Fine chromatin	Mild variation of chromatin within cells and between cells	Moderate-to-marked variation both within and between cells with hyperchromasia
Nucleoli	Nucleoli absent	Nucleoli absent or inconspicuous	Nucleoli inconspicuous	Nucleoli multiple and prominent may be present
Mitotic figures	Mitoses absent	Mitotic figures rare, basal	Mitotic figures occasional, at any level	Mitotic figures usually frequent, at any level
Umbrella cells	Uniformly present	Present	Usually present	May be absent

- Cystoscopy reveals a polypoid and pedunculated lesion with smooth contour. Histologic examination of tumor specimen shows invagination of the urothelial epithelium, which usually lacks atypical features. Papillae are absent, or focally present.

Flat Urothelial Lesions with Atypia

Diagnostic categories of flat urothelial lesions with atypia include reactive urothelial atypia, urothelial atypia of unknown significance, urothelial dysplasia and urothelial carcinoma *in situ*. Urothelial cells show nuclear enlargement, chromatin clumping and loss of polarity (Fig. 23.4).

- Urothelial carcinoma *in situ* refers to areas of urinary bladder mucosa, where normal urothelium has been replaced by neoplastic epithelium that does not invade through the basement membrane. Compared with normal urothelium, nuclei are more often rounded and enlarged with coarse chromatin and thick nuclear membrane and presence of atypical mitotic figures in carcinoma *in situ*.
- Carcinoma *in situ* is a recognized precancerous lesion, but usually remains asymptomatic. It is detected as a flat lesion in 90% of cases in association with other urothelial neoplasms. Most cases of urothelial carcinoma *in situ* progress to invasive urothelial carcinoma.

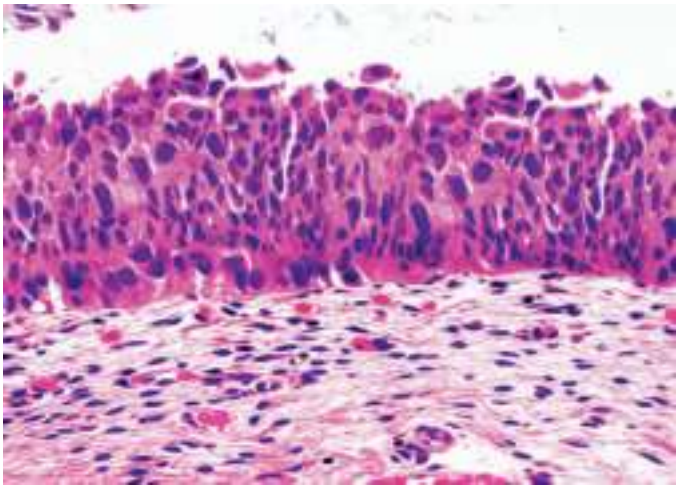


Fig. 23.4: Urothelial dysplasia shows good preservation of cellular polarity with uniform nuclear size and shape. There is presence of mitotic figures in the urothelium (400X).

Surgical Pathology: Flat Urothelial Lesions with Atypia	
Light Microscopy	
Histologic examination of biopsy specimen shows cellular atypia of the entire mucosa, from the basal layer to the surface (Fig. 23.5).	
Immunohistochemistry Technique	
Immunohistochemistry study shows positivity for CK20, CD44, p53, Ki-67, CK5/CK6 and SAT B2.	
Markers	**Expression**
▪ CK20	▪ Positive
▪ CD44	▪ Positive
▪ p53	▪ Positive (89%)
▪ Ki-67	▪ Positive
▪ CK5/CK6	▪ Positive
▪ SAT B2	▪ Positive

Invasive Urothelial Carcinoma

Invasive urothelial carcinoma is a high-grade tumor, which may have papillary, polypoid, nodular or ulcerative configurations. Cystoscopy may reveal multiple, red, velvety, flat patches. Pathologic stage depends on anatomic depth of invasion in the urinary bladder wall by tumor. There are several different histologic patterns: urothelial carcinoma, squamous cell carcinoma, adenocarcinoma, small cell carcinoma; and urothelial carcinoma with squamous differentiation (20%) or glandular differentiation (6%) and trophoblastic differentiation (hCG positivity and elevated serum hCG).

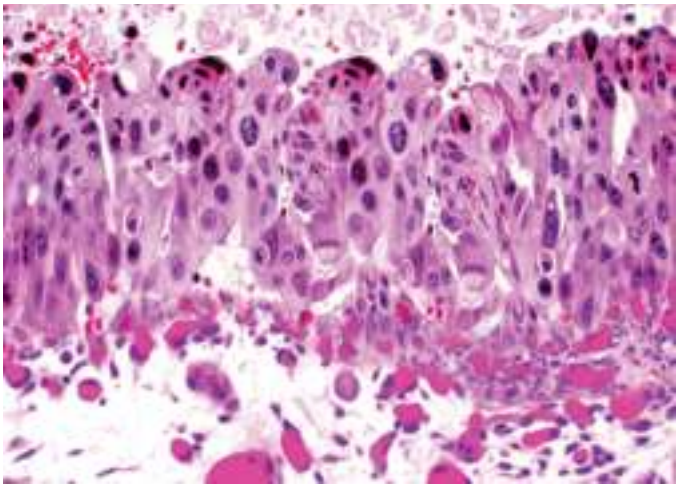


Fig. 23.5: Carcinoma *in situ*. Urothelial cells are pleomorphic with high nucleocytoplasmic ratio, irregular nuclear contours, hyperchromatic nuclei and prominent nucleoli. Cellular polarity is lost. Mitotic figures are atypical. Diagnosis of urothelial carcinoma *in situ* does not require full-thickness involvement. The superficial umbrella cell layer may be intact (400X).

Risk Factors

Pathogenesis of urothelial carcinoma and serum and urine markers is shown in Fig. 23.6. Indirect acting chemical carcinogens linked to urothelial carcinoma and other tumors are given in Table 23.4. Types of metaplasia in urothelium of urinary bladder and their clinical significance are given in Table 23.5.

- Tobacco smoking is the most important risk factor for urothelial carcinoma. Tobacco smoke

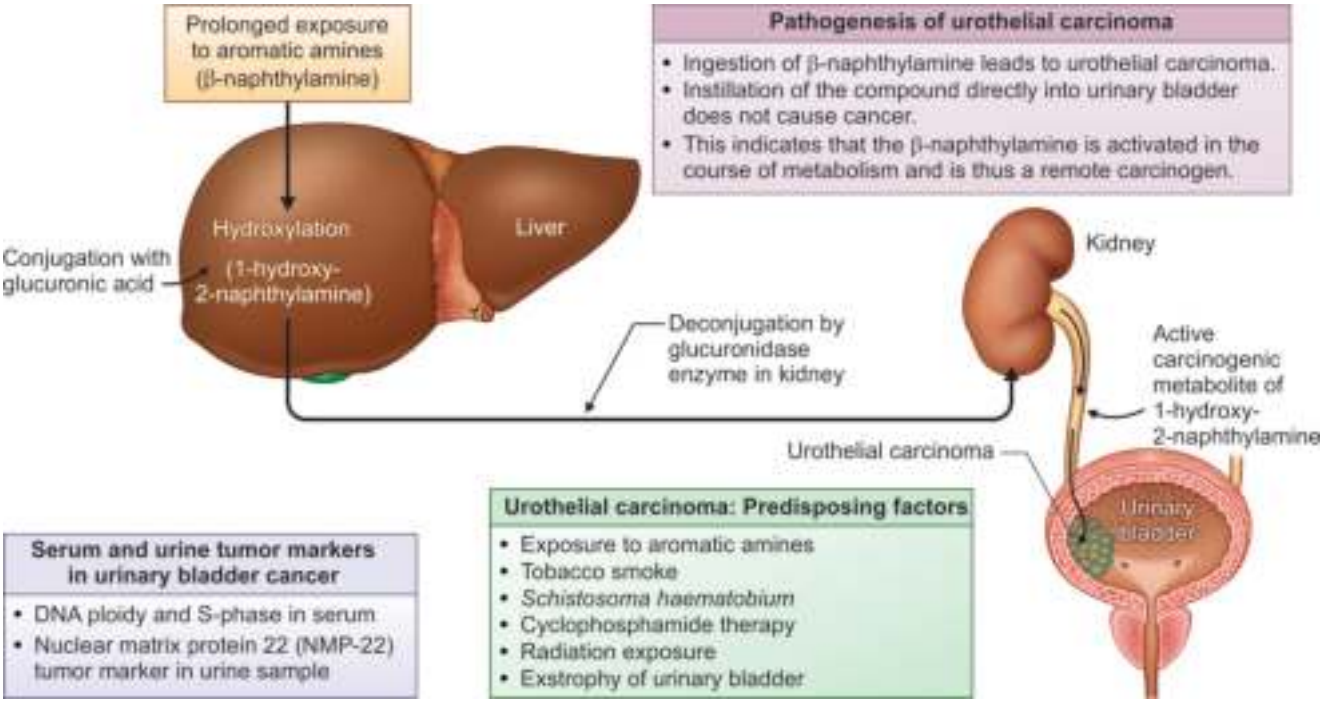


Fig. 23.6: Urothelial carcinoma: Pathogenesis of urothelial carcinoma and serum and urine markers.

Table 23.4 Indirect acting chemical carcinogens linked to urothelial carcinoma and other tumors

Chemical Carcinogen	Exposure to Carcinogens	Associated Cancers
Polycyclic hydrocarbons	▪ Tobacco smoke ▪ Consumption of smoked meat and fish	▪ Urothelial carcinoma ▪ Oral carcinoma ▪ Esophagus carcinoma (middle and distal) ▪ Pancreas carcinoma (lung carcinoma) ▪ Renal pelvis transitional cell carcinoma
Aromatic amines	▪ Aniline dye and rubber industries, benzidine-O, anisidine-O and toluidine ▪ Unlike polycyclic hydrocarbons, aromatic amines produce no local carcinogenic effect	▪ Urothelial carcinoma ▪ Mechanism: Mucosal glucuronidase in the urinary bladder converts β-naphthylamine glucuronide (aromatic amine) to the carcinogenic molecule β-naphthylamine results in urinary bladder cancer
Aromatic hydrocarbons	Benzene presents in crude oil (petrochemical), benz(a) anthracene and benz(a) pyrene	▪ Urothelial carcinoma ▪ Acute leukemia ▪ Hodgkin's disease

Table 23.5 Types of metaplasia in urothelium of urinary bladder and their clinical significance

Type of Metaplasia	Comments on Metaplasia	Clinical Significance
Intestinal metaplasia in cystitis glandularis/cystitis cystica	Intestinal metaplasia without dysplasia	▪ No risk for urothelial carcinoma ▪ Increased risk for urothelial carcinoma
Squamous metaplasia	▪ Nonkeratinizing metaplasia in trigone region of urinary bladder ▪ Keratinizing metaplasia in trigone region of urinary bladder	▪ No risk for squamous cell carcinoma of urinary bladder ▪ Increased risk for squamous cell carcinoma of urinary bladder
Nephrogenic metaplasia/ adenoma	Nephrogenic metaplasia/adenoma	▪ Benign lesion in urinary bladder ▪ Tubular component of nephrogenic metaplasia/adenoma involves the superficial muscularis mucosa mimicking malignant lesion ▪ Immunohistochemical markers positive P504S, CK7 and PAX2

containing polycyclic hydrocarbons are converted to ultimate carcinogen (benzo(a)pyrene), which may produce lung carcinoma at local site and urothelial carcinoma and renal cell carcinoma at distant sites.

- Persons working in aniline dye and rubber industries are heavily exposed to β-naphthylamine, which is absorbed by gastrointestinal tract resulting in urothelial carcinoma. In the liver, hydroxylation of β-naphthylamine forms 1-hydroxy-2-naphthylamine (water-soluble active carcinogenic metabolite), that combines with glucuronic acid and excreted in the urinary tract. Molecule is deconjugated by urinary bladder mucosal enzyme glucuronidase, thus exposing the urothelium to the active carcinogenic metabolite resulting in urothelial carcinoma.
- ***Schistosoma haematobium*** infection (in Egypt, Middle East and parts of Africa) is linked to urothelial carcinoma and squamous cell carcinoma of urinary bladder. Parasite causes foci of squamous metaplasia of urinary bladder epithelium resulting in squamous cell carcinoma of the bladder.
- Administration of cyclophosphamide and analgesics increase risk of urothelial carcinoma. Radiation therapy for cervical, prostate, or rectal cancer may increase the risk of urinary bladder carcinoma.
- Exstrophy of urinary bladder is absence of the anterior surface of the urinary bladder and abdominal wall, that can be recognized at the time of birth. In patients with exstrophy of urinary bladder, posterior wall of urinary bladder undergoes mechanical trauma resulting in squamous or glandular metaplasia and recurrent infections. Despite surgical repair, there is an increased risk for squamous cell carcinoma or adenocarcinoma of urinary bladder in 50–60 years of age group.

Molecular Genetic Alterations: Urothelial Carcinoma

Genetic profile of urothelial carcinoma is complex. There are two independent pathways of urothelial carcinoma development. However, occasional overlaps between these two pathways can occur.

RAS-MAPK Signaling Pathway

- RAS-MAPK signaling pathway is represented by low-grade papillary urothelial neoplasm including urothelial neoplasm of low malignant potential (PUNLMP) and low-grade papillary urothelial carcinoma. It is characterized by frequent local recurrence but no metastatic potential.
- PUNLMP and low-grade papillary urothelial carcinoma occasionally progress to high-grade papillary urothelial carcinoma or muscle-invasive urothelial carcinoma. Low-grade papillary urothelial carcinomas show activation of RAS-MAPK pathway, due to amplification of fibroblast growth factor receptor 1 (FGFR1) gene (60–80%) or H-RAS gene (15–30%).
- H-RAS is a signal-transducing protein, that participates in intracellular guanosine triphosphate signaling.
- Chromosome 9q deletion is common with several candidate genes have been implicated, including TSC1 (tuberous sclerosis complex 1), PTCH (patched homolog) and DBC1 (deleted in bladder cancer 1). PI13KCA gene mutation is demonstrated in 10–27% of cases.

Second Molecular Signaling Pathway

- Second molecular signaling pathway is less common pathway and represented by urothelial carcinoma *in situ* (CIS) and ≥pT2 urothelial carcinoma.
- Urothelial carcinoma *in situ* has tendency to progress into ≥pT2 in 60–80% of cases within five years. Urothelial carcinoma *in situ* most often demonstrates mutations in the tumor suppressor genes such as TP53 (70%), RB gene on locus 13p14 (37%) and PTEN (35%), frequently accompanied by deletion of chromosome 9.
- Similar changes are demonstrated in smooth muscle invasive urothelial carcinomas (pT12–pT4).
- But there are additional cytogenetic alterations such as deletions of chromosomes 3p, 5q, 6q, 10q, 11p and 18q.

Third Molecular Signaling Pathway

- Third molecular signaling pathway may be involved in high-grade papillary urothelial carcinoma and pT1 urothelial carcinoma. It may evolve from flat dysplasia or low-grade papillary urothelial carcinoma.
- High-grade papillary urothelial carcinoma and pT1 urothelial carcinoma demonstrate deletion of chromosome 9, with homologous deletion of CDKN2A (INK4A) gene, which encodes p16. FGFR3 and TP53 mutations may be present.
- Other commonly reported genetic abnormalities involve TERT and NOTCH.
- Oncogenes and tumor suppressor genes associated with urothelial carcinoma and other cancers are given in Table 23.6. Chromosomal abnormalities in urothelial carcinoma are given in Table 23.7.

Clinical Features

The earliest sign of invasive urothelial carcinoma is sudden painless hematuria in urine, which may be red- or rust-colored. In the later stages, the urinary bladder may become irritable with painful urination.

Table 23.6 Oncogenes and tumor suppressor genes associated with urothelial carcinoma and other cancers

Category	Proto-oncogene	Mechanism	Associated Malignancies
Growth factors			
Fibroblast growth factors (FGF)	▪ HST1 ▪ FGF3	▪ Overexpression ▪ Amplification	▪ Urothelial carcinoma ▪ Osteosarcoma ▪ Gastric carcinoma ▪ Breast carcinoma ▪ Melanoma
Signal-transduction proteins			
GTP-binding (G) proteins	H-RAS	Point mutation	▪ Urothelial carcinoma ▪ Renal cell carcinoma
Mitogenic signaling pathway inhibitor			
RB tumor suppressor gene	Retinoblastoma gene (locus 13q)	Deletion and nonsense mutation	▪ Urothelial carcinoma ▪ Retinoblastoma (familial or sporadic) ▪ Osteosarcoma (familial or sporadic) ▪ Breast carcinoma ▪ Colon carcinoma ▪ Prostatic carcinoma ▪ Lung carcinoma

Table 23.7 Chromosomal abnormalities in urothelial carcinoma

Chromosome Number	Comments
Chromosome 1 aberration	Represents secondary change in urothelial carcinoma
5p isochromosome	▪ Urothelial carcinoma (40% cases) ▪ Isochromosome arises from transverse division of the centromere during meiosis instead of the normal longitudinal division
Chromosome 9q deletion or monosomy	▪ Monosomy of chromosome 9 in superficial papillary tumors (50%) ▪ 9q deletion in advanced bladder cancer 67% of cases
Chromosome 11p deletion	▪ Urothelial carcinoma (40% cases) ▪ Represents secondary change related to invasiveness of urothelial carcinoma
Chromosome 17p deletion	▪ Chromosome 17p deletion results in p53 tumor suppressor gene mutation ▪ 17p deletion demonstrated in invasive urothelial carcinoma (63%)
Chromosome 7 trisomy	▪ Trisomy of chromosome 7 demonstrated in few cases of urothelial carcinoma ▪ Amplification of epidermal growth factor (EGF) located on chromosome 7 is demonstrated in invasive urothelial carcinoma (80%) and superficial urothelial lesions (27%)

Surgical Pathology: Invasive Urothelial Carcinoma

Gross Morphology

The lesions in urinary bladder may show various morphologic patterns: flat (carcinoma *in situ*), papillary (small lesion), exophytic (cauliflower-like) or ulcerated lesions. Invasive urothelial carcinoma often presents as ulcerated growth with indurated margins, that usually invades into the deeper layers of the urinary bladder and extends into the surrounding structures and pelvic organs (Fig. 23.7A and B).

Light Microscopy

Histologic examination of invasive urothelial carcinoma reveals cellular atypia of the entire mucosa, from the basal layer to the surface invading the lamina propria. The prognosis of urothelial carcinoma depends on the grade and stage of the tumor.

- Well-differentiated invasive urothelial carcinoma is grade 1 tumor associated with good prognosis (Fig. 23.8A and B).
- Papillary invasive urothelial carcinoma is grade 2 neoplasm and composed of broad papillae and solid areas associated with intermediate prognosis. Solid anaplastic invasive urothelial carcinoma is classified as grade 3 tumor associated with poor prognosis (Fig. 23.9A to D).

Histologic Variants: Invasive Urothelial Carcinoma

- **Micropapillary invasive urothelial carcinoma:** Micropapillary invasive urothelial carcinoma histologically mimics ovarian serous carcinoma. Tumor is composed of neoplastic cells arranged in nests with back to back pattern. Micropapillary carcinoma invades muscularis propria at diagnosis and metastases to regional lymph nodes and distant organs in majority of cases.
- **Plasmacytoid invasive urothelial carcinoma:** Tumor is morphologically similar to lobular carcinoma of breast and diffuse-type gastric carcinoma (linitis plastica).
  - Tumor is composed of monomorphic round discohesive neoplastic cells with homogenous eosinophilic cytoplasm and eccentrically placed nucleus arranged either scattered or in clusters. Intracytoplasmic vacuoles are also common in scarred neoplastic cells.
  - Plasmacytoid invasive urothelial carcinoma has a pattern of spread distinct from typical urothelial carcinoma.
  - Tumor involves serosal surfaces, ovary and causes recurrent malignant effusions. A recent study has demonstrated frequent somatic CDH-1 loss of function mutations in plasmacytoid carcinoma.
  - Immunohistochemical analysis of plasmacytoid invasive urothelial carcinoma demonstrates positivity for CD138, CK20 and GATA3. But p63 is less sensitive marker.
- **Nested invasive urothelial carcinoma:** Tumor is composed of rounded aggregates of cytologically bland urothelium invading in subepithelial tissue. Some tumors demonstrate central cystic change (microcystic variant) mimicking cystitis glandularis or cystitis cystica, but former invades subepithelial tissue.
- **Lymphoepithelioma-like invasive urothelial carcinoma:** Tumor is a nonkeratinizing carcinoma associated with heavy inflammatory infiltrate. Some tumors are pure masked by heavy inflammatory infiltrate.
- **Sarcomatoid (spindle cell, metaplastic) invasive urothelial carcinoma:** Tumor is a high-grade neoplasm of urinary bladder, in which malignant epithelial component with exhibiting urothelial, glandular, squamous differentiation or undifferentiation or spindled sarcoma-like appearance (such as osteosarcoma, chondrosarcoma, liposarcoma, pleomorphic rhabdomyosarcoma, or pleomorphic undifferentiated sarcoma) is present in the neoplasm.
  - The tumor is most often large and polypoid lesion, which usually affects elderly males, which involves ureter and renal pelvis and usually metastasizes to regional lymph nodes and distant organs.
  - Tumor at metastatic site is composed of epithelial component or the sarcoma-like component.

Immunohistochemistry Technique

Invasive urothelial carcinoma shows positivity for p63, thrombomodulin, uroplakin III, GATA, CK7 and CK20.

Markers	Expression
▪ p63	▪ Positive (81–96%) (more specific but less sensitive)
▪ Thrombomodulin	▪ Positive (69–91%)
▪ Uroplakin III	▪ Positive (57%)

▪ GATA (most sensitive marker)	▪ Positive
▪ CK7	▪ Positive (65–100%)
▪ CK20	▪ Positive (65–100%)
▪ Prostate-specific antigen (PSA)	▪ Negative
▪ Prostate-specific acid phosphatase	▪ Negative
▪ Prostein (P501S)	▪ Negative
▪ Prostate-specific membrane antigen	▪ Negative

Treatment and Prognosis

Treatment strategy for invasive urothelial carcinoma depends on age of the patient, surgical risk, the extent of stage and histologic grade of tumor, the presence of urothelial carcinoma *in situ* elsewhere in the urinary bladder. Recommended treatment for extensive urothelial carcinoma *in situ* is total cystectomy. Small and apparently localized urothelial lesions are treated

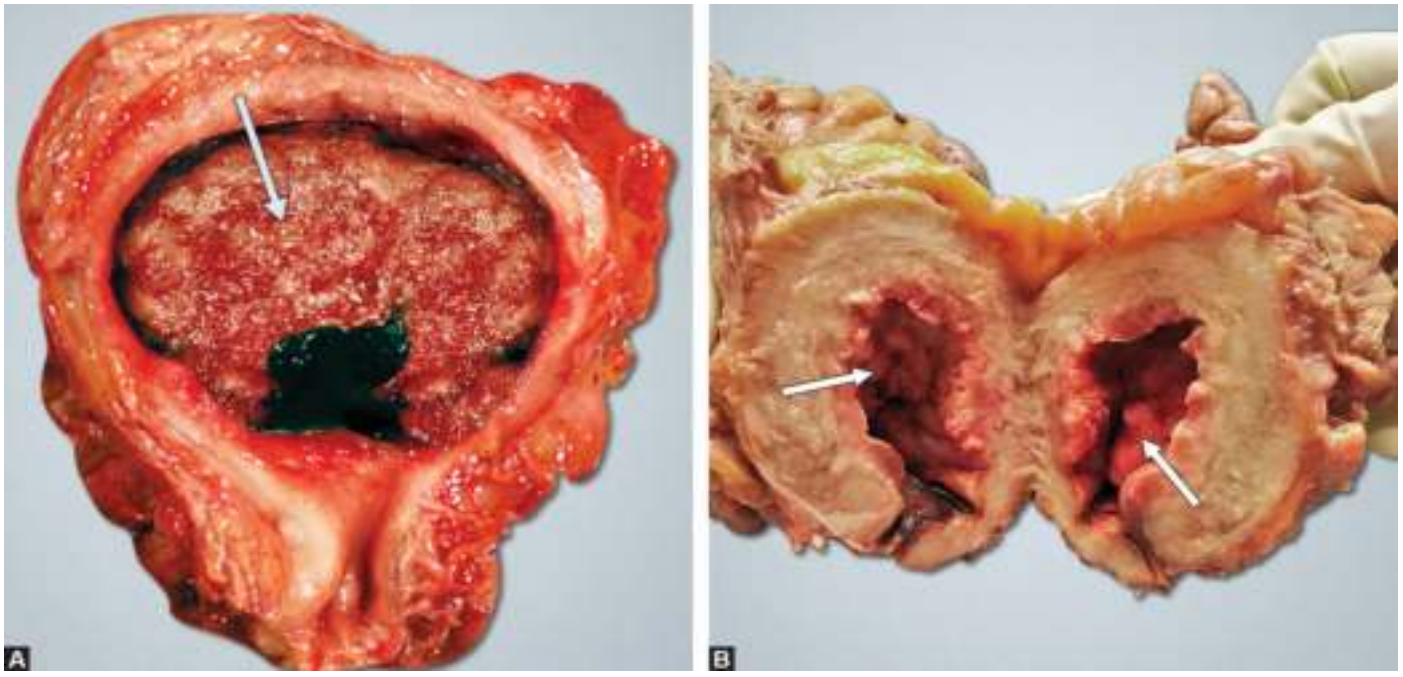


Fig. 23.7: Urothelial carcinoma. (A) Cut opened urinary bladder specimen shows exophytic (cauliflower-like) growth filling cavity with areas of hemorrhage (arrow). Macroscopic findings range from subtle urinary bladder wall thickening to obvious exophytic mass. (B) Cut opened cystectomy specimen shows a tumor arising from the lining epithelium forms thickening of the wall of urinary bladder, and at places forming fungating tumor mass (arrows). (*Courtesy*: Senior Professor and Head, Department of Pathology, Govt. Medical College, Srinagar, J&K.)

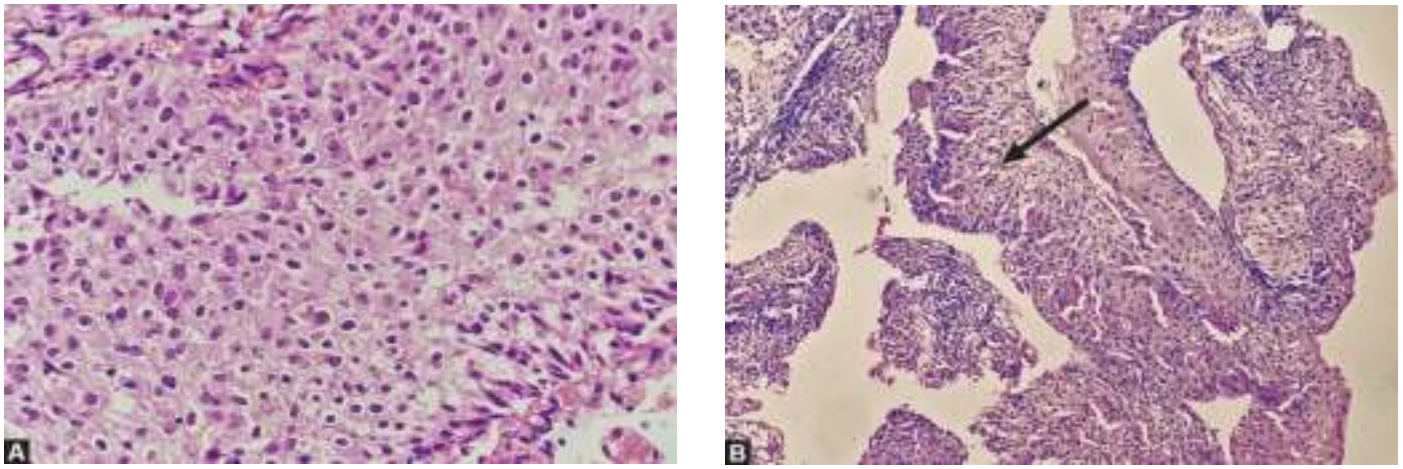


Fig. 23.8: (A) Low-grade urothelial carcinoma. Neoplastic proliferation of the urothelium shows papillary configuration without invasion through the basement membrane. Neoplasm demonstrates low-grade architectural and cytologic features such as slight variation in cells and few mitotic figures, without high-grade features such as irregular pleomorphic nuclei, with frequent, prominent nucleoli and mitoses (400X), (B) low-grade urothelial carcinoma with squamous differentiation. Tumor is composed of neoplastic cells forming papillary configuration without invasion through the basement membrane. Tumor is composed of neoplastic cells, which demonstrate slight variation in cells, few mitotic figures, and squamous metaplasia (arrow) (400X). (*Courtesy*: Senior Professor and Head, Department of Pathology, Govt. Medical College, Srinagar, J&K.)

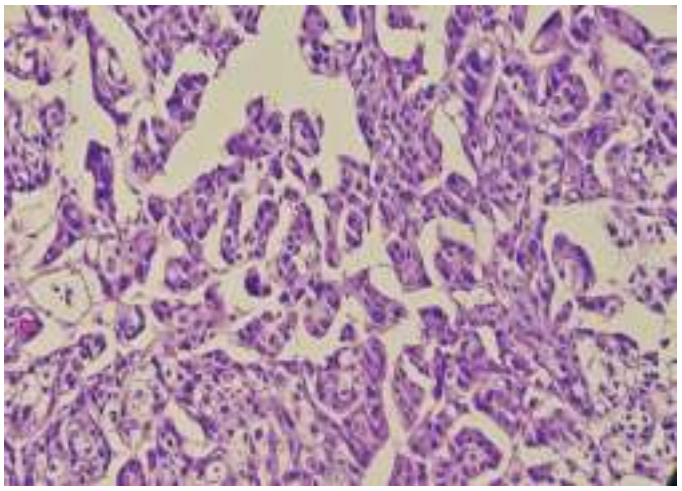


Fig. 23.9A: Micropapillary urothelial carcinoma. On histologic examination, tumor mimics ovarian serous carcinoma. It is composed of neoplastic cells arranged in nests with back-to-back pattern. Most micropapillary carcinoma invades muscularis propria at diagnosis and metastases to regional lymph nodes and distant organs (400X). (*Courtesy*: Senior Professor and Head, Department of Pathology, Govt. Medical College, Srinagar, J&K.)

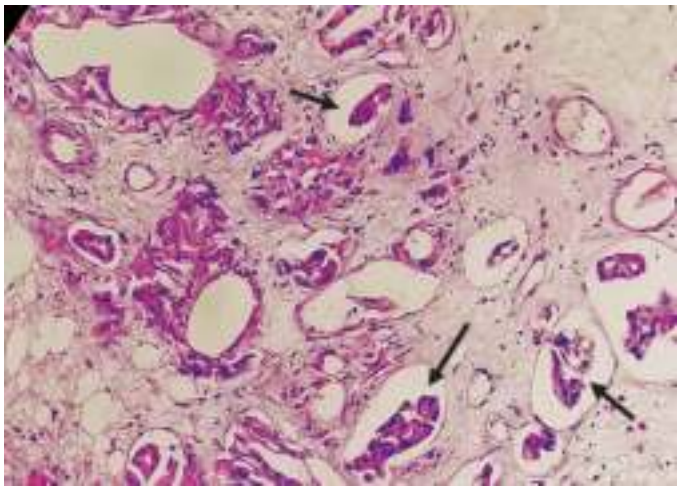


Fig. 23.9B: Vascular invasion by micropapillary urothelial carcinoma. On histologic examination, tumor mimics ovarian serous carcinoma. Tumor is composed of neoplastic cells arranged in nests with back-to-back pattern invading blood vessels. Most micropapillary carcinoma invades muscularis propria at diagnosis and metastases to regional lymph nodes and distant organs (400X). (*Courtesy*: Senior Professor and Head, Department of Pathology, Govt. Medical College, Srinagar, J&K.)

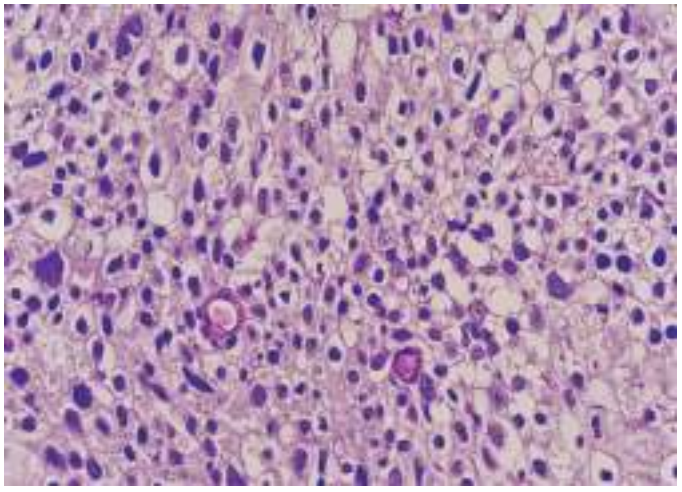


Fig. 23.9C: High-grade urothelial carcinoma. Neoplastic cells are arranged in irregular nests or single cells invading lamina propria and muscularis propria. Tumor is composed of pleomorphic cells with high-grade nuclear features such as hyperchromasia, high nucleocytoplasmic ratio with frequent mitotic figures. It is worth mentioning that nested urothelial carcinoma demonstrates bland low-grade cytological features (400X). (*Courtesy*: Senior Professor and Head, Department of Pathology, Govt. Medical College, Srinagar, J&K.)

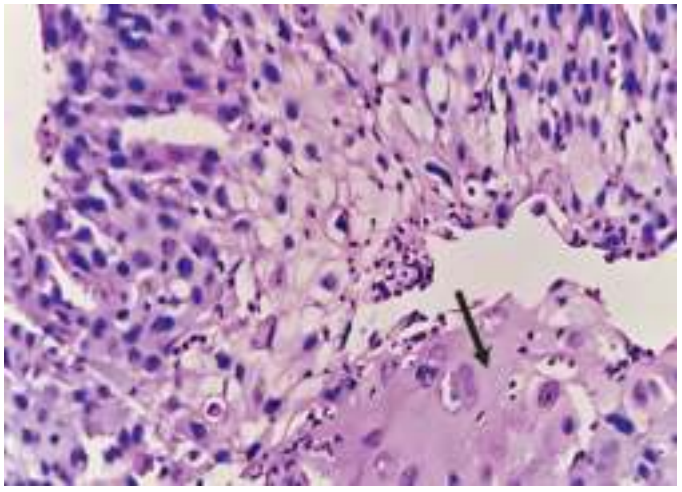


Fig. 23.9D: High-grade urothelial carcinoma with squamous differentiation. Neoplastic cells are arranged in irregular nests or single cells invading lamina propria and muscularis propria. Tumor shows high-grade nuclear features such as nuclear pleomorphism, hyperchromasia, high nucleocytoplasmic ratio with frequent mitotic figures and squamous differentiation (400X). (*Courtesy*: Senior Professor and Head, Department of Pathology, Govt. Medical College, Srinagar, J&K.)

by intravesical chemotherapy. Urothelial carcinoma without invasion of muscularis propria is usually treated initially by transurethral resection.

High-grade invasive urothelial carcinoma is treated by intravesical administration of chemotherapy. Radical cystectomy in the males with invasive urothelial carcinoma includes the urinary bladder, prostate, seminal vesicles and the adjacent perivesical tissue. Radical cystectomy in the females with invasive urothelial carcinoma includes urinary bladder, uterus, fallopian tubes, ovaries, anterior vagina, and urethra. The addition of neoadjuvant chemotherapy to the radical cystectomy has improved survival of patients with advanced invasive urothelial carcinoma.

Urothelial Neoplasms Reporting Protocol: Transurethral Resection and Cystectomy Specimens

- Urothelial neoplasms should be sampled with a cold biopsy instrument. Ideally, the biopsy should include tumor and a portion of the underlying muscle tissue and normal adjacent mucosa from three other sites (one lateral to each ureteral orifice and one from the upper posterior wall).
- These biopsies should be submitted separately in different containers and processed.
- Pathologist must report whether the tumor is invasive (depth of invasion of muscularis propria or muscle) or noninvasive, histologic grade, lymphovascular invasion and foci of urothelial carcinoma *in situ*.
- Detection of residual tumor cells in the biopsy specimens from patients having had a prior biopsy or transurethral resection can be challenging.
- Immunohistochemical stains for cytokeratins should be performed only with great care since reactive myofibroblasts are usually positive as well. Panel of immunohistochemical markers in the urinary bladder tumors is given in Table 23.8.

PRIMARY SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma (SCC) is the most common tumor in urinary bladder, across world, where *Schistosoma haematobium* is endemic (e.g. Egypt linking Middle East and North East of Africa). It represents only 5% of urinary bladder tumors in the Western countries.

- Predisposing factors for SCC of urinary bladder include chronic cystitis, long-term indwelling catheters, tobacco smoking, diverticula, nonfunctioning and exstrophy of urinary bladder, and renal transplant cases. Exstrophy of the urinary bladder may predispose to SCC or adenocarcinoma.
- Squamous cell carcinoma of urinary bladder presents as invasive carcinoma and has a worse prognosis than invasive urothelial carcinoma.
- Squamous metaplasia and urothelial carcinoma *in situ* are commonly demonstrated in areas adjacent to invasive SCC, supporting the notion that SCC in the urinary bladder arises through usual metaplasia–dysplasia sequence.

Surgical Pathology: Squamous Cell Carcinoma of Urinary Bladder

Gross Morphology

Cystectomy specimen reveals large fungating solid necrotic tumor that can fill the entire bladder. Verrucous squamous cell carcinoma (exophytic growth) is exclusively associated with *Schistosoma haematobium*.

Light Microscopy

Invasive squamous cell carcinoma of urinary bladder is well-differentiated tumor. It shows well-defined islands of squamous cells with keratinization, keratin pearls, prominent intercellular bridges, and minimal nuclear pleomorphism (Fig. 23.10A).

Table 23.8 Panel of immunohistochemical markers in the urinary bladder tumors

Immunohistochemical Markers	Expression
Normal urothelium or reactive urothelial atypia	
p53	Negative
CK20	Positive (umbrella cells only)
E-cadherin	Positive (all layers)
CD44	Positive (basal layers)
Urothelial carcinoma *in situ*	
p53	Positive (89%)
CK20	Positive (all layers)
E-cadherin	Negative or low expression
CD44	Negative or low expression
Ki-67	Positive
Invasive urothelial carcinoma	
CK903 (HMWCK)	Positive
p63	Positive (more specific but less sensitive)
Thrombomodulin	Positive
Uroplakin III	Positive
GATA3	Positive
CK7	Positive (strongly positivity suggests urothelial carcinoma)
CK20	Positive (strongly positivity suggests urothelial carcinoma)
Prostate-specific antigen (PSA)	Negative
Prostatic-specific acid phosphatase	Negative
Prostein (P501S)	Negative
Prostate-specific membrane antigen (PSMA)	Negative
Primary adenocarcinoma of urinary bladder	
SATB2	Positive (50%)
CDX2	Positive/negative
CK20	Positive/negative
CK7	Positive/negative
Thrombomodulin	Positive/negative
Racemase	Negative
Prostate-specific antigen	Negative
Prostate-specific acid phosphatase	Negative
Prostein	Negative
Prostate-specific membrane antigen	Negative
β-Catenin (nuclear)	Negative

Contd...

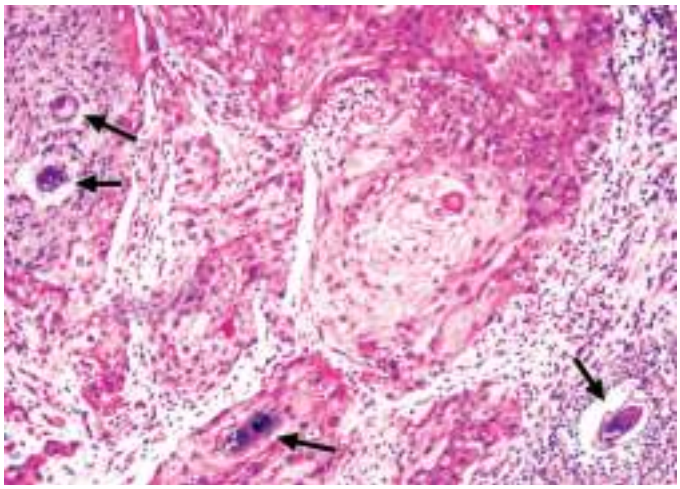


Fig. 23.10A: Invasive well differentiated squamous cell carcinoma of urinary bladder. It shows well-defined islands of squamous cells with keratinization, keratin pearls, prominent intercellular bridges, and minimal nuclear pleomorphism. It demonstrates *Schistosoma haematobium* (arrows) (400X).

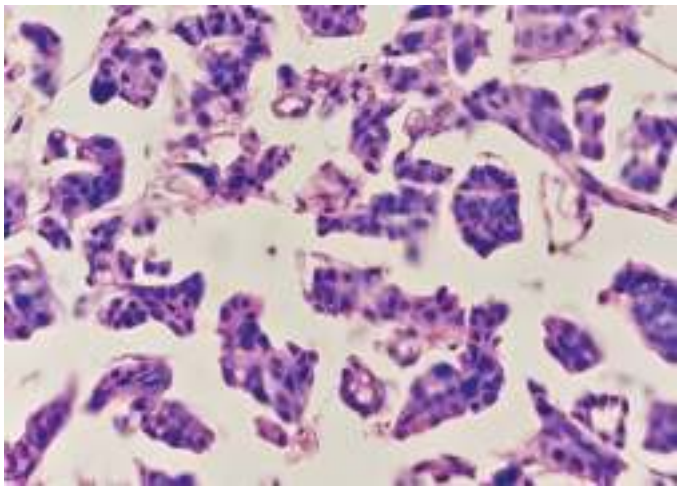


Fig. 23.10B: Adenocarcinoma of urinary bladder. Histologic variants of urothelial adenocarcinoma include intestinal type, mucinous type, signet ring cell type and associated with *in situ* component. On histologic examination, the tumor resembles colonic adenocarcinoma. Neoplastic glands are lined by pleomorphic mucin producing pseudostratified columnar epithelium. Central necrosis is commonly demonstrated (400X). *Courtesy*: Senior Professor and Head, Department of Pathology, Govt. Medical College, Srinagar, J&K.)

PRIMARY ADENOCARCINOMA

In situ adenocarcinoma is extremely rare form of intraepithelial carcinoma, which is associated with an increased risk for development of high-grade urothelial carcinoma, small cell carcinoma or micropapillary carcinoma.

Adenocarcinoma accounts for 2% of all malignant tumors of the urinary bladder. Most important risk factor is exstrophy of urinary bladder. Tumor arises from foci of cystitis glandularis or intestinal metaplasia, remnants of urachal epithelium in urinary bladder dome and urinary bladder diverticula. Tobacco smoking is associated with urothelial carcinoma and squamous cell carcinoma but not adenocarcinoma.

Surgical Pathology: Primary Adenocarcinoma of Urinary Bladder

Gross Morphology

Advanced adenocarcinoma of the urinary bladder usually grows as a polypoidal mass in the trigone region, that may undergo ulceration of mucosa and invasion of the urinary bladder wall. The mucosal surface of the mucin-secreting tumor is covered by gelatinous material.

Light Microscopy

Tumor exhibits wide range of glandular differentiation with intestinal metaplasia. Tumor may be superficial or deep invading the muscle. Sometimes, tumor behaves like a signet ring carcinoma, with diffuse infiltration of urinary bladder wall and associated with fatal outcome. Histology of adenocarcinoma of urinary bladder is shown in Fig. 23.10B.

Immunohistochemistry Technique

A recent study documented SATB2 nuclear expression in 50% of cases.

Markers	Expression
▪ SATB2	▪ Positive (50%)
▪ CDX2	▪ Positive/negative
▪ CK20	▪ Positive/negative
▪ CK7	▪ Positive/negative
▪ Thrombomodulin	▪ Positive/negative
▪ β-Catenin (nuclear)	▪ Negative
▪ Racemase	▪ Negative
▪ Prostate-specific antigen	▪ Negative
▪ Prostate-specific acid phosphatase	▪ Negative
▪ Prostein	▪ Negative
▪ Prostate-specific membrane antigen	▪ Negative

Prognosis

Overall prognosis of adenocarcinoma of urinary bladder is poor with five-year survival in 18% of cases. Stage of tumor is the most important prognostic factor.

BOTRYOID RHABDOMYOSARCOMA

Botryoid rhabdomyosarcoma of urinary bladder most often affects in children. Cystoscopy reveals grape-like polypoidal lesions on mucosal surface of urinary bladder trigone. Almost all cases in genitourinary tract show embryonal rhabdomyosarcoma variant.

Surgical Pathology: Botryoid Rhabdomyosarcoma of Urinary Bladder

Gross Morphology

Most embryonal rhabdomyosarcomas of the urinary bladder present as polypoidal intraluminal masses resembling cluster of grapes hence named botryoid type.

Light Microscopy

Botryoid rhabdomyosarcoma tumor of urinary bladder is composed round to oval cells admixed with spindle cells in loose myxoid stroma. A cambium layer of tightly packed undifferentiated neoplastic cells is demonstrated beneath mucosal lining of urinary bladder (Fig. 23.11A and B).

Immunohistochemistry Technique

Tumor cells show positivity for desmin, myogenin, myoglobin and MyoD1.

Markers	Expression
▪ Desmin	▪ Positive
▪ Myogenin	▪ Positive
▪ Myoglobin	▪ Positive
▪ MyoD1	▪ Positive

Treatment

Combined radiation therapy and chemotherapy increase survival rates in these children with botryoid rhabdomyosarcoma.

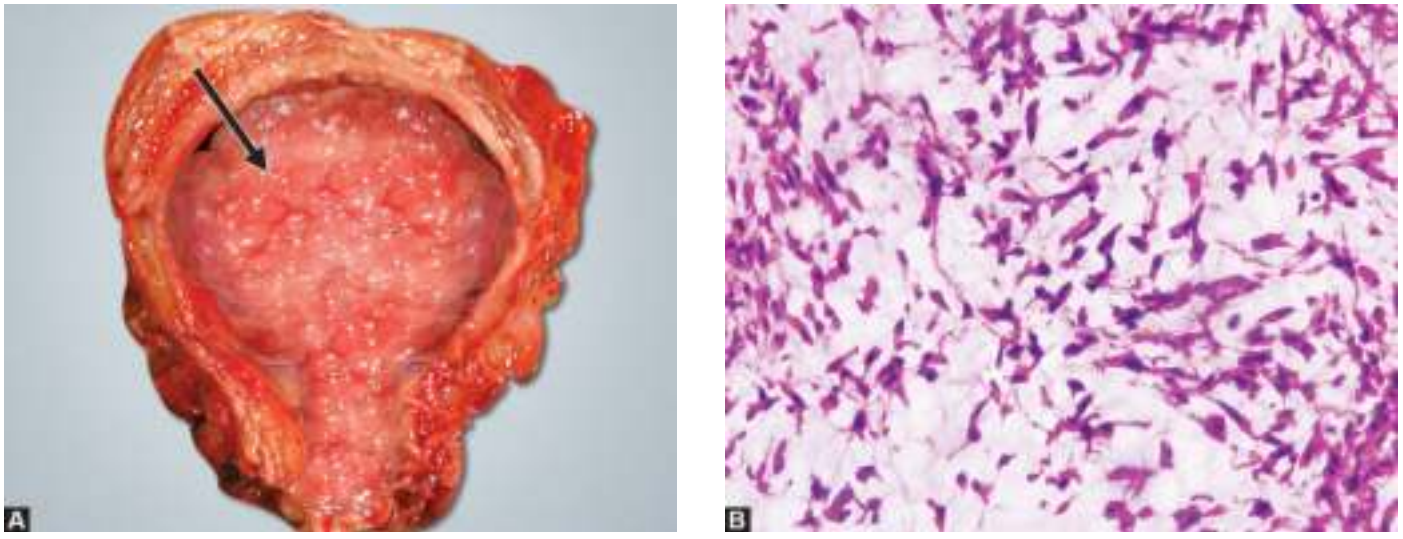


Fig. 23.11: Botryoides rhabdomyosarcoma of urinary bladder. (A) Embryonal rhabdomyosarcoma in the cut opened urinary bladder demonstrates polypoidal intraluminal masses resembling cluster of grapes hence named botryoid type (arrow), (B) tumor is composed of round to oval cells admixed with spindle cells in loose myxoid stroma (400X).

DISORDERS OF TESTES

TESTES: OVERVIEW

Scrotum is a pouch containing extra-abdominal testis, which helps in maintaining the low temperature of testes 2–2.5°C less than the body temperature, necessary for spermatogenesis. Each testis is connected with abdomen or pelvic cavity by the inguinal canal. Testes descend from abdominal cavity through inguinal canal and pulls blood vessels, nerves, ductus deferens and cremasteric muscle and connective tissue forming spermatic cord.

ANATOMY

Testes are covered by tunica vaginalis and enclosed in scrotum-site of production of spermatozoa. Excretory genital tract comprises ductus epididymis, ductus deferens or vas deferens, urethra (prostatic part, membranous part and penile part), which opens into penile meatus.

- Accessory glands comprise seminal vesicles, prostate gland and bulbourethral glands. Their secretion is rich in fructose, calcium and enzymes.
- Secondary sexual organs in males include seminal vesicles, prostate gland and bulbourethral glands and penis. External genitalia (penis) is erectile copulatory organ, which consists of three columns of erectile tissue (two cylinders of corpus cavernosa dorsally and one cylinder of urethral corpus spongiosum ventrally) covered by tunica albuginea. Glans penis is the extended tip part of penis.

- Each testis measures approximately 4.5×2.5×3 cm and weighs 20 g. Testicular size is measured by commercially available plastic testes models orchidometer.
- Each testis is surrounded by a small extension of peritoneal cavity, the tunica vaginalis, with visceral layer of peritoneal sac lying on the tough fibrous capsule of the testis known as tunica albuginea. A small amount of fluid is present between parietal and visceral layers lined by mesothelium.

HISTOLOGY

Testis is composed of compound tubular glands enclosed in scrotum and covered by tunica vaginalis and enclosed in tough tunica albuginea. Testis consists of 250 testicular lobules.

- Each testicular lobule contains 1–3 highly coiled seminiferous tubules lined by 4–8 layers of spermatogenic cells and surrounded by a basement membrane (tunica propria) and supported by a delicate fibrovascular stroma, in which Leydig cells are present.
- Sertoli cells are elongated and pyramidal cells; apical end extends into the lumen of the seminiferous tubules, which support spermatogenic cells and provide nutrition to the spermatozoa act as barrier to infectious agents. Sertoli cells synthesize estrogens, some androgens and androgen-binding proteins (ABP), which bind testosterone and keep its concentration high in the seminiferous tubules. Sertoli cells also secrete inhibin, which inhibits FSH, so help in the rate of spermatogenesis. Normal germ cell to Sertoli cell ratio is 13:1 in seminiferous tubules.
- Leydig cells (interstitial cells) discovered by Franz von Leydig are present between seminiferous tubules, which synthesize testosterone and other androgenic hormones involved in growth and development; protein anabolism, spermatozoa maturation and development of secondary sex characters.
- The seminiferous tubules converge to empty into rete testis at the testicular hilum, and these in turn anastomose with the efferent ductules, external to the testis, coil to form head of epididymis. At the hilum, the rete seminiferous tubules are supported by a fibrous tissue stroma and together comprise the testicular mediastinum.
- The epididymis is present on the posterior surface of testis, and it gives rise to the ductus (vas) deferens, which empties into the seminal vesicle.
- Male genital system produces spermatozoa and transfers of spermatozoa to female reproductive system.

SPERMATOGENESIS

Germinal elements account for over 75% of testicular mass. Under physiologic state, various hormones, i.e. growth hormone, testosterone synthesized by Leydig cells play an important role in spermatogenesis. Sertoli cells convert some portion of testosterone into estrogens.

- Estrogens play vital role in spermiogenesis by maturation of spermatids into spermatozoa. During spermatogenesis, spermatogonium (stem cells) (2n) form primary spermatocytes (2n), secondary spermatocytes (n), maturation of spermatids (n) into spermatozoa (n). Sertoli cells synthesize androgen binding protein, which binds and transports both testosterone and estrogens into seminiferous tubules, which promote maturation of spermatozoa.
- Pathway of semen from seminiferous tubules in testis to penile meatus is: tubuli recti (short segments of straight tubules), rete testis (highly anastomosing labyrinth of cuboidal epithelium lined channels), ductus efferent (10–20 fine tubules, intragenital duct system), epididymis (tail, body and head), vas ductus (vas deferens 6 meters long), enclosed in spermatic cord along with blood vessels, nerves, cremasteric muscles pass through inguinal canal and joins ejaculatory ducts and opens into urethra passing through prostate gland.
- Urethra consists of prostatic part, membranous part, and penile part, that opens into penile meatus. Tearing of inguinal canal results in the bulging of a loop of intestine into scrotum is called inguinal hernia.
- Spermatozoa consist of head, neck, midpiece and tail. Head region of spermatozoa contains nucleus with haploid number of chromosomes. Neck portion consists of proximal and distal centrioles. Midpiece consists of mitochondria/ tail (axoneme) consists of microtubule. Various enzymes are produced by acrosome of spermatozoa. Hyaluronidase acts on ground substance of follicular cells. Corona penetrating enzyme dissolves corona radiata. Zona lysine and acrosin digest zona pellucida. These enzymes are collectively called 'sperm lysins'.
- Human male ejaculates 3–4 ml of semen and 200–300 million spermatozoa during coitus. Semen is composed of seminal plasma and sperms. About 60% of spermatozoa have normal shape and at least 40% of them must show vigorous motility. Secretions of epididymis, vas deferens, seminal vesicle and prostate are essential for the maturation and motility of spermatozoa. Capacitation refers to activation of spermatozoa, that takes 5–6 hours.

- Storage of semen at –196°C preserves spermatozoa for many years. Spermatozoa are stored mainly in alkaline medium. In temperate regions, spermatozoa motility is increased but lifespan decreased. Life span in female genital system is 1–2 days. The various stages of development of spermatozoa (Fig. 23.12A and B).

Johnsen Score System for Quantifying Spermatogenesis

Johnsen defined 10-point scoring system for quantifying spermatogenesis according to the profile of the cells encountered along seminiferous tubules. Johnsen score of 10 indicates maximum spermatogenesis activity, i.e. formation of mature spermatozoa, whereas score of 1 indicates complete absence of germ cells in seminiferous tubules.

SEMEN (SEMINAL FLUID)

Semen is the male reproductive fluid containing spermatozoa in suspension. Volume of the ejaculate is contributed mainly by seminal vesicle (60%), prostate gland (30%), small amount from the bulbourethral glands and epididymis.

- Precise analysis of volume is essential in any evaluation of semen analysis, because it allows the total number of spermatozoa and non-sperm cells in the ejaculate to be calculated.
- Prostatic fluid is milky white with alkaline pH, containing citrate ion, calcium, acid phosphatase and profibrinolysin. Alkaline pH neutralizes acidic pH of vas deferens and vaginal acidic pH (3.5–4).
- Seminal vesicle synthesizes fructose, prostaglandins and fibrinogen. Fructose provides nutrition to sperms.
- Prostaglandins play an important role in fertilization of spermatozoa with ovum by reacting with cervical mucus to make susceptible to sperms and possibly cause reverse peristaltic contraction in the uterus and fallopian tube to move spermatozoa upwards towards ovaries.

Semen Analysis

Normal sperm count is 60–120 million per ml. In case of infertility, two or more specimens are necessary with two days abstinence.

- Freshly ejaculated specimen is a coagulum. Prostatic lysozyme and α-amylase liquefy the semen within 30 minutes.
- Coagulum is not formed in patients with underdeveloped seminal vesicle or lacking vas deferens.
- Fructose levels are below normal in these patients. Normal semen values as suggested by WHO are given in Table 23.9. Causes of male infertility are given in Table 23.10.

Cryopreservation of Spermatozoa

Semen from healthy fertile donors may be stored at –196°C for future use. These donors should be ejaculated, scrutinize by 'sperm bank' and their spermatozoa used anonymously. Alternatively, the recipients may know the donors. The donors' spermatozoa can be used for IVF (*in vitro* fertilization). IVF is one of the more widely known types of assisted reproductive technology, which works by using a combination of medicines and surgical procedures and can help the fertilized egg implant in uterus.

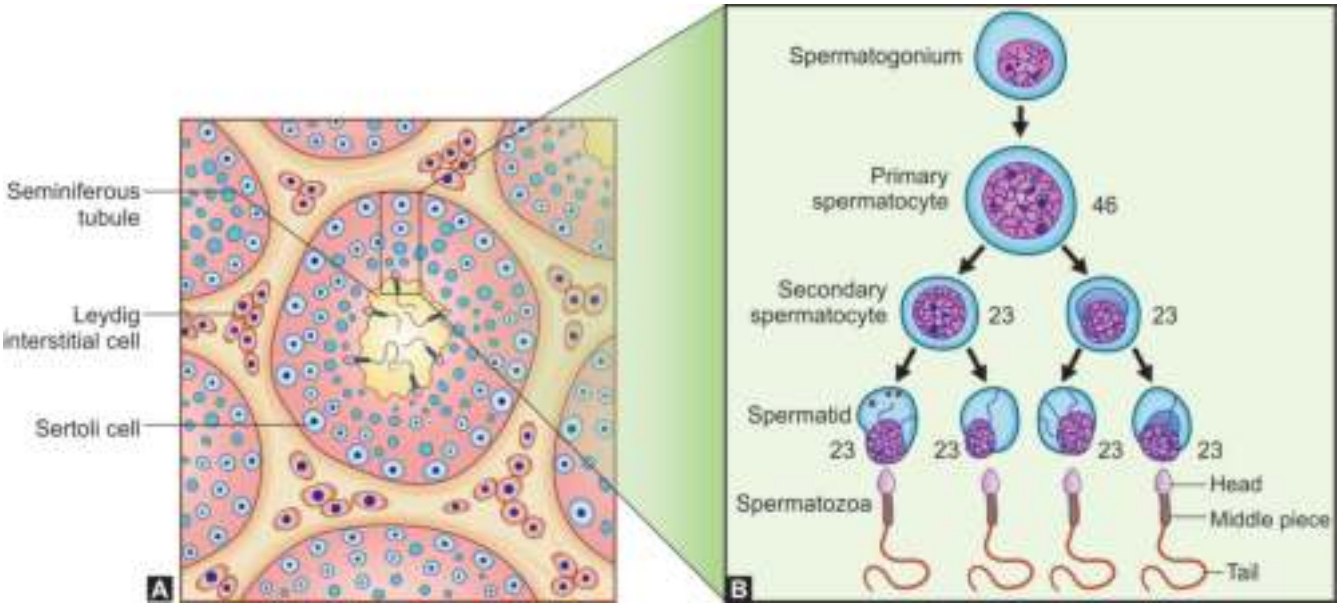


Fig. 23.12: The various stages of spermatogenesis. (A) Cross-section of seminiferous tubule, (B) stages of development of spermatozoa.

Table 23.9 Normal semen values as suggested by WHO

Parameters	Normal Spermiogram	Comments
Volume	≥2–6 ml	≥2.0 ml
Color and odor	Milky-cloudy, chestnut blossom aroma	Homogenous, grey-opalescent (less opaque due to low spermatozoa)
pH (measured after 30 minutes)	7.2–7.8	▪ ≥8 (acute prostatitis, acute vesiculitis and bilateral acute epididymitis) ▪ 6.6–7 (chronic prostatitis, chronic vesiculitis, bilateral chronic epididymitis, occlusion of ejaculatory duct)
Unprepared spermiogram		
Motility of spermatozoa	≥50% overall motility, at least 30% progressive motility	Actively motile, moderately motile and immotile spermatozoa must be analyzed
Counting chamber used analyze number and density of spermatozoa		
Number of spermatozoa per ejaculate	40–800 × 10^6 spermatozoa	Must be assessed after liquefaction of semen after 30 minutes
Spermatozoal density	20–250 × 10^6 spermatozoa	▪ Oligospermia (<20 million/ml) ▪ Polyspermia (>250 million/ml) ▪ Cryospermia (spermatozoa not discovered until after sedimentation) ▪ Azoospermia (no spermatozoa)
Stained smear to analyze morphology of spermatozoa		
Morphology of spermatozoa	50% normal morphology of spermatozoa	% of abnormal morphology with malformation of head, midpiece and tail must be analyzed
Vitality of spermatozoa by eosin technique		
Vitality of spermatozoa using eosin-nigrosin technique	80% live spermatozoa	Test increases the contrast between the background and spermatozoa heads
Fructose level	>1200 μg/ml or 1.2 g/liter	Low fructose value indicative of an obstruction of the seminal vesicle
Leukocytes, lymphocytes	<500,000/ml	Pyospermia (leukocytospermia) weakens genetic material of spermatozoa

Table 23.10 Causes of male infertility

Causes	Comments
Chronic alcoholism	Decreased gonadotropin and testosterone levels
Drugs	Heroin and methadone adversely affect hypothalamic-hypophyseal axis, ejaculation volume and spermatogenesis
Exposure to diethylstilbestrol *in utero*	Epididymal cysts and obstructive changes
Exposure to DDT (dichlorodiphenyltrichloroethane) and chlordecone	Decreased spermatogenesis
Colchicine, alkylating agents and nitrofurantoin	Spermatozoa structure and motility abnormality
Diabetes mellitus	Partial or complete retrograde ejaculation or impotence
Varicocele	Poor quality and retrograde ejaculation (spermatozoa in urine)
Vasectomy and hernia repair and decreased blood supply	Sterility in men
Tuberculous epididymo-orchitis	Cause epididymis obstruction
Mumps orchitis	Testicular atrophy
Renal diseases	Oligospermia
Fertilization of ovum with sperm from undescended testes	Spontaneous abortion

INFLAMMATORY DISORDERS OF TESTIS

BACTERIAL ORCHITIS

Bacterial epididymitis is more common than orchitis, which is often associated with epididymitis. It is sexually acquired infection due to *Neisseria gonorrhoeae* (gonorrhea) or *Chlamydia trachomatis*. *Escherichia coli* associated urinary tract infection.

- Patient presents with intrascrotal pain and tenderness, with or without associated fever. Bilateral, orchitis may result in sterility due to atrophy of the seminiferous tubules.
- Serum testosterone is decreased, whereas pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are increased.

MUMPS ORCHITIS

Mumps orchitis begins 4–8 days after swelling of the parotid gland, and is caused by the mumps virus transmitted via the respiratory route by inhalation or oral contact with infected respiratory droplets or secretions. Patient presents with painful parotitis and orchitis after puberty. The virus is highly neurotropic, with laboratory evidence of central nervous system (CNS) infection in approximately half of cases (Fig. 23.13).

TUBERCULOUS EPIDIDYMO-ORCHITIS

Tuberculous epididymo-orchitis is usually a complication of pulmonary and renal tuberculosis (Fig. 23.14). The epididymis is enlarged and indurated and usually associated with testicular involvement by tuberculosis. Isolated tuberculous epididymo-orchitis may closely mimic testicular tumor particularly in patients with no history of systemic tuberculosis thereby posing a diagnostic and treatment challenges. Adequate evaluation of patients with testicular mass by means of abdominal and scrotal ultrasound coupled with fine needle aspiration cytology is critical in diagnostic accuracy, optimal treatment and possibility of avoiding surgery in those patients with testicular tuberculosis. Histologic examination reveals caseous necrosis, epithelioid cell granulomas and occasional Langhans' giant cells.

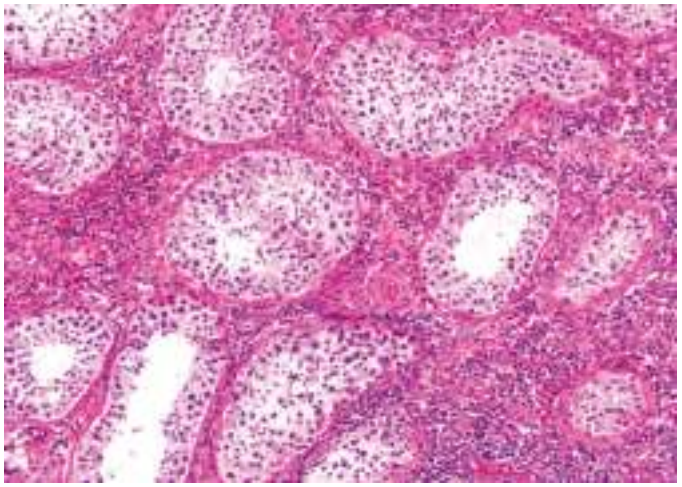


Fig. 23.13: Mumps orchitis. Testes become painful and swollen 4–6 days after parotitis. Testes show widespread foci of acute and chronic inflammatory cells destroying seminiferous tubules. Bilateral testes involvement may result in infertility (100X).

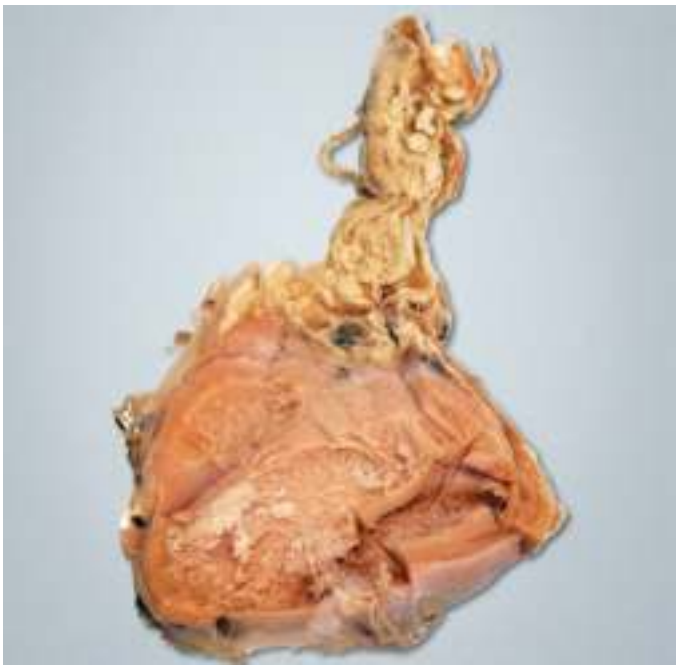


Fig. 23.14: Tuberculous epididymo-orchitis. The testis contains multiple rounded granulomatous lesions. The epididymis is almost completely replaced by similar tissue. The patient had disseminated tuberculosis.

GRANULOMATOUS ORCHITIS

Granulomatous orchitis is usually associated with trauma to testis. Some consider it as an autoimmune disorder, which affects middle-aged males.

- Some cases are associated with urinary tract infections, history of prostatectomy, inguinal hernia repair and trauma.
- Testicular involvement may be total or partial resulting in vaguely nodular, firm painful testicular enlargement associated with fever.
- Granulomatous orchitis is usually accompanied by sudden onset of painful testicular enlargement, tenderness in scrotal region and fever.
- Histologic examination reveals lymphocytes, plasma cells, macrophages, fibroblasts and scattered multinucleated giant cells.

SYPHILITIC ORCHITIS

Syphilitic orchitis is a rare manifestation of gumma in tertiary syphilis. Histologic examination of syphilitic

orchitis reveals multiple discrete epithelioid cell granulomas with central necrosis and peripheral fibrosis.

- Initially, there is edema of the tunica albuginea and interstitium, lymphocytic infiltration in interstitium, congestion and hemorrhage progressing to necrosis of the seminiferous tubules sparing Sertoli cells.
- The seminiferous tubules are infiltrated by lymphocytes, plasma cells and histiocytes.
- Clinically, syphilitic orchitis can resemble a neoplasm, which is confirmed by immunohistochemistry and PCR testing for *Treponema pallidum* DNA polymerase 1 gene using paraffin-embedded tissue sections.

NEOPLASTIC DISORDERS OF TESTIS

Testicular tumors are one of the most common neoplasms that occurs in males, which include germ cell tumors, sex cord-gonadal stromal tumors and secondary testicular tumors. About 95% of testicular tumors are germ cell tumors derived from primordial germ cells, which arise in midline locations such as gonads, retroperitoneum, mediastinum, and central nervous system (called germinoma). Spermatocytic seminoma arises from primary spermatocytes.

- Germ cell tumors are classified as seminomatous and nonseminomatous tumors. Seminoma is an example of seminomatous germ cell tumor. Nonseminomatous germ cell tumors include embryonal carcinoma, yolk sac tumor, choriocarcinoma, teratoma. Germ cell tumors exist in pure form in 40% and mixed form in 60% of cases. Seminoma is most radiosensitive germ cell tumor.
- Testicular germ cell may present as a painless scrotal mass as an incidental radiologic finding, post-traumatic symptom, scrotal pain. Diagnosis of testicular germ cell tumors involves the evaluation of serum tumor markers α-fetoprotein, human chorionic gonadotropin and lactate dehydrogenase, but clinically several types of immunohistochemical markers are more useful and more sensitive in germ cell tumors but not in teratoma.
- These new biomarkers are genes expressed in primordial germ cells/gonocytes and embryonic pluripotency related cells but not in normal adult germ cells , which include PLAP, OCT3/4 (POU5F1), NANOG, SOX2, REX1, AP-2γ (TFAP2C) and LIN28. Revised 2024 WHO classification of tumors of the testes is given in Table 23.11. Cystic lesions in testes during childhood are given in Table 23.12. Classification of testicular tumors is shown in Fig. 23.15.

Table 23.11 Revised 2024 WHO classification of tumors of the testes

Germ Cell Tumors derived from Germ Neoplasia *in situ*

- **Non-invasive germ cell neoplasia**
  - Germ cell neoplasia *in situ*
  - Specific forms of intratubular germ cell neoplasia
  - Intratubular seminoma
  - Intratubular embryonal carcinoma
  - Intratubular trophoblastic tumor
  - Intratubular yolk sac tumor
  - Intratubular teratoma
  - Gonadoblastoma
- **Germinoma family of tumors**
  - Seminoma
  - Seminoma with syncytiotrophoblastic element
- **Nonseminomatous tumors**
  - Embryonal carcinoma
  - Yolk sac tumor, post-pubertal type
  - Choriocarcinoma
  - Placental site trophoblastic tumor of the testis
  - Epithelioid trophoblastic tumor
  - Cystic trophoblastic tumor
  - Teratoma postpubertal type
  - Teratoma somatic type malignancy
- **Mixed germ cell tumors of the testis**
  - Mixed germ cell tumors
  - Polyembryoma
  - Diffuse embryoma
- **Germ cell tumors of unknown type**
  - Regressed germ cell tumors

Germ Cell Tumors Unrelated to Germ Cell Neoplasia *in situ*

- Spermatocytic seminoma
- Spermatocytic seminoma with sarcomatous differentiation
- Teratoma prepubertal-type
- Dermoid cyst
- Epidermoid cyst
- Yolk sac tumor, prepubertal type
- Well-differentiated neuroendocrine tumor (monodermal teratoma)
- Mixed teratoma and yolk sac tumor prepubertal-type

Sex Cord-Stromal Tumors

- **Leydig cell tumors**
  - Leydig cell tumor
  - Malignant Leydig cell tumor
- **Sertoli cell tumors**
  - Sertoli cell tumor
  - Malignant Sertoli cell tumor
  - Large cell calcifying Sertoli cell tumor
- **Granulosa cell tumors**
  - Adult granulosa cell tumor
  - Juvenile germ cell tumor
- **Fibroma thecoma family of tumors**
  - Thecoma
  - Fibroma

Contd...

Table 23.11 Revised 2024 WHO classification of tumors of the testes (*Contd...*)

- **Mixed and other sex cord-stromal tumors**
  - Mixed sex cord-stromal tumor
  - Signet ring stromal tumor
  - Mixed gonadal stromal tumor
  - Sex cord stromal tumor, NOS

Tumors of the Testicular Adnexa

- **Ovarian-type tumors of the collecting ducts and rete testis**
  - Serous cystadenoma, NOS
  - Serous borderline tumor, NOS
  - Serous cystadenocarcinoma
  - Mucinous cystadenoma
  - Mucinous borderline tumor
  - Mucinous cystadenocarcinoma
  - Endometrioid borderline tumor
  - Endometrioid adenocarcinoma
  - Clear cell adenocarcinoma
  - Brenner tumor
- **Tumors of the colleting ducts and rete testis**
  - Adenoma
  - Adenocarcinoma
- **Paratesticular mesothelial tumors**
  - Adenomatoid tumor
  - Well differentiated papillary mesothelial tumor
  - Mesothelioma
  - Epithelioid mesothelioma
  - Sarcomatoid mesothelioma
  - Biphasic mesothelioma
- **Tumors of the epididymis**
  - Cystadenoma of the epididymis
  - Papillary cystadenoma
  - Adenocarcinoma of the epididymis
  - Squamous cell carcinoma
  - Melanotic neuroectodermal tumor

Hematolymphoid Tumors

- Diffuse large B cell lymphoma
- Follicular lymphoma, NOS
- Extranodal NK/T cell lymphoma, nasal type
- Plasmacytoma
- Myeloid sarcoma
- Rosai-Dorfman disease

GERM CELL TUMORS OF TESTIS

Tumor confined to seminiferous tubule is known as intratubular neoplasia. Seminoma directly originates from primordial germ cell. Seminoma is not end stage neoplasm, but may also act as precursor of other germ cell tumors such as embryonal carcinoma. Germ cell differentiates into totipotent stem cells. Tumors arising from totipotent stem cells may show embryonic differentiation (e.g. embryonal carcinoma and teratoma), and extra-embryonic differentiation (e.g. yolk sac tumor and choriocarcinoma). Occurrence of germ cell tumors of testes in various age groups is given in Table 23.13.

Table 23.12 Cystic lesions in testes during childhood

Yolk sac tumor
Teratoma
Dermoid cyst
Epidermoid cyst
Granulosa cell tumor (juvenile type)
Cystic lymphangioma
Cystic dysplasia of rete ridges
Simple cyst

- **Etiopathogenesis:** Although the cause of testicular germ cell tumors is unknown, there is a possible genetic link and geographic risk for acquiring these tumors. Approximately 95% of testicular germ cell tumors arising in the testis are germ cell tumors. Risk factors for testicular germ cell tumors are given in Table 23.14.
- **Molecular genetic alterations:** Molecular genetic alterations in germ cell tumors include isochromosome 12, random gain of chromosomes, novel gene, DAD-R gene, aneuploid DNA content and increased telomerase activity (Table 23.15).
- **Clinical features:** Often the first sign of testicular germ cell tumor is a slight enlargement of the testicle that may be accompanied by some degree of discomfort. There may be an ache in the abdomen or groin or a sensation of dragging or heaviness in the scrotum. Pain may be experienced in the later stage as germ cell tumor grows rapidly.
- **Mode of spread:** Testicular germ cell tumors spread by lymphatic and hematogenous routes (Table 23.16), which can spread when the tumor may be barely palpable. Signs of metastatic spread include swelling of the lower extremities, back pain, neck mass, cough, hemoptysis, and/or dizziness.
- **Diagnostic approach:** Thorough urologic clinical history and physical examination are essential for diagnosis of a painless testicular mass. Conditions that produce an intra-scrotal mass similar to testicular germ cell tumor include epididymitis, orchitis, hydrocele, or hematocele.
  - Clinical examination for testicular lumps should include palpation of the testes and surrounding structures, transillumination of the scrotum, and abdominal palpation.
  - Testicular ultrasonography can be used to differentiate testicular masses. CT scan and MRI are used in assessing metastatic spread. Serum tumor markers are used to evaluate testicular mass for diagnosis,

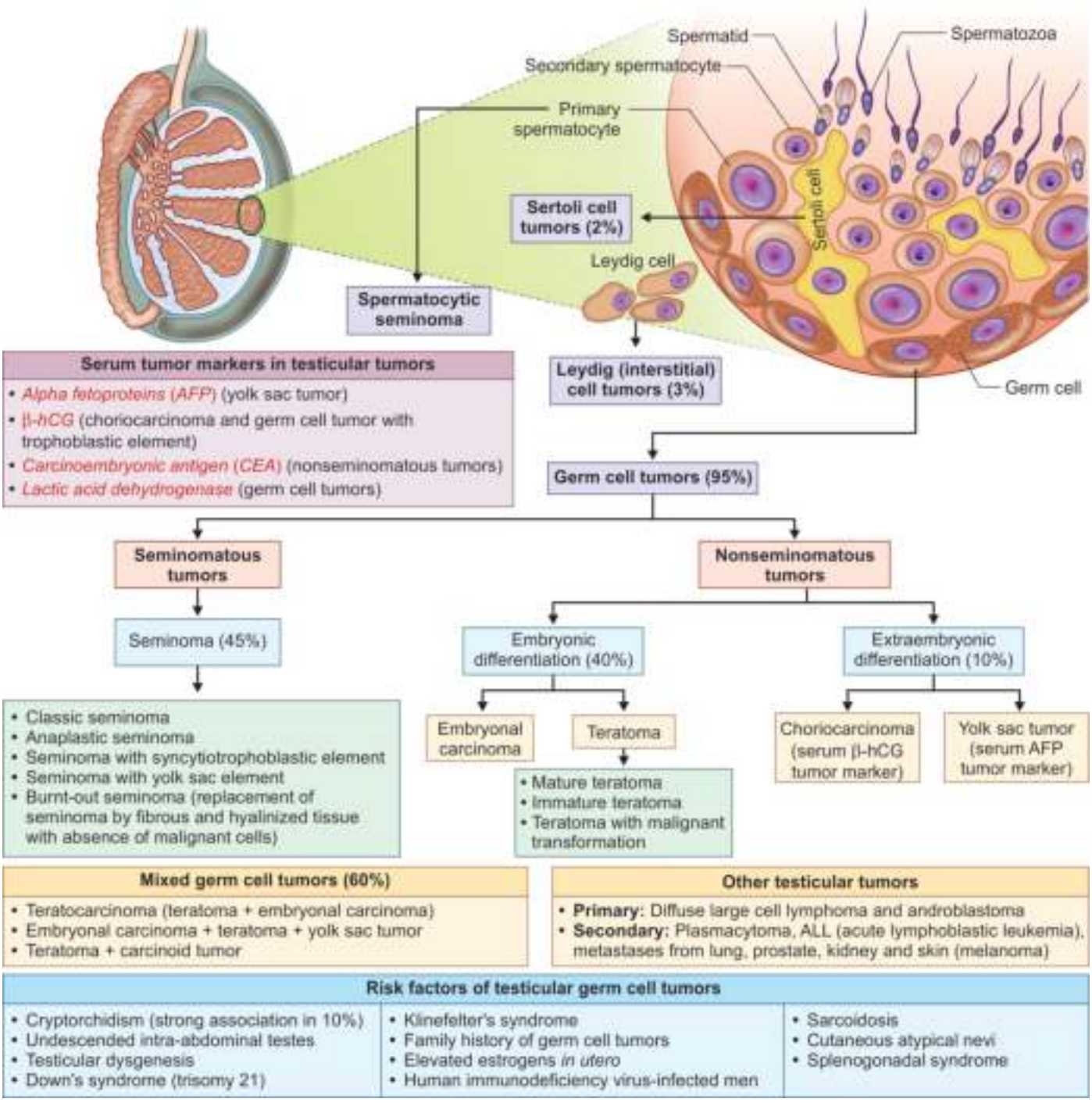


Fig. 23.15: Classification of testicular tumors.

staging, monitor response to therapy, detect relapse and assess tumor burden.

- A potential diagnostic pitfall of new biomarkers is their expression in fetal germ cells. Tumor markers that measure protein antigens produced by malignant cells provide information about the existence of a testicular germ cell tumor and the type of tumor present. These biomarkers may detect tumors that are too small to be found on physical examination or radiographs (Table 23.17).
- **Molecular diagnostics:** Transcription factor encoded by OCT 3/4 gene is expressed in primordial germ cells as well as in seminoma and embryonal carcinoma analyzed by immunoperoxidase technique. Testicular germ cell tumors show inactivation of X chromosome detected by polymerase chain reaction (PCR) technique.

Table 23.13 Occurrence of germ cell tumors of testes in various age groups

Germ Cell Tumors	Age Group	Protein Synthesized by Germ Cell Tumor	Effect of Radiation Therapy
Seminoma	▪ 30–40 years (most common) ▪ Prepuberty and >70 years (rare)	hCG (15% of cases)	Radiosensitive
Spermatocytic seminoma	>40 years of age	None	Unknown
Embryonal carcinoma	▪ 20–30 years (mean 30 years) ▪ Prepuberty and >50 years (rare)	AFP (in mixed tumor)	Radioresistant
Yolk sac tumor	▪ Prepubertal yolk sac tumor (3 to 39 months) ▪ Postpubertal yolk sac tumor (25 to 30 years)	AFP	Radioresistant
Choriocarcinoma	20–30 years	hCG	Radioresistant
Teratoma	▪ Prepubertal teratoma (20 months) ▪ Postpubertal teratoma (20–30 years and mean age 18 years)	None	Radioresistant
Mixed germ cell tumors	▪ 28 years (if NSGCT component present) ▪ 33 years (if seminomatous component present)	Variable	Radioresistant

AFP (α-fetoprotein), hCG (human chorionic gonadotropin)

Table 23.14 Risk factors for testicular germ cell tumors

Cryptorchidism (strong association in 10%)
Undescended intra-abdominal testes
Testicular dysgenesis
Down's syndrome (trisomy 21)
Klinefelter's syndrome
Family history of germ cell tumors
Elevated estrogens *in utero*
Human immunodeficiency virus-infected men
Sarcoidosis
Cutaneous atypical nevi
Splenogonadal syndrome

- **Clinical staging:** The clinical staging (TNM classification) for testicular germ cell tumor is as follows: stage I, tumor is confined to testes, epididymis, or spermatic cord; stage II, tumor spreads to retroperitoneal lymph nodes below the diaphragm; and in stage III, metastases occur outside the retroperitoneal nodes or above the diaphragm. In stage IV, germ cell tumor has spread beyond the abdomen to other parts of the body. Staging procedures include CT scans of the chest, abdomen, and pelvis; ultrasonography for detection of bulky inferior nodal metastases; and occasionally lymphangiography.
- **Treatment:** The basic treatment of all testicular germ cell tumors includes orchiectomy, which is done at the time of diagnostic exploration. Surgical excision is advantageous, because it enables precise diagnosis of the disease.

Table 23.15 Molecular genetic alterations in germ cell tumors of testes

Features	Comments
Isochromosome 12p	Germ cell tumors (90%)
Random gain of chromosomes 1, 7, 9, 12, 17, 21, 22, X	Germ cell tumors (70%)
Novel DAD-R gene amplification	Germ cell tumors (majority cases)
DNA ploidy (abnormal number of chromosomes analyzed by flow cytometry)	▪ Germ cell tumors (majority cases) ▪ Hyperdiploid (triploid or tetraploid) ▪ Mean DNA indices higher in seminoma than NSGCT ▪ Spermatocytic seminoma is diploid and or near hyperdiploid
Telomerase activity	▪ Increased in all germ cell tumors except teratoma ▪ Inverse relationship between telomerase activity and differentiation stage of germ cell tumors

Table 23.16 Mode of spread of testicular germ cell tumors

Mode of Spread	Sites of Metastases of Germ Cell Tumors
Lymphatic route	▪ Para-aortic, iliac lymph nodes and later to mediastinal and left supraclavicular lymph nodes ▪ Para-aortic lymph nodes involved in embryonal carcinoma (adults) and yolk sac tumor (children) ▪ Retroperitoneal lymph nodes below renal vessels involved on same side (80–85%) and both sides (13–20%) ▪ Inguinal lymph nodes involved even without invasion of skin or scrotum by germ cell tumor ▪ Inguinal lymph nodes can also be involved in recurrence of germ cell tumor in a cutaneous scar, or a history of previous operation in the area
Hematogenous route	▪ Seminoma metastasizes to lungs, liver, brain, and bone ▪ Embryonal carcinoma metastasizes early to distant organs ▪ Choriocarcinoma metastasizes to lungs, liver, brain, and bones

Mature teratoma in the postpubertal testis is accompanied by metastases. Conversely, mature teratoma of the prepubertal testis has never been shown to metastasize.

Table 23.17 Immunohistochemical markers of germ cell tumors of testes

Immunohistochemical Markers	Seminoma	Embryonal Carcinoma	Yolk Sac Tumor	Spermatocytic Seminoma
LIN28 (RNA binding protein)	Positive (>80%)	Positive (>80%)	Positive (>80 %)	Positive (<20%)
SALL4 (nuclear staining)	Positive (>80%)	Positive (>80%)	Positive (>80 %)	Positive (variable)
PLAP (placental alkaline phosphatase)	Positive (>80%)	Positive (>80%)	Positive (>80 %)	Positive (<20%)
c-KIT (CD117)	Positive (>80%)	Positive (<20%)	Positive (<20%)	Positive (variable)
OCT4	Positive (>80%)	Positive (>80%)	Positive (<20%)	Positive (<20%)
CD30	Positive (<20%)	Positive (>80%)	Positive (variable)	Positive (<20%)
Vimentin	Positive (strong staining)	Positive	Positive	Positive
LDH (lactic dehydrogenase)	Positive (strong staining)	Negative	Negative	Negative
Podoplanin	Positive (strong staining)	Negative	Negative	Negative
α_1-Antitrypsin	Negative	Negative	Positive (strong staining)	Negative
α-Fetoprotein (AFP)	Negative (positive in seminoma with yolk sac tumor element)	Negative	Positive (strong staining)	Negative
hCG	Negative (positive in seminoma with syncytiotrophoblastic element)	Negative	Negative	Negative
Cytokeratin (in choriocarcinoma +)	Negative	Positive	Positive (strong staining)	Negative

α-Fetoprotein (AFP) is synthesized by fetal gut, liver and yolk sac. One year after birth, it is less than 16 ng/ml in serum and undetectable except by most sensitive methods. It is increased in cases of yolk sac tumor.

β-hCG is increased in choriocarcinoma and seminoma with syncytiotrophoblastic cells.

Testicular germ cell tumors show inactivation of X chromosome detected by polymerase chain reaction (PCR).

- Recommendations for further therapy of testicular germ cell tumors (e.g. retroperitoneal lymph node dissection, chemotherapy, radiation therapy) are based on the pathologic findings from the surgical procedure.
- Treatment of germ cell tumor of testes after orchiectomy depends on the histologic characteristics of the tumor and the clinical stage of the disease. With appropriate treatment, the prognosis for men with testicular germ cell tumor is excellent. Comparison of seminomatous and nonseminomatous germ cell tumors is given in Table 23.18.

▪ **Prognosis:** Men with stage I and stage II disease do very well. Men, who have retroperitoneal lymph node dissection may experience retrograde ejaculation or failure to ejaculate because of severing of the sympathetic plexus. Infertility may result from retrograde ejaculation or the toxic effects of

Table 23.18 Comparison of seminomatous and nonseminomatous germ cell tumors

Characteristics	Seminomatous Tumors	Nonseminomatous Tumors (NSGCTs)
Germ cell tumors	Seminoma	▪ NSGCTs with embryonic differentiation (embryonal carcinoma and teratoma) ▪ NSGCTs with extraembryonic differentiation (yolk sac tumor and choriocarcinoma)
Diagnosed in stage	70% of patients are diagnosed in stage I	Majority of patients are diagnosed in stage II/III
Mode of spread	Lymphatic spread (retroperitoneal/para-aortic, mediastinal, and supraclavicular lymph nodes) followed by hematogenous route (in lungs, liver, brain and bone)	Lymphatic, hematogenous route (lungs, liver, bones and brain)
Metastasis	Late metastases	Early metastases
Management	Seminoma is radiosensitive tumor. Stage I and II seminomas are treated by irradiation of retroperitoneum up to diaphragm. Bulky retroperitoneal or distant metastases of seminoma are treated by chemotherapy	Nonseminomatous germ cell tumors are radioresistant tumors and treated by chemotherapy, or retroperitoneal lymph node dissection
Prognosis	▪ Good prognosis ▪ Poor prognosis (tumor >4 cm and rete ridges invasion)	▪ Good prognosis (teratoma) ▪ Poor prognosis (vascular invasion, S phase fraction >29%)

chemotherapy or radiation therapy. Sperm banking should be considered for men undergoing treatment of testicular germ cell tumors.

Seminoma of Testis

Seminoma is most common malignant testicular germ cell tumor, which is analogous to dysgerminoma, a germ cell ovarian tumor. It accounts for 50% of all testicular germ cell tumors. The tumor replaces the whole testis and rarely involves tunica vaginalis. Seminoma occurs commonly between the ages of 25–50 years with peak incidence at mid-30s age group. Seminoma is virtually nonexistent before puberty and rare in adolescence. Undescended (cryptorchid) testis is major predisposing factor for seminoma. Burnt out seminoma refers to replacement of seminoma by fibrous and hyalinized tissue (sometimes calcification in scar) and absence of malignant cells.

Clinical Features

Patient presents with painless unilateral testicular swelling; and about 10% of patients experience local discomfort and pain. Clinical manifestations occur due to metastases of seminoma include supraclavicular and retroperitoneal lymphadenopathy.

- Seminoma with syncytiotrophoblastic element is associated with gynecomastia due to increased serum human chorionic gonadotropin (hCG). The serum human chorionic gonadotropin level can also be elevated in choriocarcinoma and combined germ cell tumors.
- Prognosis is poor in patient with seminoma >4 cm and infiltration of tumor cells in the epithelium of rete testis. The risk of tumor relapse after orchidectomy is directly proportional to the tumor diameter.
- Patient treated with chemotherapy often experience late complications such as metabolic syndrome, secondary neoplasms and myocardial infarction.

Surgical Pathology: Seminoma of Testis

Gross Morphology

- Small seminoma is well-circumscribed and homogenous. Large seminoma replaces entire testis in 50% of cases.
- The tumor is well circumscribed, solid, rubbery-firm, bulky mass, sometimes attains 10 times the size of normal testis.
- Tunica albuginea is mostly intact without penetration by the tumor.
- Epididymis is usually uninvolved. Occasionally extension of tumor involves epididymis, spermatic cord or scrotal sac.
- On cut section, tumor bulges above the testicular parenchyma and appears lobulated, well circumscribed but without a capsule, gray-white to pinkish, homogenous, and inconspicuous necrosis and hemorrhages.
- Presence of extensive areas of hemorrhage, firm or cystic areas are strongly suggestive of an NSGCT component. Extensive sampling is required if >4 cm size tumor shows these morphologic features. Gross morphology of seminoma is shown in Fig. 23.16.

Light Microscopy

Histologic variants of seminoma are classic seminoma (85–90%), anaplastic seminoma, seminoma with syncytiotrophoblastic element and seminoma with yolk sac tumor element (Table 23.19).

Classic Seminoma

- Classic seminoma shows lobules composed of a few dozen to 100 uniform, round tumor cells separated by thin fibrous

septa and arranged in sheets. The monomorphic cells resemble immature spermatogonia. The cytoplasm is rich in glycogen and lipids and therefore appears water clear in formalin-fixed specimen. The tumor cells contain centrally located nuclei and prominent nucleoli with distinct cell borders. The nucleolus has characteristics amphophilic staining pattern, apparent multiplicity, elongated shape, and irregular contours. The number of mitoses is highly variable.

- Fine to thick fibrous septa separate lobules of tumor cells. These fibrous septa are infiltrated by numerous lymphocytes (CD8+ cytotoxic T cells and CD4+ helper T cells and some NK cells and B cells), plasma cells, macrophages and eosinophils. Noncaseating granulomatous reaction containing Langhans' type multinucleated giant cells and epithelioid cell granulomas may also be present in 25% of cases. The noncaseating granulomatous reaction is patchy but immunologic response can obscure the morphology of neoplastic cells. It is therefore crucial to examine closely any testis with an extensive noncaseating granulomatous reaction for a residual seminoma tumor cells or intratubular germ cell neoplasia before accepting such as granulomatous orchitis (Fig. 23.17).
- Intratubular germ cell neoplasia is a precursor lesion known as intratubular germ cell neoplasia (ITGCN) often identified in the background seminiferous tubules (Fig. 23.18A).

Anaplastic Seminoma

Anaplastic seminoma is a histologic subtype of seminoma with marked cellular pleomorphism with 6 or more atypical mitotic figures per high power field. Prognosis of anaplastic seminoma is worse than classic seminoma (Fig. 23.18B).

Seminoma with Syncytiotrophoblastic Component

- Presence of multinucleated giant cells of syncytiotrophoblastic element around blood vessels reveals foci of hemorrhages around them within 7% of seminomatous tumors. Prognosis of this variant is relatively poor (Fig. 23.19).
- Serum hCG levels, elaborated by syncytiotrophoblastic element present in seminoma, are elevated in about 15% of cases, but these elevations are not as high as those seen in choriocarcinoma.

Seminoma with Yolk Sac Component

- Seminoma exhibits foci consistent with yolk sac differentiation, by virtue of the architectural arrangement, hyaline globules, and immunoreactivity for **α-fetoprotein** (AFP).
- Sometimes yolk sac element is missed during reporting. Seminoma associated with yolk sac component is usually associated with increased serum AFP.

Histochemistry

The tumor cells in seminoma are rich in glycogen. It can be demonstrated by periodic staining (PAS) with diastase pretreatment.

Immunohistochemistry Technique

- Panel of markers is used in surgical specimens of seminoma is mentioned as follows:

Markers	Expression
▪ LIN28 (RNA binding protein)	▪ Positive (>80%)
▪ SALL4 (nuclear staining)	▪ Positive (>80%)
▪ PLAP (placental alkaline phosphatase)	▪ Positive (>80%)
▪ c-KIT (CD117)	▪ Positive (>80%)
▪ OCT4	▪ Positive (>80%)
▪ CD30	▪ Positive (<20%)
▪ Vimentin	▪ Positive (strong staining)
▪ LDH (lactic dehydrogenase)	▪ Positive (strong staining)
▪ Podoplanin	▪ Positive (strong staining)
▪ α-Fetoprotein (AFP)	▪ Positive (seminoma with yolk sac tumor element)
▪ hCG	▪ Positive (seminoma with syncytiotrophoblastic element)
▪ α_1-Antitrypsin	▪ Negative
▪ Cytokeratin (in choriocarcinoma +)	▪ Negative

Seminoma with syncytiotrophoblastic element shows positivity for human hCG. The prognosis of this variant is relatively more aggressive.

DNA Ploidy and Cytogenetics in Seminomas

All seminomas are aneuploid, mostly with hypertetraploid stem cells. Like all germ cell tumors, seminomas demonstrate consistent gain of isochromosome p12; the number of copies is however lower than in nonseminomatous germ cell tumors (NSGCTs). Seminomatous and nonseminomatous germ cell tumors (NSGCTs) also show gain of chromosomes 1,

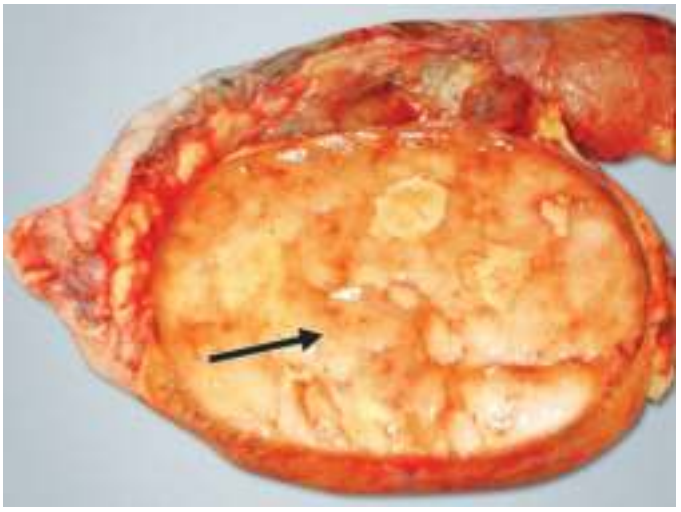


Fig. 23.16: Seminoma of the testis. Large seminoma replaces entire testis in 50% of cases. The tumor is well circumscribed, solid, rubbery-firm, bulky mass, sometimes attains 10 times the size of normal testis. Tunica albuginea is mostly intact without penetration by the tumor. On cut section, tumor bulges above the testicular parenchyma and appears lobulated, well circumscribed but without a capsule, gray-white to pinkish, homogenous, and inconspicuous necrosis and hemorrhages (arrow).

Table 23.19 Features of histologic variants of seminoma

Histologic Variants of Seminoma	Characteristics
Classic seminoma	▪ Monomorphic tumor cells are arranged in sheets divided by fibrous septa. Fibrous septa are infiltrated by chronic inflammatory cells ▪ A granulomatous reaction containing Langhans' type multinucleated giant cells and epithelioid cells may also be present in 25%
Anaplastic seminoma	▪ Marked cellular pleomorphism with 6 or more mitotic figures per high power field ▪ Prognosis of this variant is worse than classic seminoma
Seminoma with syncytiotrophoblastic element	Syncytiotrophoblastic element synthesizes hCG leading to increased serum levels
Seminoma with yolk sac element	Tumor cells show immunoreactivity for α-fetoprotein (AFP)

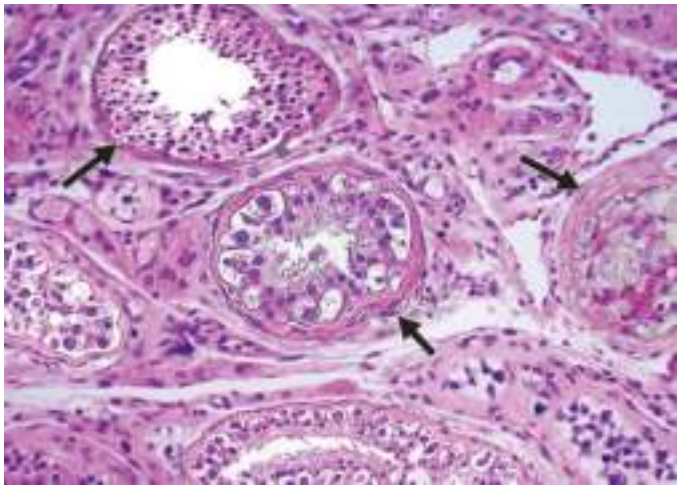


Fig. 23.17: Intratubular seminoma with syncytiotrophoblastic cells. The seminiferous tubules are entirely filled with seminoma cells. In most instances, invasive foci are observed in the specimen (arrows) (400X).

2, 7, 8, 20, and X; and loss of chromosomes 4, 5, 9, 11q, 13 and 18. Additionally, seminomas can demonstrate gain of chromosomes 3p, 6p, 10, and 22; and loss of chromosomes 1q, 9p, 11p, 16p, and 17p.

Management

Seminomas are exclusively sensitive to radiation and chemotherapy (carboplatin) even when the metastases are present in the abdominal para-aortic and paracaval lymph nodes. These modalities usually represent the primary forms of treatment following orchidectomy. Cure rates exceeding >95% can be expected in these cases.

- The most important prognostic factor of seminoma is tumor stage, which includes tumor bulk. Seminoma with syncytiotrophoblastic component and high hCG may have poor prognosis and survival.
- Presence of a lymphoid infiltrate and prominent noncaseating granulomatous reaction in seminoma

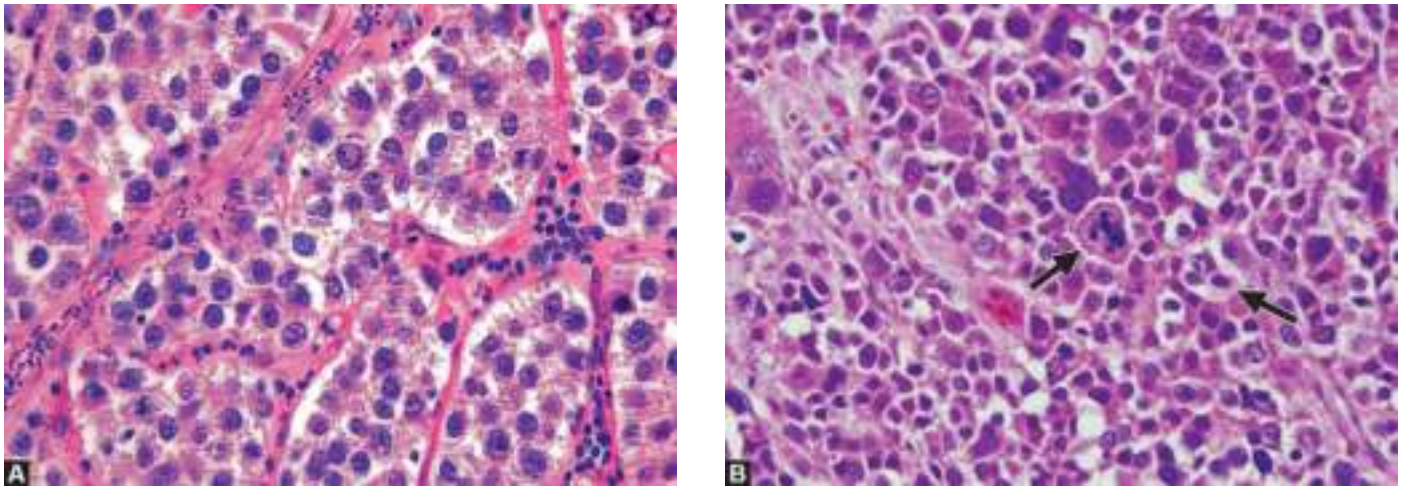


Fig. 23.18: Classic seminoma. (A) Seminoma shows lobules composed of a few dozen to 100 uniform, round neoplastic cells with clear cytoplasm, contain centrally located nuclei and prominent nucleoli with distinct cell borders separated by thin fibrous septa and arranged in sheets. Fibrous septa are infiltrated by numerous lymphocytes, plasma cells, macrophages and eosinophils. Noncaseating granulomatous reaction containing Langhans' type multinucleated giant cells and epithelioid cells may also be present in 25% of cases. (B) Anaplastic seminoma shows marked cellular pleomorphism with 6 or more mitotic figures per high power field. Prognosis of this variant is worse than classic seminoma (arrows) (400X).

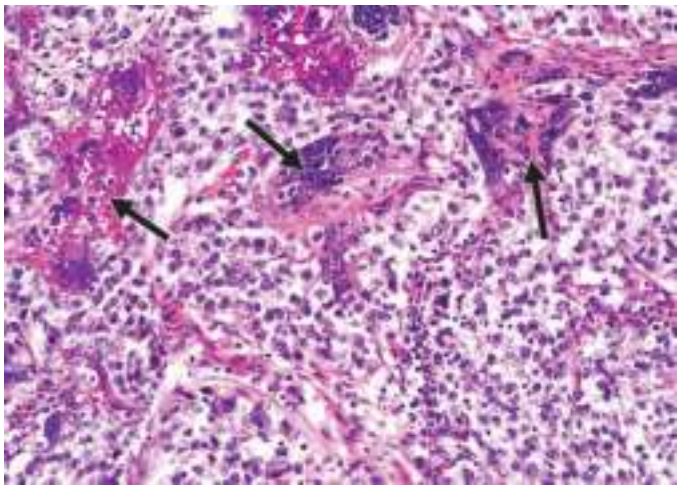


Fig. 23.19: Seminoma with syncytiotrophoblastic element. Presence of multinucleated giant cells of syncytiotrophoblastic element around blood vessels reveals foci of hemorrhages around them within 7% of tumors (arrows) (100X).

may be associated with borderline favorable prognosis. Anaplastic seminoma is associated with poor prognosis.

Spermatocytic Seminoma of Testis

Spermatocytic seminoma is a rare slow-growing tumor seen in older men (average age >65 years), which constitutes <1% of all germ cell tumors. However, it can affect younger patients between 30 and 40 years of age. Spermatocytic seminoma originates from primary spermatocyte and not primordial germ cells.

- There is no predisposing factor such as cryptorchidism for spermatocytic seminoma.
- In contrast to classic seminoma, spermatocytic seminoma is never seen in combination with non-seminomatous germ cell tumor (NSGCT). Metastases of spermatocytic seminoma are rare; hence prognosis is excellent following simple orchidectomy.

Clinical Features

Patient presents with painless swelling in the scrotal region. Metastases are practically non-existing. Sometimes, spermatocytic seminoma exhibits highly malignant component of sarcomatous element of skeletal muscle differentiation resulting in widespread metastases with associated fatal outcome.

Surgical Pathology: Spermatocytic Seminoma of Testis

Gross Morphology

The tumor measures 3–4 cm in size but sizes up to 15 cm. The outline of the tumor well is wall-circumscribed, soft and grayish white in color. Tumor bulges out on cut surface. Cut surface is soft, friable, edematous and gelatinous/mucinous appearance. Hemorrhage and necrosis are limited (Fig. 23.20).

Light Microscopy

- The tumor cells resemble primary spermatocytes showing marked degree of pleomorphism. Tumor is composed of three distinct cell types: small cells (6–8 micron), medium cells (15–20 micron) and scattered single mononucleated or multinucleated giant cells (50–150 micron).
- Tumor is composed of tumor cells with filamentous nuclear chromatin resembling spermatocytes. The cytoplasm is devoid of glycogen. Main part of the tumor shows an expansile growth pattern, surrounding seminiferous tubules are greatly extended and filled with neoplastic cells. Tubular rupture in more than one area explains the occurrence of multiple distinct nodules in one testis.
- In contrast to other germ cell tumors (GCTs); carcinoma *in situ* (CIS) cannot be demonstrated in the adjoining seminiferous tubules in spermatocytic seminoma. These seminiferous tubules may show normal spermatogenesis. The stroma is conspicuous and lacking lymphocytic infiltration.
- Tumor demonstrates large edematous areas similar to those of microcystic seminomas.
- Tumor may demonstrate skeletal muscle differentiation (rhabdomyosarcomatous component) associated with poor prognosis (Fig. 23.21). Differences between classic seminoma and spermatocytic seminoma are given in Table 23.20.

Immunohistochemistry Technique

Panel of markers is used for work up of spermatocytic seminoma.

Markers	Expression
LIN28 (RNA binding protein)	Positive (<20%)
SALL4 (nuclear staining)	Positive (variable)
PLAP (placental alkaline phosphatase)	Positive (<20%)
c-KIT (CD117)	Positive (variable)
OCT4	Positive (<20%)
CD30	Positive (<20%)
Vimentin	Positive
NUT (recent marker)	Positive (variable)
GAGE7 (recent marker)	Positive (variable)
NY-ESO-1 (recent marker)	Positive (variable)
LDH (lactic dehydrogenase)	Negative
Podoplanin	Negative
α_1-Antitrypsin	Negative
α-Fetoprotein (AFP)	Negative
hCG	Negative
Cytokeratin	Negative

DNA Ploidy and Cytogenetics in Spermatocytic Seminoma

Numerical chromosomal aberrations are common. Gain of chromosome 9 and loss of isochromosome 12p are characteristics in spermatocytic seminoma. Tumor expresses NY-ESO-1 (the product of a cancer testis gene), like normal spermatogonia, primary spermatocytes, and

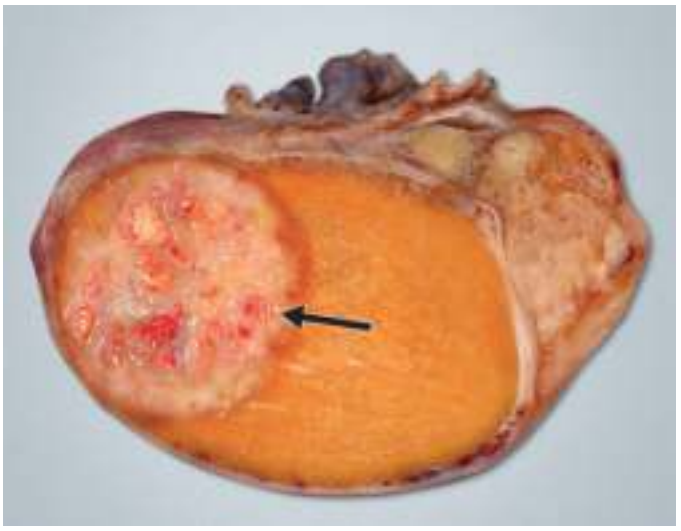


Fig. 23.20: Spermatocytic seminoma. The tumor measures 3–4 cm in size but sizes up to 15 cm. The outline of the tumor well is well-circumscribed, soft and grayish white in color. Tumor bulges out on cut surface. Cut surface is soft, friable, edematous and gelatinous/mucinous appearance. Hemorrhage and necrosis are limited in extent (arrow).

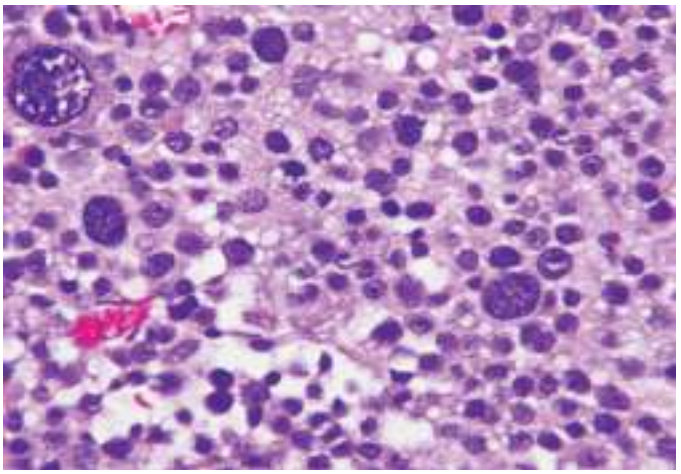


Fig. 23.21: Spermatocytic seminoma. The tumor cells are of three types. Small (6–8 mm), medium (15–20 mm) and large cells (50–100 mm) exhibit a filamentous or "spireme" chromatin distribution (400X).

the cells of intratubular germ cell neoplasia (IGCN), in contrast to classic seminoma and nonseminomatous germ cell tumors.

Table 23.20 Differences between classic seminoma and spermatocytic seminoma

Parameters	Classic Seminoma	Spermatocytic Seminoma
Origin	Primordial germ cell	Primary spermatocyte
Peak age	4th decade	>65 years of age
Frequency	Most common (40–50%)	Uncommon (1–2%)
Sites	Testes, mediastinum, retroperitoneum (seminoma testes counterpart of dysgerminoma ovary)	Testes only
Bilateral involvement	Bilateral testes (2%)	Bilateral testes (9%)
Association with cryptorchidism	Yes	No
Combined with other germ cell tumors	Yes (≤15%)	Never
Combined with germ cell neoplasia *in situ* (GCNIS)	Yes (>90%)	Never
Intercellular edema	Uncommon	Common
Molecular genetic alterations	Gain of isochromosome p12, 1, 2, 7, 8, 20 and X, 3p, 6p	NY-ESO-1 (the product of a cancer-testis gene)
Growth	Rapid growth	Slow growth
Gross morphology		
Replacement of testis	Complete replacement of testes in 50% of cases	Partial replacement of testis
Tumor size	Well circumscribed, solid, rubbery-firm, bulky mass, sometimes attains 10 times the size of normal testis	The tumor measures 3–15 cm
Cut surface of tumor	Cut surface appears lobulated gray-white homogenous	Tumor bulges on the cut surface and soft, friable, edematous and gelatinous/mucinous appearance
Histologic features		
Histologic variants	Classic seminoma, anaplastic seminoma, seminoma with syncytiotrophoblastic element and seminoma with yolk sac component	Spermatocytic seminoma

Contd..

Table 23.20 Differences between classic seminoma and spermatocytic seminoma (*Contd...*)

Parameters	Classic Seminoma	Spermatocytic Seminoma
Cell morphology	Monomorphic tumor cells are divided by fibrous septa	Tumor consists of polymorphous triphasic cells with eosinophilic cytoplasm and round dark nuclei, and lacks fibrous septa
Stroma	Scanty prominent	Stroma scanty
Lymphoid reaction	Lymphoid reaction prominent	Lymphoid reaction scanty
Granulomas	Often present	Extremely rare
Sarcomatous transformation	Absent	Occasional
Histochemistry		
Glycogen in tumor cells	Abundant (PAS positive)	Absent to scanty (PAS negative)
Immunohistochemistry Technique		
LIN28 (RNA binding protein)	Positive (>80%)	Positive (<20%)
SALL4 (nuclear staining)	Positive (>80%)	Positive (variable)
PLAP (placental alkaline phosphatase)	Positive (>80%)	Positive (<20%)
c-KIT (CD117)	Positive (>80%)	Positive (variable)
OCT4	Positive (>80%) diffuse staining	Positive (<20%)
CD30	Positive (<20%)	Positive (<20%)
Vimentin	Positive (strong staining)	Positive
NUT (recent marker)	Negative	Positive (variable)
GAGE7 (recent marker)	Negative	Positive (variable)
NY-ESO-1 (recent marker)	Negative	Positive (variable)
LDH (lactic dehydrogenase)	Positive (strong staining)	Negative
Podoplanin	Positive (strong staining)	Negative
α_1-Antitrypsin	Negative	Negative
α-Fetoprotein (AFP)	Negative (positive in seminoma with yolk sac tumor element)	Negative
hCG (human chorionic gonadotropin)	Negative (positive in seminoma with syncytiotrophoblastic element in 10%)	Negative
Cytokeratin	Negative	Negative
Chromosomal abnormality		
Chromosome	Inversion p12	Gain of chromosome 9
Prognosis		
Metastasis	Frequent	Extremely rare

Young age does not exclude the diagnosis of spermatocytic seminoma.

Prognosis

Spermatocytic seminoma may involve bilateral testes. Metastases are practically nonexistent associated with excellent prognosis and, therefore, simple orchiectomy may be sufficient therapy.

Embryonal Carcinoma of Testis

Embryonal carcinoma is a highly malignant nonseminomatous germ cell tumor (NSGCT), which is analogous to a similar tumor occurring in the ovary. Tumor is composed of primitive cells that retain the ability to differentiate toward somatic or extraembryonic structures. It most often occurs in younger age group between 25 and 35 years of age. This tumor is rare in children and persons over 50 years of age. Pure embryonal carcinoma accounts for 2–10% of all germ cell tumors. But embryonal carcinoma is very frequent (80%) in mixed germ cell tumors with more than one germ cell tumor component. It most often occurs mixed with teratoma in 40% of cases and known as teratocarcinoma, that shows an increase in both carcinoembryonic antigen (CEA) and α-fetoprotein (AFP) in 90% of cases.

Clinical Features

Patient presents with painless unilateral testicular swelling. Tumor invades the testis, epididymis, blood vessels and metastasizes to retroperitoneal para-aortic lymph nodes (40%), supradiaphragmatic lymph nodes (20%), lungs, liver, brain and other organs. Teratocarcinoma often metastasizes as embryonal carcinoma, and the reverse also occurs. About 10% of patients suffer from systemic manifestations such as fever, weight loss, night sweats, headache.

Surgical Pathology: Embryonal Carcinoma of Testis

Gross Morphology

- Embryonal carcinoma is the smallest pure form of germ cell tumor measuring 2.5 cm and not replacing entire testis.
- Cut surface of tumor reveals variegated, soft, gray-white, granular or smooth, bulging surface with areas of hemorrhage and necrosis.
- Approximately, 20% of embryonal carcinomas have invaded the adjacent epididymis or tunica albuginea at the time of excision (Fig. 23.22).

Light Microscopy

- Pure embryonal carcinoma is composed of highly pleomorphic polygonal, cuboid or columnar epithelial cells with hyperchromatic nuclei, prominent large single or multiple nucleoli and indistinct cell borders that are similar to cells from early embryos. The color of the cytoplasm changes from clear to eosinophilic, or basophilic.
- Tumor cells are arranged in glandular, papillary structures or solid pattern; and variable areas of undifferentiated sheets of tumor cells exhibiting syncytial growth surrounded by dilated vascular channels filled with red blood cells.
- Tumor shows high mitotic activity, tumor giant cells and areas of hemorrhage.
- Syncytiotrophoblastic component may be present all over the tumor.
- Peritumoral invasion of blood vessels and lymphatics is important feature to document (Fig. 23.23).

Immunohistochemistry Technique

Panel of markers is used on surgical specimens of embryonal carcinoma.

Markers	Expression
LIN28 (RNA binding protein)	Positive (>80%)
SALL4 (nuclear staining)	Positive (>80%)
PLAP (placental alkaline phosphatase)	Positive (>80%)
c-KIT (CD117)	Positive (<20%)
OCT4	Positive (>80%)
CD30	Positive (>80%)
Vimentin	Positive
LDH (lactic dehydrogenase)	Negative
Podoplanin	Negative
α_1-Antitrypsin	Negative
α-Fetoprotein (AFP)	Negative
hCG	Negative
Cytokeratin	Positive

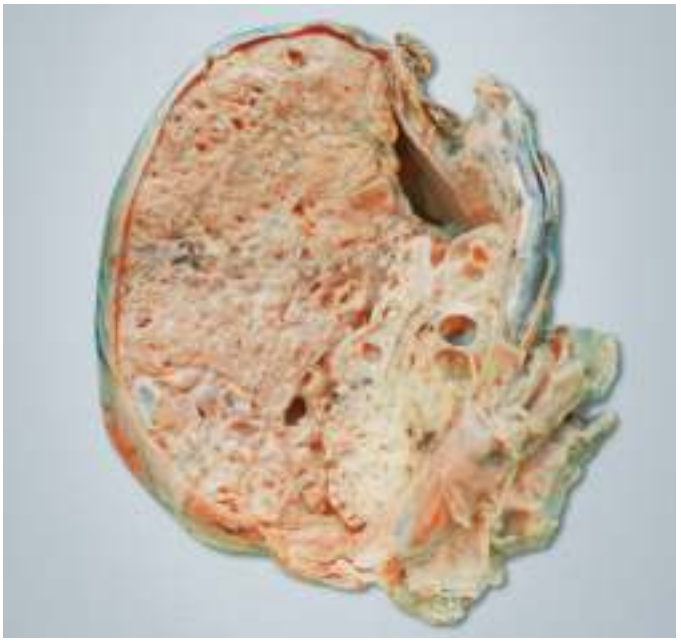


Fig. 23.22: Embryonal carcinoma. In adults, embryonal carcinoma is almost invariably a component of a mixed germ cell tumor. Cut surface reveals variegated, gray-white, granular or smooth, bulging surface with areas of hemorrhage and necrosis.

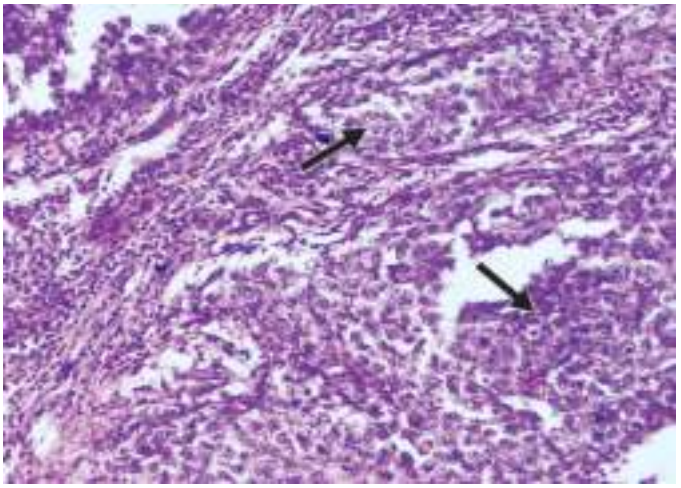


Fig. 23.23: Embryonal carcinoma. Tumor architecture is variable. Tumor cells often grow in diffuse sheets and may be arranged to resemble poorly formed glands. Tumor cells are large and tend to overlap one another, and cell membranes are difficult to discern, exhibiting a syncytial appearance to the tumor. Tumor cell nuclei are vesicular with irregular outlines, coarse chromatin, and prominent nucleoli. Atypical mitotic figures and apoptotic cells are abundant (arrows) (400X).

DNA Ploidy and Cytogenetics in Embryonal Carcinoma

Embryonal carcinomas are triploid or hypoploid neoplasms. Cytogenetic analysis has confirmed the presence of isochromosome 12p in many cases. There is a correlation between the aggressiveness of the tumor and the number of isochromosome copies.

Prognosis

Embryonal carcinoma is less sensitive to radiation than seminoma and hence more aggressive and lethal. The prognosis is much worse than for seminoma. Embryonal carcinoma is highly sensitive to chemotherapy, and the cure rates are now over 90%.

Yolk Sac Tumor

Yolk sac tumor is the most common malignant nonseminomatous germ cell tumor in infants and children under 34 years of age, but rare in adults. Telium first described this entity and named 'endodermal sinus tumor'. Yolk sac tumor arises from primordial germ cell showing differentiation towards yolk sac and allantoic membrane. It is analogous to endodermal sinus tumor of the ovary.

- Serum α-fetoprotein (AFP) can be identified in the cytoplasm of most yolk sac tumors with the frequency from 55 to 100%. Levels of AFP are elevated in most preoperative cases of yolk sac tumor. AFP is oncofetal glycoprotein synthesized during intrauterine fetus by liver and the visceral endoderm of the yolk sac.
- Hypermethylation of the RUNX3 gene promoter is thought to play a pathogenetic role in yolk sac tumor. Yolk sac tumor may be a component of mixed germ cell tumor.

Clinical Features

A painless testicular tumor and elevated serum α-fetoprotein (AFP) are the features of pure yolk sac tumor in infants and children under the age of 3 years. About 80% of prepubertal patients present with stage I disease of yolk sac tumor. Disorders associated with elevated serum α-fetoprotein (AFP) are given in Table 23.21.

Table 23.21 Disorders associated with elevated serum α-fetoprotein (AFP)

Yolk sac tumor of gonads
Conventional hepatocellular carcinoma
Hepatoblastoma
Cirrhosis
Massive hepatocellular necrosis with regeneration
Chronic hepatitis
Pregnancy

Surgical Pathology: Yolk Sac Tumor of Testis

Gross Morphology

Yolk sac tumor measures 2–6 cm in size. Tumor is lobulated and demarcated from surrounding tissues. Cut surface reveals grayish white to yellow solid tumor with mucinous texture and cystic areas. Hemorrhage and necrosis may be seen (Fig. 23.24).

Light Microscopy

- Yolk sac tumor is composed of interlacing strands of cuboidal to columnar epithelial cells surrounded by loose myxoid connective stroma. About 80% of tumors are composed of microcystic (reticular) and solid patterns. Solid pattern has intracellular hyaline globules known as basement deposits.
- The classic finding is the 'Schiller-Duval bodies' attempting to form the yolk sac. The lobular arrangement of cells, surrounded by empty spaces, results in the formation of glomeruloid structures containing a central fibrovascular core referred to as Schiller-Duval bodies.
- The presence of α-fetoprotein (AFP) in the tumor cells is highly characteristic of differentiation of tumor into yolk sac cells (Fig. 23.25A and B).

Histologic Patterns

Yolk sac tumor has various histologic patterns: endodermal sinus, endometrioid, glandular-alveolar, hepatoid, macrocystic, microcystic/reticular, myxomatous, papillary, parietal, sarcomatoid/spindle cell, solid pattern and vitelline.

Immunohistochemistry Technique

Tumor cells contain hyaline globules, which stain with diastase-resistant PAS. Panel of markers is used in surgical specimen of yolk sac tumor.

Markers	Expression
LIN28 (RNA binding protein)	Positive (>80%)
SALL4 (nuclear staining)	Positive (>80%)
PLAP (placental alkaline phosphatase)	Positive (>80%)
c-KIT (CD117)	Positive (<20%)
OCT4	Positive (<20%)
CD30	Positive (variable)
α_1-Antitrypsin	Positive (strong staining)
α-Fetoprotein (AFP)	Positive (strong staining)
Cytokeratin	Positive (strong staining)
Vimentin	Positive
LDH (lactic dehydrogenase)	Negative
Podoplanin	Negative
hCG	Negative



Fig. 23.24: Yolk sac tumor. This is the commonest testicular tumor in children, the majority of whom are <2 years old. In children, yolk sac tumor is typically pure in its makeup. It has completely replaced the normal testicular tissue. It tends to be bulging, shiny, or mucoid (creamy), and may show areas of cystic degeneration. The cut surface of embryonal carcinoma is often somewhat granular in consistency; necrosis and hemorrhage are common and may be extensive. It is typically smaller than seminoma and its interface with adjacent normal parenchyma may be somewhat indistinct (arrow).

DNA Ploidy and Cytogenetics in Yolk Sac Tumors

The DNA stem line of yolk sac tumor is peritetraploid and occasionally even diploid. Anomalies of 1p, 6q and 13q are present in pediatric yolk sac tumors, whereas isochromosome 12p is missing.

Treatment

The treatment of adult patients with yolk sac tumor is similar to that of other nonseminomatous germ cell tumors.

- Prognosis is excellent in children under 3 years of age following timely orchidectomy in a 95% of cases. Retroperitoneal lymph node dissection is no longer performed in children.
- Serum AFP analysis can be used to monitor the recurrence of disease following surgery, which is one of the major oncofetal antigens. Many children with yolk sac tumors are managed by close follow-up after orchidectomy.

Teratoma of Testis

Teratoma is composed of mature or immature (fetal/embryonal) tissue components derived from all three embryonic germinal layers: ectoderm, endoderm and mesoderm.

- Multiple tissues can be present in the tumor: neural tissue, cartilage islands, ciliated epithelium of bronchi, thyroid tissue, liver cells, embryonic gut, or striated muscle embedded in fibrous or myxoid stroma. Because of the different histogenesis and prognosis, the new WHO classification distinguishes a prepubertal and a postpubertal teratoma.
- Prognosis is good prepubertal teratomas in infants and children (4–12 years). But in postpubertal period, all teratomas are regarded as malignant and capable of metastatic behavior regardless of elements are mature or immature.
- A few teratomas may contain carcinoid tumor component (argyrophil and secretory granules containing neuron-specific enolase (NSE), chromogranin, synaptophysin, but not associated with the carcinoid syndrome).

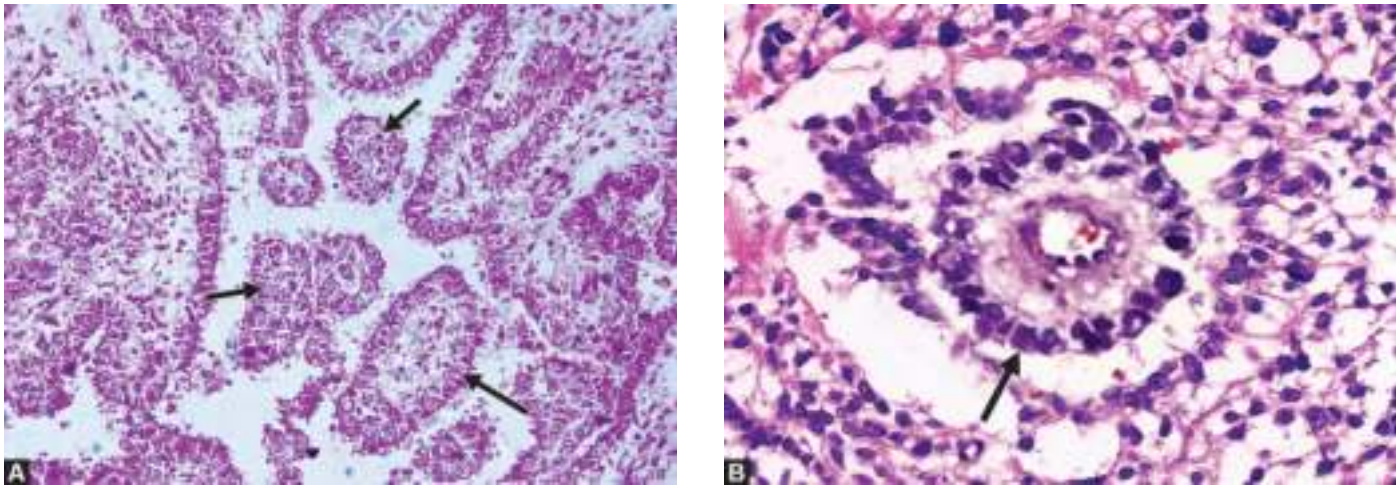


Fig. 23.25: Yolk sac tumor. (A) The endodermal sinus pattern of yolk sac tumor is characterized by the presence of irregular interconnecting labyrinthine-like spaces and Schiller-Duval bodies, several of which are nicely illustrated (arrows), (B) tumor is showing Schiller-Duval bodies (arrow) (400X).

Epidemiology

After yolk sac tumors, teratomas are the second most frequent nonseminomatous germ cell tumors accounting for 14–20% during childhood and 66% before the age of two years. Pure teratomas account for 3–7% of germ cell tumors during 20–30 years of age group. Teratoma can be present as a tumor component in 50% of all mixed germ cell tumors. Mixed teratomas consist of two components in 45% of cases: teratoma and embryonal carcinoma.

Clinical Features

Patient presents with painless testicular swelling. Since prepubertal teratomas do not metastasize, hence parenchyma sparing surgery is performed for small teratoma. Postpubertal teratomas metastasize first to the retroperitoneal lymph nodes and then to distant organs (lung, liver, brain, bones). These patients are resistant to chemotherapy and radiotherapy. Surgical excision of the lymph nodes and organ metastases. Prognosis is better in stage I and poor in stage III.

Surgical Pathology: Teratoma of Testis

Gross Morphology

On macroscopic examination, teratoma measures 5–10 cm in diameter. Cut surface has a variegated appearance with cystic and solid areas. The cysts are filled with hair mucus, or sebaceous material. Cartilage takes the shape of a glassy nodule. Presence of pigmented areas simulates retina. Presence of hemorrhage necrosis indicates admixture of teratoma with embryonal carcinoma or choriocarcinoma or both. Mature teratoma is shown in Fig. 23.26.

Light Microscopy

Teratomas are classified according to the degree and type of differentiation seen.

- **Mature teratoma:** Mature teratoma is well differentiated tumor composed of tissue components derived from ectoderm, mesoderm, and endoderm.
  - Tumor contains cysts lined with squamous or glandular epithelium of the ciliated-type respiratory or enteric-type epithelium. The cysts can be filled with ferritin or mucus.
  - Organized structures simulating bronchus or gut are composed of the corresponding epithelium encircled by smooth muscle.
  - Organized structures may contain scattered neuroendocrine cells producing gastrin, somatostatin and bombesin.
  - Mesodermal tissue derived components such as smooth muscle, cartilage and bone with or without hematopoietic bone marrow embedded in fibrous or myxoid stroma are present.
  - Neuroglia with ependymal differentiation is most often present.
  - Pigmented retinal epithelium may be seen in occasional tumor.
  - Differentiated liver, prostate, thyroid and pancreas are rarely present.
  - Mature teratoma is almost always malignant with metastatic potential, whereas the analogous ovarian tumor is almost always benign (Fig. 23.27).
- **Immature teratoma:** Immature teratoma is less differentiated tumor composed of primitive elements such as undifferentiated spindle cells and small immature glandular structures.
  - Squamous epithelium is arranged in solid nests without keratinization.
  - Other components of immature teratoma are primitive neuroepithelium, rhabdomyoblasts, renal blastema, and fetal adipose tissue with lipoblasts. Immature tissue is more often encountered in adult patients than in children. Gross morphology and light microscopy of immature teratoma are shown in Figs 23.28 and 23.29.
- **Teratoma with somatic malignant transformation:** Teratoma may contain malignant tissue such as squamous cell carcinoma, mucin secreting adenocarcinoma and sarcoma.

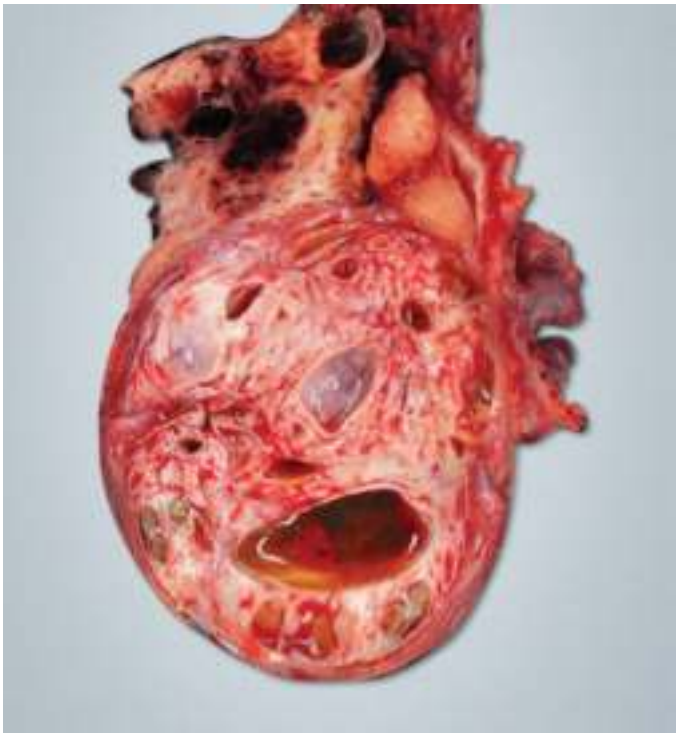


Fig. 23.26: Mature teratoma—teratomas typically have both solid and cystic components. The solid component is sometimes recognizable as cartilage. The material within the cysts may be serous, mucoid, or keratinous. This tumor arose in the testis of a 2-year-old boy, and as such was considered benign. However, teratomas in 20–30 years of malignant behavior.

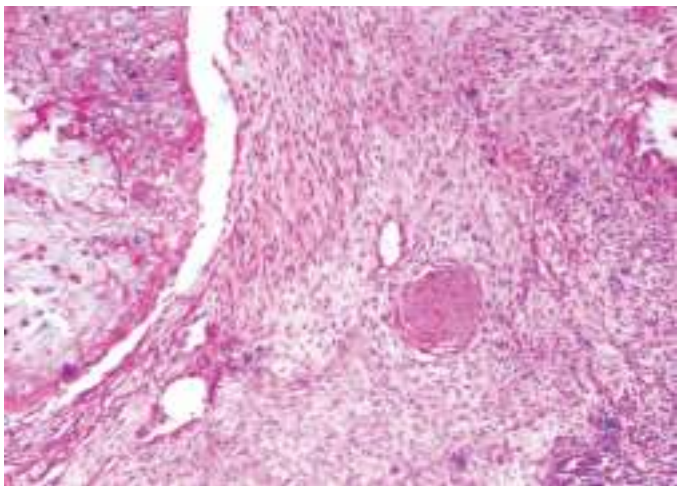


Fig. 23.27: Mature teratoma. Mature teratoma is well differentiated tumor composed of components derived from ectoderm, mesoderm and endoderm. It shows cartilage and an island of squamous epithelium set in a fibrous stroma (400X).

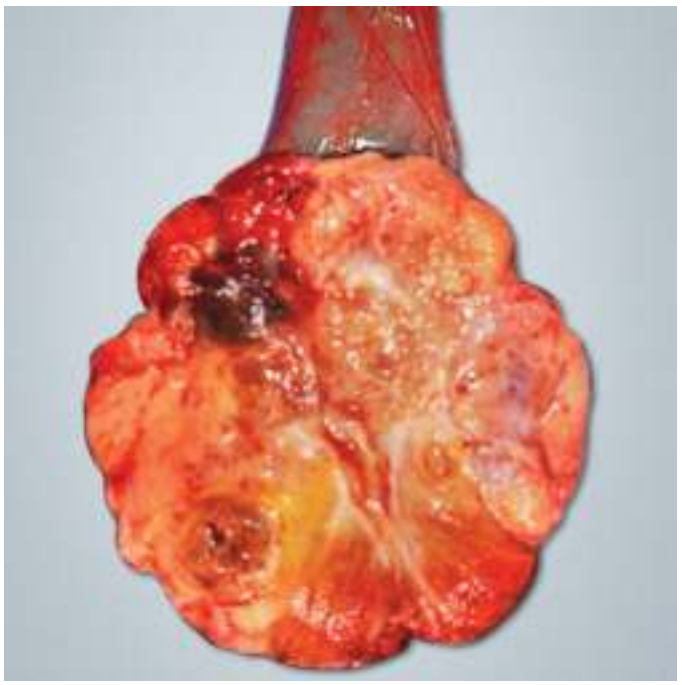


Fig. 23.28: Teratoma with immature elements. This teratoma has a very extensive component of immature elements in the form of primitive neuroectodermal tumor, perhaps accounting for its fleshy bulging encephaloid appearance and the areas of hemorrhage.

DNA Ploidy and Cytogenetics in Teratomas

Prepubertal teratomas are diploid and postpubertal teratoma as hypotriploid. Postpubertal teratoma demonstrates isochromosome 12p. Teratomas with somatic malignant transformation can demonstrate chromosomal aberrations (translocation) typical of the respective somatic tumor as well as isochromosome 12p that is specific for germ cell tumors.

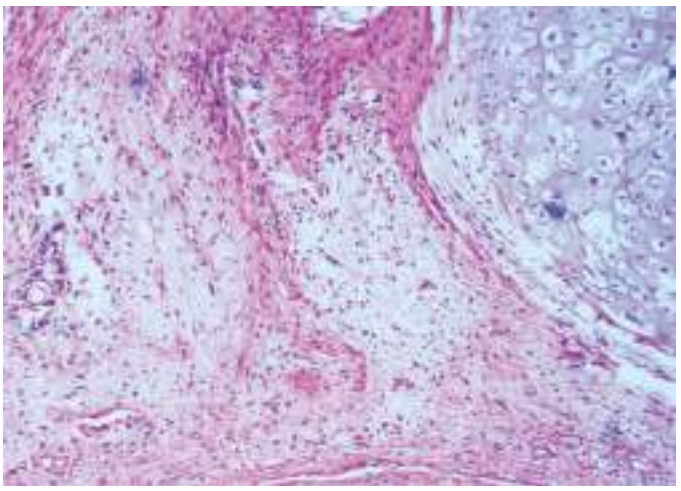


Fig. 23.29: Teratoma with immature elements. This teratoma with mature adipose, cartilage and squamous elements has an area of immature tissue at upper right. Immature elements, if limited, appear to have no influence on prognosis (100X).

Mode of Spread

The majority of nonseminomatous germ cell tumors (e.g. teratoma) spread via lymphatic route to retroperitoneal, para-aortic, mediastinal and supraclavicular lymph nodes, and via hematogenous routes to lungs, liver, brain, bones and are not radiosensitive, hence their natural history is markedly different from that of seminomas. Teratocarcinoma often metastasizes as embryonal carcinoma, and the reverse also occurs.

Choriocarcinoma of Testis

Choriocarcinoma is highly malignant rapidly growing nonseminomatous germ cell tumor. This tumor is analogous to choriocarcinoma of the ovary. Identical choriocarcinomas are also seen in placenta, ovary, sequestrated nests of totipotent cells in mediastinum and abdomen. Tumor synthesizes β-hCG representing germ cell differentiation to an extraembryonic structure, namely the placenta. Choriocarcinoma is highly chemosensitive tumor. It most often occurs in the second to third decades.

Clinical Features

Patient presents with widespread metastases via hematogenous routes to lungs (100%), liver (86%), brain (56%), gastrointestinal tract (70%) and bones with fatal outcome. Tumor is detected as a small palpable nodule without testicular enlargement. The high serum hCG levels >100,000 IU/L cause gynecomastia. Choriocarcinoma is a very aggressive tumor treated by chemotherapy. Profuse intracranial, pulmonary and gastrointestinal bleeding with substantially increased serum β-hCG (>50,000 IU/L) is referred to as 'choriocarcinoma syndrome'.

Surgical Pathology: Choriocarcinoma of Testis

Gross Morphology

- Choriocarcinoma is usually small size tumor surrounded by extensive areas of hemorrhage and necrosis.
- Sometimes, primary tumor is replaced by fibrous scar but widespread metastases occur.
- The invasive growth of trophoblastic cells in the tumor is associated with hemorrhage (Fig. 23.30).

Light Microscopy

- Choriocarcinoma is composed of syncytiotrophoblastic cells, which cap a cluster of cytotrophoblastic cells giving the impression of a placental villus.
- Cytotrophoblastic element cells are more regular polygonal cells with distinct cell membrane, abundant vacuolated cytoplasm and a vesicular nucleus arranged in cords and masses.
- Syncytiotrophoblastic element cells are large multinucleated with many irregular, hyperchromatic vesicular nuclei with abundant eosinophilic vacuolated cytoplasm.
- Since the tumor does not have its own stroma, the syncytiotrophoblastic giant cells directly invade the local blood vessels (Fig. 23.31).

Immunohistochemistry Technique

Mononuclear cytotrophoblastic cells of choriocarcinoma of tests are variable positive for SALL-4 staining.

Markers	Expression
All three layers: syncytiotrophoblastic, cytotrophoblastic and intermediate trophoblastic cells show positivity.	
CK7	Positive
CK8	Positive
CK19	Positive
CAM 5.2	Positive
AE1/AE3	Positive
α-Inhibin	Positive
Cytotrophoblastic cells show positivity	
hCG	Positive
EMA (epithelial membrane antigen)	Positive
Syncytiotrophoblastic cells and intermediate trophoblasts show positivity	
Human placental lactogen	Positive
Pregnancy-specific β_1-glycoprotein	Positive
Proliferative marker	
MIB-1	Positive

SALL-4 reacts with mononuclear tumor cells, but not for syncytiotrophoblastic and cytotrophoblastic cells.

Ploidy and Cytogenetics in Choriocarcinoma

Choriocarcinoma demonstrates isochromosome 12p.



Fig. 23.30: Choriocarcinoma—the testis is replaced by a bloody bulging mass. More typically, choriocarcinoma tends to be somewhat smaller, and may not significantly distort the testis (arrows).

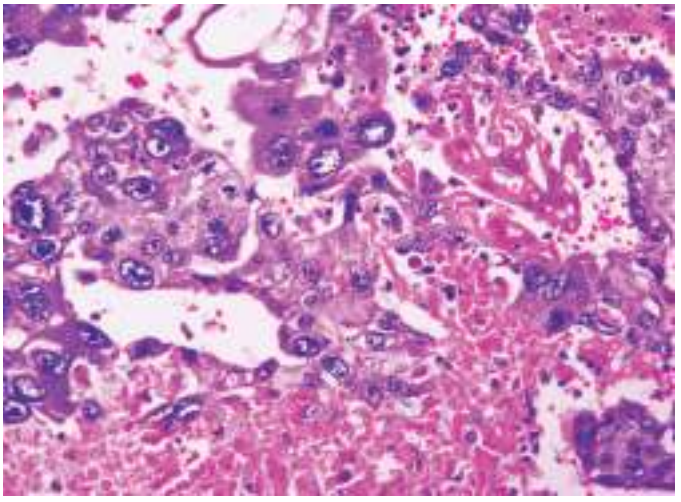


Fig. 23.31: Choriocarcinoma—the tumor is composed of multinucleated syncytiotrophoblastic and cytotrophoblastic cells. The multinucleated syncytiotrophoblasts have voluminous eosinophilic cytoplasm and large darkly staining irregular nuclei with prominent nucleoli. The smaller mononuclear cytotrophoblasts are fairly uniform in size with amphophilic cytoplasm and distinct cellular outlines resembling to the tumor cells of solid yolk sac tumor (400X).

Treatment

Choriocarcinoma is treated similarly to other nonseminomatous tumors. Orchidectomy is primary treatment for nonmetastatic choriocarcinoma. Retroperitoneal lymph node dissection or chemotherapy for pT_2 or higher. A choriocarcinoma syndrome with hemorrhagic visceral metastases is associated with poor prognosis.

Mixed Germ Cell Tumors of Testis

Mixed germ cell tumors most often occur in adults (20–30 years), which account for 30–55% of all germ cell tumors. Mixed germ cell tumors are as frequent as seminomas.

- The gross morphology and histologic features of mixed germ cell tumors are dependent on the number of different components. Some preferred combinations of mixed germ cell tumors occur.
- Embryonal carcinoma is most frequent tumor with other germ cell tumor in 80% of cases. Teratocarcinoma is composed of teratoma and embryonal carcinoma in 45% of cases.
- Seminoma can be present with teratoma, embryonal carcinoma, yolk sac tumor or choriocarcinoma in 15% of cases. For the purpose of treatment, it is important that seminomas are extensively sampled to rule out nonseminomatous component.
- High serum α-fetoprotein (AFP) in seminoma indicates that a nonseminomatous component has been overlooked.
- The invasion of the lymph nodes and blood vessels is the powerful predictor of metastases or relapse. A large embryonal carcinoma component also negatively influences the prognosis. Presence of teratoma is marker of favorable prognosis. Teratoma elements may be mature or immature (fetal/embryonal).
- The combination of percentage embryonal carcinoma and vascular invasion permits correct prediction for the final pathologic stage in 90% of patients.
- A few teratomas may contain carcinoid tumor component (argyrophil and secretory granules containing neuron-specific enolase, chromogranin, synaptophysin, but not associated with the carcinoid syndrome).
- Mixed germ cell tumor may be composed of teratoma, embryonal carcinoma and yolk sac tumor. In combined tumor (seminoma + NSGCT), the latter component is the one most likely to metastasize. Gross morphology and light microscopy of mixed germ cell tumor are shown in Figs 23.32 and 23.33.

Burnt-out Germ Cell Tumors of Testis

Burnt-out germ cell tumors refer to a germ cell tumor in extragonadal tissues with spontaneous regression of an intragonadal tumor.

- Extragonadal germ cell tumors are usually found in the retroperitoneal region.
- Many studies suggested that germ cell tumors could regress spontaneously.
- Conclusive evidence for spontaneous regression was established by Azzopardi and his associates, who conducted autopsy on patients, who died of metastatic germ cell tumor but lacked primary testicular germ cell tumor. Out of germ cell tumors of testes, seminoma regresses spontaneously.

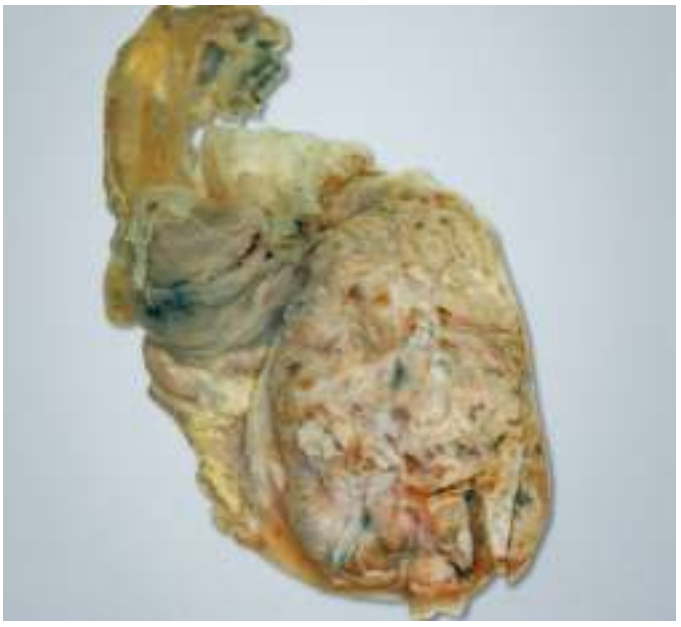


Fig. 23.32: Mixed germ cell tumor of testis. Tumor consists of teratoma, yolk sac and embryonal carcinoma.

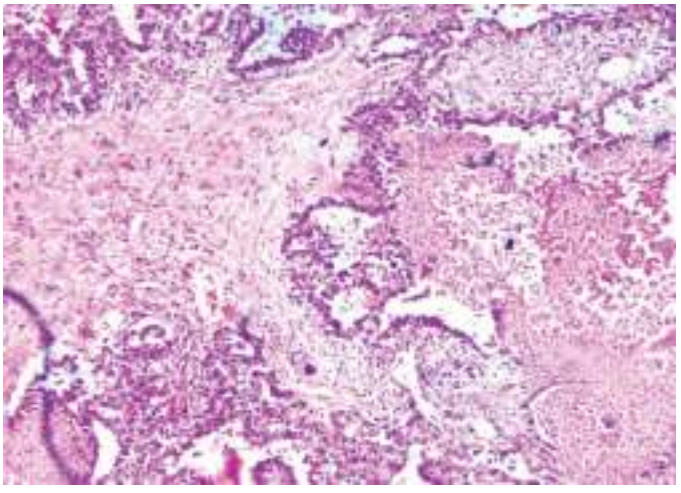


Fig. 23.33: Teratocarcinoma. Tumor is composed of components of teratoma and embryonal carcinoma (100X).

Surgical Pathology: Burnt-out Germ Cell Tumors of Testis
Gross Morphology
The burnt-out germ cell tumor consists of single or multiple well demarcated nodular scars and sometimes cystic or cartilaginous areas.
Light Microscopy
▪ Aggregates of proliferated Leydig cells in the surrounding atrophic testis are demonstrated. ▪ The seminiferous tubules of the surrounding parenchyma are atrophic and hyalinized with microliths. Foci of carcinoma *in situ* (CIS) may be identified.

Clinical Features

The prognosis and management of regressed germ cell tumor depend on the morphologic features of the primary tumor. Retroperitoneal metastases in young males are always suspicious for a primary germ cell tumor in the testis. Histologic examination of biopsy or surgical exploration with intraoperative frozen section is essential to establish diagnosis.

SEX CORD-STROMAL TUMORS OF TESTIS

Normally, Sertoli cells support primordial germ cells and form basement membrane of seminiferous tubules, which synthesize estrogens and up to some extent androgens. Leydig cells produce testosterone and other androgens. Sex cord/gonadal stromal tumors arise in the gonads of both genders, which account for 3–5% of testicular tumors. Over 30% of infants and children are affected by sex cord/gonadal stromal tumors.

Sertoli Cell Tumor (Androblastoma) of Testis

Sertoli cell tumor is a non-germ cell tumor derived from the sex cord stroma of testis. It is characterized by a paucity of endocrine manifestations. Sertoli cell tumors are most often benign, but malignant in 10% of cases metastasizing to iliac and para-aortic lymph nodes.

Clinical Features

Patient presents with testicular swelling. If Sertoli cell tumor synthesizes estrogen, clinical manifestations are loss of libido and impotence. Gynecomastia occurs more frequent in malignant Sertoli cell tumor. Malignant Sertoli cell tumors have also been reported in children. Orchidectomy is the only therapeutic option.

Surgical Pathology: Sertoli Cell Tumor of Testis

Gross Morphology

Tumor is small size (average size 3.5 cm), firm, encapsulated and well circumscribed nodule. Cut surface is gray white to yellow, homogenous cut surface with focal cystic areas.

Light Microscopy

- Well differentiated Sertoli cell tumors are lined by Sertoli cells having clearly visible basement membrane, oval vesicular nuclei and prominent reddish nucleoli and eosinophilic cytoplasm sometimes large amount of lipids.
- Tumor cells are arranged in distinctive trabeculae with tendency to form tubular structures resembling well-formed or poorly formed tubules lined by elongated cells having the appearance of Sertoli cells (Fig. 23.34).
- Less differentiated Sertoli cell tumor is composed of spindle-shaped cells growing in solid sheets.

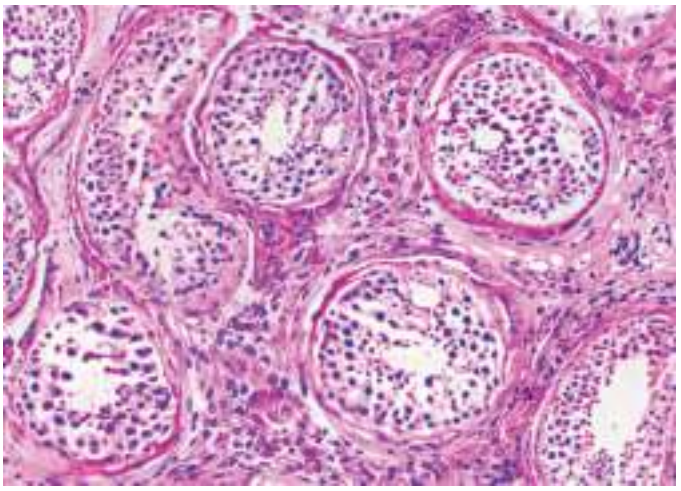


Fig. 23.34: Sertoli cell tumor. Tumor is composed of Sertoli cells or may have a component of granulosa cells. Tumor cells contain vesicular nuclei, prominent reddish nucleoli and eosinophilic cytoplasm sometimes large amount of lipids. These are arranged in distinctive trabeculae with tendency to form tubular structures resembling well-formed or poorly formed tubules lined by elongated cells having the appearance of Sertoli cells (400X).

- Tumor also consists of bands of fibrous tissue in variable proportion.
- Malignant Sertoli cell tumor is quite large measuring 7–15 cm, which shows nuclear pleomorphism and occasionally invasion of the lymphatic channels.

Immunohistochemistry Technique

Panel of markers is performed on paraffin-embedded sections from Sertoli cell tumor. FOXL2, β-catenin and WT1 are best markers. In many cases, immunohistochemistry technique is not very helpful in establishing correct diagnosis.

Markers	Expression
FOXL2	Positive (83%)
β-Catenin	Positive (83%)
WT1	Positive (82%)
Cytokeratin	Positive (78%)
SF1	Positive (75%)
Inhibin	Positive (67%)
Melan A	Positive (67%)
Calretinin	Positive (56%)
CD99	Positive (56%)
Synaptophysin	Positive (24%)
S-100	Positive (28%)

DNA Ploidy and Cytogenetics in Sertoli Cell Tumors

In various studies, Sertoli cell tumors demonstrate gain of X chromosome and loss of chromosome 2. Mutation of CTNNB1 (cadherin-associated protein β1) gene is likely to be involved in the pathogenesis of Sertoli cell tumor resulting in nuclear accumulation of β-catenin and expression of cyclin D1.

Leydig Cell Tumor of Testis

Leydig cell tumor (LCT) arises from Leydig cells, which accounts for 3% of testicular tumors. Leydig cell tumor is composed of more or less well-differentiated Leydig cells. The average age of LCT diagnosis is between 30 and 50 years in 20% of cases. However, LCT may occur in children under 10 years and adults. Tumor most often involves unilateral testis in 97% and bilateral testis in 3% of cases. Cryptorchidism and Klinefelter syndrome are considered predisposing factors. Approximately 25% of all LCTs show evidence of malignant behavior in the form of metastatic disease, particularly to lymph nodes, lung and liver.

Clinical Features

About 50% of patients present with unilateral testicular swelling and gynecomastia. Leydig cell tumors can synthesize androgens, estrogens and sometimes corticosteroids. Approximately 25% of adult patients suffer from loss of libido and impotence due to synthesis of estrogens by LCT.

- Prepubertal Leydig cell tumor synthesizes testosterone, in which penile growth, pubic hair and precocious puberty are the most common presenting symptoms, followed by deep voice and facial acne.
- About 20% of patients with malignant LCTs have already metastasizing to retroperitoneal lymph nodes and via hematogenous route to lungs (43%), liver (38%) and bones (28%) at the time of diagnosis.
- Malignant LCT is resistant to chemotherapy. Hence, the patient is treated by orchidectomy with retroperitoneal lymph nodes dissection (RPLND) and chemotherapy. Patient has fatal outcome within six months.

Surgical Pathology: Leydig Cell Tumor of Testis

Gross Morphology

- On gross examination, Leydig cell tumors are on average 2–3 cm (range: 0.5–10 cm).
- Tumor is well circumscribed nodule due to presence of fine thin fibrous capsule. It forms a golden-brown nodule within the body of the testis and homogenous on cut surface; and also, at metastatic sites.
- Tumor with a diameter of ≥5 cm is almost without exception malignant.

Light Microscopy

Benign Leydig Cell Tumor

- Benign Leydig cell tumor is composed of large round or polygonal cells arranged in sheets or tubules or trabecular pattern.
- Tumor cells have distinct borders, abundant granular deeply eosinophilic cytoplasm and round to oval nuclei with prominent nucleoli. Multiple nuclei may be present in few tumor cells.
- Tumor cells contain intracytoplasmic rod-shaped Reinke crystals demonstrated with Masson trichrome stain.
- The stroma is scant with a network of capillaries typical of endocrine tumors. Some tumors are composed of spindle cells with abundant intracytoplasmic lipids.
- Psammoma bodies and calcifications can be demonstrated in these tumors (Fig. 23.35).

Malignant Leydig Cell Tumor

- Malignant Leydig cell tumor measures about 7 cm in size. Tumor is composed of polymorphic spindled cell shape tumor cells with nuclear atypia and frequent mitoses, areas of necrosis, vascular invasion and invasion of surrounding tissues.
- The number of proliferating tumor cells is also more in malignant Leydig cell tumor demonstrated by proliferative Ki-67/MIB-1 marker.
- Diagnostic criteria for malignancy in Leydig cell tumor and Sertoli cell tumor are given in Table 23.22.

Immunohistochemistry Technique

Panel of markers is performed on paraffin-embedded sections from Leydig cell tumor.

Markers	Expression
Inhibin and calretinin	Positive (97%)
Melan A	Positive (94%)
SF1	Positive (92%)
CD99	Positive (74%)
Synaptophysin	Positive (65%)
β-Catenin	Positive (50%)
S-100	Positive (28%)
Cytokeratin	Positive (22%)
FOXL2	Positive (21%)

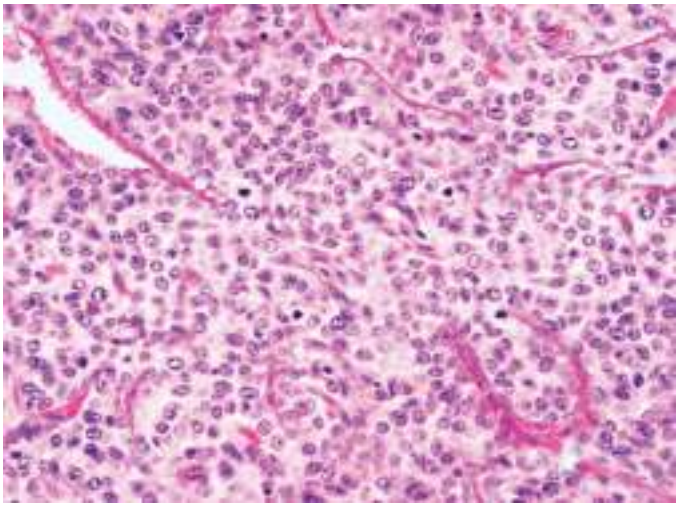


Fig. 23.35: Leydig cell tumor. Leydig cell tumor is composed of large round or polygonal cells arranged in sheets or tubules or trabecular pattern. The tumor cells have distinct borders, abundant granular deeply eosinophilic cytoplasm and round to oval nuclei with prominent nucleoli. Multiple nuclei may be present in few tumor cells. These cells contain intracytoplasmic rod-shaped Reinke crystals demonstrated with Masson trichrome stain. The stroma is scant with as network of capillaries typical of endocrine tumors. Some tumors are composed of spindle cells with abundant intracytoplasmic lipids (400X).

Table 23.22 Diagnostic criteria for malignancy in Leydig cell tumor and Sertoli cell tumor

Features	Leydig Cell Tumor	Sertoli Cell Tumor
Gross morphology		
Tumor size >5 cm	Present	Present
Histologic features		
High mitotic count numerous (normal average 1.9/HPF)	Present	Present
Spindled shape pleomorphic malignant cells	Present	Present
Nuclear atypia	Present	Present
Areas of necrosis	Present	Present
Vascular invasion by tumor cells	Present	Present
Invasion of surrounding tissues by tumor cells	Present	Present

DNA Ploidy and Cytogenetics in Leydig Cell Tumor

Leydig cell tumors can demonstrate gain of chromosome X, or 19p, and loss of chromosomes 8 and 16. LH-receptor gene mutations may be detected.

Clinical Behavior

About 90% of Leydig cell tumors are benign in adults, while 10% have a potential for invasion and metastases. Only the diameter of tumor is a reliable prognostic factor in adults and not in infants. Poor prognostic factors are tumor size >5 cm, high mitotic count (normal 1.9/HPF), spindled cell shape, nuclear atypia and polymorphism, necrosis, vascular invasion and invasion of surrounding tissue.

OTHER MALIGNANT TUMORS OF TESTIS

Testicular Lymphoma

Primary testicular lymphomas constitute 5% of testicular neoplasms. Primary testicular lymphoma most commonly represents dissemination from extratesticular sites, although is occasionally in primary testicular neoplasm.

- Testicular lymphoma is a disease of elderly persons with mean age of 60–70 years. Patient presents with testicular swelling, fever and weight loss. Children are more to develop follicular lymphoma than adults.
- Bilateral testicular involvement in a patient over 60 years should suggest systemic non-Hodgkin's lymphoma rather than primary germ cell tumors.
- Treatment involves multimodality approach involving surgery, chemotherapy and radiotherapy. Outcome remains poor in spite of aggressive therapy.
- Diffuse large B cell lymphoma (DLBCL) is relatively common and represents 30–58% of non-Hodgkin's lymphomas. Most DLBCLs originate in lymph nodes, but 40% initially present in extranodal sites such as testes and central nervous system. DLBCL is diagnosed by clinical history, physical examination, molecular analysis, and immunophenotyping.

Surgical Pathology: Testicular Diffuse Large B Cell Lymphoma

Gross Morphology

On gross examination, the diffuse large B cell lymphoma tumor replaces testicular parenchyma as fleshy gray to tan pink neoplasm that resembles seminoma.

Light Microscopy

- The most important clue to the diagnosis occurs at scanning magnification where an interstitial pattern, with relative tubular preservation is identified.
- The tumor cells of DLBCL are large 45 times diameter of small lymphocytes with round to oval nucleus, with either 2–3 nucleoli located adjacent to the nuclear membrane or a single nucleolus centrally placed and moderate pale or basophilic cytoplasm.
- Mitoses are frequent. Tumor cells are arranged in diffuse pattern. More anaplastic tumors may contain multinucleated Reed-Sternberg-like cells with large, inclusion-like nucleoli, and these may be termed immunoblastic lymphoma (Fig. 23.36A).

Immunophenotyping

The tumor cells often mark with CD19, CD20, CD22, CD45 and CD79a. Most express surface immunoglobulin (Fig. 23.36B).

Markers	Expression
CD19	Positive
CD20	Positive
CD22	Positive
CD45	Positive
CD79a	Positive

Treatment

Diffuse large B cell lymphoma of the testis is treated with orchiectomy and chemotherapy, sometimes combined with radiation therapy.

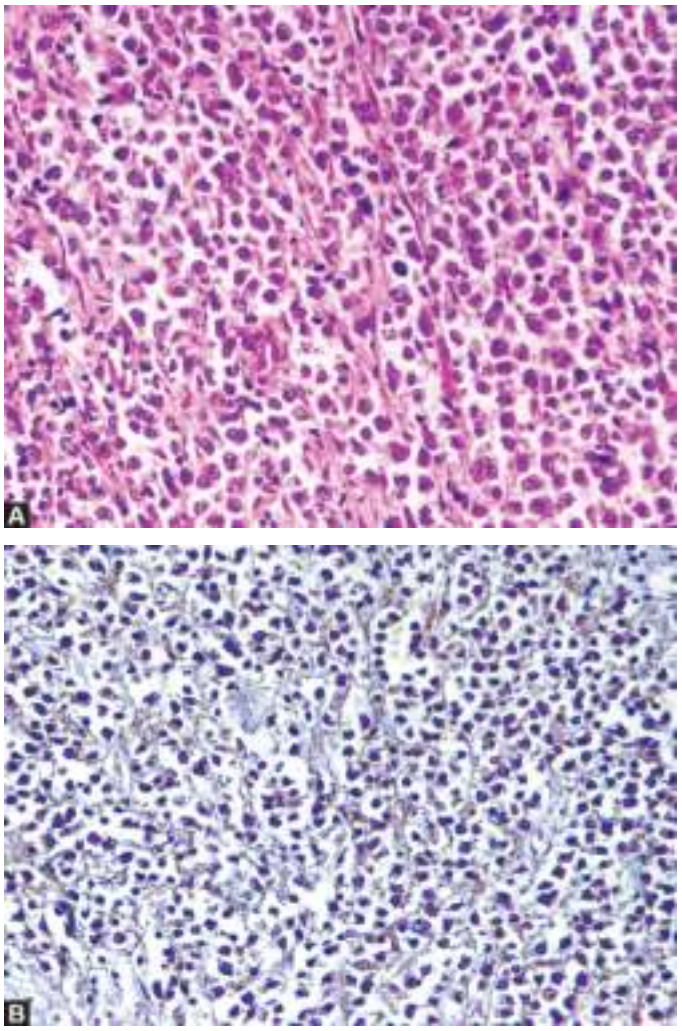


Fig. 23.36: (A) Diffuse large lymphoma of testis (H&E staining 400X), (B) neoplastic cells show CD45+ staining (immunohistochemistry).

Testicular Plasmacytoma

Testicular plasmacytoma is rare neoplasm. It is most commonly a manifestation of diagnosed case of multiple myeloma in 2% of cases.

- Testicular plasmacytoma is occult and apparently an isolated finding on autopsy. Mean age is approximately 55 years.
- Diagnosis of multiple myeloma is based on the combination of clinical, morphologic, radiological and immunological findings.
- On gross examination, testicular plasmacytoma is usually soft and fleshy, tan to gray white and often with foci of hemorrhage.
- Histologic features of testicular plasmacytoma are similar to those of plasmacytomas at other sites. Mature clonal plasma cells measure 20–30 μm in diameter with abundant cytoplasm and eccentrically placed nuclei with dense clumped chromatin giving cartwheel appearance. The seminiferous tubules are effaced centrally but preserved at the periphery of the tumor.
- Some seminomas may have a plasmacytoid morphology and also show intratubular growth, potentially mimicking plasmacytoma.
- The presence of germ cell *in situ* neoplasia indicates seminoma over plasmacytoma.
- The presence of OCT3/4 indicates seminoma over plasmacytoma.

Leukemic Infiltration and Myeloid Sarcoma of Testis

Leukemic infiltration in the testes most often occurs in the course of acute myelogenous/lymphoblastic leukemias (40–65%) of patients and chronic lymphocytic leukemia (20–35%).

- Testicular involvement is generally occult. It is clinically evident in 5% of cases. Testicular involvement at clinical presentation is seen in 2% of cases.
- Boys with acute lymphoblastic leukemia are more prone to develop bilateral testicular involvement with a positive biopsy even in otherwise complete clinical and bone marrow remission in 5% of cases.
- The major differential diagnosis includes orchitis, plasmacytoma, lymphoma and seminoma.
- On gross examination of testes in acute lymphoblastic leukemia resembles lymphoma (Fig. 23.37).
- On histologic examination, myeloid sarcoma may be mistaken for lymphoma and plasmacytoma.
- Radiotherapy is most effective in controlling the testicular involvement, but bone marrow relapse will develop in most cases.
- Myeloid sarcoma is tumorous myelogenous leukemia infiltrates in testes and other tissues outside bone marrow.

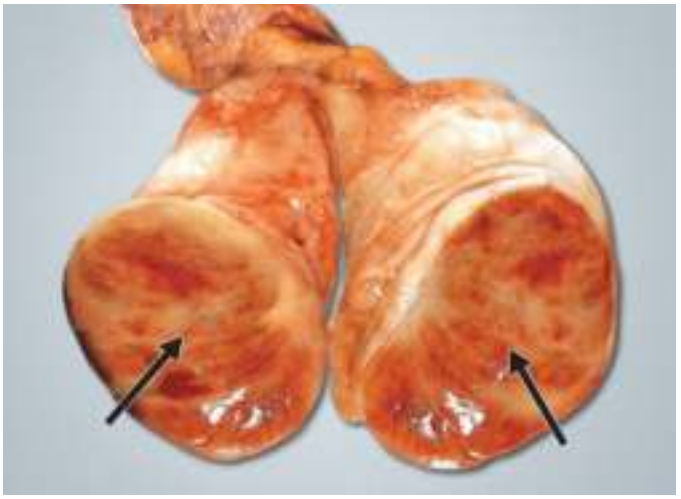


Fig. 23.37: Acute lymphoblastic leukemia infiltrating both testes. The normal testicular tissue has been completely replaced by creamy, slightly hemorrhagic tumor tissue. When patients have responded to chemotherapeutic treatment of acute lymphoblastic leukemia, the first manifestation of recurrence is frequently in the testes (arrows).

Metastases in Testis

Metastases of the testis frequently occur in patients with a known malignancy in prostate, lung, stomach, colon, kidney, urinary bladder, pancreas and others including melanoma.

- Neuroblastoma, rhabdomyosarcoma and Wilms' tumor in children may also metastasize to testes. In some instances, testicular mass may be presenting feature of an occult neoplasm.
- Prostatic adenocarcinoma may extensively involve the rete testis. Prostatic adenocarcinoma metastasizing to testes has better prognosis than involving penis. Some high-grade prostatic carcinomas may prominently grow within the seminiferous tubules as well as the interstitium and resemble seminoma. Intratubular growth of urothelial carcinoma may mimic intralobular embryonal carcinoma.
- Metastatic renal cell carcinoma of clear cell can be misinterpreted as Sertoli cell tumor. Melanoma may have foamy cytoplasm that resembles that of Leydig cell tumor. Melanoma with discohesive cells between tubules may simulate lymphoma. Most frequent sites of primary tumors metastasizing to the testes are given in Table 23.23.

NEOPLASTIC DISORDERS OF MESOTHELIUM

ADENOMATOID TUMOR

Adenomatoid tumor is the most common benign tumor of testicular adnexa, which represents almost 60% of cases. Tumor is of mesothelial origin. Adenomatoid tumor arises in upper or lower pole of the epididymis.

Table 23.23 Most frequent sites of primary tumors metastasizing to the testes

Primary Tumors	Frequency
Malignancy in adults	
Prostatic adenocarcinoma	35–60%
Lung carcinoma	5–15%
Melanoma	9–15%
Colon carcinoma	5–9%
Renal cell carcinoma	5–9%
Gastric carcinoma	4%
Miscellaneous tumors	1–10%
Malignancy in children	
Neuroblastoma	Some patients
Rhabdomyosarcoma	Some patients
Wilms' tumor	Some patients

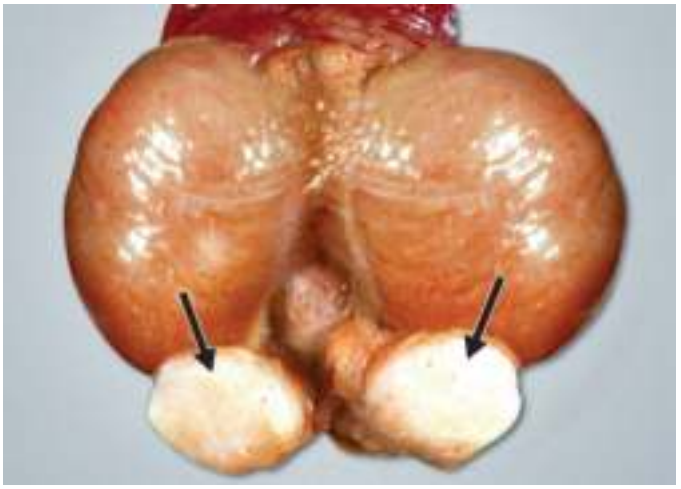


Fig. 23.38: Adenomatoid tumor of the epididymis. There is a creamy, well circumscribed tumor in the epididymis (arrows).

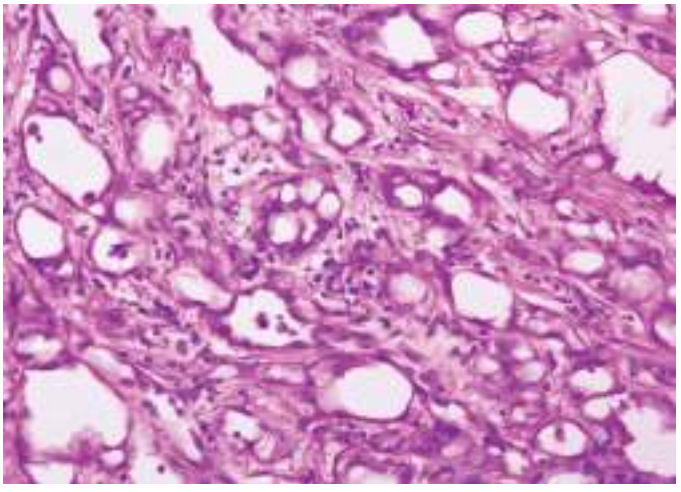


Fig. 23.39: Adenomatoid tumor of the epididymis. Tumor is composed of round to oval or slit-like tubules in fibrous, hyalinized, and/or muscular stroma. The lining cells are columnar or flat having vacuolated cytoplasm (400X).

Surgical Pathology: Adenomatoid Tumor
Gross Morphology
Tumor is solitary, round to oval in shape measuring 5 cm in diameter (Fig. 23.38).
Light Microscopy
Tumor is composed of round to oval or slit-like tubules in fibrous, hyalinized, and/or muscular stroma. The lining cells are columnar or flat having vacuolated cytoplasm (Fig. 23.39).
Immunohistochemistry Technique
Tumor cells are immunoreactive for expression of cytokeratin, calretinin, EMA and WT1. Tumor cells are negative for factor VIII-related antigen and CD31, a profile indicating mesothelial origin rather from vascular endothelium.

Markers	Expression
▪ Cytokeratin	▪ Positive
▪ Calretinin	▪ Positive
▪ Epithelial membrane antigen (EMA)	▪ Positive
▪ WT1	▪ Positive
▪ Factor VIII-related antigen	▪ Negative
▪ CD51	▪ Negative

NON-NEOPLASTIC DISORDERS OF MESOTHELIUM

TESTICULAR TORSION

Testicular torsion is a true surgical emergency with a peak presentation in adolescence between 12 and 16 years of age.

- Faulty anchoring of testis on wall of the scrotum allows testis to rotate. As the testicle twists around the spermatic cord, venous blood flow is cut off, resulting in venous congestion, hemorrhage and ischemic necrosis of the testicle. The testicle will become tender, swollen.
- Ultrasonography is the primary diagnostic modality beyond physical examination of testicular torsion. It is approximately 93% sensitive and 100% specific. Doppler can be employed to evaluate for both venous and arterial flow.
- Patient presents with excruciating sudden pain in the testis, swelling scrotal region, nausea and vomiting.
- On physical examination, scrotal swelling and diffuse testicular tenderness is universal. Cremasteric reflex is absent on affected side.
- On gross examination, torsion testis becomes enlarged, necrotic and hemorrhagic (Fig. 23.40).
- Histologic examination reveals hemorrhage and necrosis of seminiferous tubules and interstitial tissue.



Fig. 23.40: Testicular torsion. On gross examination, torsion testis becomes enlarged, necrotic, hemorrhagic with blackish discoloration.

HYDROCELE

Hydrocele is an abnormal collection of serous fluid between the visceral and parietal layers of the tunica vaginalis covering the testes in the scrotum and/or along the spermatic cord.

- In the normal scrotum 1–2 ml of serous fluid may be observed in the potential tunica vaginalis cavity and should not be mistaken for hydrocele. In most cases, underlying cause of hydrocele is idiopathic.
- Congenital hydrocele occurs due to persistence of continuity of the tunica vaginalis with the peritoneal cavity. It is the most common cause of scrotal swelling in infants often associated with inguinal hernia.
- Acquired hydrocele occurs in adults secondary to infection, lymphatic obstruction by tumor or trauma affecting scrotum.
- Positive transillumination test differentiates hydrocele from testicular tumors. Preoperative ultrasound is widely used to diagnose hydrocele in adults.

HEMATOCELE

Hematocele is accumulation of blood between the visceral and parietal layers of the tunica vaginalis covering the testes in the scrotum and/or along the spermatic cord. Hydrocele is commonly associated with a history of scrotal trauma, usually characterized by scrotal pain and acute onset. It may be caused in the setting of testicular tumors and infections. Rarely, hematocele can be idiopathic with a mild onset, following spontaneous bleeding.

SPERMATOCELE

Spermatocele is a spermatozoa-containing cyst formed from the protrusions of widened efferent ducts of the rete testis or epididymis. The exact cause of spermatoceles is not clear, but they might be due to a blockage in one of the tubes that transport spermatozoa. Spermatocele is located above and posterior to the testis and attached to the epididymis, but separate from the testes. Most spermatoceles are small. A spermatocele causes no symptoms and might remain stable in size. Large size spermatocele may cause pain or discomfort in the affected testis. Spermatocele is unlikely to cause complications. Large size spermatocele is treated by surgery.

VARICOCELE

Varicocele results from dilation of the multiple veins of the spermatic cord. Patient presents with nodularity on the lateral side of the scrotum. Most patients are asymptomatic and discovered during routine physical examination.

- Varicocele is palpated as a bag of worms. It is considered a common cause of male infertility and oligospermia due to unknown reason.
- Surgical resection of varicocele by ligation of the internal spermatic vein often improves reproductive function.

MALE HYPOGONADISM

Male hypogonadism refers to failure of the Leydig cells to produce testosterone, failure of seminiferous cells to generate spermatozoa, or an abnormality in androgen receptors.

- Approximately 90% of male infertility is due to hypogonadism resulting in impaired spermatogenesis in the presence of normal production of testosterone (seminiferous tubule dysfunction).
- Primary hypogonadism involves testicular problems in synthesizing testosterone in the Leydig cells (increased LH levels, decreased spermatozoa count, normal FSH) or generating spermatozoa in the seminiferous tubules [increased FSH (decreased inhibin), low sperm count, normal LH and testosterone].
- Secondary hypogonadism refers to disorders in the pituitary hypothalamic axis or to receptor deficiencies.

MALE IMPOTENCE

Male impotence is defined as persistent inability to obtain an erection during attempted sexual intercourse, which occurs in 10–35% of adult men. Testosterone deficiency accounts for 15–20% of cases. Other causes of male impotence include psychological problems, autonomic neuropathy, endocrine disease (hypothyroidism, hypopituitarism) or drugs.

- The most useful finding that excludes an organic cause of male impotence is the presence of nocturnal penile tumescence (erections that normally occur during sleep).
- Drugs associated with male impotence include tranquillizers (phenothiazines), lithium, sedatives (barbiturates or benzodiazepines), antihypertensive drugs (methyl dopa or debrisoquine or clonidine), alcohol, estrogens and drug abuse (heroin or methadone).

CRYPTORCHIDISM

In majority of these infants, the testis will descend into the scrotum during the first year of life. Cryptorchidism is developmental failure of a testis to descend into the scrotum especially in premature newborns. According to their location, the cryptorchid testes can be classified as abdominal, inguinal, or upper scrotal. Anorchia refers to congenital absence of testes. Cryptorchidism is associated with testicular atrophy and sterility. There is increased incidence of germ cell tumors such as seminoma and embryonal carcinoma, even if the testis is surgically moved from its ectopic location back to the scrotum. It is the most common urologic disorder requiring surgical treatment in infants.

TESTICULAR ATROPHY

Testicular atrophy occurs due to mumps orchitis, trauma, Klinefelter's syndrome (47,XXY) as a result of meiotic nondisjunction during oogenesis, chronic debilitating disease, cryptorchidism, old age and hormonal imbalance. Hormonal excess or deficiency occurs due to disorders of hypothalamus or pituitary, cirrhosis of liver and estrogen therapy. Klinefelter's syndrome is characterized by infertility, and the testicular atrophy and loss of meiotic and postmeiotic germ cells.

On gross examination, testes are small and brown in adults. On histologic examination, the testicular seminiferous tubules are atrophic, and their basement membrane is greatly thickened and hyalinized. Scattered patent seminiferous tubules with markedly thickened walls are present, but may contain only Sertoli cells and absence of germ cell elements. The Leydig cells are prominent; some of them may be present inside the tubules.

MALE PSEUDOHERMAPHRODITISM

Male pseudohermaphroditism is congenital disorder also known as testicular feminization syndrome, which occurs due to a congenital deficiency of the androgen receptor. Males have a normal 46XY karyotype. Patient presents with cryptorchid testes, but the external genital organs appear feminine or ambiguously female, with signs of virilization. Patients with congenital adrenal hyperplasia have a normal 46XX karyotype.

DISORDERS OF PROSTATE GLAND

ANATOMY AND HISTOLOGY—PROSTATE

Prostate gland is located at the base of urinary bladder. The seminal vesicles are located posterior to the prostate. The urethra exits from the urinary bladder and traverses the prostate gland before exiting to the penile urethra. A thin layer of connective tissue surrounding prostate merges with surrounding soft tissues, including nerves. There is no distinct capsule. Normal weight of prostate gland is 20 g for 20–50 years and 30 g for 60–80 years.

- Prostate gland comprises glandular and nonglandular components. Based on presence of group of glands, the prostate gland is subdivided into three main glandular zones: (a) peripheral zone, (b) the central zone, and (c) the transition zone, and in addition a smaller periurethral gland region.
  - The peripheral zone constitutes 70% of the prostate gland. The ducts protrude from the lateral side and posteriorly from the urethral wall. Most prostatic carcinomas arise in the peripheral region.
  - The central zone forms about 25% of the prostate gland and the ducts surround the ejaculatory ducts which lie in the distal urethra. Prostatic carcinoma rarely arises in the central zone. The transition zone constitutes 5% of the prostate.
- Benign nodular hyperplasia (BPH) occurs in the transition zone which lies in the angular region of the urethra between the verumontanum and the urinary bladder neck. The periurethral gland region consists of small ducts and acini, which stain for prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP). The nonglandular component of prostate gland is composed of periprostatic sphincter, striated sphincter, anterior fibromuscular stroma and prostatic gland capsule (Fig. 23.41).
- The basic function of the prostate gland is to synthesize proteins into the seminal vesicle fluid.
- The prostate gland develops from the urogenital sinus during embryogenesis, and differentiation process requires the interaction of epithelial cells with the mesenchyme.
- Dihydrotestosterone (**DHT**) is the primary androgen responsible for stimulation of glands and stroma in the prostate via androgen signaling pathway.
- Prostate gland is composed of tubuloalveolar glands and supporting fibromuscular stroma in equal proportions. The glands are radiating from the urethra. The luminal glands are lined by two cell layers, i.e. outer cuboidal cells and inner tall columnar mucin-secreting epithelium, forming inward papillary projections. The glandular component synthesizes laminated concretions of prostatic gland secretions known as corpora amylacea within the glandular lumina. The prostate epithelium consists of three types of cells: luminal, basal and neuroendocrine glands.
  - The luminal cells are secretory cells, which synthesize seminal vesicle fluid. Luminal cells also demonstrate androgen receptor, cell surface markers such as CD57 and PSA.
  - The basal cells do not possess secretory function. Basal cells express antiapoptotic genes such as BCL-2 and DNA damage protecting genes such as p53 related protein, c-Met, and prostatic stem cell antigen (PSCA).
  - Neuroendocrine cells are also present in small proportion in all prostatic gland zones.
- Most common prostatic gland sampling is done by needle biopsy, transurethral resection of prostate

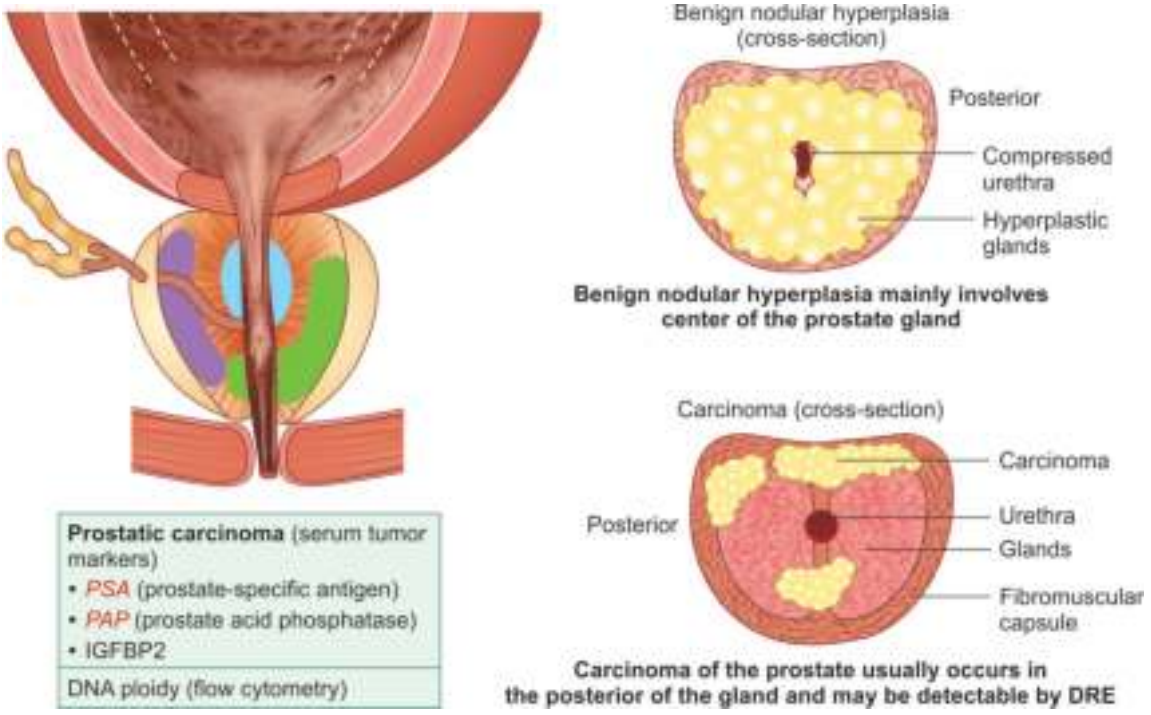


Fig. 23.41: Prostate gland shows site of benign nodular hyperplasia and prostatic adenocarcinoma with serum tumor markers (prostate-specific antigen, prostate acid phosphatase, IGFBP2) and flow cytometry DNA ploidy.

(TURP), fine needle aspiration cytology, open simple and radical prostatectomy. Needle biopsy and prostate chips are fixed in 10% neutral buffered formalin.

INFLAMMATORY DISORDERS—PROSTATE

Inflammatory disorders of prostate gland include acute bacterial prostatitis, asymptomatic inflammatory prostatitis, chronic prostatitis and nonspecific granulomatous prostatitis. Clinical manifestations and elevated serum PSA may mimic prostatic carcinoma. Physician must perform digital rectal examination. Urine examination should be performed to rule out urinary tract infection.

ACUTE BACTERIAL PROSTATITIS

Acute bacterial prostatitis is the most common urologic disease in males younger than 50 years usually associated with urinary bladder outlet obstruction secondary to benign nodular hyperplasia or an immunocompromised person. Urinary tract infection by coliform organisms as well as *Neisseria gonorrhoeae* results into acute prostatitis and may be associated with some dysuria. On histologic examination, the prostatic glands are filled with neutrophils, and the intervening prostatic stroma may also contain a few neutrophils (Fig. 23.42).

ASYMPTOMATIC INFLAMMATORY PROSTATITIS

Asymptomatic inflammatory prostatitis is characterized by the incidental finding of prostatic inflammation without genitourinary complaints. The disorder is diagnosed during a workup for infertility and elevated serum PSA level. The disorder can produce elevated white blood cells in the ejaculate (leukocytospermia) and can cause male infertility, if left untreated.

CHRONIC PROSTATITIS

Chronic prostatitis affects men under the age of 50 years with or without clinical symptoms, which has negative impact on quality of life, as it difficult to treat owing to its wide symptomatology. It may follow acute bacterial prostatitis. *Chlamydia trachomatis* bacteria may also cause chronic prostatitis. Patient presents with dysuria along with low-grade pelvic pain or low back pain. Prostatic stroma is infiltrated by lymphocytes, plasma cells, and macrophages (Fig. 23.43).

NONSPECIFIC GRANULOMATOUS PROSTATITIS

Nonspecific granulomatous prostatitis probably occurs in response to prostatic gland secretions released into the prostatic stroma due to rupture of the ducts and acini. It consists of a lobular inflammatory infiltrate composed of lymphocytes, plasma cells, foamy histiocytes, and small number of neutrophils. In the early stages, a dilated prostatic duct is evident.

- The disorder causes symptoms of obstruction and infection of the urinary tract.
- In nonspecific granulomatous prostatitis, prostatic nodularity on palpation is accompanied by elevated PSA clinically mimic prostatic carcinoma.
- It is worth mentioning that nonspecific granulomatous prostatitis must be differentiated from other specific cause of prostatitis. Mycobacterial prostatitis produces caseating granuloma. Allergic prostatitis is associated with allergic disorders such as bronchial asthma. Post-biopsy granulomas consist of a central region of fibrinoid necrosis surrounded by palisaded histiocytes in prostate gland. Nonspecific granulomatous prostatitis is shown in Fig. 23.44.

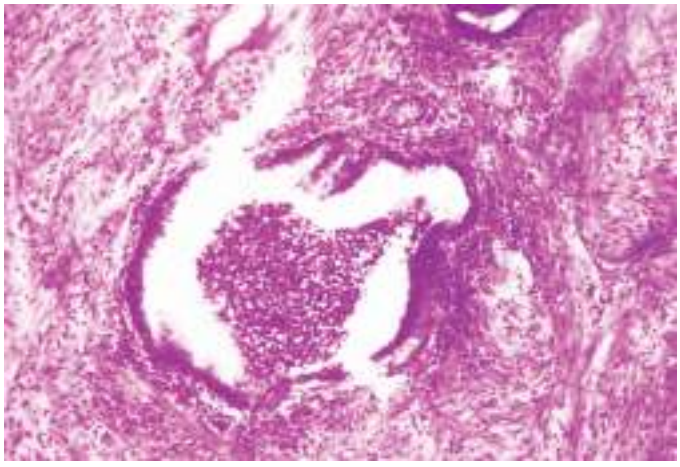


Fig. 23.42: Acute prostatitis. Prostatic glandular luminal space is filled with neutrophils. Neutrophilic infiltrate is also seen in the prostatic stroma (400X).

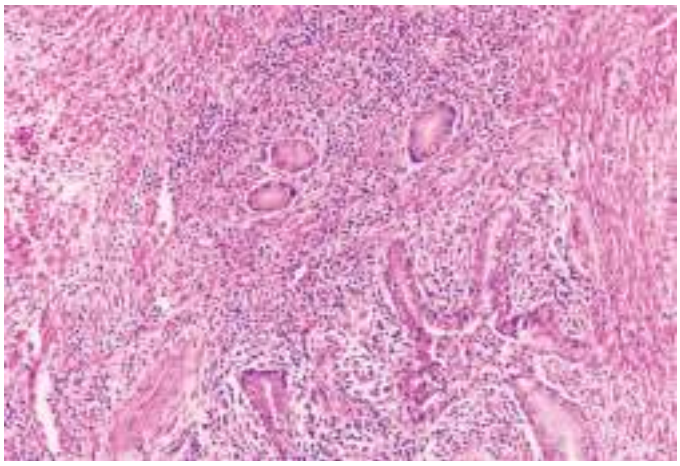


Fig. 23.43: Chronic prostatitis, no specific. There is prominent periductal inflammation. The inflammatory infiltrate is composed of lymphocytes and histiocytes (400X).

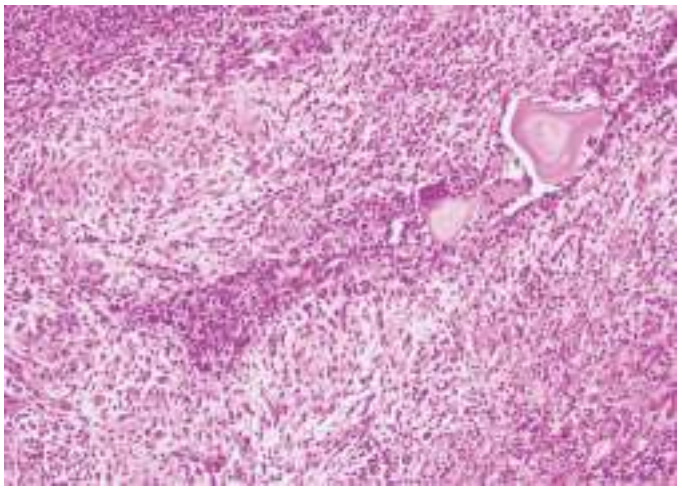


Fig. 23.44: Nonspecific granulomatous prostatitis. There is polymorphous infiltrate of lymphocytes, macrophages, plasma cells, neutrophils, multinucleated giant cells and occasional eosinophils (400X).

XANTHOGRANULOMATOUS PROSTATITIS

Xanthogranulomatous prostatitis is benign inflammatory rare disorder of prostate gland that can clinically and even biochemically with elevated PSA mimic prostatic carcinoma.

- A high degree of suspicion and close co-operation with histopathologist is necessary for the diagnosis of xanthogranulomatous prostatitis.
- Generally, xanthogranulomatous inflammatory prostatitis is observed in kidney and gallbladder and very rarely in the prostate gland. In xanthogranulomatous prostatitis, histiocytes are observed in large amounts of inflammatory infiltrate, that is accompanied by numerous lymphocytes and plasma cells.
- Presence of numerous histiocytes in the prostatic biopsy may be misdiagnosed as prostatic adenocarcinoma with hypernephroid pattern (Gleason 4B).

BENIGN NODULAR HYPERPLASIA OF PROSTATE GLAND

Benign nodular hyperplasia of prostate gland is also known as benign prostatic hypertrophy (BPH). It is detected in 20% at age 40 years, 50% at age 50 years and 70% at age 60 years. It primarily affects periurethral and transitional zones. Involvement of periurethral portion of the prostate gland compresses urethra side to side resulting in a vertical slit.

It is the most common cause of urinary tract obstruction. Digital rectal examination reveals a firm, enlarged, and nodular prostate gland.

PATHOGENESIS

It has been established that benign nodular hyperplasia of prostate gland occurs in persons with intact testes and it is considered as an androgen-dependent disorder. It has been suggested that benign nodular hyperplasia of prostate gland may result from an initial activation of mesenchymal clones and embryonal functions that stimulate its development of the glandular component. In turn, it may result from dihydrotestosterone (DHT) within the prostate gland due to decreased catabolism of DHT molecule and enhanced intracellular binding to nuclear for DHT receptors and triggering prostatic hyperplasia of both glandular and fibromuscular stromal elements.

- Dihydrotestosterone is a major growth factor for prostate gland. DHT is derived from testosterone by the action of 5α-reductase enzyme.
- Therapeutic administration of drug inhibiting 5α-reductase enzyme is useful in the medical management of nodular hyperplasia of prostate gland.
- Age-related increase in estrogens level also plays an indirect role by stimulating the production of DHT receptors. The estrogens promote expression of nuclear receptors for DHT in prostate gland.
- The α-adrenergic blockers cause relaxation of smooth muscle in prostate and help in relieving obstruction.

CLINICAL FEATURES

Patient's symptoms are related to urethral obstruction of urine outflow. Patient presents with frequency of micturition, difficulty in urination, nocturia, dribbling, incomplete emptying of urinary bladder and dysuria.

- Persistent urethral obstruction to urine outflow may result in urinary bladder distension, muscular hypertrophy, and formation of diverticulum and trabeculae.
- Urine retained inside a diverticulum of urinary bladder is often infected, a complication that may lead to the formation of urinary bladder stones. There is risk for urinary tract infection, hydroureter, hydronephrosis and renal failure (Fig. 23.45).

Surgical Pathology: Benign Nodular Hyperplasia of Prostate Gland

Gross Morphology

- Benign nodular hyperplasia of prostate gland occurs in periurethral and transitional zones, that compresses urethra side to side resulting in a vertical slit space. The nodular lesion can be large, discrete in periurethral region, weighing 50–100 g (normal 20–30 g).
- Cut surface of benign nodular hyperplasia of prostate gland shows solid and cystic areas, yellow-pink and soft in consistency with oozing of prostatic fluid.
- Prostatic stromal hyperplasia is gray-white nodules of variable sizes with rubbery consistency (Fig. 23.46).

Light Microscopy

Histologically, benign nodular hyperplasia of prostate gland is diagnosed on samples like transurethral resection of prostate (TURP) chips, simple or radical prostatic specimens. It should not be diagnosed in needle biopsy samples.

- The earliest change in benign nodular hyperplasia of prostate gland is a stromal proliferation about small sinusoidal spaces in the periurethral regions and to a lesser degree, in the periductal and intralobular areas. The lesion begins around urethra where ejaculatory ducts enter the prostatic transitional or periurethral zone. Stromal changes include increase in smooth muscle and reduced elastic tissue.
- Benign nodular hyperplasia of prostate gland is composed of hyperplasia of glands and stroma in variable proportions forming papillary buds, infoldings and cysts.
  - The lining epithelium ranges from flat to columnar, sometimes facing each other in the same gland.
  - Basal layer is demonstrated immediately above a sell-developed epithelium. It is associated with squamous metaplasia and infarction.
  - The glands are dilated or even cystic and containing an inspissated secretion of glycoprotein nature (corpora amylacea), which is sometimes calcified.
  - Small clusters of lymphocytes are demonstrated in the interstitial tissue and around the ducts.
  - Many morphologic variants occur due to proliferation of one component over the other component (Fig. 23.47A and B).
- Mixed epithelial (glandular) and stromal hyperplasia in various proportions are present. The glands show irregular sized glandular spaces lined by epithelium, i.e. inner columnar layer and outer flattened myoepithelial cell layer.
- Pure stromal nodules are uncommon and composed of hyalinized leiomyomatous nodules with spindled or myxoid (stellate cells).

TREATMENT

The treatment of benign nodular hyperplasia of the prostate gland includes medical or surgical treatment. Medical treatment includes administration of medicines aimed at blocking the actions of androgens such as dihydrotestosterone (DHT) by preventing their secretion or their conversion to active form in tissue, or at relaxing stromal muscle cells. The nodular region of prostate is excised by various techniques such as transurethral resection of prostate (TURP), suprapubic prostatectomy and laser enucleation. It is worth mentioning that these procedures remove only the newly formed nodules.

NEOPLASTIC DISORDERS OF PROSTATE

The prostate gland is the most common site of neoplastic disorders in men. Prostatic intraepithelial neoplasia lesion is regarded as a precursor lesion, which undergoes neoplastic transformation of prostatic epithelium. Intraductal prostatic carcinoma can undergo invasive and metastatic prostatic carcinoma, which is characterized by unpredictable biologic behavior, and a long latency before diagnosis.

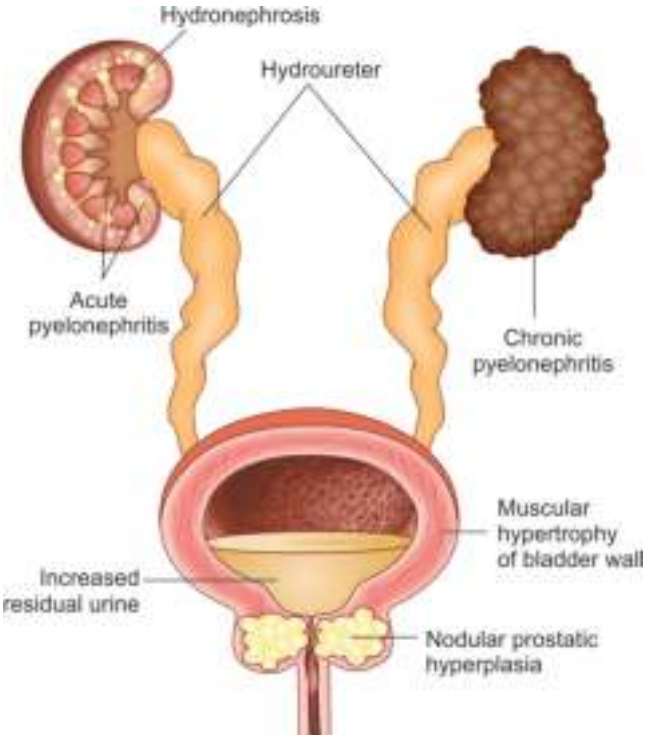


Fig. 23.45: Benign nodular hyperplasia of prostate. Persistent obstruction to urine outflow has produced hydroureter, hydronephrosis and acute pyelonephritis.



Fig. 23.46: Nodular hyperplasia of prostate. The prostate is considerably enlarged. Its cut surface shows a creamy, lobulated solid and cystic areas appearance.

PROSTATIC INTRAEPITHELIAL NEOPLASIA

Proliferation of luminal cells of the ducts with dysplasia results in prostatic intraepithelial neoplasia (PIN). PIN

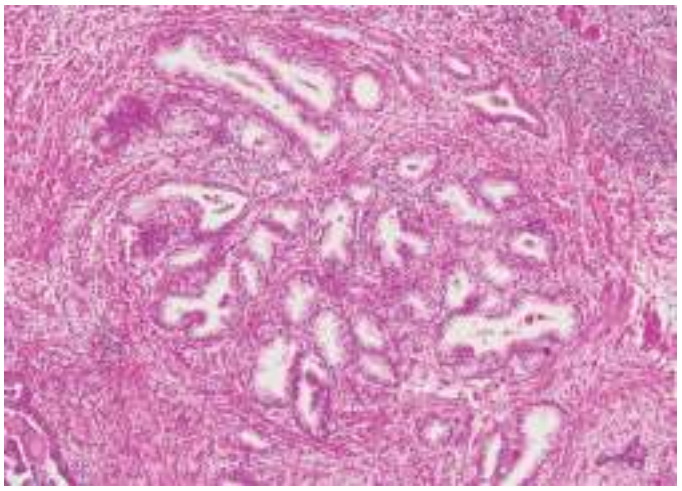


Fig. 23.47A: Benign nodular hyperplasia prostate. The acini are relatively uniform and evenly spaced. The acini are lined by columnar secretory cells. The basal cell layers may be inconspicuous, but may be highlighted by high molecular weight cytokeratin immunohistochemical stain (400X).

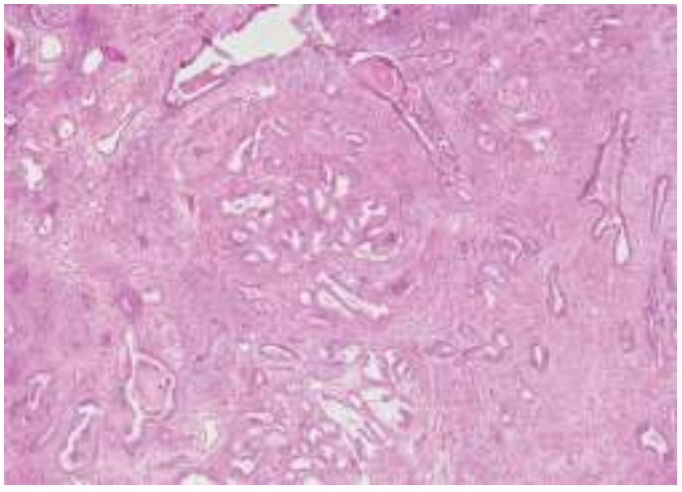


Fig. 23.47B: Benign nodular glandular hyperplasia of prostate gland. The lesion is composed of variably sized glandular structures lined by basal and secretory cells. Glands demonstrate dilatation with papillary infoldings and cysts, often containing corpora amylacea and sometimes calcification. Epithelial lining ranges from flat to columnar, with pink pale cytoplasm, regular, centrally located nuclei and inconspicuous nucleoli (400X).

occurs in two forms: low-grade PIN (**LGPIN**), or high-grade PIN (**HGPIN**). The basal layer is intact in LGPIN but absent in HGPIN. A key immunohistochemical marker protein to indicate the presence or absence of the basal cell layer is the antikeratin stain 34bE12.

- In general, HGPIN is considered to be a precursor of invasive prostatic adenocarcinoma. Changes accumulating in LGPIN and HGPIN evolve in intraductal and invasive prostatic adenocarcinoma.
- Digital rectal examination, transrectal ultrasound scan (TRUS), transurethral resection of prostate (TURP), and PSA analysis may be used to detect PIN.

Genetic Alterations and Immunohistochemical Markers: Transition of HGPIN to Prostatic Adenocarcinoma

- Some of the immunohistochemical marker, oncoproteins are amplified and involved in the transition of HGPIN to prostatic adenocarcinoma, which include BCL-2, C-erb B2, C-erb B3, c-Met, racemase, telomerase, AMACR and PSCA.
- Secretory immunohistochemical marker proteins such as prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) are downregulated in advanced PIN and invasive prostatic adenocarcinoma.
- Common allelic loss in chromosomal loci of 8p, 10q, and 16q are demonstrated in prostatic intraepithelial neoplasia (PIN) and invasive prostatic adenocarcinoma. Epigenetic regulation of GSTP1 where promoter is hypermethylated observed in both PIN and invasive prostatic adenocarcinoma.

INTRADUCTAL AND INVASIVE PROSTATIC ADENOCARCINOMA

It is generally accepted that dysplastic precursor lesion, i.e. HGPIN in prostatic ducts progresses to intraductal and invasive prostatic adenocarcinoma.

- In intraductal prostatic adenocarcinoma, the lumen of ducts and acini are filled with malignant cells with preservation of basal cells with solid or dense cribriform pattern or loose cribriform or micropapillary pattern with either marked nuclear atypia and comedonecrosis. In invasive prostatic adenocarcinoma, neoplastic cells invade basal cell layer as compared to HGPIN and intraductal prostatic adenocarcinoma. Invasive prostatic adenocarcinoma most often arises from the peripheral group of glands (ducts and acini) of prostate gland in elderly persons of >50 years of age. There is no evidence that prostatic adenocarcinoma originates from nodular hyperplasia prostate.
- The primary known major risk factors for prostatic adenocarcinoma are older age, strong family history, obesity, dietary factors (excessive consumption of saturated fat, milk products, calcium), obesity, hypertension, lack of exercise, persistently elevated testosterone levels, exposure to 'agent orange' (rainbow herbicide chemical used in warfare by US army), occupational exposure to cadmium (used in batteries and metal paintings), ethnicity and gene mutations. Dietary consumption of vitamin D, selenium, soya products and tomatoes product containing lycopene prevent prostatic adenocarcinoma. Hereditary and familial genetic factors are linked to prostatic adenocarcinoma.
- Hormones are integral part of the prostate, which regulate its growth, differentiation and maintenance. Androgenic hormones such as dihydrotestosterone (DHT) are demonstrated in the prostate gland.

- Testosterone diffuses into the prostate gland and gets converted to dihydrotestosterone (DHT) in the prostate gland.
- Androgen ablation therapy is commonly used to counter the growth prostatic adenocarinoma which results in the blockage of the androgen supply. However, it has not been established that androgenic hormones are the cause of prostatic adenocarcinoma.

Molecular Genetic Alterations

Many molecular genetic alterations have been described in prostatic adenocarcinoma. Allelic loss in chromosomes 8p, 10q, 13q, 16p, and 17p, which are the sites of tumor suppressor genes have been demonstrated in 50% of cases.

- Approximately 50% of prostatic adenocarcinomas demonstrate fusion of the androgen-responsive serine protease gene TMPRSS2 (21q22.2) with one of the ETS transcription factor gene family members such as ERG (21q22.2), ETV1 (7p21.2), ETV4 (17q21) and ETV (3p27), with ERG accounting for >90% of cases. TMPRSS2-ERG fusion gene most often results from an insertional deletion of 21q22.2-22.3 which causes juxtaposition of the two genes.
- Point mutation in SPOP is demonstrated in 10% of prostatic adenocarcinomas. Loss of PTEN tumor suppressor gene (10q23) is demonstrated by FISH in 10–70% of cases. TP53 tumor suppressor gene mutation is associated with aggressive behavior of prostatic adenocarcinoma. RB gene mutation is seen in significant number of cases. BRCA2 gene mutation has been linked to prostatic adenocarcinoma.
- BRCA2 gene (13q) mutation has been associated with prostate adenocarcinoma. BRCA2 regulates DNA repair by binding to RAD51, a molecule that mediates DNA double-strand repair breaks. BRCA2 encodes protein involved in checkpoint functions related to progression of the cell cycle into S phase.
- HER2/neu gene amplification is demonstrated in 33% of prostatic adenocarcinoma. HOXB13 gene mutation also predisposes to prostatic adenocarcinoma. Patients with mutation of mismatch repair genes in HNPCC (Lynch syndrome) have increased risk for prostatic adenocarcinoma. Molecular genetic alterations associated with increased risk for prostatic adenocarcinoma are given in Table 23.24.
- Hereditary and familial genetic factors account for 10% of prostatic adenocarcinoma. Risk of prostatic adenocarcinoma increases with first degree relative with prostate adenocarcinoma especially at an early age.
- Besides genetic mutations, many epigenetic alterations occur in prostatic adenocarcinoma. The term epigenetics refers to the external modification of DNA bases which are inherited but do not cause a change in the DNA sequence.
- Epigenetic modifications vary from methylation of DNA to acetylation, phosphorylation and methylation of histones, which have been linked to aberrant activity of the enzymes and reader proteins involved in these epigenetic processes, leading to the search for dedicated inhibitory compounds.
- Genes implicated in hereditary prostatic adenocarcinoma differ from those affecting sporadic prostatic adenocarcinoma.
- In hereditary prostatic adenocarcinoma, some of the genes implicated in hereditary prostatic

Table 23.24 Molecular genetic alterations associated with increased risk of prostatic adenocarcinoma

Cytogenetic and Molecular Genetic Alterations	Comments	Frequency
Chromosomal allelic losses	Chromosomes 8p, 10q, 13q, 16p, and 17p, which are the sites of tumor suppressor genes	50% cases
TMPRSS2-ERG fusion	Fusion of the androgen-responsive serine protease gene TMPRSS2 (21q22.2) with one of the ETS transcription factor gene family members	>90% cases
SPOP gene	Point mutation	10% cases
PTEN tumor suppressor gene (10q23)	Loss of PTEN	10–70% cases
RB gene mutation	Gene mutation	Significant cases
BRCA2 gene	Gene mutation	Significant cases
HER2/neu gene	Gene mutation	33%
TP53 tumor suppressor gene	Gene mutation	Significant cases associated with aggressive behavior
HOXB13 gene	Gene mutation	Significant cases
Mismatch repair genes	Gene mutations in HNPCC (Lynch syndrome)	Significant cases

Table 23.25 Genes implicated in hereditary and familial prostatic adenocarcinoma

Genes	Locus on Linkage Analysis	Comments
HPC1 (hereditary prostate cancer gene 1)	1q24-25	RNASEL gene with normal apoptotic function mapped to the HPC2 locus, is mutated in hereditary prostatic adenocarcinoma
HPCX	Xq27-28	Hereditary prostatic adenocarcinoma (some cases)
PCAP	1q42-43	Hereditary prostatic adenocarcinoma with early onset disease in families
HPC2/ELAC	17p11	HPC2 encodes a hydroxylase which acts like DNA repair. Mutation responsible for high risk prostatic adenocarcinomas in families
NKX3.1	8q21	Tumor suppressor gene mutation associated with hereditary prostatic adenocarcinoma

adenocarcinoma are HPC1, HPCX, PCAP, HPC2/ELAC and NKX3.1.

- Signaling pathways are activated in the process of development of prostatic adenocarcinoma.
- The signaling pathways are interconnected and ligands affect more than the immediate downstream target in their respective signaling pathways. Genes implicated in hereditary and familial prostatic adenocarcinoma are given in Table 23.25.

Clinical Features

Patient is asymptomatic in the initial phase and becomes symptomatic, when prostatic adenocarcinoma spreads beyond the prostate gland invading surrounding structures, i.e. ejaculatory ducts in the space between the seminal vesicles or perivesicular fascia, urinary bladder and rectum. Local invasion of tumor into the urethra and urinary bladder neck may cause frequency of micturition and difficulty in passage of urine. This is followed by involvement of draining lymph nodes and metastases to bones. When disseminated, the prostatic adenocarcinoma may respond to endocrine therapy because tumor growth is partially related to the activity of androgens.

Diagnostic Modalities

Prostatic adenocarcinoma is most often diagnosed by rectal examination, ultrasound-guided (TRUS) prostatic tissue biopsies and serum PSA.

- Serum prostate-specific antigen is widely used for early detection of prostate adenocarcinoma. Newer diagnostic modalities include total and free PSA levels, PCA3 urine testing, prostate health index (PHI) scoring, the "4K" test, genomic analysis, MRI imaging, PIRADS scoring, and MRI-TRUS fusion-guided biopsies. When prostatic adenocarcinoma is limited to the prostate gland, it is considered localized and potentially curable.
- Outcomes depend on age, associated health problems, tumor histology and the extent of prostatic adenocarcinoma.

Serum Tumor Markers in Prostatic Adenocarcinoma

Both prostate-specific antigen (PSA) and serum prostate-specific acid phosphatase are increased in prostatic adenocarcinoma. Concentration of prostate-specific antigen values in different age groups is given in Table 23.26. Percentage of total PSA levels and free PSA in serum is given in Table 23.27.

- **Prostate-specific antigen:** PSA is a product of prostatic epithelium normally synthesized in the semen. It is serine protease, whose function is to cleave and liquefy the seminal vesicle coagulum formed after ejaculation. In most laboratories, normal cut off point of serum level of prostate-specific antigen is 4 ng/ml. Serum PSA levels may be increased in prostatic adenocarcinoma, nodular hyperplasia of prostate, prostatitis, and prostate infarct. Prostatic adenocarcinoma is associated with increased total PSA levels in serum and decreased free form. On the other hand, increased total PSA levels in serum with

Table 23.26 Prostate-specific antigen (PSA) values in different age groups

Age Group (Years)	Serum Prostate-specific Antigen (ng/ml)
40–49	2.5 ng/ml
50–69	3 ng/ml
70–79	6.5 ng/ml

Older men have higher serum PSA level than younger one.

Table 23.27 Percentage of total prostate-specific antigen (PSA) levels and free PSA in serum

Prostate Disorders	Total Serum PSA Levels	Serum-free Levels
Prostate carcinoma	High	<10%
Benign nodular hyperplasia	High	>25%

Percentage of free PSA/total PSA levels in serum is lower in men with prostate carcinoma than with benign nodular hyperplasia.

a proportionate increase in the fraction of free PSA suggests nodular hyperplasia of prostate.

- **Serum prostate-specific acid phosphatase:** Its level increases when prostatic adenocarcinoma invades capsule and surrounding tissues, but it lacks sensitivity in early disease. Therefore, estimation of serum prostate-specific acid phosphatase along with PSA is useful in the follow-up cases of widespread metastases.
- **Serum alkaline phosphatase:** Serum alkaline phosphatase is an indicator of bone osteoblastic metastases in advanced disease.

Surgical Pathology: Prostatic Adenocarcinoma

Gross Morphology

Tumor shows gray-white to yellowish poorly delineated and firm areas when compared to the native parenchyma (Fig. 23.48).

Light Microscopy

Prostatic carcinomas can be divided into two major histologic categories: acinar and ductal patterns, which may coexist in the same prostatic carcinoma. The large majority of prostatic carcinomas demonstrate acinar histologic type. The diagnosis of prostatic adenocarcinoma is based on the careful analysis of hematoxylin and eosin stained slides using a systemic approach. Histologic types of prostatic adenocarcinoma is given in Table 23.28.

- **Prostatic adenocarcinoma with acinar pattern:** This variant exhibits a wide spectrum of histologic appearances, ranging from highly differentiated neoplasms to rare anaplastic prostatic carcinoma. Four major cytoarchitectural patterns are: medium-sized glands, small-sized glands, diffuse individual cell infiltration and cribriform. Presence of prostatic glands within perineural spaces is common in these tumors. This finding is strong indicator of malignancy. An additional glomeruloid pattern of growth has recently been described, which is characterized by the presence of intraluminal ball-like clusters of tumor cells.
  - Prostatic adenocarcinoma is composed of medium-sized glands are detected on low power examination by virtue of the closely spaced back-to-back arrangement of glands, the prominent luminal cytoplasm with orientation of normochromatic nuclei against the basement membrane, smooth luminal surface and the scanty intervening stroma. Atypical mitoses are rare in well-differentiated neoplasm composed of medium-sized glands (Fig. 23.49A and B).
  - Prostatic adenocarcinoma is composed of small glands exhibits individual glands with round configuration containing hyperchromatic nuclei and prominent nucleoli. Atypical mitoses are also of significance, but these are rarely present in well-differentiated neoplasm composed of small glands.
  - Prostatic adenocarcinoma composed of diffuse cell infiltration resembles somewhat that of invasive lobular carcinoma of the breast.
  - Prostatic adenocarcinoma composed of cribriform pattern varies in the dimension of the epithelial aggregates ranging from anastomosing epithelium to small- or medium-sized glands with complex intraluminal proliferations.
- **Prostatic adenocarcinoma with ductal pattern:** It is usually seen in a periurethral location. Cystoscopy reveals polypoid villous or infiltrative urethral component. Tumor is composed of papillary and cribriform architecture lined by columnar pseudostratified malignant epithelium. Many prostatic carcinomas present as cystadenocarcinomas.
- **Prostatic carcinoma of other histologic types:** These are prostatic adenocarcinoma with neuroendocrine differentiation, small cell prostatic carcinoma, mucinous prostatic adenocarcinoma, signet ring prostatic carcinoma, adenosquamous prostatic carcinoma, squamous cell carcinoma, adenoid basal carcinoma, basal cell carcinoma with adenoid cystic carcinoma, lymphoepithelioma-like carcinoma and sarcomatoid carcinoma.

Immunohistochemistry Technique

- Panel of immunohistochemical markers in the assessment of benign/noninvasive lesions and invasive prostatic adenocarcinoma is shown in Table 23.29 and Fig. 23.50.
- Prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP) immunohistochemical markers are used to demonstrate prostatic epithelium in benign and malignant process. But these are of great use in identifying the prostatic origin of metastatic tumors. NXK3.1 is a recently described nuclear marker for prostatic adenocarcinoma that has improved sensitivity and specificity over PSA and PSAP.
- Alpha-methylacyl-CoA racemase (AMACR) is a new immunohistochemical marker for prostatic adenocarcinoma that can be very helpful in difficult cases when limited amount of tumor is present in biopsies.
- The distinction between poorly differentiated prostatic carcinoma and urothelial carcinoma is challenging. GATA3 is the most specific sensitive immunohistochemical marker for urothelial carcinoma.
- Other markers for urothelial carcinomas are p53, thrombomodulin, uroplakin III and cytokeratins. Immunohistochemical markers of prostatic adenocarcinoma and urothelial carcinoma are given in Table 23.30.

Markers	Expression
PSA (prostate-specific antigen)	Positive (94–100%)
PSAP (prostate-specific acid phosphatase)	Positive (89–100%)
Prostate-specific membrane antigen (PSMA)	Positive (92%)
NXK3.1	Positive (95%)
AMACR	Positive (new marker)
P504S	Positive (new marker)
Prostein (P501S)	Positive (100%)
p63 (basal cell marker)	Positive (0–3%
Cytokeratins (high molecular weight) detected by antibody 34bE12	Positive (0–10%)

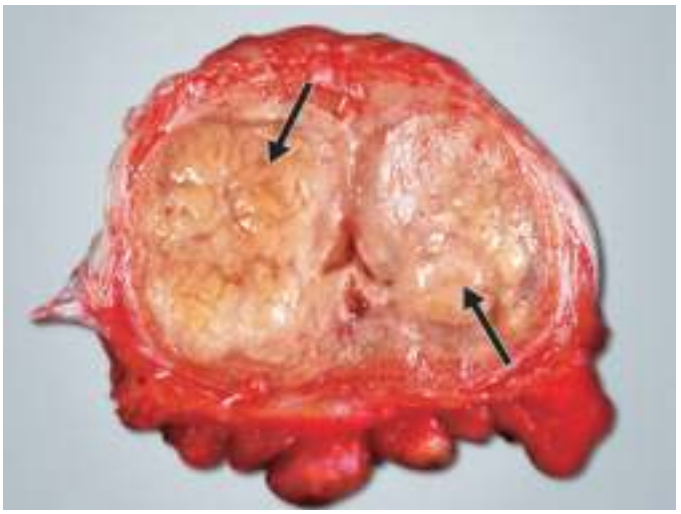


Fig. 23.48: Prostatic adenocarcinoma. It shows a large yellow tumor presents in the posterolateral aspect of the prostate gland (arrows).

Gleason Grading System

Gleason system of grading of prostatic adenocarcinoma is done based on histologic architecture of tumor rather than nuclear atypia.

- Most pathologists grade prostatic adenocarcinoma according to the Gleason score, which assigns a grade (1–5) based on how the malignant cells look compared with normal prostatic cells.
- Gleason system of grading based on differentiation of prostatic adenocarcinoma helps in prediction of course. The higher the Gleason score, the more likely that prostatic adenocarcinoma will grow and metastasize rapidly.
- Most prostatic adenocarcinomas detected by needle biopsies are given Gleason score of 6 or 7. Prostatic adenocarcinoma with Gleason score 7 or higher needs treatment.
- Prostatic adenocarcinoma with a high Gleason score is more aggressive and associated with poor prognosis. Gleason system of grading of prostatic adenocarcinoma is given in Table 23.31.

Gleason Staging System

After the diagnosis of prostatic adenocarcinoma, it is important to know its spread to the draining lymph nodes and distant organs. This process is called staging, which helps in determining the seriousness of prostatic adenocarcinoma. Staging of prostatic adenocarcinoma is based on diagnostic tests such as PSA and histologic examination of the biopsy. American Joint Committee on Cancer (AJCC) TNM staging system, which was most recently updated in 2018.

- The TNM system for prostatic adenocarcinoma is based on five pieces of information: (i) the extent of the primary tumor (T category), (ii) whether the

Table 23.28 Histologic types of prostatic adenocarcinoma

Histologic Type	Comments
Prostatic adenocarcinoma of acinar type (most common)	Four major cytoarchitectural patterns are: Medium-sized glands, small glands, diffuse individual cell infiltration and cribriform ranging from well-differentiated to anaplastic prostatic carcinoma
Prostatic adenocarcinoma of ductal type	Located as polypoid villous or infiltrative in periurethral location detected on cystoscopy. Tumor is composed of papillary and cribriform architecture lined by columnar pseudostratified malignant epithelium or as cystadenocarcinomas
Prostatic adenocarcinoma with neuroendocrine differentiation	This variant expresses neuroendocrine markers by histochemistry and is associated with good prognosis
Mucinous prostatic adenocarcinoma	This variant is accompanied by the formation of large amount of intracellular and extracellular mucin comprising 25% or more of the tumor. Tumor expresses PSAP and PSA. It is associated with poor prognosis
Signet ring prostatic adenocarcinoma	This variant is highly malignant and composed of neoplastic cells laden with mucin arranged in a solid, acinar, or single file pattern
Adenosquamous prostatic carcinoma	This variant may arise in *de novo* or after radiation or hormonal therapies in prostatic carcinomas
Squamous cell carcinoma	This variant may arise in *de novo* or after radiation or hormonal therapies in prostatic carcinomas and present as a well-circumscribed nodule in the prostatic transition zone
Adenoid basal carcinoma	This variant resembles adenoid cystic or basal cell carcinoma of salivary gland and presents as expansile pattern of growth. PSA and PSAP staining is negative or focally positive
Basal cell carcinoma with adenoid cystic carcinoma	This variant is rare and highly aggressive neoplasm, in which MYB rearrangement may be demonstrated
Lymphoepithelioma-like carcinoma	This variant is analogous to nasopharyngeal lymphoepithelioma
Sarcomatoid carcinoma	This variant combines histologic features of recognizable prostatic carcinoma with sarcomatoid elements

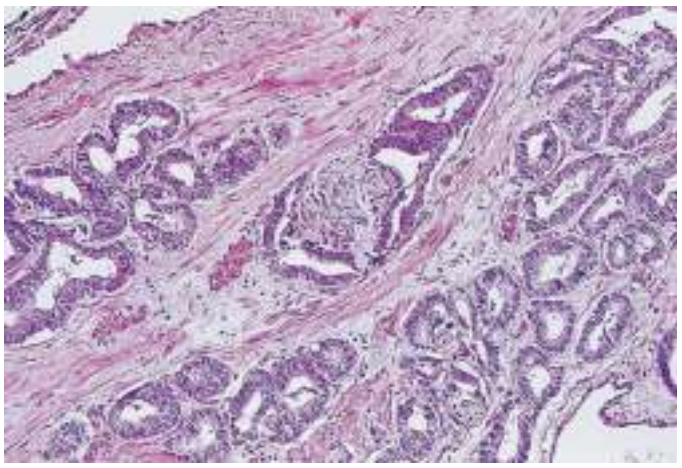


Fig. 23.49A: Prostatic adenocarcinoma with perineural invasion. Perineural invasion finding is strong indicator of malignancy. Acid sulfated and nonsulfated mucins are often present in malignant acini, appearing as amorphous or delicate thread-like faintly basophilic intraluminal secretions. Tumor is composed of medium sized glands with back-to-back arrangement of glands containing normochromatin nuclear against basement membrane (400X).

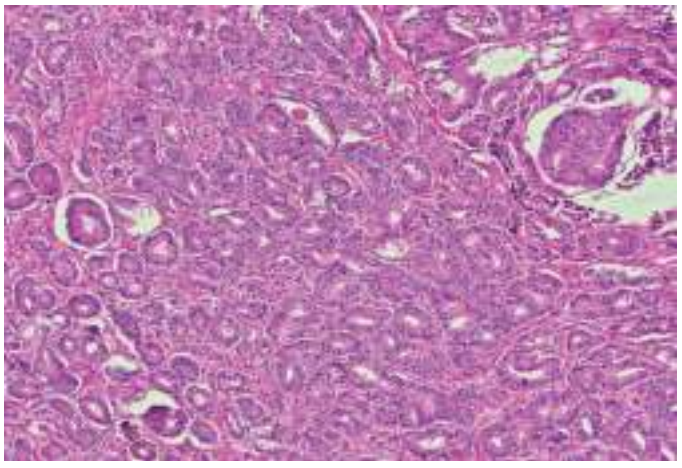


Fig. 23.49B: Prostatic adenocarcinoma. Histologic patterns depend on Gleason grading. Invasive prostatic adenocarcinoma is composed of closely packed small- to medium-sized glands (Gleason grade 3) irregular outline, smooth luminal surface, splitting stromal fibers. Neoplastic cells contain nuclear enlargement, hyperchromasia and prominent nucleoli. Neoplastic cells are arranged in papillary or cribriform glands or solid growth or single cells (400X).

Table 23.29 Panel of immunohistochemical markers in the assessment of benign/noninvasive lesions and invasive prostatic adenocarcinoma

Prostatic Cells	Immunohistochemistry (Benign/Noninvasive Lesion)	Immunohistochemistry (Invasive Neoplasm)
Prostatic basal cells	▪ Prostatic basal cell present ▪ CK903+ (cytoplasm) ▪ p63 negative	▪ Prostatic basal cell absent ▪ CK903 negative ▪ p63 negative
Prostatic acinar cells	▪ Prostatic acinar cell present ▪ AMACR negative	▪ Prostatic acinar cell present ▪ AMACR positive (cytoplasmic staining)

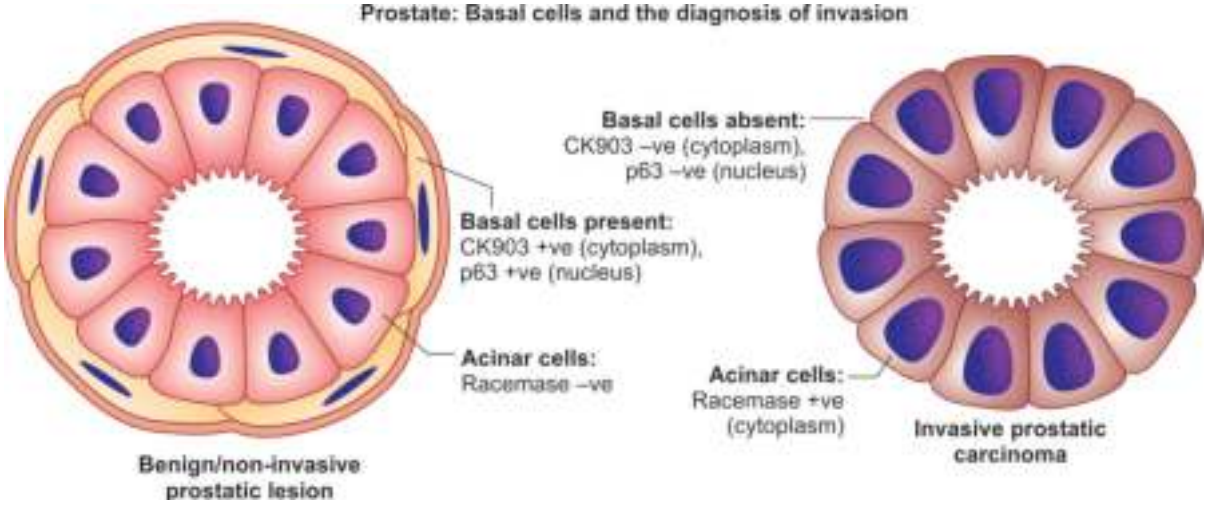


Fig. 23.50: Panel of immunohistochemical markers in the assessment of benign/noninvasive lesions and invasive prostatic adenocarcinoma.

prostatic adenocarcinoma has spread to nearby draining lymph nodes (N category), (iii) whether the prostatic adenocarcinoma has metastasized to distant organs (M category), and (iv) the prostate-specific acid phosphatase high level in prostatic adenocarcinoma at the time of diagnosis.

- The grade group based on the Gleason score, which indicates rapid growth and metastasis of prostatic carcinoma. There are two types of T categories for prostatic carcinoma: (i) the clinical T category is based on the extent of disease on clinical examination including digital rectal examination and

Table 23.30 Immunohistochemical marker of prostatic adenocarcinoma and urothelial carcinoma

Markers	Prostatic Carcinoma (% of Cases Expression)	Urothelial Carcinoma (% of Cases Expression)
PSA (prostate-specific antigen)	Positive (94–100%)	Negative
PSAP (prostate-specific acid phosphatase)	Positive (89–100%)	Negative
PSMA (prostate-specific membrane antigen)	Positive (92%)	Negative
NXK3.1	Positive (95%)	Negative
Prostein	Positive (100%)	Positive (6%)
GATA	Negative	Positive (most specific marker)
p63	Positive (0–3%)	Positive (81–96%)
Thrombomodulin	Negative	Positive (69–91%)
Uroplakin III	Negative	Positive (57%)
Cytokeratins (high molecular weight) detected by antibody 34bE12	Negative/positive (0–10%)	Positive (65–100%)

Mean of 3% prostatic carcinoma cases are positive. Up to 20% of cases of metastatic prostatic carcinoma can be positive.

Table 23.31 Gleason grading of prostatic adenocarcinoma

Parameters	Pathologic Features
Histologic patterns of prostatic carcinoma	
Gleason pattern 1 (rarely demonstrated)	▪ Prostatic adenocarcinoma closely resembles normal prostate tissue. It is composed of closely packed small, well-formed uniform, round to oval medium-sized acini. The glandular acini are larger than pattern 3 ▪ Pattern 1 closely resembles well differentiated prostatic adenocarcinoma
Gleason pattern 2 (rarely demonstrated)	▪ Prostatic adenocarcinoma is fairly circumscribed prostatic nodule with periphery showing minimal infiltration ▪ Prostatic carcinoma has well-formed, loose, and uniform glands with increased stroma between the glands; but not as uniform demonstrated in Gleason pattern 1
Gleason pattern 3 (well differentiated tumor most common)	▪ Prostatic adenocarcinoma is composed of small glands with darker cells than demonstrated in Gleason pattern 1 and Gleason pattern 2 ▪ At higher magnification, some of these cells have left the prostatic glands and are beginning to invade the surrounding tissue or having an infiltrative pattern. This corresponds to a moderately differentiated prostatic adenocarcinoma
Gleason pattern 4 (moderately differentiated tumor)	▪ Prostatic tissue has few recognizable microacinar glands of ill-defined glands with poorly formed glandular lumina. Many neoplastic cells invading the surrounding tissue are arranged in clusters ▪ This histologic appearance corresponds to a poorly differentiated prostatic carcinoma
Gleason pattern 5 (poorly differentiated tumor)	▪ Prostatic tissue lacks glandular differentiation with central necrosis, however may demonstrate a few recognizable glands. It demonstrates neoplastic cells arranged in papillary or cribriform or solid sheets throughout the surrounding tissue ▪ This histologic appearance corresponds to an anaplastic prostatic carcinoma
Primary, secondary and tertiary grades	
Primary grade	Primary grade is assigned to the dominant pattern of prostatic carcinoma. Dominant pattern of the tumor must to be >50% of the total pattern.
Secondary grade	Secondary grade is assigned to the next—most frequent pattern of prostatic carcinoma. Dominant pattern of the tumor must be <50%, but at least 5% of the total prostatic carcinoma
Tertiary grade	Tertiary grade is assigned to the increasingly pattern of prostatic carcinoma, where there is small component of a third generally more aggressive pattern

Final score is the combination of the predominant and minor architectural patterns (e.g. Gleason grade 3 plus minor component of grade 4 = 3 + 4)

prostatic biopsy and any imaging tests, and (ii) the pathology T category (written as pT) is more accurate than clinical T category and based on histologic examination of the prostate. Staging of prostatic adenocarcinoma is given in Table 23.32.

Prognostic Factors

Patient with well differentiated prostatic adenocarcinoma with free resected margins, lack of invasion of nerves, blood vessels and Gleason's capsule has good prognosis.

Therapeutic Correlation

Tumor cells of prostatic adenocarcinoma exhibit androgen-dependent growth. Chemical castration by the administration of androgenic antagonists (e.g. leuprolide) is used in the treatment of prostate adenocarcinoma.

Table 23.32 Staging of prostatic adenocarcinoma

Stage	Comments
Stage I	▪ Stage T1a and T1b are clinically unsuspected tumors found in transurethral resection of prostate (TURP) or enucleation post-mastectomy specimens removed for benign prostatic hyperplasia. There is absence of metastasis in lymph node or distant organs ▪ Stage T1c is a nonpalpable prostatic carcinoma diagnosed on needle biopsy
Stage II	Prostatic adenocarcinoma is confined to the prostate
Stage III	Prostatic adenocarcinoma is extending out of the prostate gland: Stage T3a without involvement of seminal vesicle and stage T3b with involvement of seminal vesicle

DISORDERS OF PENIS

ANATOMY OF PENIS

The penis, an external organ of male genital system consists of shaft and glans is used for sexual activity and urination.

- Glans (i.e. head or tip) is the most sensitive part of the penis.
- Penile shaft contains three columns of erectile tissue, i.e. two columns of spongy corpus cavernosa dorsally, and one column of corpus spongiosum ventrally, containing urethra wrapped in dartos fascia that also wraps foreskin, and scrotum.
- Male urethra is a tube inside the penis that runs from the urinary bladder to the glans penis.
- Meatus is the opening at the tip of the glans penis through which urine or semen exits the body.
- Prepuce is a loose fold that covers the glans penis. Surgical removal of the prepuce is referred to as circumcision.
- Deep structures of the penis are supplied by internal pudendal artery, a branch of the internal iliac artery. Venous drainage of the penis happens via one of the three systems: superficial, intermediate, and deep venous system.
- The penis is innervated by terminal branch of pudendal nerve, dorsal nerve, and branch of the ilioinguinal nerve. The sympathetic and parasympathetic afferent fibers of penis run together with the pudendal nerves arise in S2–S4 or T12–L1.
- When there is an increased flow of blood into the erectile tissues of corpus cavernosa and corpus spongiosum stiffen, causing an erection of penis. The corpus spongiosum prevents the urethra from closing during an erection.
- Ejaculation is the discharge of semen from the penis that involves a series of simultaneous involuntary muscle contractions.

CONGENITAL ANOMALIES OF PENIS AND MISCELLANEOUS DISORDERS

Congenital anomalies of the penis may range from the absolute lack of penis to segmentary anomalies. Hypoplasia and hyperplasia of the penis are usually associated with endocrine abnormalities.

- Epispadias occurs when the location of urethral meatus opens on the dorsal surface of the penis, which is less common than hypospadias.
- Glandular epispadias occurs when the opening of the urethra opens in the dorsal aspect of the glans.
- Hypospadias refers to location of urethral meatus opens on the ventral surface of the penis anywhere from the glans to the perineum, which results from incomplete closure of the urethral folds of the urogenital sinus. The classification of hypospadias depends on the anatomic location of the defect. It can be glandular (most common), coronal, penile, penoscrotal or perineal. Hypospadias is repaired by surgical procedure.

- In phimosis, abnormal tight foreskin of penis leads to narrowing of the orifice of prepuce resulting in difficulty to retract over the glans penis. Phimosis may be congenital or result from inflammation or from trauma. In paraphimosis, forceful retraction of narrow prepuce may strangulate the glans and obstruct venous blood flow.
- Normally, bone is absent in penis. Presence of heterotopic bone in penis is usually demonstrated in the Peyronie disease, in which subcutaneous fibrosis occurs in the dorsum of the penis in the elderly persons.
- Priapism is an intractable, often painful erection of penis due to venous thrombosis of the corpora cavernosa, sickle cell anemia, hypercoagulable states, spinal injuries, chronic myelogenous leukemia and some drugs.

INFLAMMATORY DISORDERS OF PENIS

BACTERIAL INFECTIONS

Bacterial infections of the genitourinary tract occur in especially uncircumscribed (phimosis) males at any age.

- Pathogenic bacteria adhere and colonize the mucosal surface of the prepuce. Bacterial inflammation of the glans penis is known as balanitis. Extension of inflammation from glans to the foreskin is called balanoposthitis. Patient presents with mild burning, pruritus, itching, swelling, erythematous patches, and plaques or bullae involving the glans penis, satellite eroded pustules and moist curd-like accumulations.
- Balanitis has worse clinical presentation in diabetic and immunocompromised patients, with fulminating edema or ulcers in severe cases.
  - Significant complications of balanitis are stricture of the meatus, phimosis, and paraphimosis. *Neisseria gonorrhoeae* infection is sexually transmitted.
  - Patient presents with urethral discharge, typically purulent and greenish yellow. Deep infections of the penis are rare caused by *Neisseria gonorrhoeae*. Tuberculosis of the penis can be involved by direct spread from nearby areas or via hematogenous route in generalized tuberculosis.
- Fournier gangrene, a necrotizing fasciitis of the genitalia, perianal and perianal region, is a life-threatening disorder. There is involvement of dartos and penile fascia. Thrombosis of the small blood vessels, known as '**obliterative endarteritis**' plays important role in the pathogenesis of Fournier gangrene, which is caused by polymicrobial infection especially in diabetes mellitus. Most important organisms are Streptococci, Staphylococcus, gram-negative bacteria and anaerobic bacteria. The source of infection may be urologic, colorectal or cutaneous.
- Gangrenous balanitis is known as '**Corbus disease**', which is a rapidly progressing necrotizing inflammatory disorder caused by anaerobic bacteria especially in diabetes mellitus. Disorder is characterized by total necrosis of the glans penis.

FUNGAL INFECTIONS

Fungal infections of the penis are probably more common especially in immunocompromised persons and diabetes mellitus. Mycotic infections of penis can be superficial or deep. Superficial infections are caused by dermatophytes and *Candida albicans*.

- Dermatophytes usually infects the penis via local spread of infection in groin region.
- *Candida albicans* is sexually transmitted and involving penis. In superficial fungal infections, numerous neutrophils are present in the squamous epithelium and keratin layer.
- Deep mycotic infections of the penis may be caused by *Histoplasma capsulatum* and *Cryptococcus neoformans*, but these are rare.

SEXUALLY TRANSMITTED INFECTIONS

Penile Syphilis

Treponema pallidum causes penile syphilis by sexual transmission of the organism. Patient presents with an elevated, painless, superficially ulcerated, firm papule (chancre) on the glans penis or prepuce that ordinarily heals within 2–6 weeks. *Treponema pallidum* is demonstrated by dark field examination and polymerase chain reaction (PCR).

Granuloma Inguinale

Calymmatobacterium granulomatis is a gram-negative bacterium, that causes granuloma inguinale. Patient presents with small, relatively painless nodule on the prepuce, glans, penile shaft or scrotum, that later ulcerates resulting in exuberant granulation tissue and pseudoepitheliomatous hyperplasia. Granuloma inguinale spreads by peripheral extension and attains a large size. On histologic examination, numerous plasma cells, neutrophils and mononuclear cells with Donovan bodies are present in the granulation tissue. There is absence or paucity of lymphocytes.

Lymphogranuloma Venereum

Chlamydia trachomatis is sexually transmitted disease. Patient presents with infrequent primary penile lesion, secondary lymphadenitis with bubo formation leading to fibrosis, edema and fistula formation.

- Lymphadenopathy occurs within one to two weeks after the appearance of primary lesion.

- On histologic examination, primary penile lesion demonstrates nonspecific granulation tissue infiltrated by plasma cells and lymphocytes. There is presence of noncaseating epithelioid cell granulomas composed of epithelioid cells and a few giant cells surrounded by plasma cells. Lymph node shows focal accumulation of neutrophils in the necrotic foci in early stage. Later, lymphoid hyperplasia with massive plasma cell infiltration distorts the architecture of lymph node resulting in formation of sinus tracts.
- Diagnosis can be confirmed by culture of skin biopsy or lymph node and serology.

Genital Chancroid

Haemophilus ducreyi is a gram-negative organism causing chancroid, which requires special media, not easily available in most laboratories, to ensure its growth. Organism results in papule formation.

- Gene clusters have been observed in *Haemophilus ducreyi* which encode for a cytolethal distending toxin, that causes irreversible cell death of epithelial cells and may lead to the development of the skin breakdown and subsequent painful necrotizing genital ulcer development accompanied by inguinal lymphadenopathy and inguinal abscess.
- *Haemophilus ducreyi* can cause widespread necrosis and destruction of external genitalia and forming giant chancroid.
- On histologic examination, there is a zonation phenomenon at the base of the ulcer.
  - The uppermost layer shows necrosis, fibrin and numerous neutrophils.
  - Middle layer demonstrates abundant granulation tissue with prominent blood vessels, some of which have potential thrombosis.
  - The deepest layer shows an intense infiltration by plasma cells and lymphocytes. Fusobacterium organisms can cause phagedenic chancroid.

Anogenital Condyloma Acuminatum

Human papillomavirus (HPV) is associated with anogenital wart known as '**condyloma acuminatum**'. Low risk types of HPV 6 and 11 are associated with condyloma acuminatum'. High risk types of HPV 16, 18, 31, 33, 39, 45, 52, 58 and 59 have been linked to cervical, vulvar, penile and oropharyngeal carcinomas. HPV is a double-stranded DNA virus primarily spread through sexual contact. Condyloma acuminatum can occur on the glans, prepuce and shaft of penis, that may extend to the meatus.

- Patient presents with soft, fleshy plaques with cobblestone or filiform appearance.
- The lesions vary from small flat and patchy to large cauliflower-like growth, which may occur sporadically or in the setting of immunosuppression state. There is increased risk for penile condyloma acuminatum in men, whose life partners have cervical HPV infection.
- HPV can be analyzed by immunohistochemistry, polymerase chain reaction (PCR) and fluorescence *in situ* hybridization techniques. Penile condyloma acuminatum may attain >8 cm size.

Surgical Pathology: Condyloma Acuminatum

Light Microscopy

- On histologic examination, surface of the condyloma acuminatum shows exophytic lesion characterized by acanthosis, papillomatosis, and a sharp fairly regular bulbar base.
- The hallmark of condyloma acuminatum is the presence of cytopathic viral effect, namely koilocytosis. Cellular changes are more marked on upper levels of epithelium.
- Koilocytes contain enlarged wrinkled nuclei surrounded by a perinuclear halo. Binucleated and multinucleated forms and dyskeratotic cells may be present (Fig. 23.51).

Genital Ulcers

Herpes simplex virus 2 (HSV-2) is a DNA virus that is efficiently sexually transmitted through intimate genital tract contact and causes persistent infection that cannot be eliminated. HSV-2 may cause frequent symptomatic self-limited genital ulcers, but in most persons infection is subclinical. Patient presents with multiple millimeter sized vesicles that rupture and form genital painful ulcers. Patient can also develop fissures and ulcerations. On histologic examination, intraepithelial vesicles contain prominent rounded acantholytic keratinocytes, demonstrate cytopathic changes that consist of nuclear ground glass appearance and molding. There is also presence of multinucleated keratinocytes with classic nuclear changes. Well-defined acidophilic inclusions can be present.

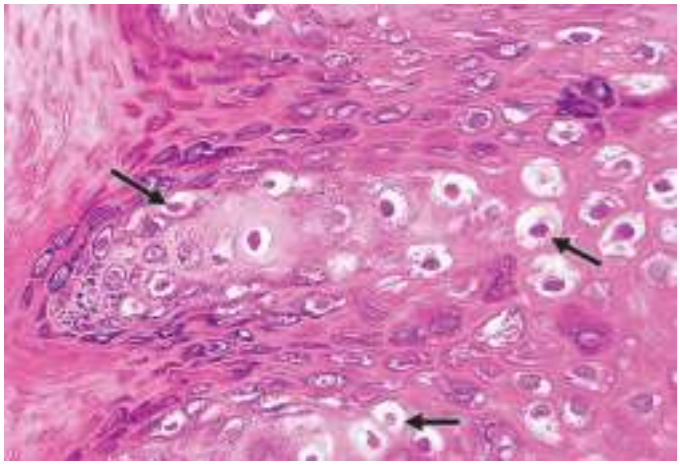


Fig. 23.51: Condyloma acuminatum. It shows vacuolization of epidermal cells (koilocytic change) in upper epithelium with wrinkled nuclei surrounded by clear halo (arrows) (400X).

Genital Molluscum Contagiosum

Genital molluscum contagiosum is a viral cutaneous infection in childhood that occurs worldwide and caused by a DNA poxvirus. Genital lesions are mainly transmitted by sexual contact.

- MCV-1 is transmitted by direct skin-skin contact or sharing infected clothes. MCV-2 is transmitted via sexual contact in adults. DNA poxvirus most often occurs in children and adolescents. Patient presents with umbilicated, dome-shaped papules over body, arms and legs.
- Average incubation period is 6 weeks. Umbilication of lesion occurs as a result of intracytoplasmic viral inclusions extruding infected cell on the surface with central pore.
- On histologic examination, epidermis grows deeper down into the dermis and form closely packed lobules. Epidermal cells contain large intracytoplasmic eosinophilic stained inclusion bodies known as molluscum bodies (**Henderson-Paterson bodies**), displace the nuclei at the periphery of epidermal cells (Fig. 23.52).
- In the center of the lesion, stratum corneum disintegrates and release the molluscum bodies together with keratinous debris resulting in formation of central crater.
- Dermis shows prominent acute and chronic inflammatory infiltrate and foreign body giant cells.

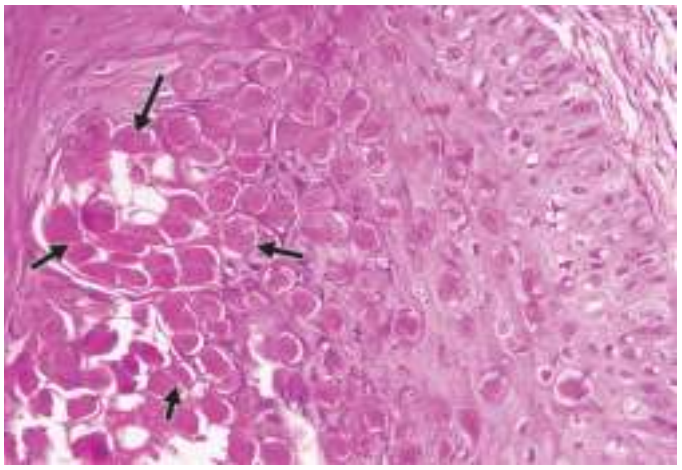


Fig. 23.52: Molluscum contagiosum. Epidermal cells contain large intracytoplasmic eosinophilic stained inclusion bodies known as molluscum bodies (arrows) (400X).

NEOPLASTIC DISORDERS OF PENIS

PENILE INTRAEPITHELIAL NEOPLASIA

Penile carcinomas occur in the form of carcinoma *in situ* or invasive squamous cell carcinoma, which mainly show a bimodal pathway of carcinogenesis: HPV related and HPV unrelated.

- Penile intraepithelial neoplasia refers to microscopically full-thickness atypia of the squamous epithelium. The term penile intraepithelial neoplasia (**PeIN**) is preferred over old term squamous intraepithelial lesion (SIL), erythroplasia of Queyrat, and Bowenoid disease.
- Both erythroplasia of Queyrat, and Bowenoid disease are synonymous with carcinoma *in situ*.
  - Erythroplasia of Queyrat is a disease of shaft to penis characterized by solitary or multiple, shiny, soft, erythematous plaques on the glans and foreskin.
  - Bowen disease is characterized by a sharply demarcated erythematous plaque or grayish plaque most often on the shaft of the penis or on the scrotum in younger persons, that may progress to invasive carcinoma. PeINs may be solitary or multiple.
- Gross appearance of penile intraepithelial neoplasia (PeIN) is heterogenous and does not allow one to distinguish between two types. Lesions vary from flat to slightly elevated, pearly white or moist erythematous, black to dark brown macules, papules, or plaques. The contours may be subtle or sharp and irregular.
- Histologic examination of PeIN shows thickened epithelium, usually associated with elongated and anastomosing rete ridges, subtle abnormal maturation (enlarged keratinocytes with abundant eosinophilic cytoplasm), whirling and keratin pearl formation usually in the deep rete ridges, prominent intercellular bridges and atypical basal cell with hyperchromatic nuclei. Parakeratosis is frequent finding.

PENILE INVASIVE SQUAMOUS CELL CARCINOMA

Penile invasive squamous cell carcinoma is an aggressive tumor of the skin of the glans or of the inner layer of the prepuce, which occurs in the sixth to seventh decades of life. It is characterized by invasive growth and early metastatic spread to lymph nodes. About 50% of tumors originate from nonkeratinized epithelium of the glans or inner foreskin of prepuce near coronal sulcus as a slow-growing, irregular mass. In high incidence areas, tumor can involve multiple anatomical compartments of penis in ≤50% of cases.

Predisposing Factors

Predisposing factors for penile squamous cell carcinoma include poor personal hygiene, phimosis, accumulation of smegma, chronic inflammation (balanoposthitis), lichen sclerosus, psoriasis treated with UVB radiation, penile rash >1 month, immunosuppression, radiation therapy, uncircumcised men, tobacco smoking, and

venereal disease. Disease is often associated with HPV infection serotypes 16, 18, 31, 33, 45, 56, and 65 in 66% of cases.

HPV Positive or Negative Penile Carcinoma

There are two different pathogenic pathways involved in the development of penile carcinomas. Approach to tumor classification of penile carcinoma is related to the presence or absence of human papillomavirus (HPV).

- Immunohistochemical detection of p16 is used as a surrogate parameter for HPV association. Most common serotypes are HPV 16, 18, 31, 33, 45, 56, and 65.
- Penile carcinomas related to presence of HPV infection include basaloid, warty and warty-basaloid carcinomas in 33% of cases.
- Penile carcinomas related to absence of HPV infection include verrucous, pseudohyperplastic and cuniculatum carcinomas. HPV is rarely present in papillary and sarcomatoid carcinomas. The second pathogenic pathway involved in the pathogenesis of penile carcinomas includes lichen sclerosis or chronic inflammation of the foreskin related to phimosis.
- Molecular techniques such as polymerase chain reaction (PCR) or immunohistochemistry are used to detect HPV.
- Because of these two different pathogenic pathways of penile carcinoma, the WHO classification distinguishes between HPV-associated and non-HPV-associated penile carcinomas in various histologic subtypes. Revised 2024 WHO classification of penile squamous cell carcinoma is given in Table 23.33.

Tumor Growth Patterns of Penile Carcinoma

Classification of penile squamous cell carcinoma according to growth pattern is related to prognosis.

Table 23.33 Revised 2024 WHO classification of tumors of the penis and scrotum

Benign and Precursor Squamous Lesions

- Condyloma accuminatum
- Squamous cell carcinoma precursors, HPV associated
- High-grade squamous intraepithelial lesion
- Squamous cell carcinoma precursors, HPV independent
- Differentiated penile intraepithelial neoplasia

Invasive Epithelial Tumors of the Penis and Scrotum

- **Invasive squamous epithelial tumors**
  - Squamous cell carcinoma, HPV associated
  - Squamous cell carcinoma, HPV independent
  - Squamous cell carcinoma, usual type
  - Squamous cell carcinoma, NOS
  - Basaloid squamous cell carcinoma
  - Warty basaloid carcinoma
  - Clear cell squamous cell carcinoma
  - Lymphoepithelial carcinoma
  - Verrucous carcinoma (including carcinoma cuniculatum)
  - Papillary squamous cell carcinoma
  - Sarcomatoid squamous cell carcinoma
- **Other epithelial tumors**
  - Adenosquamous carcinoma
  - Mucoepidermoid carcinoma
  - Paget disease, extramammary

Others

- Basal cell carcinoma
- Unclassified carcinoma

About 33% of penile carcinomas show mixed pattern of growth in clinically advanced stages. Various growth patterns of penile carcinoma include superficial spreading, vertical, verruciform, multicentric. Growth patterns of penile carcinoma are given in Table 23.34.

Table 23.34 Growth patterns of penile carcinoma

Pattern of Growth	Features
Superficial spreading growth pattern	▪ Minimally invasive tumor throughout epithelium involving foreskin ▪ Usual type SCC slow growing in superficial layers of the sulcus, and/or glans ▪ Prognosis better
Vertical growth pattern	▪ Deeply invasive carcinoma large fungating and/or ulcerative growth invading corpus spongiosum with frequent penetration of the tunica albuginea and corpora cavernosa ▪ Basaloid, sarcomatoid and high-grade usual squamous cell carcinoma ▪ Metastases in regional lymph nodes ▪ Poor prognosis
Verrucous growth pattern	▪ Histologic types: Verrucous, warty (condylomatous), papillary SCC ▪ Slow growing, large granular, exophytic growth on glans (most common site)
Multicentric growth pattern	▪ Presence of two or more independent foci of carcinoma separated by benign tissues ▪ Recurrence common unless all compartments are excised
Mixed and multicentric growth patterns	▪ Histologic types: Mixed hybrid/verrucous and common SCC ▪ Advanced stage penile carcinoma (30% cases)

Clinical Features

Penile squamous cell carcinoma with or without keratinization is the most common aggressive subtype tumor in 70–75% of cases by early metastatic potential. Other aggressive penile tumors include basaloid, sarcomatoid and warty subtypes in 10% of cases. In contrast, the verrucous and condylomatous subtypes only spread in exceptional cases and have a much better prognosis.

- Patient presents with fungating or ulcerative growth most often on the shaft of penis. Occasionally, patient presents with inguinal lymph node metastases with occult penile carcinoma due to severe phimosis or very small primary carcinoma. About 5% of patients have metastases at the time of diagnosis.
- Common sites of metastases are inguinal and pelvic lymph nodes, liver, lung, heart or bone. Local recurrence in one-third is due to insufficient surgery or positive margins, which also increases risk of regional inguinal and pelvic nodal metastases.
- Early suspicion of penile carcinoma, biopsy is necessary to prevent delays in treatment initiation.

Surgical Pathology: Penile Squamous Cell Carcinoma

Gross Morphology

Grossly examined growth patterns of penile carcinoma may have prognostic implications.

- **Superficial spreading penile carcinoma:** Tumor is limited to lamina propria or superficial corpus spongiosum and usually extend horizontally through multiple anatomical compartments.
- **Vertical growth penile carcinoma:** Tumor invades deep anatomical levels, surface is non-verruciform and frequently ulcerated.
- **Verruciform penile carcinoma:** Tumor is exophytic and papillomatous with a cauliflower-like aspect; and may be limited to surface (verrucous) or invade deep anatomical levels (cuniculatum). Invasive penile exophytic growth may involve glans and coronal sulcus completely obstructing urethra (Fig. 23.53).
- **Mixed patterns penile carcinoma:** Tumor is observed in 10–15% of all cases. In some cases, multicentric tumors (2 or more independent foci of carcinomas) are identified.

Light Microscopy

Usual penile squamous cell carcinoma is an infiltrative carcinoma that can be classified with three-grade system based on the level of keratinization, cellular pleomorphism and the presence of regular nests or sheets of tumor cells. Features to report include depth of invasion measured from deepest malignant cells to highest overlying dermal papilla; resection margin.

- **Well differentiated SCC (grade 1):** Tumor is composed of well differentiated squamous cells, almost undistinguishable from normal squamous cells except for the presence of minimal basal/parabasal cell atypia. Tumor cells are arranged in nests or sheets with central keratinization and keratin pearls. It is less common than grade 2 (Fig. 23.54).
- **Moderately differentiated SCC (grade 2):** All tumors are not fitting into criteria for grade 1 or 3. Tumor is composed of cells with high nuclear to cytoplasmic ratio, abundant cytoplasm and monomorphic nuclei and irregular outlines arranged in small nests with scant keratinization. This grade is more common along with transition to grade 3.
- **Poorly differentiated SCC (grade 3):** Tumor is composed of anaplastic cells arranged in nests, cords and solid sheets with focal areas containing spindle, trabecular and clear cells.



Fig. 23.53: Invasive penile carcinoma. There is exophytic growth involving glans and coronal sulcus completely obstructing urethra (arrow).

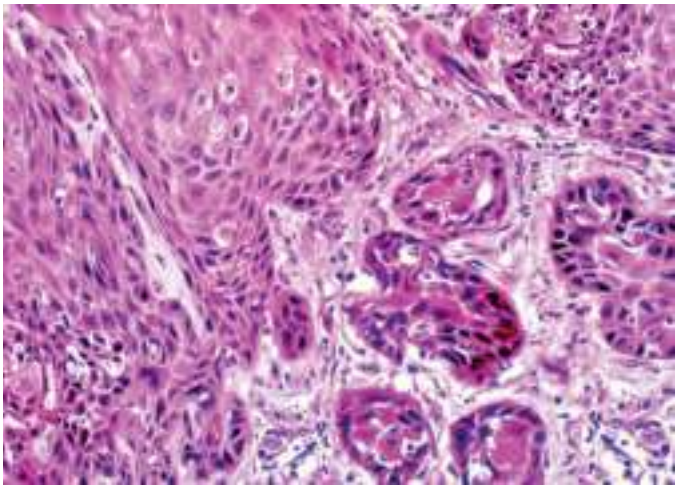


Fig. 23.54: Penile well differentiated squamous cell carcinoma. Tumor is composed of well differentiated squamous cells, almost undistinguishable from normal squamous cells except for the presence of minimal basal/parabasal cell atypia. Tumor cells are arranged in nests or sheets with central keratinization and keratin pearls (400X).

Molecular Genetic Alterations

HPV-DNA has been demonstrated in 40–45% of penile carcinomas. High risk HPV-16 is most frequent type associated with penile carcinoma, followed by HPV-18. Two viral oncoproteins, E6 and E7, appear crucial in the process of carcinogenesis.

- Oncoprotein E6 interferes with the p53 pathway leading to suppression of p53 normal inhibitory function of cell cycle. Oncoprotein E7 interferes with $p16^{INK4a}$/cyclin D/retinoblastoma pathway. Oncoproteins E6 and E7 work independently and together to cause enhanced immortalization of human cells.
- Particularly common genetic alterations are losses of alleles in the regions 9p21 and 17p, coding the tumor suppressor genes p16 and p53, respectively. Inactivation of p16 and p53 is also affected by the HPV oncogenes E6 and E7. P16 promoter hypermethylation and LOH as well as other changes of other tumor suppressor genes (KAI1, nm23H1) are associated with metastatic spread of penile carcinoma.

Clinical Course

Low-grade penile carcinoma with ≤6 mm superficial invasion has no regional lymph node metastases. Intermediate grade of penile carcinoma with 5–10 mm invasion has 15% risk of metastases. High-grade penile carcinoma with 8–10 mm deep invasion into corpus spongiosum, dartos or corpora cavernosa is associated with 80% rate of metastases.

Prognostic Factors of Penile Carcinoma

Poor prognostic factors of penile squamous cell carcinoma are based on depth of tumor invasion (anatomic levels—epithelium, lamina propria, corpus spongiosum, corpus cavernosum and glans penis), high-grade tumor, advanced stage of tumor, lymphovascular invasion, perineural invasion, histologic subtypes of tumor such as anaplastic, basaloid, pseudoglandular, sarcomatoid or solid; and lymph node density. Prognostic factors for squamous cell carcinoma of the penis are given in Table 23.35.

Table 23.35 Prognostic factors for squamous cell carcinoma of the penis

Prognostic Factors	Comments
Histologic subtypes	▪ Broad correlation with histologic subtypes and rates of regional or systemic dissemination ▪ Low risk: Verrucous, papillary, warty (condylomatous), pseudohyperplastic and cuniculatum carcinomas ▪ High-grade: Basaloid, sarcomatoid, adenosquamous, and solid poorly differentiated carcinomas associated with high-grade histology, deep invasion of corpora cavernosa, and frequent lymph node metastases
Lymph node status	▪ Positive lymph node in pelvis and mediastinum: Poor prognosis ▪ Negative lymph node: Better prognosis
Histologic grade	▪ Grade 1 well differentiated SCC: good prognosis ▪ Grade 2 moderately differentiated SCC: intermediate prognosis ▪ Grade 3 poorly differentiated SCC: poor prognosis
Depth of invasion and anatomical levels of invasion	Prognosis depends on depth of tumor and anatomical level of invasion in penis
Urethral invasion	Adverse prognostic factor
Perineural invasion	Independent prognostic factor associated with 5–10 mm depth of invasion
Lymphovascular invasion	Lymphovascular embolization associated with poor prognosis
Modality of tumor growth, front pattern of invasion	▪ High mortality associated with vertical growth pattern ▪ Verrucous tumors belong to low-risk group ▪ Usual SCC and pleomorphic variant belong to intermediate risk group ▪ High-grade, deeply invasive tumors such as basaloid, sarcomatoid and adenosquamous carcinomas and poorly differentiated carcinoma belong to high-risk group
Proliferative index	Ki-67 positive tumor cells in tumor and nodal metastases

Contd...

Table 23.35 Prognostic factors for squamous cell carcinoma of the penis (*Contd...*)

Prognostic Factors	Comments
Molecular factors	Accumulation of p53 protein independent predictive factor associated with poor outcome
Positive resection margins of tumor	Positive resection margins of tumor associated with poor prognosis
Staging status	▪ Risk of metastases to lymph nodes and distant organs increases in tumors infiltrating >5 mm in the glans, dermis in the skin ▪ Stage 1 is superficially invasive (better prognosis) ▪ Stage 2 invades the corpus spongiosum or cavernosum ▪ Stage 3 invades urethra or multiple positive lymph nodes (poor prognosis) ▪ Stage 4 invades pelvis or distant metastases (worst prognosis)

Mode of Spread

Penile squamous cell carcinoma can spread by local invasion, lymphatic route and hematogenous route.

- Superficial spreading tumors may spread from one mucosal compartment to the other; whereas arising from foreskin may spread to the skin of shaft, coronal sulcus, or glans; or tumor originating from glans may spread to the coronal sulcus and foreskin.
- Vertical spreading tumor involves from surface to deep regions in glans involving lamina propria, corpus spongiosum, albuginea and corpus cavernosum; the foreskin involving lamina propria, dartos and outer skin; and the sulcus involving lamina propria and fascia.
- Lymphatic or perineural invasion may be demonstrated at the margins.
- Penile squamous cell carcinoma may spread to the bilateral inguinal lymph nodes. The first site of metastases is in the '**sentinel lymph node**' demonstrated by preoperative lymphoscintigraphy. Systemic dissemination of penile squamous cell carcinoma may involve retroperitoneal lymph nodes, lungs, liver and bones via hemato genous route.

Management

Penile squamous cell carcinoma is treated by local resection or partial/total penectomy. Local excision and partial penectomy are inadequate for sarcomatoid and basaloid carcinomas (outcomes with metastatic disease).

There is higher risk for recurrence if node positive or partial penectomy. Tumor confined to glans may be treated by brachytherapy, that involves placing radioactive material inside body. Patients with prognostic index scores of 5–7 may benefit from prophylactic groin dissection.

CHAPTER

24

Female Reproductive System

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

- Female genital system: overview
  - Embryology of female genital system
  - Anatomy of female genital system
  - Fertilization and development of embryo
  - Pathologic disorders

DISORDERS OF VULVA

- Inflammatory disorders of vulva
  - Human papillomavirus (HPV) infection
  - Lymphogranuloma venereum
  - Granuloma inguinale
  - Genital herpes simplex virus 2 (HSV-2) infection
- Non-neoplastic disorders of vulva
  - Vulvar cysts
  - Bartholin cyst
  - Carbuncle
  - Toxic shock syndrome
  - Lichen sclerosus
- Neoplastic disorders of vulva
  - Papillary hidradenoma of vulva
  - Angiomyofibroblastoma of vulva
  - Leukoplakia of vulva
  - Bowen's disease of vulva
  - Squamous cell carcinoma of vulva
  - Paget's disease of vulva
  - Melanoma of vulva

DISORDERS OF VAGINA

- Inflammatory disorders of vagina
  - Bacterial vaginosis
  - *Candida albicans* infection
  - *Trichomonas vaginalis* infection
  - *Gardenerella vaginalis* infection
  - *Chlamydia trachomatis* infection
  - *Treponema pallidum* infection (syphilis)
  - *Haemophilus ducreyi* infection (chancroid)
  - Pelvic inflammatory disease (PID)
- Neoplastic disorders of vagina
  - Vaginal intraepithelial neoplasia
  - Squamous cell carcinoma of vagina
  - Verrucous carcinoma of vagina
  - Clear cell adenocarcinoma of vagina
  - Botryoid rhabdomyosarcoma of vagina
  - Leiomyosarcoma of vagina
  - Melanoma of vagina

DISORDERS OF UTERINE CERVIX

- Uterine cervix: overview
- Inflammatory disorders of uterine cervix
  - Acute cervicitis
  - Chronic cervicitis
  - Viral cervicitis
- Neoplastic disorders of uterine cervix
  - Endocervical polyp
  - Cervical intraepithelial neoplasia
  - Cervical carcinoma
  - Neuroendocrine tumors of cervix

DISORDERS OF UTERINE ENDOMETRIUM

- Menstrual cycle
- Menstrual cycle disorders
  - Amenorrhea
  - Dysmenorrhea
  - Dysfunctional uterine bleeding
- Inflammatory disorders of uterine endometrium
  - Acute endometritis
  - Chronic endometritis and infertility
- Non-neoplastic disorders of uterine endometrium
  - Endometrial hyperplasia
  - Arias-Stella reaction
  - Adenomyosis uteri
  - Endometriosis
- Neoplastic disorders of uterine endometrium
  - Endometrial polyp
  - Endometrial carcinoma
  - Endometrial stromal tumors
  - Mixed Müllerian tumor

DISORDERS OF UTERINE MYOMETRIUM

- Neoplastic disorders of uterine myometrium
  - Leiomyoma (fibroid/fibromyoma/myoma)
  - Smooth muscle tumor of uncertain malignant potential (STUMP)
  - Leiomyosarcoma

DISORDERS OF FALLOPIAN TUBES

- Fallopian tubes: anatomy
- Inflammatory disorders of fallopian tubes
  - Acute salpingitis
  - Chronic salpingitis
  - Salpingitis isthmica nodosa
- Non-neoplastic disorders of fallopian tubes
  - Tubal endometriosis
  - Tubal ectopic pregnancy
- Neoplastic disorders of fallopian tubes
  - Adenomatoid tumor of fallopian tubes
  - Adenocarcinoma of fallopian tubes

DISORDERS OF OVARIES

- Inflammatory disorders of ovaries
  - Acute oophoritis
  - Autoimmune oophoritis
  - Noninfectious granulomatous oophoritis
- Ovarian cysts
  - Follicular cyst
  - Corpus luteal cyst
  - Polycystic ovary syndrome (Stein-Leventhal syndrome)
  - Surface epithelial inclusion cysts
  - Paraovarian or paratubal cysts
  - Endometriosis (chocolate cysts)
  - Theca luteal cysts

NEOPLASTIC DISORDERS OF OVARY

- Krukenberg's tumor (metastatic tumor)
- Surface epithelial tumors of ovary
- Germ cell tumors of ovary
- Sex cord-stromal tumors of ovary
- Sertoli-Leydig cell tumors of ovary
- Laboratory diagnosis of ovarian tumors

GESTATIONAL TROPHOBLASTIC NEOPLASTIC DISORDERS AND RELATED TUMOR-LIKE LESIONS

- Gestational trophoblastic disease
- Hydatidiform mole
  - Complete hydatidiform mole
  - Partial hydatidiform mole
  - Invasive hydatidiform mole
- Gestational trophoblastic neoplastic disorders
  - Gestational choriocarcinoma
  - Placental site trophoblastic tumor
  - Epithelioid trophoblastic tumor

PREGNANCY ASSOCIATED NON-NEOPLASTIC DISORDERS

- Toxemia of pregnancy
- Ectopic pregnancy
- Thromboembolism
- Amniotic fluid embolism
- Gestational diabetes mellitus
- Sheehan's syndrome

FEMALE GENITAL SYSTEM: OVERVIEW

EMBRYOLOGY OF FEMALE GENITAL SYSTEM

A central principle of genital tract development in both sexes holds that the Müllerian tubes develop along female lines unless specifically impeded by embryonic testicular factors. In males, Sertoli cells in the developing testis produce Müllerian-inhibiting substance, a protein that causes the Müllerian ducts to regress. Müllerian ducts are the precursors of the fallopian ducts, uterus, and upper-third of the vagina. Formation of the ovary and vulva is not affected by Müllerian inhibitory protein.

ANATOMY OF FEMALE GENITAL SYSTEM

Organs of female genital system include ovaries, fallopian tubes, uterus (corpus cervix), vagina and external genitalia (labia majora, labia minora and clitoris).

- Functions of female genital system are conception, delivery of the baby, synthesis of estrogen and progesterone. Ovaries participate in ovulation.
- Fertilization takes place in fallopian tubes. Fertilized ovum is transported from fallopian tubes to uterine cavity. Implantation of fertilized ovum and placenta formation occurs within uterine cavity.
- Ovaries and placenta function as hormones producing organs to maintain the pregnancy.
- Mammary glands are integrated structurally and functionally to support the process of ovulation, fertilization, pregnancy, baby birth and childcare.
- Induction of labor and delivery of baby occur through vagina. Organs of female genital system are shown in Fig. 24.1.

Ovaries

Ovaries are located in pelvic cavity and attached to pelvic wall and uterus by ligaments. Each ovary measures 3 × 1.5 × 1.0 cm. Outer surface is lined by epithelium and covered by mesovarium, a layer of peritoneum.

- Cut section of ovary has cortex and medulla. Cortex contains large number of graafian follicles. A large number of primary graafian follicles degenerate from birth to puberty. Only 60,000–80,000 primary graafian follicles are left in each ovary. Tunica albuginea consists of dense connective tissue.
- Oogenesis occurs under the influence of endocrine hormones during embryonic development to form oocytes by mitosis, when the female fetus is 25 weeks old. Each ovary consists of two lacs graafian follicles. Total number of ova produced during reproductive period is 450. In each menstrual cycle, one ovum is released. Ovulation occurs between 14th day to 21st day of menstrual cycle. In an oocyte, equatorial division is completed only if fertilization occurs.

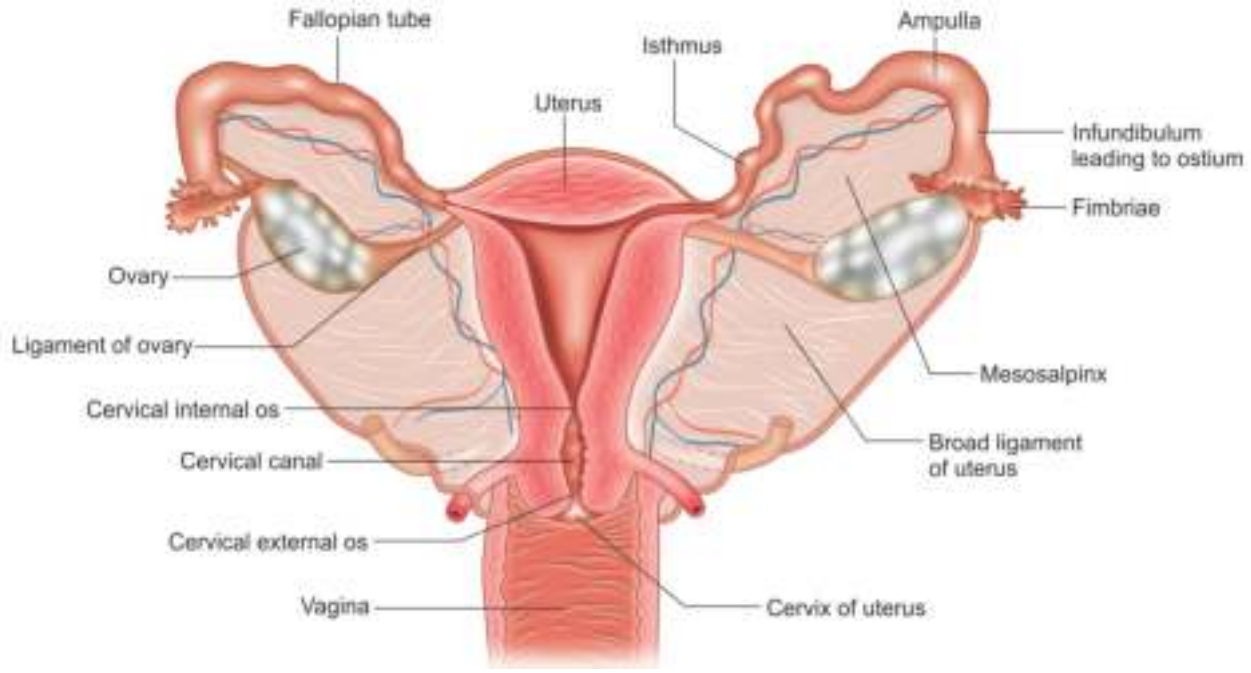


Fig. 24.1: Organs of female genital system. These include ovaries, fallopian tubes, uterus (corpus cervix), vagina and external genitalia (labia majora, labia minora and clitoris).

- After ovulation, corpus luteum secretes progesterone; which prepares the endometrium for receiving blastocyst. Corpus luteum inhibits the contraction of uterus and development of new graafian follicle. If fertilization does not take place, then rising level of progesterone inhibits the release of GnRH, which in turn inhibits production of FSH, LH and progesterone. As the progesterone level drops, the corpus luteum degenerates to become corpus albicans.
- Zona pellucida layer (glycoprotein in nature) secreted by follicular cells or granulosa cells surrounds ovum or oocyte.
- Corona radiata is formed by elongation of granulosa cells in the vicinity of the ovum and zona pellucida. Fluid accumulates in graafian follicle forming liquor follicle.
- Ovum or oocyte is attached with a pedicle called cumulus oophorus formed by granulosa cells. After liberation of the ovum from the graafian follicle, the remaining structure is called corpus luteum.
- Corpus luteum is a mass of granulosa cells and interstitial cells of ovary after ovulation, which is a temporary endocrine gland; which synthesizes progesterone in large quantity and oestrogens.
- Progesterone is essential for maintenance of endometrium. Such an endometrium is necessary for implantation of fertilized ovum and other events of pregnancy. In the absence of fertilization, the corpus luteum degenerates, which causes disintegration of endometrium leading to menstruation, marking a new menstrual cycle.
- Follicular atresia is regression and disappearance of graafian follicles due to phagocytosis, which occurs if ovum does not get fertilized. After follicular atresia, some thecal cells are formed from the stroma and located around the graafian follicle; these are called interstitial cells. The interstitial cells secrete small amount of estrogens.

Fallopian Tubes

Fallopian tubes are also called uterine tubes or oviducts. Each fallopian tube is 10–12 cm in length. Its wall is composed of mucosa, muscular layer and serous layer. Region of fallopian tube close to ovary is called infundibulum, which possesses finger-like projections called fimbriae, which collects ovum from the ovary after ovulation. Region of fallopian tube close to uterus is called isthmus. Wider region of fallopian tube is called ampulla lying between isthmus and infundibulum. Fallopian tube is lined by ciliated columnar epithelium as well as secretory cells. Secretory cells produce viscous liquid medium, which provides nutrition and protection to the ovum.

Uterus

Normal uterus is a pear-shaped hollow organ, which weighs 40–80 g in adult women. Uterus is divided into upper corpus and lower cervical segments. The uterine cavity is triangular measuring 6 cm in length. Uterus is composed of inner endometrial lining consisting of endometrial glands, middle muscular wall and outer serous coat. Serous coat extends to the peritoneal reflection. The peritoneal reflection is short in anterior region than in posterior region. Therefore, it may be used for orienting hysterectomy specimens.

- Uterine cervix has internal os and external os, that projects slightly into an elastic muscular tube, the vagina, which is a checkpoint of vaginal bacterial flora, which secretes mucin to control bacterial growth in vagina. Cervix prevents preterm labor by acting as stopper for conceptus before term.
- Tissue sampling of uterus includes endometrial biopsy and curettage specimens, products of conception, and hysterectomy specimens. The samples are processed and examined by light microscopy to establish diagnosis.
- Menstrual cycle has three phases: menstrual phase, proliferative phase and secretory phase.
  - Menstrual phase occurs for 3–4 days due to disintegration of uterine mucosa, which is sloughed off and discharged from uterus as menstrual bleeding.
  - Proliferative phase occurs from 5th day to 13th day of menstrual cycle. Estrogen levels are high in this period.
  - Secretory phase starts at 14th day and continues till 28th day of menstrual cycle. Progesterone levels are high.

Vagina

Vagina consists of mucosa, muscular layer and serous layer without glands, which is covered by hymen, a thin ring of tissue. Vagina is composed of labia majora, labia minora and accessory genital glands.

External Genital Organs

External genital organs include mons pubis and clitoris. Mons pubis is fatty tissue covered by skin and pubic hair. Clitoris is tiny finger-like structure lined by stratified squamous epithelium, which is analogous to the male's glans penis.

FERTILIZATION AND DEVELOPMENT OF EMBRYO

Fertilization can occur only if the ovum and spermatozoa are transported simultaneously to the ampullary-isthmic junction. The process of fusion of ovum and sperm is called fertilization.

- Ejaculation of semen in the vagina triggers an increase in motility by muscular contraction of uterus and

oviducts, and secondly by secretions liberated by secretory cells of epithelial lining of oviduct mucosa. This phenomenon is called 'capacitation'.

- Spermatozoa comes in contact with zona pellucida layer of ovum and induces changes in its membrane that blocks the entry of additional spermatozoa.
- Activated spermatozoa undergo acrosomal reaction and release various chemicals like substances synthesized by acrosome of spermatozoa such as hyaluronidase, corona penetrating enzyme and zona lysin or acrosin: (a) hyaluronidase that acts on the ground substances of follicle cells, (b) corona penetrating enzyme dissolves corona radiata, and (c) zona lysin or acrosin helps to digest the zona pellucida.
- Secretion of the acrosome helps spermatozoa entry into the cytoplasm of the ovum through zona pellucida and plasma membrane. This induces the completion of meiotic division of the secondary oocyte. About 3–4 ml of semen contains 80–100 million spermatozoa, out of which only one will succeed in entering ovum. Ovum becomes activated and then undergoes depolarization of its membrane.
- During fertilization, genetic material from a spermatozoa cell merges with that of an oocyte to form a single nucleus. Fertilization takes place in ampullary portion of fallopian tube. Spermatozoa pronucleus and ovum pronucleus fusion takes place to form zygote.
- Progesterone is a pregnancy hormone required continuously during entire period of pregnancy or gestation. Other hormones synthesized by placenta during pregnancy are human choriogonadotropin hormone, human placental hormone, estrogens, progesterone, cortisol, prolactin and thyroxine.
- During embryonic stage, holoblastic cleavage is a process in which zygote divides by mitotic division and the subsequent formation of daughter cells (blastomeres). Such cleavage is called 'holoblastic cleavage'.
  - Formation of blastomeres occurs by formation of 2 daughter cells → 4 daughter cells → 8 daughter cells → 16 daughter cells in morula stage. Morula stage is 16 celled stage of embryo. The embryo at the 16 celled stage is called the morula and named from mulberry fruit. Morula continues to divide and transforms into blastocyst.
  - Blastomeres in blastocyst are arranged into outer trophoblast (trophoectoderm cells) and inner cell mass.
  - Trophoblast layer gets attached to the endometrium; while inner cell mass develops into embryo. Peripheral cells become trophoblast or trophoectoderm cells.
- Trophoblast forms four extraembryonic membranes and part in formation of the placenta. Trophoblastic cells secrete fluid into the morula to form blastocoel and resulting in blastocyst, and inner cell mass developing into embryo proper.
- Umbilical cord connects fetus to the placenta, which consists of two umbilical arteries and one umbilical vein. Placenta consists of two parts: (a) chorion or fetal part invades uterine mucosa with chorionic villi bathed in blood, and (b) maternal part or decidua basalis.
- Placenta plays an important role in supporting gestation, which acts as a barrier of maternal immune response and fetus. Cell surface major histocompatibility complex (MHC) class II protein of placenta is involved in immune response. It is maternal–fetal interface for molecular exchanges such as gases, inorganic and organic substances, nutrients, hormones and antibodies. Placenta is physical protector of fetus. It participates in elimination of nitrogenous waste products. Placenta acts as endocrine gland by secreting human chorionic gonadotropin (hCG) hormone, estrogens, progesterone, and chorionic corticotropin. The hCG stimulates corpus luteum of pregnancy that continues to secrete progesterone until the end of the pregnancy.
- In the later phase of pregnancy, relaxin synthesized by placenta and ovary facilitates parturition by softening the connective tissue of pubic symphysis.
- Average gestation period in human is nine months. Parturition (labor) is induced by signals from 'fetal ejection reflex'. Powerful contraction of uterus occurs due to the action of oxytocin secreted by posterior pituitary gland. Opening of cervix results in rupture of amniotic fluid-watery fluid. After delivery, umbilical cord of the newborn baby is cut and tied to cease the blood. Infant breathes and lung expands.
- Ductus arteriosus (communication between arch of aorta and pulmonary artery) closes and becomes ligamentum arteriosum. Foramen ovale (communication between left and right atria) closes and becomes fossa ovalis. Normal blood circulation is established in newborn baby. Placenta is expelled after parturition.

PATHOLOGIC DISORDERS

Common reproductive health concerns for women are endometriosis, uterine leiomyomas, gynecologic cancers, HIV/AIDS, interstitial; cystitis, polycystic ovarian syndrome, sexually transmitted diseases (STDs), tubal pregnancy and molar pregnancy. Pathologic disorders of female genital tract are shown in Fig. 24.2.

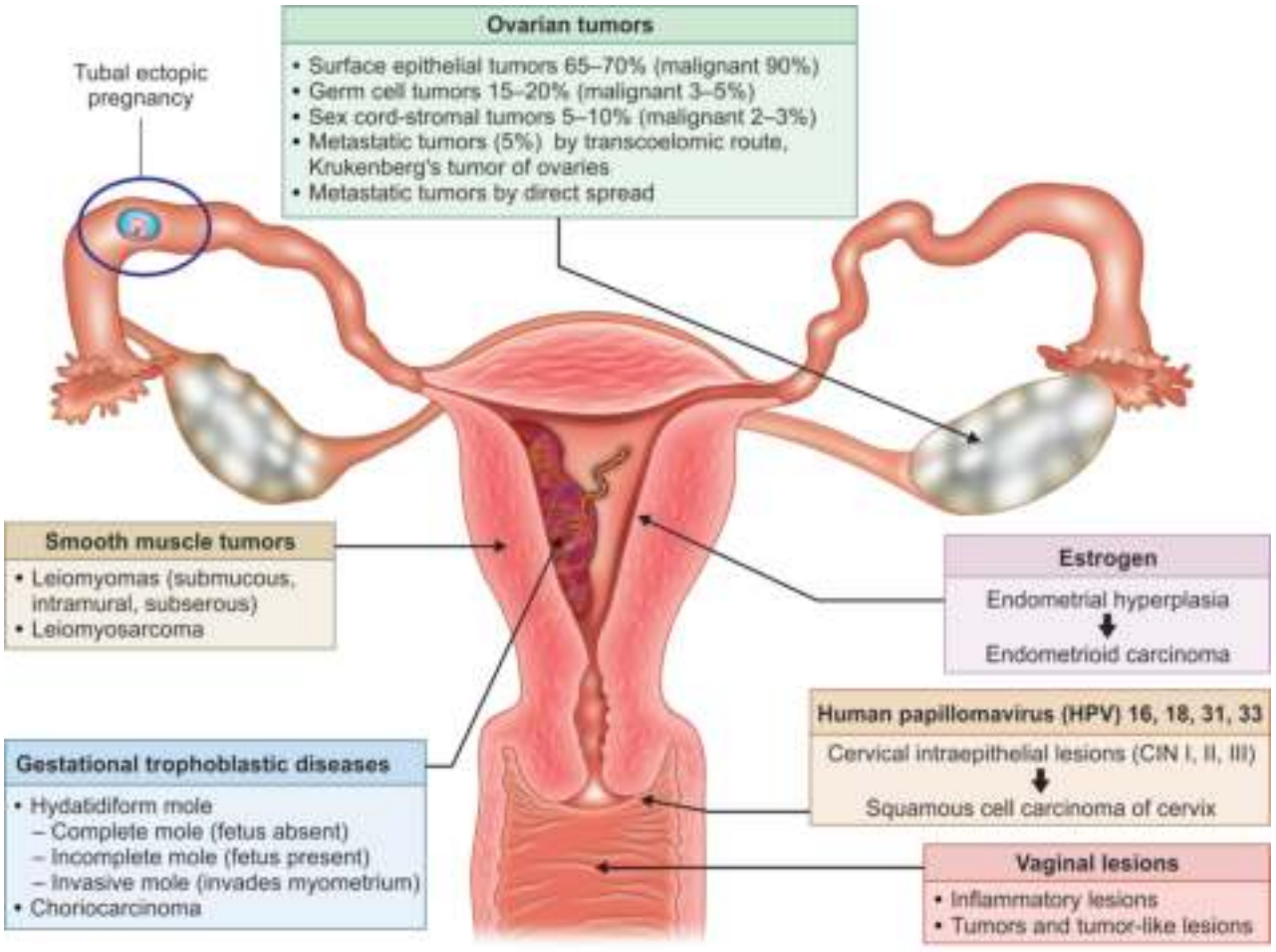


Fig. 24.2: Pathologic disorders of female genital system.

DISORDERS OF VULVA

INFLAMMATORY DISORDERS OF VULVA

Inflammation of vulva most often occurs in post-menopausal women, which is related to thin atrophy of the skin, which has very thin epithelial covering at this phase of life and is easily abraded. Inflammation during reproductive period of life frequently involves Bartholin's gland. The duct of Bartholin's glands may become blocked to form cyst, which may become infected to form Bartholin's abscess. The vulva is most often involved by infections, especially those that flourish in moist areas (e.g. *Candida albicans*), or involve hair follicles to form boils by Staphylococcus. Skin of the vulva may be involved with any other skin disease such as psoriasis or eczema.

HUMAN PAPILLOMAVIRUS (HPV) INFECTION

More than 95% of cervical carcinoma is due to the high-risk human papillomavirus (HPV) strains 16, 18, 31, 33, 45, 52 and 58. Low risk HPV strains (6 and 11) cause about 90% of genital warts on vagina and cervix, which rarely develop into cancers.

- Genital wart, also known as condyloma acuminatum, is a tumor-like exophytic, papillomatous lesion on the skin or mucous membranes of the lower female genital tract (perineum, vulva and vagina). The median incubation period from infection to first detection of HPV is 3 months. HPV types 6 and 11 are detected in over 80% of macroscopically visible condylomata.
- Transmission of low-risk HPV 6 and 11 occurs direct contact between individuals, or autoinoculation.
- Some risk factors such as young age, early age (≤15 years) at first sexual intercourse, sexual promiscuity and immunosuppression have been consistently associated with HPV infection in women. The risk of HPV (16, 18) infection increases with increasing number of recent and lifetime sexual partners, which

may contribute to the pathogenesis of squamous cell carcinoma of vulva and vagina.
- Generally, genital warts are self-limited lesions, often undergo regression spontaneously within six months to two years or even more. Untreated cases due to HPV (16, 18) infections develop cervical carcinoma in a span of 10–30 years.

Clinical Features

Most precursor and premalignant HPV infections are asymptomatic. Infections with low-risk HPV may cause condylomas and genital warts commonly seen as multiple (or single) white, flat or pedunculated, keratotic lesions that may cause pruritus or pain.

Surgical Pathology: HPV-related Epithelial Changes in Vulva

Light Microscopy

- Histologically, the changes involve only the lower 1/3rd of the epithelium due to HPV infection. Koilocytes (intracytoplasmic vacuolation caused by HPV 6 and 11 infection) are superficial or intermediate squamous cells with large and irregular, well-defined perinuclear halos with a cookie cutter border and cytoplasmic thickening. Nuclei of epithelial cells are enlarged (2–3 times of normal).
- Infected cells may demonstrate two or multiple nuclei, which are also apparent in cytopathological Papanicolaou smear. The virus particles can be demonstrated in nucleus by electron microscopy and immunohistochemistry (Fig. 24.3).

LYMPHOGRANULOMA VENEREUM

Lymphogranuloma venereum (LGV) is an ulcerative disease of the genital region due to infection by gram-negative *Chlamydia trachomatis* (L1, L2, or L3 serotypes) transmitted by sexual contact, which primarily affects the lymphatic system. Binding of *Chlamydia trachomatis* occurs via heparin sulfate receptors.

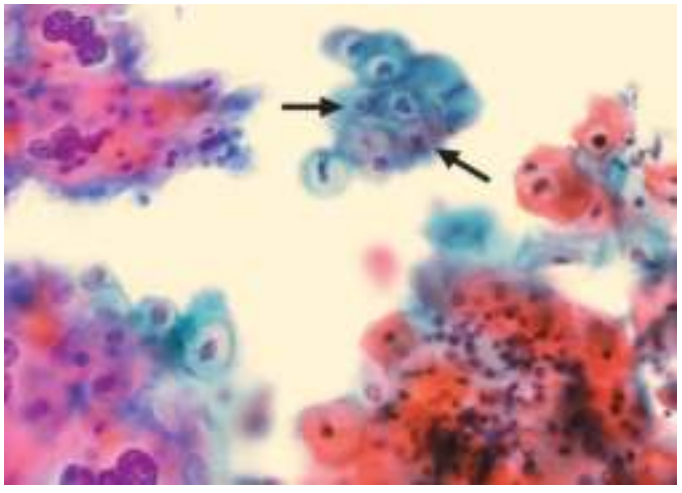


Fig. 24.3: Human papillomavirus (HPV) infection. Pap smear shows koilocytosis, the typical appearance of cervical squamous cell infected by HPV. There is a halo of cleared cytoplasm surrounding an enlarged nucleus (arrows) (400X).

Clinical Features

During clinical course, lymphogranuloma venereum disease has three stages after a month of infection: (a) primary stage is characterized by development of painless genital ulcer or papule at the site of inoculation, (b) secondary stage is characterized by unilateral or bilateral tender inguinal and/or femoral and pelvic lymphadenopathy, also called bubos resulting in chronic suppurative fistulas in the pelvic viscera, and (c) late stage is characterized by formation of rectal strictures, fibrosis due to lymphatic obstruction and fistulae in the anogenital region.

Late sequelae usually occur in untreated cases, which include necrosis and rupture of lymph nodes, fibrosis and stricture in the anogenital; and elephantiasis of the genital organs. The recommended treatment regimen is doxycycline 100 mg orally twice a day given for three weeks.

GRANULOMA INGUINALE

Granuloma inguinale is a bacterial disease caused by *Klebsiella granulomatis*. It was formerly known as *Calymmatobacterium granulomatis*. Disease begins as a papule after about 40 days of infection on the vagina, perineum or vagina, which can spread widely, resulting in extensive ulceration and destruction of tissue. It is endemic in many less-developed regions. The diagnosis of granuloma inguinale is based on the patient's sexual history and clinical examination revealing a painless, 'beefy-red ulcer' with rolled edge of granulation tissue.

Surgical Pathology: Granuloma Inguinale

Light Microscopy

- Histologic examination reveals granulomatous lesion.
- Donovan bodies represent macrophages stuffed with numerous *Klebsiella granulomatis*.

GENITAL HERPES SIMPLEX VIRUS 2 (HSV-2) INFECTION

Herpes simplex virus 2 (HSV-2) is a DNA virus, which is transmitted through intimate genital tract contact. Persistent HSV-2 infection may cause frequent, symptomatic self-limited small vesicles and shallow genital ulcers, that can involve the cervix, vagina, clitoris, vulva, urethra, and perianal skin. In most cases, HSV-2 infection is subclinical. Recent studies have demonstrated that HSV-2 is frequently shed from genital surfaces even in the absence of signs or symptoms of

clinical disease and that the HSV-2 can be transmitted during these periods of shedding, which contributes to increased risk for HIV acquisition.

Clinical Features

Patient presents with vesicles on vulva 3–7 days after sexual intercourse in 30% of women. Genital ulcers may persist for 1–3 weeks but heal spontaneously without scarring. HSV-2 migrates along the nerves to the lumbar ganglia, and remains dormant. HSV-2 can be activated, that may descend to the vulva to produce recurrent vesicles.

Surgical Pathology: Genital Herpes Simplex Virus 2 Infection
Light Microscopy
Multinucleated giant cells with viral inclusions, nuclear molding, and margination of the chromatin are demonstrated in cytological smears from lesions.

NON-NEOPLASTIC DISORDERS OF VULVA

VULVAR CYSTS

Epidermoid cysts are the most common vulvar cysts, which usually develop as a result of invagination of superficial squamous epithelium within epidermis. Vulvar cysts are usually located in the anterior half of the labia majora. Hymenal cysts are common in the newborns. Type of vulvar cysts is given in Table 24.1.

BARTHOLIN CYST

The Bartholin glands are located in the lower right and left portions at the 4 o'clock and 8 o'clock positions of the vaginal introitus, which produce a clear mucoid secretion that continuously lubricates the vestibular surface.

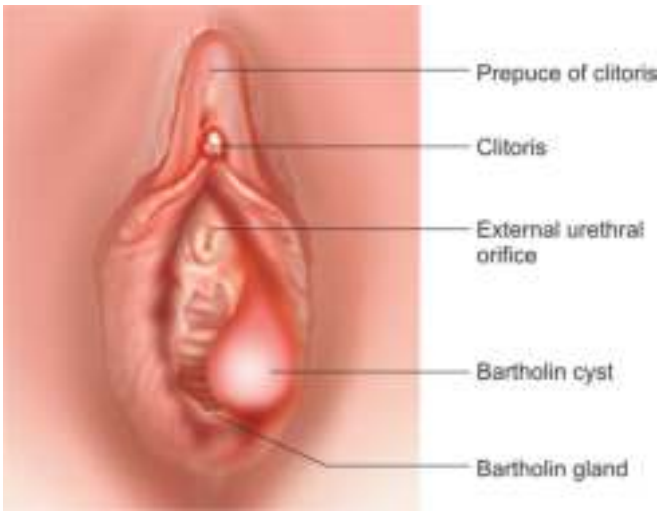


Fig. 24.4: Bartholin cyst. Obstruction of Bartholin ducts results in Bartholin cyst. The cyst usually measures 2–4 cm in diameter, that may cause dyspareunia, urinary irritation, and vague pelvic pain.

- Obstruction of Bartholin ducts results in Bartholin cyst. It may be secondarily infected by *Neisseria gonorrhoeae* or Staphylococci, or *Chlamydia trachomatis* resulting in Bartholin gland abscess. It is treated with incision and drainage.
- Bartholin cyst and abscess are often found after the onset of puberty and a decrease in incidence after menopause. The Bartholin cyst usually measures 2–4 cm in diameter, that may cause dyspareunia, urinary irritation, and vague pelvic pain.
- Bartholin cyst is usually filled with nonpurulent fluid that contains Staphylococcus, Streptococcus, and *Escherichia coli*. Asymptomatic Bartholin cyst does not require further treatment.
- Bartholin cyst or abscess is managed conservatively with sitz bath and analgesics. Bartholin cyst is shown in Fig. 24.4.

Table 24.1 Type of vulvar cysts

Type	Origin	Lining Epithelium
Epidermoid cyst	Invagination of superficial squamous epithelium within epidermis	Squamous epithelium
Bartholin duct cyst	Bartholin duct	Transitional epithelium
Pilonidal cyst	Hair follicle	Squamous epithelium
Hymenal cyst	Hymen	Squamous epithelium
Skene duct cyst	Skene duct	Transitional epithelium
Cyst of canal of Nuck	Processus vaginalis	Mesothelium
Mucinous duct cyst	Minor vestibular glands	Mucus secreting epithelium
Gartner duct cyst	Mesonephric/wolffian	Non-mucus secreting epithelium
Fox-Fordyce disease	Apocrine gland	Hair follicle wall
Endometriotic cyst	Endometriotic foci	Endometrium

CARBUNCLE

Carbuncle is an exophytic or often ulcerated, polypoid mass of 1–2 cm in diameter present near the female urethral meatus resulting in pain and bleeding. Carbuncle most often affects perimenopausal women. Histologic examination of carbuncle reveals acute and chronic inflammatory granulation tissue and hyperplasia of lining epithelium.

TOXIC SHOCK SYNDROME

Toxic shock syndrome occurs due to production of superantigens by certain strains of invasive *Staphylococcus aureus* and Streptococci infection. Patient presents with fever, shock, desquamative erythematous rash, vomiting, diarrhea, myalgias, neurologic signs and decreased platelets count. Patient may develop disseminated intravascular coagulation (DIC).

LICHEN SCLEROSUS

Lichen sclerosus is an inflammatory disease of the vulva most often associated with autoimmune disorders such as vitiligo, pernicious anemia, and Hashimoto's thyroiditis. Patient presents with dry, scaly, itchy (pruritus) white plaques and atrophic skin with a parchment paper-like or wrinkled appearance resulting in marked contracture of the vulvar tissues (dyspareunia). TP53 gene mutation has been demonstrated in some cases of Lichen sclerosus.

Surgical Pathology: Lichen Sclerosus

Light Microscopy

- Skin lesion from lichen sclerosus shows hyperkeratosis, epithelial thinning with flattening of the rete pegs and homogeneous, a cellular zone in the upper dermis.
- A band of chronic inflammatory cells typically lies beneath this layer.
- Minority of patients may develop squamous cell carcinoma in 15% of cases.

NEOPLASTIC DISORDERS OF VULVA

PAPILLARY HIDRADENOMA OF VULVA

Papillary hidradenoma is the most common well circumscribed benign tumor of the vulva, which is sometimes clinically mistaken for a malignant tumor because of its tendency to undergo ulceration. Tumor occurs in labia majora and labia minora, which arises from anogenital ectopic breast tissue or apocrine sweat glands. Patient presents with labial nodule that may ulcerate and bleed.

Surgical Pathology: Papillary Hidradenoma of Vulva

Light Microscopy

- Papillary hidradenoma tumor is composed of cells arranged in complex glandular and papillary pattern with scant stroma.
- Luminal layer is lined by cuboidal epithelium with apocrine differentiation, and basal layer of myoepithelial cells.
- Surgical excision cures the patient.

ANGIOMYOFIBROBLASTOMA OF VULVA

Angiomyofibroblastoma is a well circumscribed mesenchymal tumor derived from subepithelial myxoid stromal zone of vulva, vagina or perineal in middle-aged women.

Surgical Pathology: Angiomyofibroblastoma of Vulva

Gross Morphology

- Angiomyofibroblastoma is well circumscribed mass measuring 1–15 cm.
- Cut surface is homogeneous, pink, gray without hemorrhage or necrosis.

Light Microscopy

- Angiomyofibroblastoma is composed of spindle cells or oval cells with minimal myxoid stroma.
- The tumor cells are arranged in whirling pattern around blood vessels.

Immunohistochemistry Technique

Immunohistochemistry of tumor cells of angiomyofibroblastoma shows positivity with desmin and hormone receptors (ER/PR), CD34 expression is demonstrated in 50% of cases.

Markers	Expression
Desmin	Positive
Hormone receptors (ER/PR)	Positive
CD34	Positive (50%)

LEUKOPLAKIA OF VULVA

Leukoplakia is a firmly attached white plaque-like thickening on a mucous membrane of vulva noticed on naked eye examination. The edges of the lesion undergoes abrupt changes with time. Histologic examination shows increased keratin build up with or without abnormal cells, but is not diagnostic. Leukoplakia is associated with an increased risk for carcinoma.

BOWEN'S DISEASE OF VULVA

Bowen's disease is a multicentric lesion in vulva and also known as vulvar carcinoma *in situ*, which has the potential of progression to invasive carcinoma. Bowen's disease appears as a slow-growing flat or slightly raised,

persistent reddish-brown dry scaly patch or plaque on vulva. The vulvar lesions are normally not associated with any symptoms, but occasionally can itch, ooze pus, bleed or become crusted and/or tender.

SQUAMOUS CELL CARCINOMA OF VULVA

Squamous cell carcinoma of vulva is the most common malignant tumor of the vulva. Cytokeratins are expressed in neoplastic cells. It constitutes 85% cancers of vulva. It is the end result of a multistep process that has its origin as vulvar intraepithelial neoplasia.

- Human papillomavirus (HPV) 16, 18, 31, or 33 (HPV 16 most common) commonly infects vulva in 85% of elderly women.
- Patient develops HPV-induced premalignant vaginal intraepithelial neoplasia (grades 1, 2 and 3 lesions) resulting in vaginal invasive squamous cell carcinoma, which may also develop in association with dysplastic precursor known as 'lichen sclerosus'. Clinical photograph of squamous cell carcinoma of vulva is shown in Fig. 24.5.

Mode of Spread

Squamous cell carcinoma of vulva spreads slowly and invades contiguous skin, vagina, and rectum. Tumor metastasizes by lymphatic route to the superficial inguinal lymph nodes and then involving the deep inguinal, femoral, and pelvic lymph nodes. Tumor metastasizes to lung, and liver via hematogenous route. The frequency of metastases is related to the size and local extension of tumor.



Fig. 24.5: Clinical photograph of squamous cell carcinoma of vulva. Fungating growth with ulceration is visible.

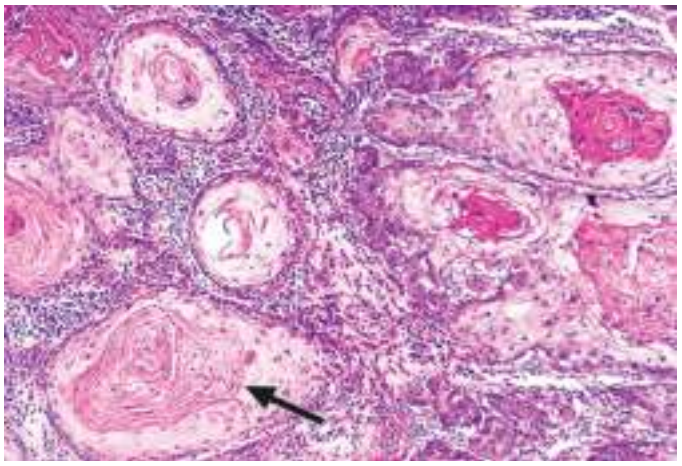


Fig. 24.6: Squamous cell carcinoma of vulva. At high magnification, nests of neoplastic squamous cells are invaded through a chronically inflamed stroma. This cancer is well-differentiated, as evidenced by keratin pearls (400X).

Surgical Pathology: Squamous Cell Carcinoma of Vulva
Gross Morphology
Growth is most often exophytic (66%) and may be ulcerative and endophytic.
Light Microscopy
▪ Tumor shows well-differentiated squamous cell carcinoma with keratin pearls in majority of cases. ▪ Some may be poorly differentiated (basaloid) carcinoma (Fig. 24.6).

PAGET'S DISEASE OF VULVA

Paget's disease of vulva is similar to Paget's disease of the breast. It is primarily an intradermal adenocarcinoma of vulva derived from multipotent cells in the epidermis, adnexa or anogenital glands, which usually occurs on the labia majora in elderly women. Diagnosis of Paget's disease of vulva must be confirmed on histologic examination. Underlying adenocarcinoma should be ruled out by complete pelvic examination, colposcopy and radiologic examination. Clinical photograph of Paget's disease of vulva is shown in Fig. 24.7.

Clinical Features

Patient presents with pruritic, eczematous raised rash in the vulvar perianal, or perineal areas, which may persist for decades and often recurs following surgical excision.

Surgical Pathology: Paget's Disease of Vulva
Gross Morphology
Paget's disease of vulva has sharply demarcated plaque.

Light Microscopy

- Intraepidermal single or clusters of large tumor cells characterized by abundant eosinophilic, clear or amphophilic cytoplasm containing glycosaminoglycans (mucin) surrounded by a clear, halo-like area of vacuolation, round/ovoid vesicular nuclei, open chromatin and small nucleoli.
- The intraepithelial cells are known as Paget's cells are arranged in nests at the epidermal–dermal interface and within the epidermis (pagetoid spread).
- Basal layer is preserved but may be attenuated. Clefting between tumor cells and the surrounding epidermis may be present, due to absence of desmosomes (Fig. 24.8).

Histochemistry

Mucopolysaccharides present in the tumor cells are stained with PAS, mucicarmine and Alcian blue.

Immunohistochemistry Technique

The tumor expresses carcinoembryonic antigen (CEA), epithelial membrane antigen and CK7. But CK20 is negative in tumor cells.

Markers	Expression
CEA	Positive
EMA	Positive
CK7	Positive
CK20	Negative

Prognostic Factors

Most important prognostic indicators of Paget's disease of vulva include clinical detection of a nodule within the primary lesion, Paget's disease of vulva, palpable lymphadenopathy, the level of tumor invasion and lymph node metastases. Histologic features of Paget's disease of vulva are associated with tumor progression and invasion include increased expression of p53, cyclin D1 and Ki-67; and decreased expression of E-cadherin. Patient with clitoris involvement may need more aggressive treatment.



Fig. 24.7: Paget's disease of vulva. Eczematous raised rash is seen in the vulvar region.

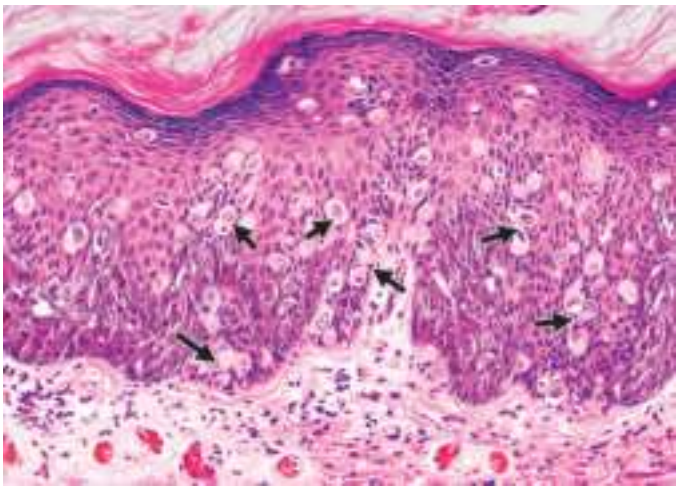


Fig. 24.8: Paget's disease of vulva. The tumor cells in the overlying epidermis are called Paget's cells. These are large cells with abundant clear cytoplasm and large nuclei with prominent nucleoli, arranged singly or in clusters; represent intraepithelial extension of an underlying carcinoma *in situ* or invasive ductal carcinoma (arrows) (400X).

Treatment

Paget's disease of vulva is treated by Mohs' micrographic surgery or wide local excision, topical imiquimod, radiotherapy, photodynamic therapy or CO_2 laser ablation.

MELANOMA OF VULVA

Melanoma of vulva is a rare lesion affecting elderly women (60–70 years), which accounts for approximately 10% of malignant tumors of the vulva. Labia (minora and majora) are the most commonly affected sites, followed by clitoris in some studies. Melanoma of vulva may be discovered during routine gynecologic examinations. Patient usually presents with spreading pigmented patch or multiple lesions (satellitosis) or sometimes nonpigmented lesions (macules, patches, nodules). Spreading pigmented patch resembles extramammary Paget's disease of vulva.

Imaging Techniques

Computed tomography (CT) and magnetic resonance imaging (MRI) and scans are done for staging of disease. Lymphoscintigraphy is performed for identification of sentinel nodes.

Surgical Pathology: Melanoma of Vulva

Gross Morphology

Preservation of specimen orientation is critical for thorough evaluation of peripheral and deep tissue margins. Lesion can be pigmented patch or multiple lesions (satellitosis).

Light Microscopy

- Melanoma of vulva is evaluated and staged similar to cutaneous melanoma involving the cutaneous surface (superficial spreading being the most common type at this site). Melanoma involving mucosal surface is classified as lentiginous type.
- Tumor is composed of melanoma cells larger than nevus cells. These cells contain large nuclei with irregular contours with eosinophilic nuclei. Chromatin is clumped at the periphery of the nuclear membrane (Fig. 24.9).

Immunohistochemistry Technique

Tumor shows positivity for S-100 protein, MART-1 (melan-A), Ki-67, CAM 5.2 and HMB-45.

Markers	Expression
Melanin antigen recognized by T cells (MART1) (melan-A)	Positive
HMB-45 (human melanoma black 45)	Positive (epithelioid melanoma)
S-100 protein (calcium binding protein)	Positive (cytoplasmic or nuclear)
Ki-67	Positive
CAM 5.2	Positive
Tyrosinase	Positive
SOX-10	Positive
MITF (microphthalmia-associated transcription factor)	Positive
SOX-100	Positive
PNL2	Positive
MUM1	Positive

Molecular Genetic Alterations

KIT mutations and amplifications appear to be more commonly demonstrated in vulvar melanomas. N-RAS mutation is also present. BRAF V600E mutations are rarely present. Vulvar melanoma may have copy number alterations in 1q and 6p.

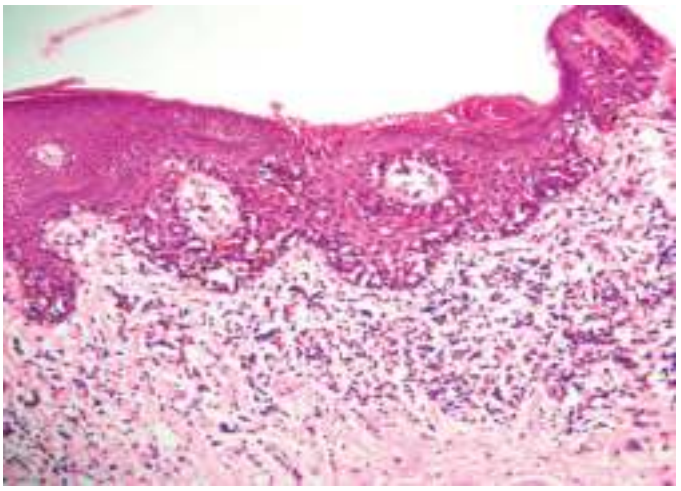


Fig. 24.9: Malignant melanoma. Tumor is composed of melanoma cells (400X).

Prognostic Factors

Histologic parameters of melanoma of vulva include Breslow depth (tumor thickness) and feasibility of complete resection with appropriate margins, mitotic rate, ulceration and margin status and stage. Free margin of ≥1.0 mm is associated with better prognosis. Patient with localized disease (stages 0, I, II) is associated with better prognosis. Presence of higher number of positive lymph nodes is associated with worse prognosis. Serum lactate dehydrogenase levels >200–225 U/L are associated with poor survival.

Treatment

Melanoma of vulva is surgical resection with adequate margins is the principal management, radiation, chemotherapy and topical imiquimod therapy.

DISORDERS OF VAGINA

INFLAMMATORY DISORDERS OF VAGINA

Vaginal discharge is a common complaint in multiparous women. Physiologic vaginal discharge is scanty, mucoid and colorless. Pathologic vaginal discharge occurs due to *Trichomonas vaginalis* or *Candida albicans* or bacterial vaginosis. Primary mode of transmission of sexually transmitted infections is through vaginal or anal sexual contact or vertical transmission from mother to fetus. There are however a number of inflammatory conditions which arise primarily in vagina.

BACTERIAL VAGINOSIS

Bacterial vaginosis is a common vaginal infection caused by an overgrowth of bacteria, which involves an imbalance in the hormonal and bacterial flora of the vagina. The *Lactobacillus* species dominates normal vaginal microbiota. Bacterial vaginosis is associated with a decline in the overall number of Lactobacillus, resulting in vaginal discharge, irritation and increased risk of transmission of sexually transmitted diseases and pelvic inflammatory diseases and complicated

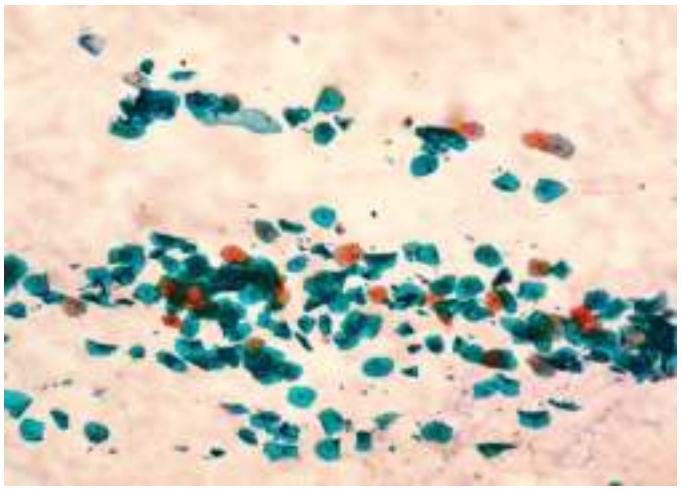


Fig. 24.10: Pap smear from normal women. Normal Pap smear shows superficial and intermediate squamous epithelial cells (400X).

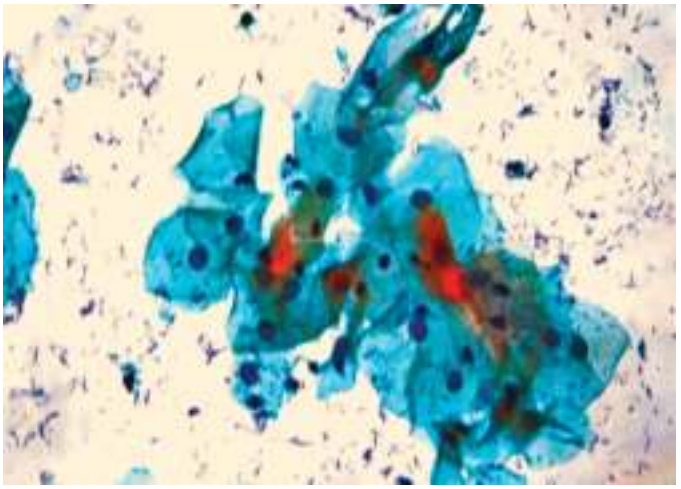


Fig. 24.11: Pap smear in bacterial vaginosis. Pap smear demonstrates clue cells (400X).

pregnancy. The hallmark of diagnosis of bacterial vaginosis relies on examination of vaginal fluid on a wet mount under light microscope. Bacterial vaginosis is characterized by the presence of clue cells, which are epithelial cells of the cervix that are embedded with rod-shaped bacteria (Figs 24.10 and 24.11).

CANDIDA ALBICANS INFECTION

Candida albicans causes vulvovaginitis affecting 10% of women. *Candida albicans* is a normal vaginal flora. Infection is commonly associated with pregnancy, diabetes mellitus, broad-spectrum antibiotic therapy, oral contraceptive use, and immunosuppression resulting in fungal growth. Patient presents with white curd-like vaginal discharge that may cause intense itching. Per vaginal examination shows white patches on the mucosal surface of vagina covered with vaginal discharge. Diagnosis is best made microscopically on wet mounts or Papanicolaou smear showing fungal pseudohyphae (Fig. 24.12).

TRICHOMONAS VAGINALIS INFECTION

Trichomonas vaginalis is the second most common cause of vaginitis. It is commonly transmitted by sexual contact. Patient presents with a profuse opaque or creamy colored frothy discharge with fishy smell. Vaginal discharge may cause vulvar irritation and burning micturition due to urethral inflammation. *Trichomonas vaginalis* is best demonstrated in freshly prepared unfixed wet smears from vaginal discharge, in which the flagellate protozoa keep moving (Fig. 24.13).

GARDNERELLA VAGINALIS INFECTION

Gardnerella vaginalis is a common cause of vaginal discharge, which grows on vagina due to loss of the normal vaginal lactobacilli. Patient presents with thin

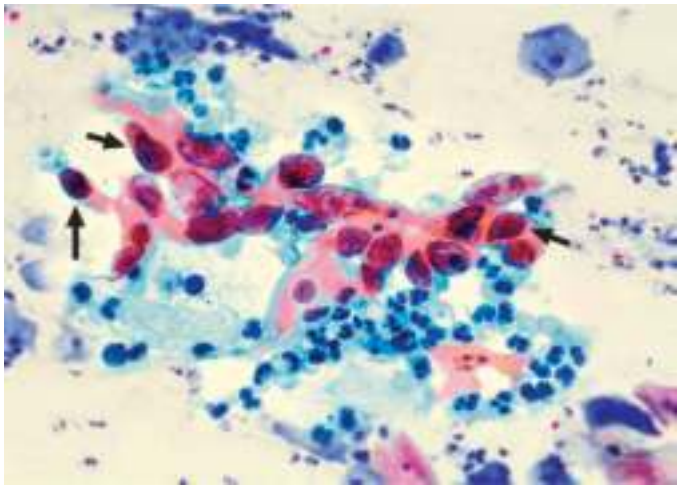


Fig. 24.12: *Candida albicans*. Pap smear demonstrates *Candida albicans* (arrows) (400X).

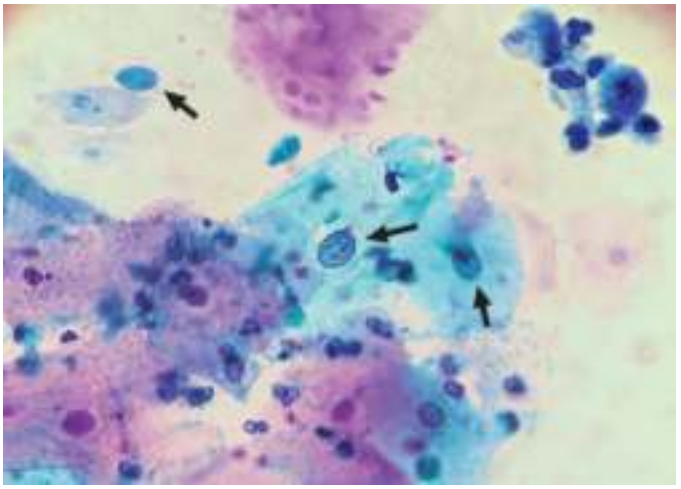


Fig. 24.13: *Trichomonas vaginalis*. Pap smear demonstrates *Trichomonas vaginalis* (arrows) (400X).

homogeneous vaginal discharge with a malodorous, fishy amine odor, especially on addition of 10% potassium hydroxide. *Gardnerella vaginalis* is best recognized on Papanicolau smear due to presence of clue cells (i.e. squamous cells covered with clumped nuclei, folded cytoplasm, and numerous bacteria attached to their surface).

CHLAMYDIA TRACHOMATIS INFECTION

Chlamydia trachomatis (serotypes D–K) is transmitted by sexual contact, which infects squamocolumnar or transitional epithelial cells leading to cervicitis and pelvic inflammatory disease (PID) in women and urethritis in men. *Chlamydia trachomatis* manipulates an array of host processes to support their obligate intracellular developmental cycle, which leads to activation of signaling pathways resulting in disproportionate influx of innate immune cells and the release of tissue damaging proteins and proinflammatory cytokines.

- *Chlamydia trachomatis*-induced pelvic inflammatory disease results in reproductive sequelae such as ectopic pregnancy, infertility and chronic pelvic pain. The disease is most often asymptomatic.
- Patient may present with vaginal discharge, vesicles and ulcers on vulva, pelvic pain or pelvic mass, and discomfort during intercourse.
- Patient is treated with azithromycin (1 g, single dose) or doxycycline (100 mg twice daily × 7 days).

TREPONEMA PALLIDUM INFECTION (SYPHILIS)

Treponema pallidum is a spirochete and the etiologic agent of syphilis. It is transmitted by sexual contact. Patient presents with firm, painless ulcer known as a chancre, which is usually not apparent clinically. During secondary syphilis, patient sometimes develops gray, flattened, wart-like lesions known as **condylomata lata**. Syphilis is hazardous during pregnancy because spirochetes can cross the placenta and resulting in fetal malformation.

HAEMOPHILUS DUCREYI INFECTION (CHANCROID)

Chancroid is an exceedingly rare sexually transmitted infection, soft and painful ulcerated lesion known as chancroid on the cervix, vagina, vulva, or perianal region after 3–5 days of sexual contact with infected partner by *Haemophilus ducreyi*. It is a small gram-negative rod which requires special media, not easily available in most laboratories, to ensure its growth.

- Chancroid is a similar lesion to the primary lesion in syphilis, but instead chancroid is painful. Chancroid is most common in tropical areas, but rare in the United States, which may be associated inguinal lymphadenopathy, fever, chills, and malaise. It sometimes causes urethral stenosis.
- Histologic examination of the chancroid lesion shows a granulomatous inflammatory reaction. Gene clusters have been observed in *Haemophilus ducreyi* which encode for a cytolethal distending toxin similar to those found in invasive enteric bacteria such as Campylobacter, Shigella, and *Escherichia coli*. This toxin causes irreversible cell death of epithelial cells and may result in development of the skin breakdown and subsequent ulcer development.

PELVIC INFLAMMATORY DISEASE (PID)

Extension of infection from vagina, fallopian tubes or ovaries into pelvis is known as pelvic inflammatory disease. It most often affects women in reproductive age group between 20 and 40 years. Pelvic inflammatory disease is most often a complication of sexually transmitted infections (40–45%) caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

- Polymicrobial infections (Staphylococci, Streptococci, or enteric bacteria) during delivery or abortion are responsible for pelvic inflammatory disease in 45–50% of cases. Other causes of PID include tuberculosis (5%), vaginal amoebiasis, and *Actinomyces israelii* in women with intrauterine contraceptive device.
- Tuberculosis and *Chlamydia trachomatis* spread via intraluminal route hence cause infertility. Patient develops acute or chronic pelvic inflammatory disease depending on the causative organisms. Papanicolau smear shows cotton ball-like pseudofilamentous appearance. *Actinomyces israelii* in woman with intrauterine contraceptive device (Fig. 24.14).

Clinical Features

Patient presents with abdominal tenderness (direct/rebound), tenderness on movement of cervix and uterus, adnexal tenderness, positive endocervical smear for

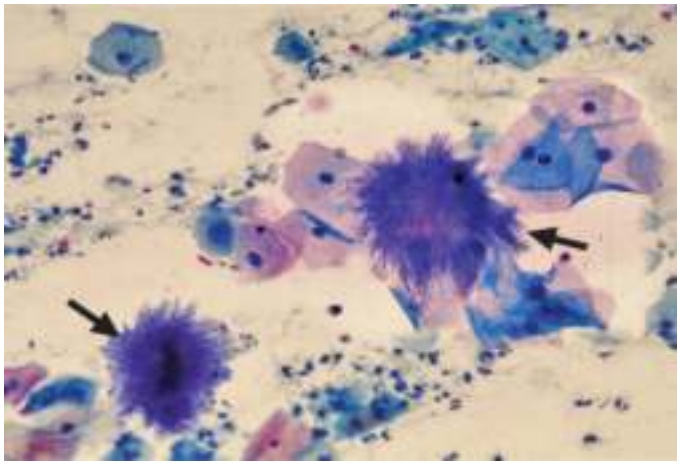


Fig. 24.14: *Actinomyces israelii.* Papanicolau smear shows cotton ball-like pseudofilamentous appearance in woman with intrauterine contraceptive device (arrows) (400X).

gonococcal infection, fever, leukocytosis, and purulent material on laparoscopy or culdocentesis. Infection spreads via periluminal route, hence chances of fertility are bright.

Complications

Infertility occurs due to occlusion of fallopian tubes. Patient with chronic nonspecific infection presents with fever, malaise, and fatigue. Pelvic mass, which is tender and often associated with pain during urinary bladder distension or defecation or sexual intercourse (dyspareunia). Patient develops peritoneal adhesions or generalized bacteremia.

Differential Diagnosis

Pelvic pain may occur due to disorders of uterus, ovaries, pelvic adhesions and endometriosis. Pelvic inflammatory disease is characterized by generalized lower abdominal shooting gripping pain more on one side. Endometriosis presents with shooting cramping pain, that builds up during menstrual period. Uterine pathology is characterized by bleeding per vagina, cramping pain in mid abdominal region before or during menstrual cycle. Ovarian cyst presents with sudden intermittent pain in iliac fossa of affected side.

NEOPLASTIC DISORDERS OF VAGINA

Tumors of vagina include vaginal intraepithelial neoplasia, squamous cell carcinoma, verrucous carcinoma, botryoides, rhabdomyosarcoma, leiomyomas and malignant melanoma.

VAGINAL INTRAEPITHELIAL NEOPLASIA

Vaginal intraepithelial neoplasia (VIN) is defined as presence of squamous cell dysplasia without invasion in upper third of the vagina, which affects women 20–80 years of age. HPV has been implicated in pathogenesis of vaginal intraepithelial neoplasia and associated with multifocal and lesions.

- Risk factors for VIN include early age at first intercourse especially with multiple sexual partners.
- Low-grade vaginal intraepithelial neoplasia is caused by low-oncogenic risk HPV 6, 11, that may undergo malignant transformation in 5% of cases. On contrary, high-grade vaginal intraepithelial neoplasia occurs due to high-oncogenic risk HPV 16, 31, 33, 35, and 39. Comparison of vaginal intraepithelial neoplasia (VIN) types is given in Table 24.2.

Clinical Features

Patient is usually asymptomatic but may present with vaginal discharge or postcoital spotting. Most common presentation of vaginal intraepithelial neoplasia is abnormal cytology. Vaginal intraepithelial neoplasia is a histologic diagnosis typical based on colposcopy assessment and biopsy of the vaginal intraepithelial neoplasia (VIN). Lugol iodine solution can be used to detect VIN and confirm boundaries prior to excision. On application of 3–5% acetic acid in the vagina, VIN appears as raised or flat white, granular epithelium with sharply demarcated borders with punctation and mosaic pattern more prevalent in vaginal intraepithelial 2 and 3. High-grade vaginal intraepithelial hyperplasia is a precursor of invasive squamous cell carcinoma of the vagina.

Surgical Pathology: Vaginal Intraepithelial Neoplasia

Gross Morphology

Vaginal intraepithelial neoplasia appears as an exophytic growth to verrucopapillary lesion.

Light Microscopy

- Squamous epithelium of vaginal intraepithelial neoplasia shows hyperchromatic nuclei and irregular nuclear membrane.
- Grading is based on the extent of thickness of squamous epithelium (VIN-1, VIN-2 and VIN-3) sharing dysplastic changes.

Table 24.2 Comparison of vaginal intraepithelial neoplasia (VIN) types

Parameters	Vaginal Intraepithelial Neoplasia Usual Type	Differentiated Vaginal Intraepithelial Neoplasia Type
Age group	Younger women	Postmenopausal women
Etiology	HPV related cytopathic effect	Not related to HPV without cytopathic effect, but associated with lichen sclerosus
Histologic features	Cells with hyperchromatic nuclei, prominent nuclear membrane and mitoses, lesion may be warty, basaloid or mixed subtypes	Cells with hyperchromatic nuclei, prominent nuclear membrane, mitoses and eosinophilic cytoplasm
Grading	VIN-1, VIN-2 and VIN-3	VIN-3
Progression to squamous cell carcinoma	Low progression to squamous cell carcinoma (SCC)	High progression to keratinized squamous cell carcinoma (SCC)

Treatment

Clinical management of VIN-1 is simple follow-up of the patient. VIN-2 and VIN-3 are treated by simple excision or laser ablation.

SQUAMOUS CELL CARCINOMA OF VAGINA

Squamous cell carcinoma originates in vagina without involvement of surrounding structures, such as cervix or vulva, which accounts for 1–3% of gynecologic cancers. About 80–90% of primary vaginal cancers are squamous cell carcinomas, which usually occur in the 6th decade of life (median age 58–68) but can also be seen in younger women associated with HPV infection.

- Most common site of squamous cell carcinoma is in the upper half of vagina. Small tumor may be missed on initial examination and involves lower two-thirds of vagina. In some cases, vaginal intraepithelial neoplasia (VIN) can be present prior to development of invasive squamous cell carcinoma.
- History of prior hysterectomy is present in 50% of cases. Vaginal squamous cell carcinoma is also associated with vaginal or uterovaginal prolapse. Squamous cell carcinoma of vagina can spread to regional lymph nodes, lungs, liver, brain and bones.

Etiopathogenesis

There is strong relationship of vaginal squamous cell carcinoma with high risk human papillomavirus, especially HPV 16 (80%), HPV 18 and HPV 31. It is more common in tobacco smokers, because tobacco smoking increases the risk for high-grade VIN in women with oncogenic HPV.

Clinical Features

Patient presents with vaginal bleeding or discharge (most common), urinary symptoms and lower abdominal pain. Small tumor is asymptomatic.

Laboratory Diagnosis

Patient is diagnosed by clinical history along with histologic examination of biopsy/resection specimen. Tumor involving both vagina and cervix should be classified as a cervical carcinoma; similarly, a tumor involving both vagina and vulva should be considered a vulvar squamous cell carcinoma. Imaging is required to determine extent of disease and to look for distant metastasis to lymph nodes and via hematogenous route to lung, liver, brain and bones.

Surgical Pathology: Squamous Cell Carcinoma of Vagina

Gross Morphology

Squamous cell carcinoma of vagina can be exophytic or ulcerative with necrosis.

Light Microscopy

- Histologically tumor is graded as well differentiated, moderately differentiated, poorly differentiated or undifferentiated.
- Well differentiated squamous cell carcinomas has polygonal squamous cells with ample eosinophilic cytoplasm, abundant keratin pearls and intercellular bridges. Nuclear pleomorphism and mitotic activity increases from well to poorly differentiated.
- Poorly differentiated squamous cell carcinomas has small cells with scant cytoplasm and hyperchromatic nuclei.
- Moderately differentiated squamous cell carcinomas has histologic features intermediate between well and poorly differentiated.
- Human papillomavirus (HPV) positive tumors are more frequently of nonkeratinizing, basaloid or warty type squamous cell carcinoma than HPV negative tumors. Histology of well-differentiated squamous cell carcinoma is shown in Fig. 24.15.

Prognostic Factors

FIGO stage of squamous cell carcinoma of vagina is most important predictor of overall survival. Prognosis is poor in patient with tumor size >4 cm and decreased local control of the disease. Total radiation dose in excess of 70 Gy is associated with improved local control of disease is associated with relatively good prognosis. Vaginal squamous cell carcinoma can directly spread to vulva, cervix, urinary bladder, rectum and through lymphatics can metastasize to obturator, hypogastric, external iliac and groin lymph nodes. Rarely, tumor may metastasize to liver, lungs, bones and brain.

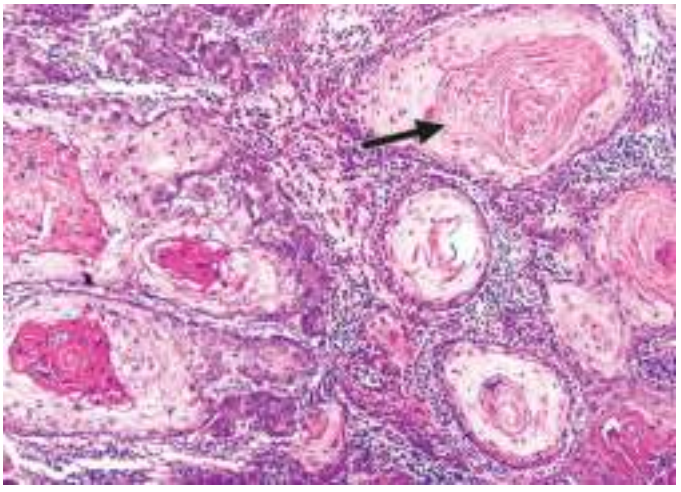


Fig. 24.15: Squamous cell carcinoma of vagina. At high magnification, nests of neoplastic squamous cells are invaded through a chronically inflamed stroma. This cancer is well-differentiated, as evidenced by keratin pearls. However, most cervical squamous carcinomas are nonkeratinizing (arrow) (400X).

Treatment

Vaginal squamous cell carcinoma can be treated with external beam radiation therapy/EBRT, interstitial brachytherapy, intracavitary brachytherapy, cisplatin based chemotherapy and surgical resection. Surgical resection is recommended for early stage of vaginal squamous cell carcinoma involving upper posterior vagina. For stage I disease, surgery consists of radical hysterectomy, upper vaginectomy and pelvic lymphadenectomy.

VERRUCOUS CARCINOMA OF VAGINA

Verrucous carcinoma of vagina is a distinct entity without HPV etiology. It is a slow-growing variant of squamous cell carcinoma, which most often occurs in the elderly postmenopausal women. Most common sites are labia majora > labia minora > posterior commissure. Local or distant metastases are very rare.

- Patient presents with slow growing firm mass, usually accompanied by pruritus and occasionally pain and vaginal discharge especially if large ulcerated or infected verrucous carcinoma.
- Histologic examination alone can be misleading due to superficial sampling of tissue or lack of obvious invasive component of the tumor. Therefore, clinical history indicating exophytic tumor in an elderly woman is often helpful. Imaging techniques may be useful to assess extent of tumor and exclude metastases of verrucous carcinoma.

Surgical Pathology: Verrucous Carcinoma of Vagina

Gross Morphology

Tumor is large, hyperkeratotic papillary or exophytic lesion, usually slow growing. Rarely multiple tumors are demonstrated in vagina.

Light Microscopy

- Exo-endophytic, well circumscribed tumor is composed of closely packed (arranged back to back), papillary structures lined by well differentiated stratified squamous epithelium, with minimal cellular atypia without invasion by single or cluster of cells. Epithelium demonstrates massive hyperkeratosis and parakeratosis, prominent acanthosis with (bulbous) expansion of rete ridges that push into the dermis/submucosa with rounded borders or a broad front.
- Tumor cells contain abundant pale, eosinophilic cytoplasm with low nuclear to cytoplasmic ratio, no/mild nuclear pleomorphism, basally located mitotic figures.
- Mild to moderate chronic inflammation in stroma is common. Lymphovascular invasion and perineural invasion are extremely rare.

Immunohistochemistry Technique

Ki-67 proliferation index is variably increased in 70–78% of cases. Ki-67 expression is localized to basal layer of squamous epithelium of verrucous carcinoma of vagina.

Prognostic Factors

Most important prognostic factors of verrucous carcinoma of vagina are depth of invasion and surgical margin status of verrucous carcinoma.Local recurrence is common (30–50%), especially after surgical resection with inadequate margins.

CLEAR CELL ADENOCARCINOMA OF VAGINA

Clear cell adenocarcinoma of vagina occurs in young daughters of below 20 years, whose mothers have taken diethylstilbestrol during pregnancy. It can also occur in postmenopausal women without exposure to DES. Most common site of tumor is anterior vaginal wall but can also occur in elsewhere in vagina. It accounts for 5–10% of primary vaginal malignancies.

Pathophysiology

Clear cell adenocarcinoma of vagina usually occurs in young women, who has been exposed *in utero* from maternal use of diethylstilbestrol (DES) used to prevent abortion during pregnancy.

- Diethylstilbestrol causes persistence of Müllerian epithelium while inducing contact between epithelium and the vaginal mesenchyme. Unopposed estrogen and obesity lead to increase in the peripheral conversion of steroid hormones to estrone by the enzyme aromatase resulting in hyperestrogenic environment.
- Endometriosis of vagina and perivaginal region may be a precursor lesion in non-DES exposed females. The frequency of vaginal clear cell adenocarcinomas arising in endometriosis ranges from 4 to 11%. Vaginal adenosis (especially tuboendometrial adenosis) is associated with clear cell adenocarcinoma in 90% of cases, suggesting adenosis is a precursor lesion.
- Vaginal adenosis is characterized by mucosal columnar epithelium lined crypts in areas normally lined by stratified squamous epithelium. Vaginal adenosis is thought to be a precursor of clear cell adenocarcinoma of vagina.

Clinical Features

Patient presents with abnormal vaginal bleeding or discharge in young daughters or postmenopausal bleeding.

- Lymphatic and vascular spread of tumor can occur and metastasize to regional lymph nodes, lungs, kidney, peritoneum, omentum, ovary, liver and brain.
- Patient can develop malignant pericardial effusion and cardiac tamponade. Tumor may recur in distant sites even in absence of pelvic disease. About 16–25% of patients remain asymptomatic.

- There is increased risk for development of malignant melanoma and other cancers in these patients. Metastases to distant organs are demonstrated by imaging techniques.

Surgical Pathology: Clear Cell Adenocarcinoma of Vagina
Gross Morphology
Superficially located polypoid, exophytic mass that typically originates from the anterior wall of the upper two-thirds of the vagina.
Light Microscopy
▪ Clear cell adenocarcinoma of vagina exhibits glandular/tubular, papillary, cystic and solid architectural patterns with focal necrosis. ▪ Tumor is composed of large cuboidal cells with distinct cell membranes, abundant clear cytoplasm, round to irregular hyperchromatic nuclei and conspicuous nucleoli. **Hobnailing** is a prominent feature with nuclei protruding into the lumen.
Electron Microscopy
▪ Clear cell carcinomas of vagina, cervix or endometrium have similar ultrastructural features. Glands have short, thick microvilli in the lumina. ▪ Glandular cells are attached by desmosomes and interdigitating cytoplasmic processes, which contain abundant glycogen granules, many uniform mitochondria and 'stacked' parallel rows of granular endoplasmic reticulum.
Immunohistochemistry Technique
Neoplastic cells of clear cell adenocarcinoma of vagina are immunoreactive to CK7, CAM 5.2, 34βE12, CEA, vimentin, BCL-2, p53 and CA125. These express variable positive staining with ER and HER2/neu. Neoplastic cells are negative for CK20 and PR.

Markers	Expression
▪ CK7	▪ Positive
▪ CAM 5.2	▪ Positive
▪ 34βE12	▪ Positive
▪ CEA	▪ Positive
▪ Vimentin	▪ Positive
▪ BCL-2	▪ Positive
▪ p53	▪ Positive
▪ CA125	▪ Positive
▪ ER	▪ Positive (variable)
▪ HER2/neu	▪ Positive (variable)
▪ CK20	▪ Negative
▪ PR	▪ Negative

Molecular Genetic Alterations

Neoplastic cells of clear cell adenocarcinoma of vagina show p53 nuclear staining of different intensity. TP53 gene mutation is not due to inactivation but rather due to persistent DNA damage or genetic instability. Persistence of wild type TP53 gene mutation in tumors may correlate with their favorable prognosis and sensitivity to radiation.



Fig. 24.16: Botryoid rhabdomyosarcoma of the vagina. Tumor resembles cluster of grapes projecting from vagina.

Treatment

Stage 1 or early stage clear cell adenocarcinoma of vagina is treated by radical hysterectomy with partial or complete vaginectomy, pelvic lymphadenectomy, vaginal reconstruction if needed.

- Small tumors are excised, evaluated for retroperitoneal lymph nodes followed by local radiation to the bed of the tumor.
- Large tumors are treated by neoadjuvant chemotherapy combined radiotherapy. Ovarian function is usually preserved in young women. Postmenopausal women should be followed up with cytologic Papanicolau smears and histologic examination of biopsy of cervix and vagina.

BOTRYOID RHABDOMYOSARCOMA OF VAGINA

Botryoid rhabdomyosarcoma is most common vaginal malignant tumor in children under 8 years of age. Most common sites of botryoid rhabdomyosarcoma are wall of hollow organs or beneath mucosal lining of urinary bladder, vagina, nasopharynx and middle ear. Clinical photograph of botryoid rhabdomyosarcoma is shown in Fig. 24.16.

Surgical Pathology: Botryoid Rhabdomyosarcoma of Vagina
Gross Morphology
Tumor resembles cluster of grapes hence named as botryoid rhabdomyosarcoma.
Light Microscopy
▪ Tumor is composed of round to oval cells with eosinophilic cytoplasm admixed with spindle cells in loose myxoid stroma. ▪ A cambium layer of tightly packed undifferentiated neoplastic cells is demonstrated beneath mucosal lining in at least one microscopic field (Fig. 24.17).

Immunohistochemistry Technique	
Tumor cells of botryoid rhabdomyosarcoma show positivity for muscle-specific actin (MSA), desmin, MyoD1, vimentin, myoglobin, and myogenin.	
Markers	**Expression**
▪ MSA ▪ Desmin ▪ MyoD1 ▪ Vimentin ▪ Myoglobin ▪ Myogenin	▪ Positive ▪ Positive (cytoplasmic staining) ▪ Positive (nuclear staining) ▪ Positive ▪ Positive ▪ Positive (nuclear staining)

Treatment and Prognosis

Botryoid rhabdomyosarcoma of vagina is fatal with 5-year survival rate in 10–35% of cases. Combined surgical excision, radiation therapy and chemotherapy increase survival rates in these children. Prognosis is most often excellent.

LEIOMYOSARCOMA OF VAGINA

Vaginal leiomyosarcoma is rare neoplasm in infants with an aggressive course, high recurrence, and undetermined ideal treatment regimen. Its diagnosis can be delayed due to its variable presentation.

Surgical Pathology: Leiomyosarcoma of Vagina	
Gross Morphology	
Tumor measures 3–5 cm in diameter. It is soft in consistency with irregular borders and areas of necrosis on gross examination or not bulging above the surface when cut.	
Light Microscopy	
▪ Tumor is composed of pleomorphic spindle-shaped cells with moderate to marked nuclear atypia and ≥5 mitoses per 10 high power fields and foci of necrosis with infiltrating margins. ▪ Single best criterion for predicting the behavior of smooth muscle tumors is mitotic activity (Fig. 24.18).	
Immunohistochemistry Technique	
Tumor cells show positivity for SMA and S-100 protein.	
Markers	**Expression**
▪ SMA ▪ S-100 protein	▪ Positive ▪ Positive

MELANOMA OF VAGINA

Primary melanoma of the vagina is a rare gynecological malignancy in postmenopausal women in the lower-third of the anterior vaginal wall. It accounts for 4–10% of all valvular malignancies. It is second most common malignant neoplasm after squamous cell carcinoma.

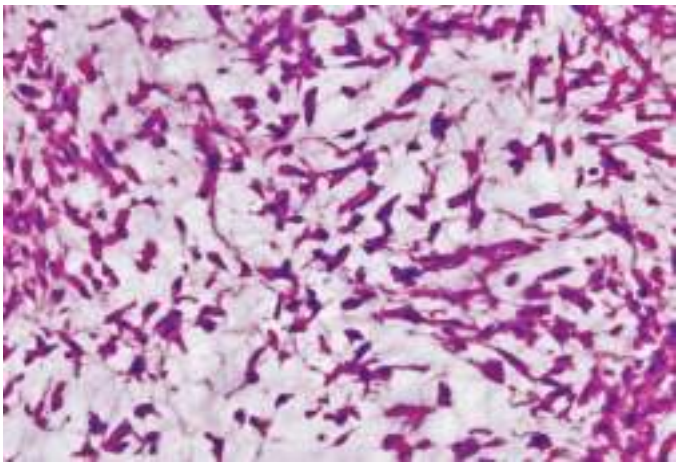


Fig. 24.17: Botryoid rhabdomyosarcoma of vagina. Tumor is composed of round to oval cells admixed with spindle cells in loose myxoid stroma (400X).

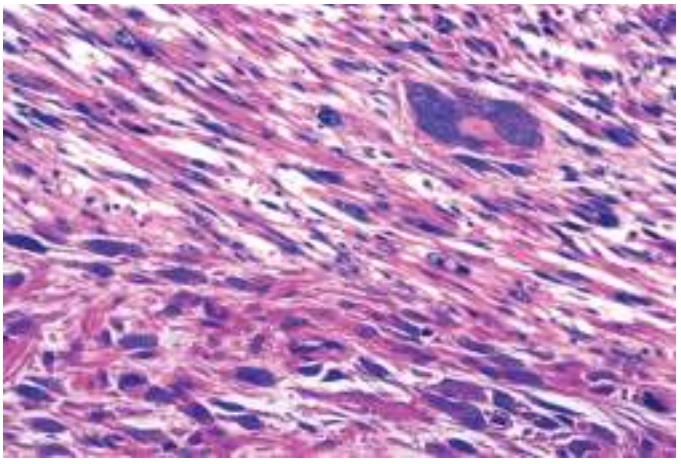


Fig. 24.18: Leiomyosarcoma. As with sarcomas in general, leiomyosarcomas have spindle cells. Several mitoses are seen here, just in this one high power field (400X).

Labia (minora and majora) are the most commonly affected sites, followed by clitoris in some studies. Tumor is associated with high risk of recurrence, distant metastases and a short survival time. Patient is treated by surgical excision, radiotherapy and chemotherapy.

Clinical Features

Primary malignant melanoma of the vagina may be discovered during routine gynecologic examinations. Patient presents with pigmented and sometimes nonpigmented lesions (macules, patches, nodules), that may be ulcerated. Patient may present with lesions (satellitosis).

Imaging Techniques

Lymphoscintigraphy for identification of sentinel nodes. CT and MRI scans are for staging the disease.

Surgical Pathology: Melanoma of Vagina

Gross Morphology

Tumor is bulky mass with or without pigmentation. Preservation of specimen orientation is critical for thorough evaluation of peripheral and deep tissue margins.

Light Microscopy

- Tumor is composed of melanoma cells larger than nevus cells. These cells contain large nuclei with irregular contours with prominent eosinophilic nucleoli (Fig. 24.19).
- Chromatin is clumped at the periphery of the nuclear membrane.

Immunohistochemistry Technique

Tumor cells show positivity for S-100 protein, MART-1 (melan-A), Ki-67, CAM 5.2, tyrosinase, SOX-10, MITF, SOX-100, PNL2, MUM1 and HMB-45. Extramammary Paget's disease shows positivity for mucin, but melanoma not.

Markers	Expression
S-100 protein (calcium binding protein)	Positive (cytoplasmic or nuclear)
Melanoma antigen recognized by T cells 1 (MART-1) (melan-A)	Positive
Ki-67	Positive
CAM 5.2	Positive
Tyrosinase	Positive
SOX-10	Positive
MITF (microphthalmia-associated transcription factor)	Positive
SOX-100	Positive
PNL2	Positive
MUM1	Positive
HMB-45 (human melanoma black 45)	Positive (epithelioid melanoma)

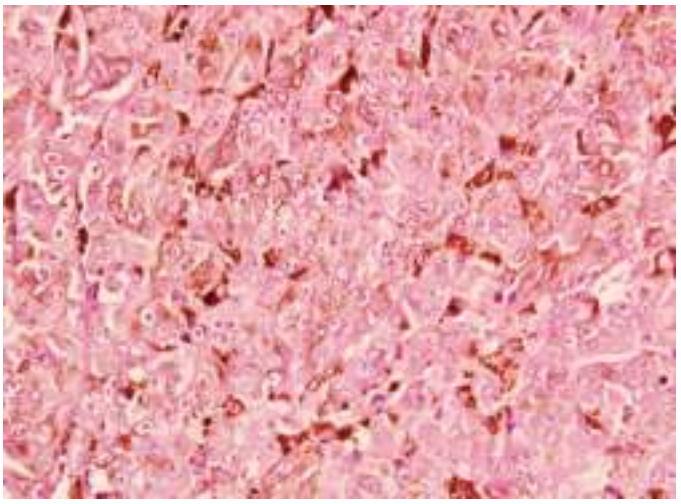


Fig. 24.19: Melanoma. Tumor is composed of melanoma cells with large nuclei with irregular contours and clumped cromatin (400X).

Prognosis

Patients of 68-year-old or younger women at diagnosis have a better prognosis. Prognosis depends on tumor thickness, feasibility of complete resection with appropriate margin status, mitotic rate and ulceration. Free surgical margin of ≥1.0 mm is associated with better prognosis. Patients with localized disease (stages 0, I, II) have a better prognosis. Higher number of positive lymph nodes is associated with worse prognosis. Strong and diffuse c-KIT expression may be associated with a worse prognosis.

Treatment

Primary vaginal melanoma is treated by surgical resection with adequate margins, radiotherapy, chemotherapy and topical imiquimod therapy.

DISORDERS OF UTERINE CERVIX

UTERINE CERVIX: OVERVIEW

The cervix is the lower portion of the uterus, that connects to the vagina through the endocervical canal. It is divided into two parts, portio vaginalis (part that protrudes into the vagina) and supravaginalis (part that lies above the vaginal vault). The outer surface of the portio vaginalis is known as the exocervix or ectocervix, and the portion related to the endocervical canal is known as endocervix.

- Ectocervix is the most readily visible portion of the cervix; endocervix is largely invisible and lies proximal to the external os. Endocervical canal is lined with glandular epithelium and the ectocervix is lined with nonkeratinizing squamous epithelium.
- The cervix has several different epithelial linings. Extocervix is lined by nonkeratinized squamous epithelium, and endocervix (endocervical canal) is lined by mucus secreting glandular epithelium. The squamous epithelium of ectocervix meets the glandular epithelium of endocervix at squamocolumnar junction (SCJ). The original squamocolumnar junction is dynamic and moves during early adolescence and during first pregnancy. The original squamocolumnar junction originates in the endocervical canal, but as the cervical remodeling process occurs during adolescence and first pregnancy, the squamocolumnar junction comes to lie on the ectocervix and becomes the new squamocolumnar junction.

- The subcolumnar reserve cells located at or near squamocolumnar junction are involved in the process of cervical intraepithelial neoplasia I, II, III (CIN I, II, III) and cervical carcinoma.
- The stroma of the cervix is composed of fibrous tissue admixed with elastic fibers and smooth muscle fibers.
- The cervix is both a sentinel for potentially serious upper genital tract infections and a target for viruses and other carcinogens, which may lead to invasive cervical carcinoma. Worldwide, cervical carcinoma is the second most common cancer in women, with an estimated 493,000 new cases each year, over 50% are fatal.
- Carcinoma of cervix arising from ectocervix is usually squamous cell carcinoma. Adenocarcinoma originates from endocervix. Cervical squamous cell carcinoma accounts for 80% and adenocarcinoma accounts for 50% in young women. Adenosquamous and small cell carcinoma of cervix are relatively rare. Primary sarcoma of the cervix has been occasionally described and malignant lymphoma of the cervix, both primary and secondary, has been reported.

INFLAMMATORY DISORDERS OF UTERINE CERVIX

Acute cervicitis is a pattern of inflammation caused by bacteria, viruses, fungi and protozoa and marked by neutrophilic infiltrate in stroma and epithelium, which shows edema and reactive epithelial atypia.

- Chronic cervicitis comprises lymphocytes and plasma cells infiltrate.
- Papillary endocervicitis refers to inflammation of endocervical mucosa forming papillary structures.
- Noninfectious cervicitis can occur due to chemical exposure, pessary, tampons and surgical trauma. Cervix may show infiltration by neutrophils or lymphocytes and plasma cells or granulomatous lesions.

ACUTE CERVICITIS

Acute cervicitis is caused by *Neisseria gonorrhoeae* (sexual transmission) and *Chlamydia trachomatis*. Patient presents with mucopurulent discharge. *Chlamydia trachomatis* may produce follicular cervicitis in chronic cases. *Actinomyces israelii* infection is associated with intrauterine device use and demonstrated in pseudofilamentous structures in Papanicolau smears. Ophthalmia neonatorum, a neonatal conjunctival infection is acquired at delivery caused by *Neisseria gonorrhoeae.*

CHRONIC CERVICITIS

Chronic cervicitis occurs due to *Candida albicans*, *Trichomonas vaginalis* and *Chlamydia trachomatis*. Chronic cervicitis caused by *Chlamydia trachomatis* is associated with reactive lymphoid follicles. Erosion of cervix is characterized by columnar epithelium replacing squamous epithelium, grossly resulting in an erythematous area. Sometimes it is a manifestation of acute cervicitis. The condition is most often asymptomatic. It may be manifested by cervical discharge. Granulomatous cervicitis is caused by *Mycobacterium tuberculosis* and *Treponema pallidum*. Clinical photograph of chronic cervicitis is shown in Fig. 24.20. Histology of chronic cervicitis is shown in Fig. 24.21.

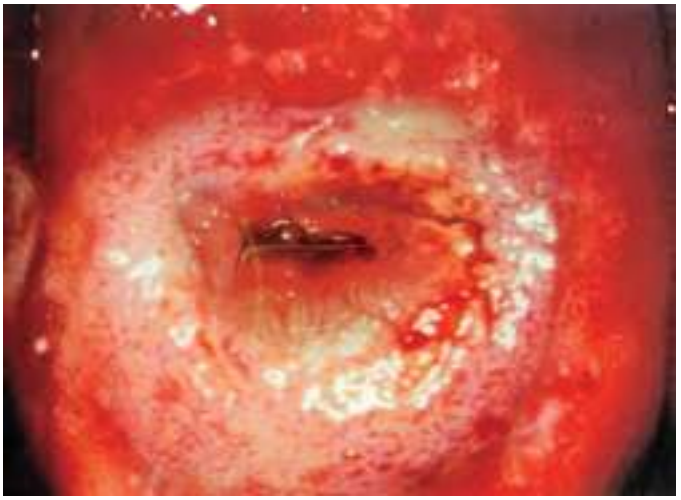


Fig. 24.20: Chronic cervicitis with ectropion of the cervix. Columnar epithelium has replaced squamous epithelium. Squamous epithelium is showing as an extensive reddened zone. The cervix is also scarred and deformed from previous deliveries.

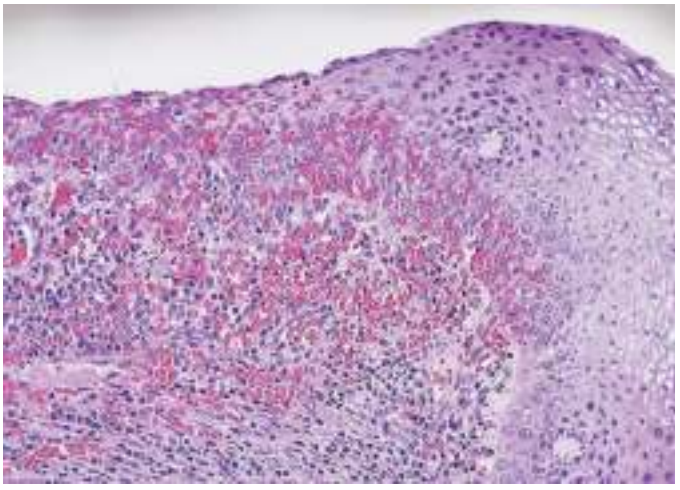


Fig. 24.21: Chronic cervicitis. It shows infiltration by numerous lymphocytes and hemorrhage (100X).

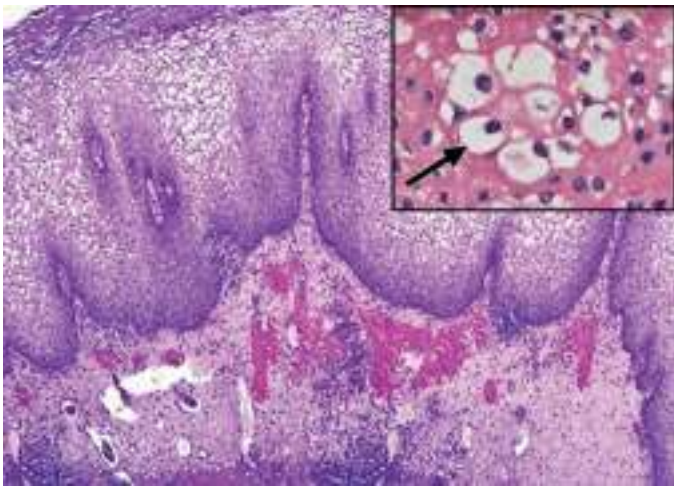


Fig. 24.22: Viral cervicitis. Cervical biopsy demonstrates a thickened squamous epithelium. Squamous cells (inset) have a vacuolated appearance showing koilocytotic change. A condyloma accuminatum would have a similar appearance (arrow) (100X).

Surgical Pathology: Chronic Cervicitis
Gross Morphology
Cervix shows extensive reddened areas.
Light Microscopy
▪ Chronic cervicitis occurs at the squamocolumnar junction of the cervix. ▪ Submucosa of cervix is infiltrated by chronic inflammatory cells.

VIRAL CERVICITIS

Herpes simplex virus type 2 causes ulceration of cervix resulting in cervicitis and characterized by enlarged epithelial nuclei, molding of nuclei, multinucleation, and margination of chromatin in virally infected at the edge of ulcer. Cytomegalovirus causes nuclear and cytoplasmic inclusions in infected cervix. Adenovirus causes smudged nuclear inclusions. Molluscum contagiosum caused by poxvirus generates large round eosinophilic inclusions in cytoplasm. Human papillomavirus (16, 18, 31, 33 or 45) infection is responsible for cervical intraepithelial neoplasia (CIN I, CIN II, CIN III) and cervical carcinoma (Fig. 24.22).

NEOPLASTIC DISORDERS OF UTERINE CERVIX

ENDOCERVICAL POLYP

Endocervical polyp is a benign exophytic proliferation composed of fibrovascular core and endocervical glandular or metaplastic squamous epithelium in variable proportion. It is common in patients over 40 years of age in cervix/endocervix. Chronic inflammation, surface erosion and reactive epithelial changes are common in endocervical polyp.

Clinical Features

Patient presents with abnormal vaginal bleeding (postcoital or contact) or discharge as a result of cervical erosions and granulation tissue. Per vaginal examination or colposcopy reveals single smooth or lobulated mass measuring <3 cm in greatest dimension protruding through the cervical os (Fig. 24.23A). Cervical Papanicolau smear may demonstrate atypical cells due to reactive changes in surface epithelial cells. Polypectomy is recommended for symptomatic and large (>3 cm) endocervical polyp.



Fig. 24.23A: Endocervical polyp. Per vaginal examination or colposcopy reveals single smooth or lobulated mass measuring <3 cm in greatest dimension protruding through the cervical os (arrow).



Fig. 24.23B: Endocervical polyp. It is usually solitary, smooth or lobulated mass measuring <3 cm in diameter protruding through the cervical os and can be detected during routine gynecologic examination or colposcopy (arrow). (*Courtesy*: Department of Pathology, Sher-E-Kashmir Institute of Medical Sciences, Srinagar, J&K.)

Surgical Pathology: Endocervical Polyp

Gross Morphology

Endocervical polyp is single smooth or lobulated mass measuring <3 cm in greatest dimension (Fig. 24.23B).

Light Microscopy

- Endocervical poly is composed of fibrovascular core containing variable sized blood vessels and chronic inflammatory cells.
- Fibrovascular core is lined endocervical by mucinous epithelium with varying degrees of squamous metaplasia, erosion and reactive changes. Mitotic activity may be demonstrated, especially in cases with marked inflammation or florid microglandular hyperplasia.
- Endocervical polyp may rarely harbor *in situ* or invasive squamous and glandular lesions (Fig. 24.24).

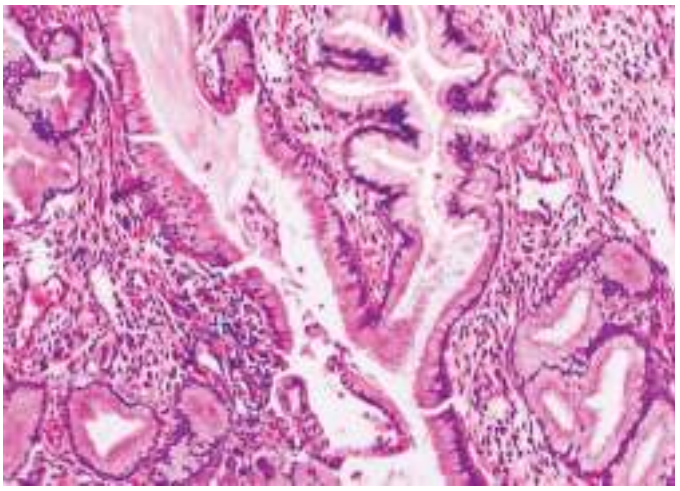


Fig. 24.24: Endocervical polyp. Endocervical poly is composed of fibrovascular core lined endocervical by mucinous epithelium with varying degrees of squamous metaplasia, erosion and reactive changes (400X).

CERVICAL INTRAEPITHELIAL NEOPLASIA

Cervical intraepithelial neoplasia (CIN) is disordered epithelial dysplastic precancerous lesion associated with both low and high risk HPV subtypes. The principal significance of CIN is its precursor role in the genesis of invasive cervical carcinoma.

- Cervical intraepithelial neoplasia is manifested by loss of polarity and nuclear hyperchromasia beginning at the basal layer and extending upwards.
- Based on the extent of involvement of cervical epithelium, cervical intraepithelial neoplasia lesions are subclassified into 3 types seen on histopathologic examination: CIN I, CIN II, and CIN III.
- Recently simplified classification has been proposed with CIN I renamed as low-grade squamous intraepithelial lesion (LSIL) and CIN II and CIN III combined into one category as high-grade squamous intraepithelial lesion (HSIL).
- The categories of cervical intraepithelial neoplasia include: (a) flat mature LSIL (flat condyloma or CIN I), (b) mature exophytic LSIL (exophytic condyloma, condyloma acuminatum), (c) extensive exophytic LSIL (giant condyloma), (d) immature exophytic LSIL (immature condyloma, squamous papilloma, papillary immature metaplasia), and (e) immature flat metaplastic LSIL.
- The recommended terminology for HPV-associated squamous lesions of the lower anogenital tract is LSIL and HSIL, which may be further classified by the applicable intraepithelial neoplasia subcategorization. Genomic integration of HPV DNA sequences, most often high risk types 16, 18, 31, 33 or 45 is associated with CIN, as well as with frank malignant change in cervix. Development of cervical intraepithelial neoplasia (CIN I, CIN II, CIN III) and invasive cervical carcinoma is shown in Fig. 24.25. Histology of CIN I, CIN II and CIN III is shown in Figs 24.26 to 24.28. Procedure for Papanicolaou smear is shown in Fig. 24.29A and B.
- Oncogenic risk of anogenital human papillomavirus associated with histologic features is given in Table 24.3. Terminologies for cervical intraepithelial neoplasia for development of cervical carcinoma is given in Table 24.4. Criteria used to diagnose squamous epithelial cell abnormalities of cervix are given in Table 24.5. Criteria used to diagnose atypical squamous cells (ASCs) are given in Table 24.6.

Pathology Pearls: Cervical Intraepithelial Neoplasia (CIN)

- **CIN I (mild dysplasia):** CIN I is a low-grade lesion. It affects 20–30 years old women. It involves the innermost one-third of the cervical epithelium affecting 20–30 years old women. More than 85% of CIN I lesions regress spontaneously, 10% progress to CIN III, and 2–3% progress to invasive carcinoma.
- **CIN II (moderate dysplasia):** CIN II involves innermost two-thirds of thickness of epithelium affecting 30–40 years old women. It may progress to CIN III in many patients.
- **CIN III (severe dysplasia):** CIN III involves the full thickness of the cervical epithelium affecting 30–40 years old women, which does not invade the basement membrane. Approximately 25% of CIN III untreated cases may progress to invasive carcinoma within 10 years of diagnosis. Such involved cervix must be excised by conization (i.e. removal of the involved cone of tissue around the endocervical canal and the transformation zone by laser vaporization or cryosurgery). It is not possible to predict whether the lesion will progress to invasive carcinoma or regress. Therefore, all patients with CINs are treated as potential cervical carcinoma.

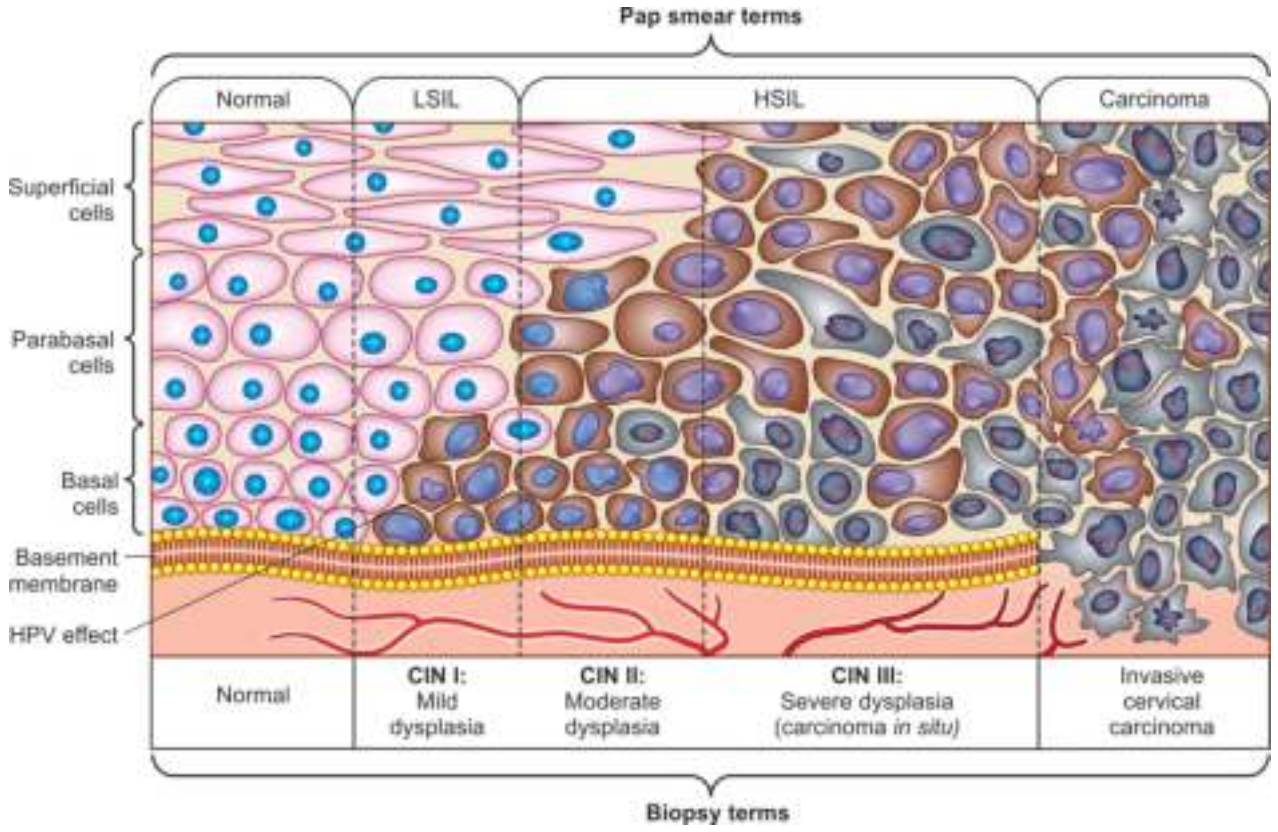


Fig. 24.25: Development of cervical intraepithelial neoplasia (CIN I, CIN II, CIN III) and invasive cervical carcinoma.

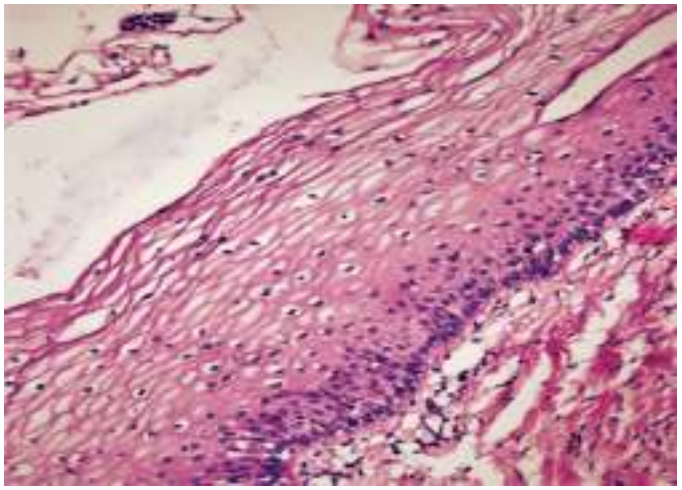


Fig. 24.26: Cervical intraepithelial neoplasia I (CIN I). Cervix shows moderate dysplastic epithelium in the lower one-third. The upper dermis is infiltrated by inflammatory cells (100X).

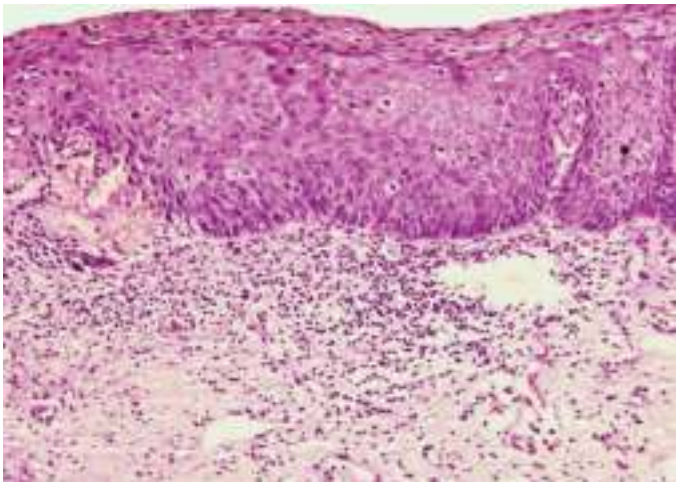


Fig. 24.27: Cervical intraepithelial neoplasia II (CIN II). Cervix shows moderate dysplastic epithelium in the lower two-thirds. The upper dermis is infiltrated by numerous inflammatory cells (100X).

CERVICAL CARCINOMA

Cervical carcinoma is most common malignant invasive tumor of the female genital tract. It has biphasic age incidence 20–39 years and >50 years and rarely occurs in younger than age 20 years. Clinical features depend on stage of the disease.

- Squamous cell carcinoma of cervix is invasive tumor, which shows squamous differentiation arising at the squamocolumnar junction of external 'os'. The squamocolumnar junction is defined as the junction between the squamous epithelium and the columnar epithelium. Its location on the cervix is variable during adolescence and first pregnancy.

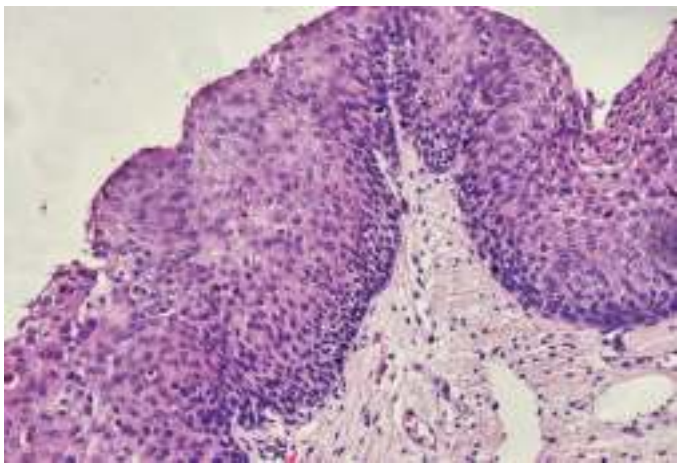


Fig. 24.28: Cervical intraepithelial neoplasia III (CIN III). This is cervical squamous dysplasia at high magnification extending from the center to the right. The epithelium is normal at the left. Note how the dysplastic cell nuclei are larger and darker, and the dysplastic cells have a disorderly arrangement (400X).

- The squamocolumnar junction is the result of a continuous remodeling process resulting from uterine growth, cervical enlargement and hormonal status.
- Risk factors for cervical carcinoma include early sexual activity and with multiple sexual partners, high risk human papillomavirus 16, 18, 31, 33, 45, human simplex virus 2, activation of RAS gene, mutation of TP53 gene, cigarette smoking and oral contraceptive use.
- Cervical carcinoma is detected clinically via white patches after application of acetic acid to cervix; cervix also has mosaic vascular patterns at colposcopy. Papanicolaou smear examination is performed to detect premalignant lesions. Recent 2020 WHO classification of tumors of the uterine cervix is given in Table 24.7.

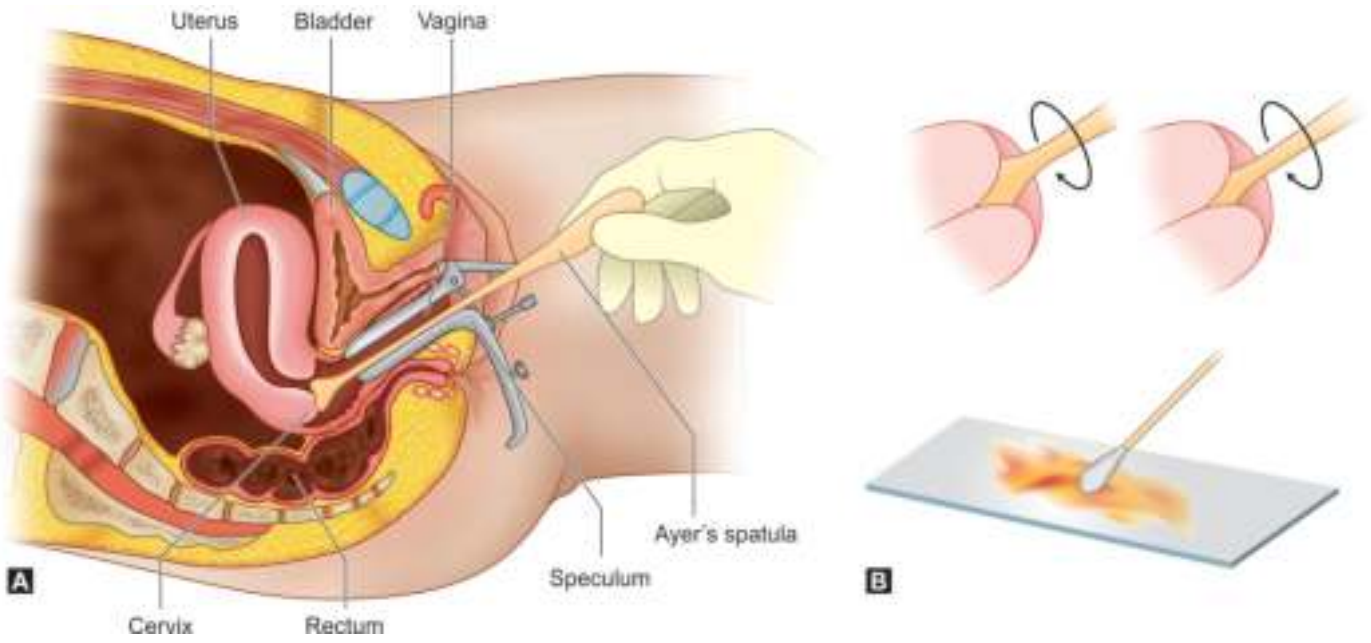


Fig. 24.29: Procedure for Papanicolaou smear. (A) Ayer's spatula is inserted per vagina for taking Pap smear, (B) preparation of Pap smear.

Table 24.3 Oncogenic risk of anogenital human papillomavirus associated with histologic features

Oncogenic Risk for Cervical Cancer	Human Papillomavirus	Histologic Features	
High oncogenic risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 68, 59, 66	▪ HSIL ▪ Koilocytosis occasional present ▪ Aneuploidy usually	▪ Abnormal mitoses frequent ▪ Undifferentiated cells and mitoses in upper two-thirds of cervical epithelium
Low oncogenic risk	6, 11, 42, 43, 44, 53	▪ LSIL ▪ Koilocytosis frequently present ▪ Diploid or polyploid most often	▪ Abnormal mitoses absent ▪ Undifferentiated cells and mitoses in lower third of cervical epithelium

Medium risk human papillomavirus includes 33, 35, 39, 51, 52, 56–59, 68; HSIL (high-grade squamous intraepithelial lesion); LSIL (low-grade squamous intraepithelial lesion).

Table 24.4 Terminologies for cervical intraepithelial neoplasia for development of cervical carcinoma

Old Classification Terminology	WHO Classification Terminology	Bethesda System Terminology
Mild dysplasia	CIN I	LSIL
Moderate dysplasia	CIN II	HSIL
Severe dysplasia/carcinoma *in situ*	CIN III	HSIL

CIN: Cervical intraepithelial neoplasia

Table 24.5 Criteria used to diagnose squamous epithelial cell abnormalities of cervix

Old Classification Terminology	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia/ Carcinoma *in situ*	Invasive Carcinoma
WHO classification terminology	CIN I	CIN II	CIN III	—
Bethesda system terminology	LSIL	HSIL	HSIL	—
Cell type and arrangement	Superficial or intermediate cells arranged in single or sheets	Parabasal cells arranged in single or sheets	Basal cells arranged in single or sheets	Basal, spindle, pleomorphic arranged in single or sheets or syncytium
Number of abnormal cells	+	++	+++	++++
Koilocytosis	+++	+	+/–	+/–
Nucleus size	+++	++	+	+
Hyperchromatic nucleus	+	++	+++	++++
Nuclear/cytoplasmic ratio	+	++	+++	++++

Table 24.6 Criteria used to diagnose atypical squamous cells (ASC)

Features	Atypical Squamous Cells of Undermined Significance (ASCUS)	Atypical Squamous Cells cannot Exclude HSIL (ASC-H)
Cells resemble	Superficial or intermediate squamous round to oval cells in size and configuration with nuclei 2–3 times the size of a normal intermediate cells	Parabasal or basal cells in size configuration
Nuclear features	▪ Nuclear contour minimally irregular ▪ Normochromatic to slight hyperchromatic nuclei	▪ Nuclear contour irregular ▪ Hyperchromatic nuclei with uneven chromatin

Clinical Pearls: Risk Factors for Cervical Carcinoma

- **Sexual activity:** The risk of both squamous cell carcinoma and adenocarcinoma of uterine cervix is strongly related to the lifetime number of sexual partners, and, independently, to age at first intercourse. Risk is increased for cervical carcinoma in women with early sexual activity below the age of 16 years; and who have sexual contact with high risk sexual partners, who had either former wife with cervical carcinoma or penile condylomas or not undergone penile circumcision.
- **Human papillomavirus:** Human papillomavirus plays an important role in the pathogenesis of cervical carcinoma. Genomic integration of HPV DNA sequences, most often types 16, 18, 31, 33 or 45 transmitted by sexual contact is associated with CIN, as well as with cervical carcinoma. Vaccines against some of human papillomavirus are available. HPV 16, 18 are associated with cervical carcinoma, HPV 16, 18 infect immature epithelial basal cells at the squamocolumnar junction. HPV (16, 18) infection produces koilocytic atypia, consisting of nuclear atypia and a cytoplasmic perinuclear halo. HPV cannot infect the mature superficial squamous cells that cover the ectocervix, vagina, or vulva.
- **Herpes simplex virus II:** Herpes simplex virus acts as a promoter. HPV viral proteins E6 and E7 bind and inactivate the gene products of p53 and Rb, respectively. HPV and herpes simplex may be synergetic.
- **Molecular genetic alterations:** Activation of the RAS oncogene and TP53 tumor suppressor gene mutation play an important role in cervical oncogenesis.
- **Cigarette smoking:** Tobacco smoking has an important co-carcinogenic role. Long duration of tobacco smoking (≥20 years) is associated with a two-fold increase in the risk of squamous cell carcinoma, but tobacco smoking is not associated with the risk of adenocarcinoma. Further studies are needed to confirm the suggestion from this and other studies of differences in risk related to smoking between squamous cell and adenocarcinomas of the cervix.

- **Oral contraceptive use:** The risk of squamous cell carcinoma and adenocarcinoma of uterine cervix is increased with increasing duration of use of oral contraceptives, and this effect is most marked in current and recent users of oral contraceptives.
- **Parity:** The risk of squamous cell carcinoma is associated with high parity and the risk of both squamous cell carcinoma and adenocarcinoma increased with early age at first birth.
- **Immunosuppression:** It increases risk of cervical carcinoma manifolds in immunosuppressive state.

Pathology Pearls: Human Papillomavirus (HPV) Oncoproteins Role in Cervical Carcinoma

- Principles of HPV E6 and E7 oncogene activity in cervical carcinoma is shown in Fig. 24.30. Human papillomavirus in cervix is shown in Fig. 24.31.
- Once, human papillomavirus enters host cervical epithelial cells, it synthesizes HPV oncoproteins E6 and E7. HPV oncoprotein E6 causes increase degradation of p53 tumor suppressor protein and HPV oncoprotein E7 leads to inactivation of regulatory retinoblastoma protein (pRb). Thus, allowing escape from p53 induced apoptosis and normal cell cycle control in G1.
  - **HPV E7 oncoprotein** increases activity of CDK4/cyclin D by inactivating CDK inhibitors (p21 and p27). It binds to the RB protein and displaces transcription factor E2F. Now E2F is free to bind to the promoters of genes (like c-Myc) that cause the cell to enter the cell cycle. Therefore, inhibition of p21 and RB-E2F causes increased activity of CDK4/cyclin D results in genome instability.
  - **HPV E6 oncoprotein** degrades p53 protein. It also stimulates the expression of TERT, the catalytic subunit of telomerase resulting in increased tyrosine kinase activity. These changes cause genome instability. The host cells enter the cell cycle resulting in increased proliferation.
- Laboratory methods to diagnose HPV infection include Papanicolau smear cytology or H&E morphology, fluorescence *in situ* hybridization (FISH), DNA HPV testing and immunohistochemistry p16 as a surrogate marker.

Pathology Pearls: Evolution of Cervical Carcinoma

Development of cervical intraepithelial neoplasia (CIN I, II, III) and invasive cervical carcinoma is shown in Fig. 24.32.

- **Cervical intraepithelial neoplasia:** Initially, patient developing cervical intraepithelial neoplasia (CIN I, CIN II, CIN III) is usually asymptomatic.
- **Microinvasive cervical carcinoma:** It refers to stromal invasion <5 mm beneath the basement membrane without evidence of lymphatic or vascular involvement.
- **Early invasive carcinoma:** It refers to invasion of basement membrane and underlying stroma by cervical carcinoma. Per vagina examination reveals fungating, ulcerative or diffusely infiltrative cervical growth resulting in barrel-shaped cervix.
- **Advanced invasive carcinoma:** It can spread directly invades adjacent pelvic structures, via lymphatic route to lymph nodes (e.g. paracervical, obturator, external iliac, internal iliac, common iliac and aortic lymph nodes), and via hematogenous route to liver, lungs, bones or brain. Cervix becomes indurated with necrosis and ulceration, resulting in formation of large fungating mass.

Table 24.7 Recent 2020 WHO classification of tumors of the uterine cervix

Squamous Epithelial Tumors
- Squamous metaplasia
- Cervical atrophy
- Condylomata accuminatum
- Low-grade squamous intraepithelial lesion
  - Cervical intraepithelial neoplasia grade I
- High-grade squamous intraepithelial lesion
  - Cervical intraepithelial neoplasia grade II
  - Cervical intraepithelial neoplasia grade III
- Squamous cell carcinoma, HPV-associated
- Squamous cell carcinoma, HPV-independent
- Squamous cell carcinoma, NOS

Glandular Tumors and Precursors
- Endocervical polyp
- Müllerian papilloma
- Nabothian cyst
- Tunnel clusters
- Microglandular hyperplasia
- Lobular laminar endocervical hyperplasia
- Mesonephric remnants and hyperplasia
- Arias-Stella reaction
- Endocervicosis (pseudoneoplastic glandular lesions)
- Tuboendometrioid metaplasia
- Ectopic prostate tissue
- Adenocarcinoma *in situ*, NOS
- Adenocarcinoma *in situ*, HPV-associated
- Adenocarcinoma *in situ*, HPV-independent
- Adenocarcinoma, NOS
- Adenocarcinoma, HPV-associated
- Adenocarcinoma, HPV-independent gastric type
- Adenocarcinoma, HPV-independent clear cell type
- Adenocarcinoma, HPV-independent mesonephric type
- Adenocarcinoma, HPV-independent, NOS
- Endometrioid adenocarcinoma, NOS
- Carcinosarcoma, NOS
- Adenosquamous carcinoma
- Mucoepidermoid carcinoma
- Adenoid basal carcinoma
- Carcinoma, undifferentiated, NOS

Mixed Epithelial and Mesenchymal Tumors
- Adenomyoma, NOS
  - Mesonephric type adenomyoma
  - Endocervical type adenomyoma
- Adenosarcoma

Germ Cell Tumors
- Mature teratoma, NOS
- Dermoid cyst, NOS
- Endodermal sinus tumor
- Yolk sac tumor, NOS
- Choriocarcinoma, NOS

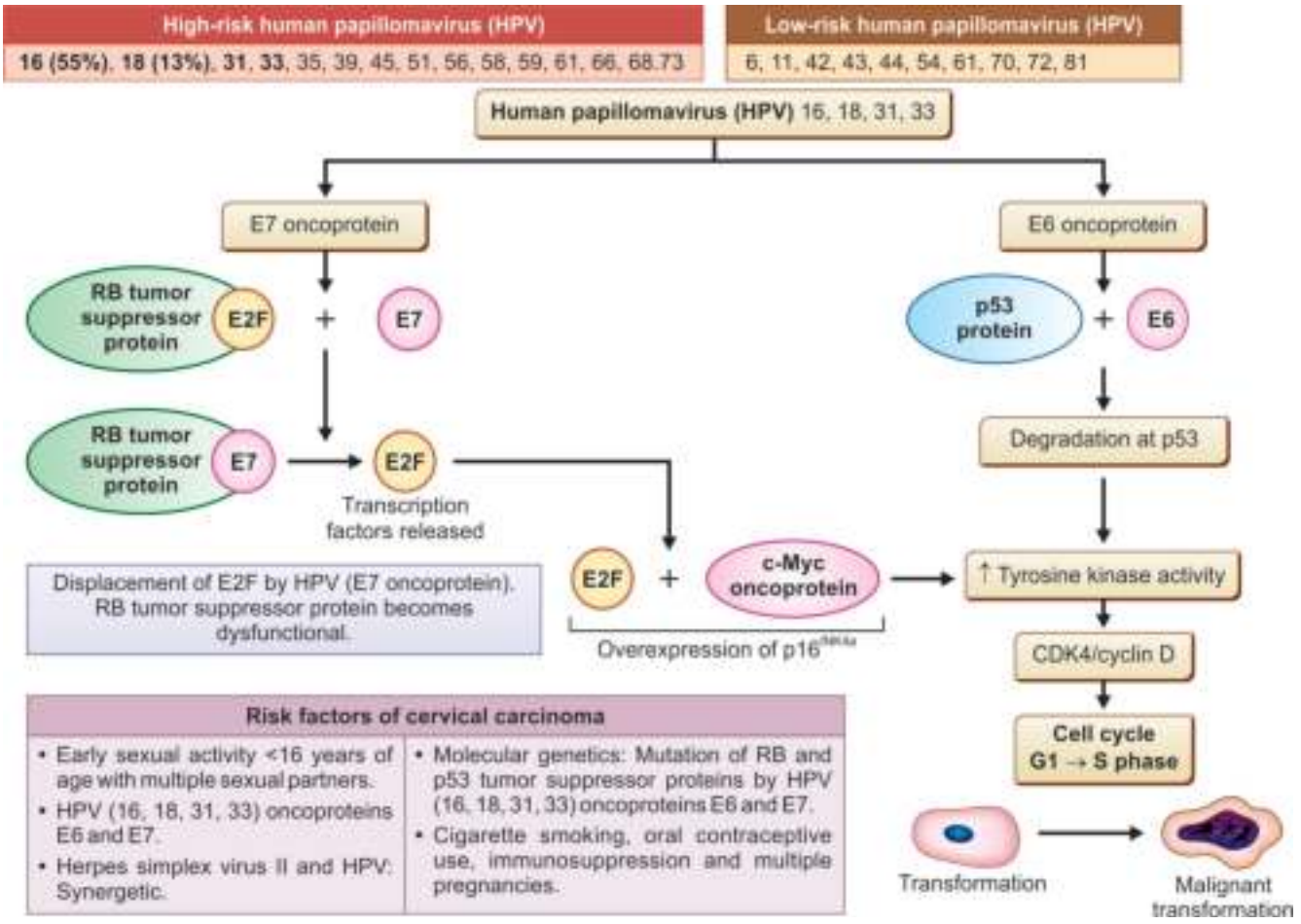


Fig. 24.30: Principles of HPV E6 and E7 oncogene activity in cervical carcinoma.

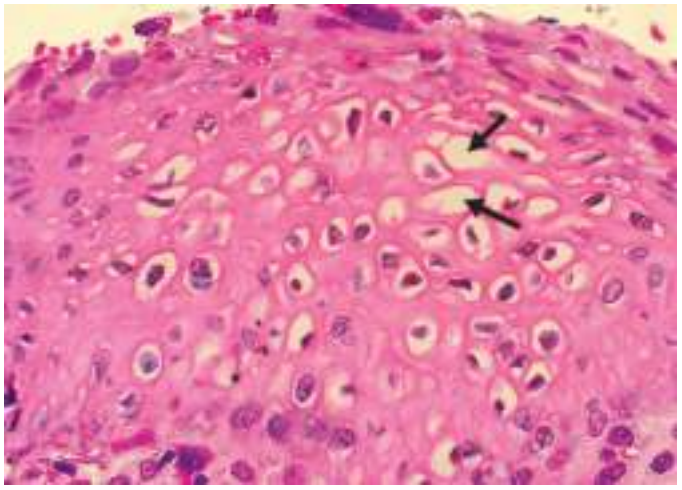


Fig. 24.31: Human papillomavirus in cervix. High power view of HPV infection characterized by cells with pyknotic, irregular nuclei and clear cytoplasm. Moderate squamous dysplasia is also present (arrows) (400X).

Clinical Features

Patient is asymptomatic in early stage of cervical carcinoma. Later, patient presents with abnormal persistent bleeding or spotting between menstrual periods or postcoital bleeding and pain. In advanced stage, patient presents with pelvic pain, foul smelling vaginal discharge (saprophytic infection), vaginal passage of stool or urine from a fistula, anorexia, cachexia, weight loss and anemia. Cytomorphologic features of cervical squamous cell carcinoma are shown in Papanicolaou stained smear (Fig. 24.33).

Surgical Pathology: Cervical Carcinoma

Gross Morphology

Early stage of cervical carcinoma often manifests as poorly defined, granular, eroded lesions or progressing to nodular and exophytic mass (Fig. 24.34).

Light Microscopy

Histologic variants of cervical carcinoma are given in Table 24.8. Invasive squamous cell carcinoma of cervix is microscopically divided into three major categories depending upon the presence or absence of keratinization and the tumor cell size: (a) large keratinizing squamous cell carcinoma (Fig. 24.35), (b) large cell nonkeratinizing squamous cell carcinoma (70%), (c) small cell nonkeratinizing squamous cell carcinoma (5%), and (d) other microscopic types of squamous

cell carcinoma such as verrucous carcinoma (well differentiated variant with a polypoid pattern of growth), spindle cell carcinoma (sarcomatoid carcinoma), basaloid carcinoma, lymphoepithelioma-like carcinoma and transitional carcinoma.

- **Squamous cell carcinoma of cervix:** It accounts for 80–90% of cases. It is further subclassified as large cell nonkeratinizing and large cell keratinizing carcinoma producing keratinizing pearls and prickle cells; and small cell variant of squamous cell carcinoma. In almost all cases, large cell keratinizing squamous cell carcinoma varies from well differentiated to moderately differentiated and highly anaplastic carcinoma.
  - **Well differentiated squamous cell carcinoma (grade 1):** Tumor is composed of mature squamous cells with abundant keratin pearls, occasional well-developed intercellular bridges, minimal pleomorphism, and minimal mitotic activity (Fig. 24.36).
  - **Moderately differentiated squamous cell carcinoma (grade 2):** Tumor is composed of cells with less distinct cell borders and less cytoplasm than well differentiated tumors; and also, more nuclear pleomorphism and more mitotic activity (Fig. 24.37).
  - **Poorly differentiated carcinoma (grade 3):** Tumor is composed of small primitive appearing cells with scant cytoplasm, hyperchromatic nuclei and marked mitotic activity; absent/rare keratinization; resembles HSIL (Fig. 24.38).
- **Adenocarcinoma of cervix:** It accounts for 10–20% of malignant cervical tumors, which arises from mucus producing endocervical glands. It affects elderly women (mean 55 years). It shares etiological factors of squamous cell carcinoma of the cervix and spreads similarly. Malignant cells are frequently infected with HPV types 16 and 18 (Figs 24.39 and 24.40).
- **Adenosquamous carcinoma of cervix:** It accounts for 4% of cervical malignant tumors. It arises from reserve cells in cervix with favorable prognosis (Fig. 24.41).
- **Small cell carcinoma of cervix:** It is rare tumor having more aggressive course than the conventional squamous cell carcinoma (Fig. 24.42).

Immunohistochemistry Technique

Neoplastic cells of squamous cell carcinoma of cervix are immunoreactive to p16, pan-keratin (CK5/CK6), AE1/AE3, CEA, p63, CD10, CD146, inhibin and β-hCG.

Markers	Expression
p16	Positive (100%)
Pan-keratin (CK5/CK6)	Positive (100%)
AE1/AE3	Positive (100%)
CEA	Positive (80%)
p63	Positive (80%)
CD10	Positive (20%)
CD146	Positive (20%)
Inhibin	Positive (20%)
β-hCG	Positive (10%)

Mode of Spread

Cervical carcinoma spreads via direct extension to vagina, uterus, parametrium, lower urinary tract, uterosacral ligaments; via lymphatic route to aortic and mediastinal lymph nodes, and via hematogenous route to lung, bones and ovary (1%). Mode of spread of cervical carcinoma is given in Table 24.9.

- **Direct invasion:** In early stage, local extension of cervical carcinoma into surrounding tissues (parametrium) leads to ureteral compression resulting in hydroureter, hydronephrosis and ultimately renal failure with fatal outcome in 50% of patients. In later stage, urinary bladder and rectal involvement by cervical carcinoma may lead to fistula formation. Spread of tumor along the uterosacral ligaments involves the sacral nerves, causing severe pain. In some cases, tumor growth encircles the 'external os', and obstructs the cervical canal leading to development of pyometra.
- **Lymphatic route:** Cervical carcinoma involves the chains of pelvic nodes (e.g. paracervical, obturator, external iliac, internal iliac, common iliac and aortic lymph nodes). The tumor most commonly extends into contiguous parametrial structures.
- **Hematogenous route:** In later stages of disease, distant metastases may occur in lungs, liver, bones or brain.

Staging and 5-Year Survival

The clinical stage of cervical carcinoma is the most important prognostic factor for survival. Staging of cervical carcinoma has been developed by FIGO as given in Table 24.10.

Prognostic Factors

Prognostic factors for cervical carcinoma include clinical stage, nodal status, size of largest lymph node and number of involved lymph nodes, tumor size, depth of invasion, endometrial extension, parametrial involvement, angiolymphatic invasion.

Laboratory Diagnosis

Various diagnostic methods adopted for cervical carcinoma include Papanicolaou smear, cervical biopsy, colposcopy, colpomicroscopy, colposcopy with directed biopsy, cone biopsy, punch biopsy and immunohistochemistry.

- **Exfoliative cytology:** Cervical carcinoma and its precursors (cervical intraepithelial neoplasia) can be detected by vaginal exfoliative cytology. Papanicolaou smears can be obtained directly or under colposcopic guidance. Positive exfoliative cytology findings need to be confirmed by biopsy. For occult lesions (contact bleeding and vaginal discharge), Papanicolaou smear, colposcopy examination and cervical biopsy are performed.

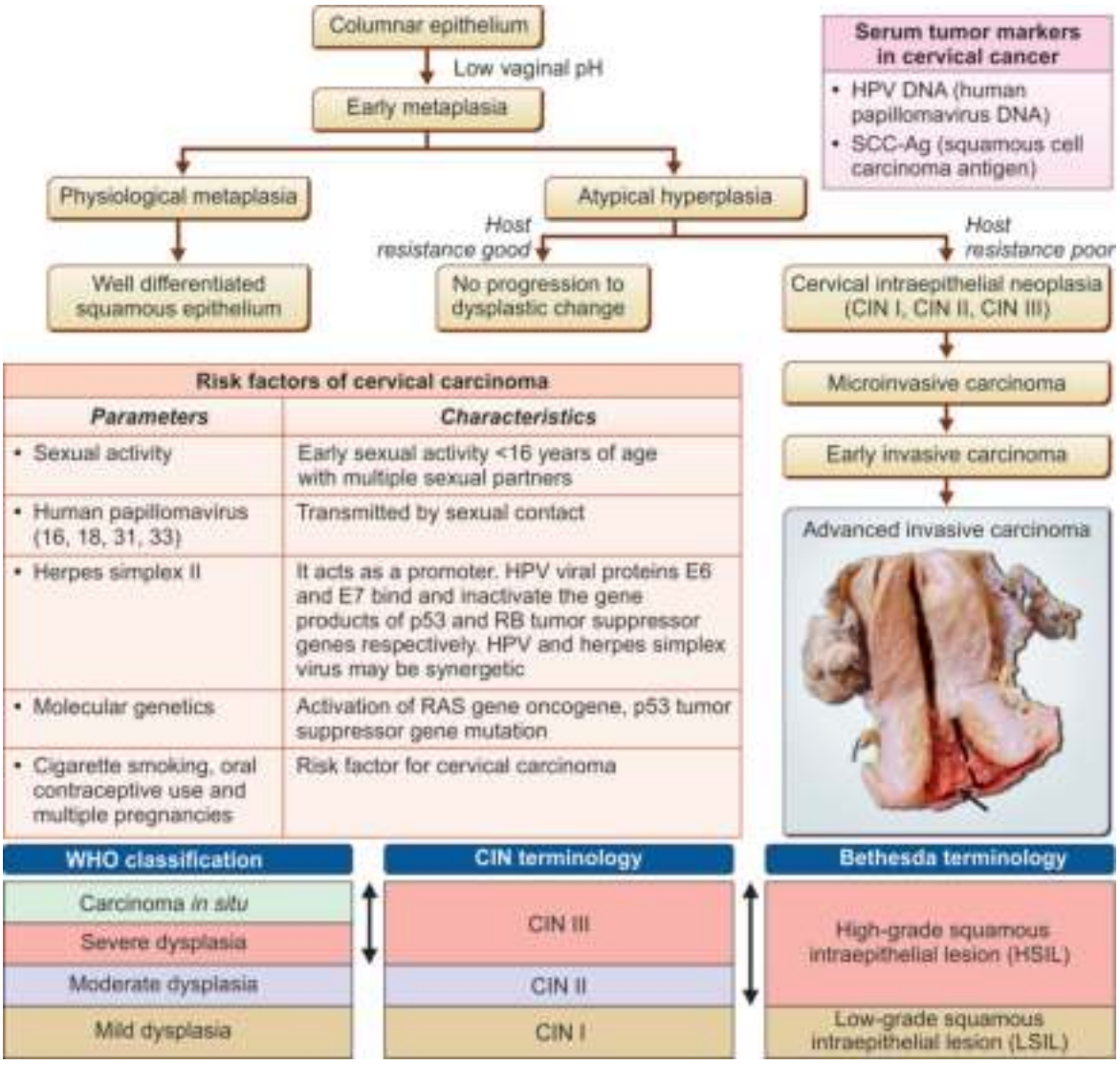


Fig. 24.32: Development of cervical intraepithelial neoplasia (CIN I, CIN II, CIN III) and invasive cervical carcinoma (arrow).

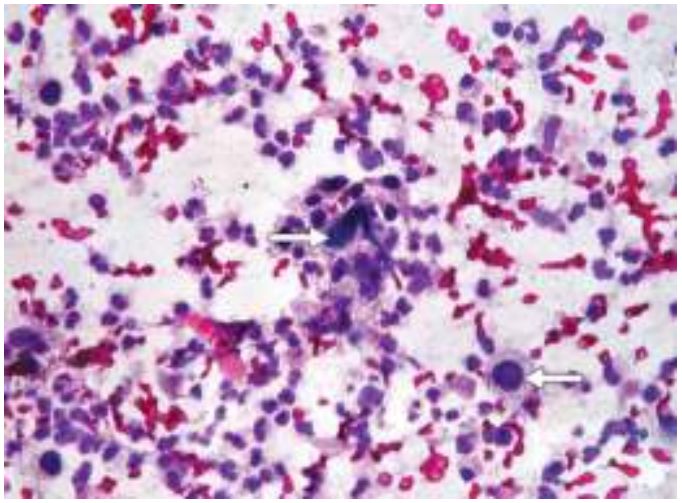


Fig. 24.33: Papanicolaou's stained smear in cervical carcinoma: It shows atypical tadpole cells with squamous differentiation in the background of inflammatory cells (arrows) (400X).



Fig. 24.34: Cervical carcinoma. Growth is seen in cervical region (arrow).

Table 24.8 Histologic variants of cervical carcinoma

Histological Variants	Frequency	Characteristics
Squamous cell carcinoma of cervix	80–90%	▪ Large cell nonkeratinizing squamous cell carcinoma (70%) ▪ Large cell keratinizing squamous cell carcinoma producing keratinizing pearls and prickle cells (25%) ▪ Small cell carcinoma nonkeratinizing carcinoma (5% associated with poor prognosis) ▪ Other microscopic types of squamous cell carcinoma such as verrucous carcinoma (well differentiated variant with a polypoid pattern of growth), spindle cell carcinoma (sarcomatoid carcinoma), basaloid carcinoma, lymphoepithelioma-like carcinoma and transitional carcinoma
Adenocarcinoma of cervix	10–20%	Adenocarcinoma arises from endocervical glands. Malignant cells are frequently infected with HPV 16 and 18
Adenosquamous carcinoma of cervix	4%	Adenosquamous carcinoma arises from reserve cells in cervix. It has favorable prognosis
Small cell carcinoma of cervix	Rare	More aggressive course than the conventional cervical squamous cell carcinoma

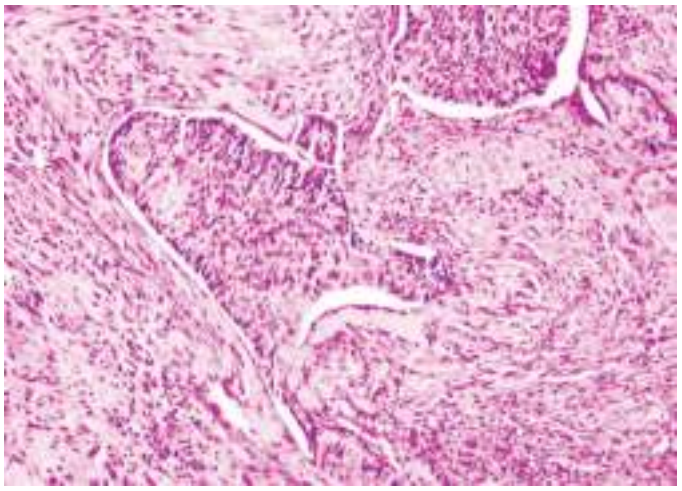


Fig. 24.35: Cervical large cell nonkeratinizing squamous cell carcinoma. Tumor is composed of cells with hyperchromatic nuclei. Cells are devoid of keratinization (400X).

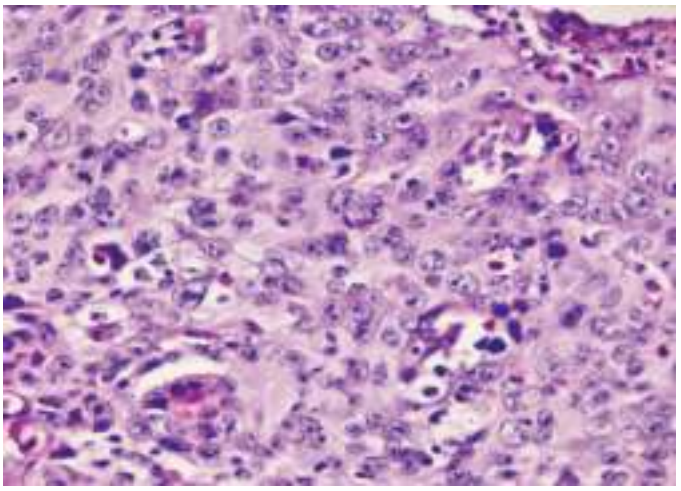


Fig. 24.37: Moderately differentiated squamous cell carcinoma (grade 2) of cervix. Tumor is composed of cells with less distinct cell borders and less cytoplasm than well differentiated tumors; and also, more nuclear pleomorphism and more mitotic activity (400X).

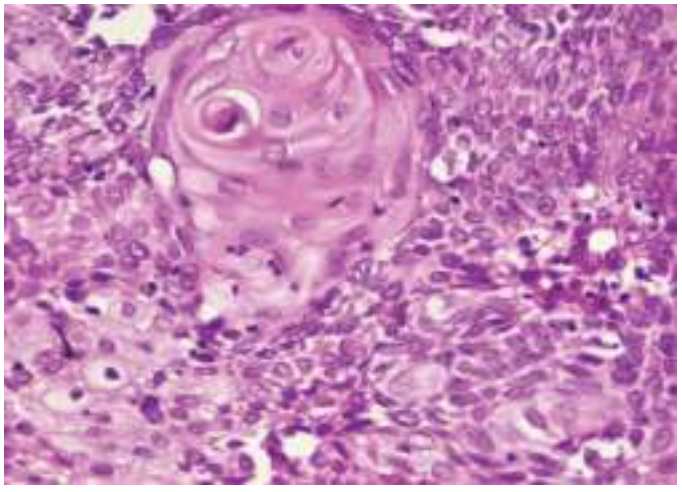


Fig. 24.36: Well differentiated squamous cell carcinoma (grade I) of cervix. Tumor is composed of mature squamous cells with abundant keratin pearls, occasional well-developed intercellular bridges, minimal pleomorphism, minimal mitotic activity (400X).

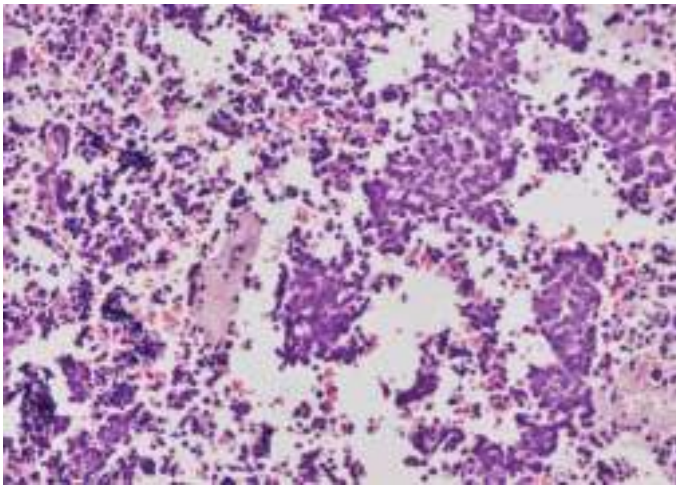


Fig. 24.38: Poorly differentiated carcinoma (grade 3) of cervix. Tumor is composed of small primitive appearing cells with scant cytoplasm, hyperchromatic nuclei and marked mitotic activity; absent/rare keratinization; resembles HSIL (400X).

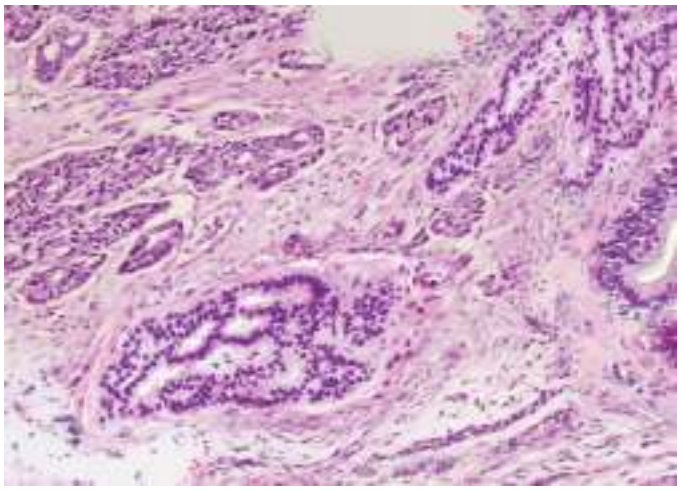


Fig. 24.39: Adenocarcinoma of cervix. Tumor arises from mucus producing endocervical glands is composed of malignant cells arranged in glandular pattern (200X).

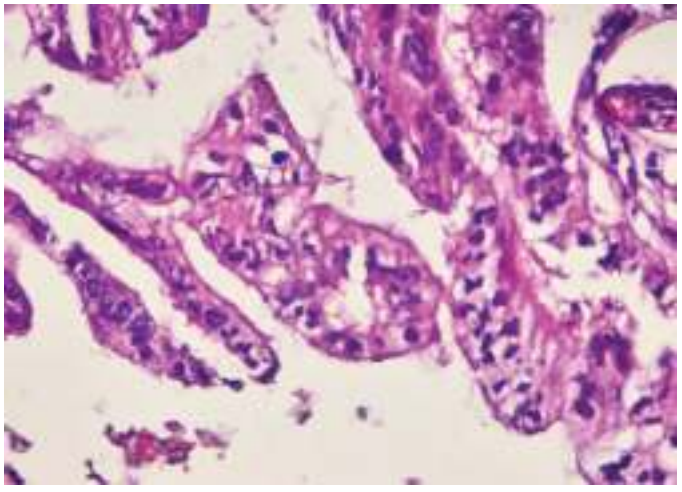


Fig. 24.40: Adenocarcinoma of cervix. Tumor arises from mucus producing endocervical glands is composed of malignant cells arranged in glandular pattern (400X).

Table 24.9 Mode of spread of cervical carcinoma

Mode of Spread	Characteristics
Direct invasion	Tumor invades parametrium and causes compression of ureter resulting in hydroureter, hydronephrosis and ultimately acute renal failure (50%)
Lymphatic route	Pelvic nodes (e.g. paracervical, obturator, external iliac, internal iliac, common iliac and aortic lymph nodes)
Hematogenous route	Distant metastases to lungs, liver, bones and brain

- **Colposcopy:** 3% Acetic acid is applied to cervical transformation zone for visualization of vascular alterations of cervical carcinoma growth.

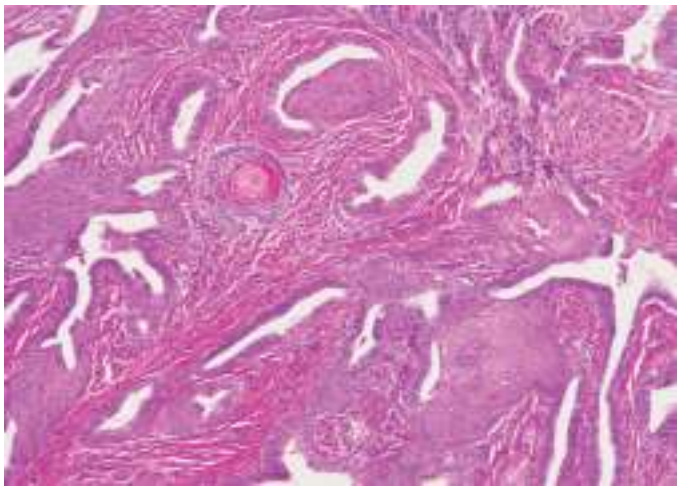


Fig. 24.41: Adenosquamous carcinoma of cervix. It arises from reserve cells in cervix with favorable prognosis. Tumor is composed of glandular and squamous components (400X).

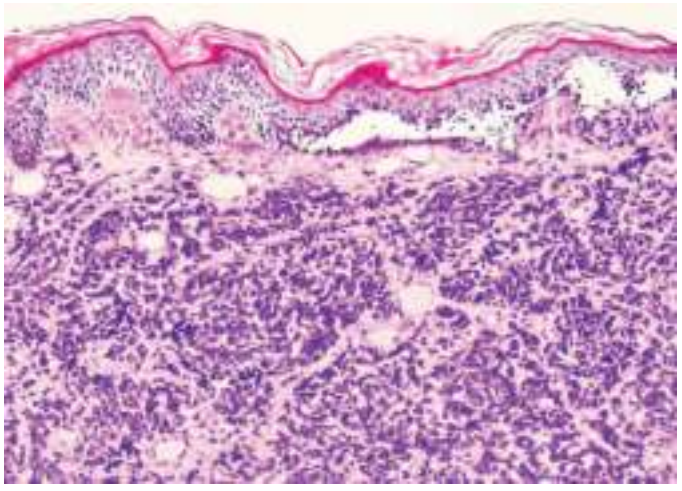


Fig. 24.42: Small cell carcinoma of cervix. Tumor is composed of small atypical cells. It has poor prognosis (400X).

- **Schiller's test:** Normal cervical epithelial cells are rich in glycogen, while absent in malignant cells. Schiller's test is performed by applying Lugol's iodine (a solution of iodine and potassium iodide). Normal cervical epithelial cells give mahogony brown discoloration; while cancerous cells of cervical carcinoma fail to take up the stain.
- **Cervical biopsy:** Punch or wedge biopsy of tumor is done at 12, 3, 6 and 9 o'clock position of cervix.
- **Endocervical curetting:** It is done with cold knife, which is useful in endocervical carcinoma when portio vaginalis appears healthy and cervix feels barrel-shaped.
- **Conization:** It is done with cold knife. It is indicated in following situations: (a) If no visible lesion is seen on colposcopy, (b) if cytologic follow-up is not possible, and (c) if there is discrepancy between vaginal cytology and directed biopsy.

Table 24.10 Staging of cervical carcinoma developed by FIGO

Stage	Cervical Carcinoma Involving Structures	5-Year Survival after Treatment
Stage 0	Cervical carcinoma involves superficial layers of the cervix	100%
Stage I	Cervical lesion (microscopic to 4 cm in diameter)	80–90%
Stage II	Extension from cervical carcinoma into the surrounding cervix area, vagina or tissues surrounding uterus	75%
Stage III	Cervical carcinoma involves lower vagina and/or the pelvic wall. Large growth blocking the outflow of urine through the urethra. Pelvic lymph nodes may be involved	36%
Stage IV	Cervical carcinoma involves urinary bladder, rectum or metastases via hematogenous route to distant organs such as liver, lungs and other organs	10%

- **Fractional curettage:** Fractional curettage also includes assessment of the endocervix, ectocervix, and squamocolumnar transformation zone. It helps to differentiate endocervical carcinoma from endometrial adenocarcinoma. Other indications of fractional curettage include abnormal uterine bleeding: irregular bleeding, menorrhagia, suspected malignant or premalignant condition.
- **Immunohistochemistry:** Immunohistochemical p16 and MIB-1 (Ki-67) markers in the differential diagnosis of squamous and glandular lesions of the uterine cervix is given in Table 24.11.

NEUROENDOCRINE TUMORS OF CERVIX

Neuroendocrine carcinoma of the cervix (NECC) is a rare variant of cervical cancer. It is associated with poor prognosis because there is no standardized therapy for this type of malignancy based on controlled trials. The most common primary treatment for neuroendocrine tumor of cervix is radical surgery combined with chemotherapy either as neoadjuvant or adjuvant chemotherapy and radiotherapy. Histologic and electron microscopic features for distinguishing neuroendocrine tumors of the uterine cervix are given in Table 24.12.

Table 24.11 Immunohistochemical p16 and MIB-1 (Ki-67) markers in the differential diagnosis of squamous and glandular lesions of the uterine cervix

Features	p16	MIB-1 (Ki-67)
LSIL (low-grade squamous intraepithelial lesion) (CIN I)	Positive/negative staining	Positive staining
HSIL (high-grade squamous intraepithelial lesion) (CIN II, III)	Positive staining	Positive staining (full thickness)
AIS (adenocarcinoma *in situ*)	Positive staining	Positive staining
AIM (atypical immature metaplasia)	Negative/positive staining	Negative/positive staining
Reactive squamous or glandular atypia	Negative/positive staining	Positive staining
Tubal metaplasia	Positive/negative staining	Negative staining

CIN: Cervical intraepithelial neoplasia

Table 24.12 Histologic and electron microscopic features for distinguishing neuroendocrine tumors of the uterine cervix

Features	Typical Carcinoid	Atypical Carcinoid Tumor	Small Cell Neuroendocrine Carcinoma	Large Cell Neuroendocrine Carcinoma
Histologic features				
Cellular patterns	Trabecular, insular, sheet-like patterns	Trabecular, insular, sheet-like patterns	Sheets, nests, trabecular and cord-like patterns	Sheets, trabecular, cord-like organ patterns
Mitotic figures	Rare	5–10/10 HPF	10/10 HPF	10/10 HPF
Nuclear atypia	Absent	Moderate atypia	Moderate atypia	Marked atypia
Necrosis	No necrosis	Focal necrosis	Extensive necrosis	Moderate necrosis
Electron microscopy				
Neurosecretory granules	Present	Present	Sometimes present	Present

Neuroendocrine markers are synaptophysin (SYN), chromogranin A (CHR), neuron-specific enolase (NSE) and CD56 (neuronal cell adhesion molecule).

DISORDERS OF UTERINE ENDOMETRIUM

MENSTRUAL CYCLE

The endometrium is the inner epithelial layer along with its mucous membrane of the uterus. It has a basal layer and a function layer. The basal layer contains stem cells which regenerate the functional layer. The functional layer thickens and then it is shed during menstruation in women during reproductive period.

- Endometrial thickness is ≤5 mm in the postmenopausal women with vaginal bleeding.
- A thin endometrium is associated with a lower implantation rate, but no absolute cut off for endometrial thickness exists.
- Normal menstrual cycle is divided into menstrual, proliferative and secretory phases.

MENSTRUAL PHASE

In an ideal 28-day menstrual cycle, menstrual phase lasts 3–4 days and is characterized by glandular and stromal shedding and inflammation. Endometrial thickness is 1–4 mm. Estrogen eventually declines, the corpus luteum involutes, and progesterone level drops, resulting in menstruation. Normal menstrual fluid is non-clotted due to the presence of **fibrinolysin**. A patient with passage of blood clots indicates uterine pathology.

PROLIFERATIVE PHASE

Proliferative phase begins on day 4 of menstrual cycle. Early proliferative phase (days 4–7) is characterized by progressive increase in the thickening of surface endometrium, mitotic activity of endometrial glands and stromal changes secondary to estrogen stimulation.

- In mid-proliferative phase (days 8–10), the surface epithelium becomes columnar, endometrial glands longer and curved, the stroma mildly edematous, and mitotic activity brisk.
- Late proliferative phase (days 11–14) is characterized by undulant surface epithelium, coiled endometrial glands with stratification, high mitotic activity, dense stroma, and appearance of subnuclear vacuoles in fewer than 50% of endometrial glands. Interval epithelium is defined as late proliferative with coiled endometrial glands and subnuclear vacuoles in fewer than 50% of glands.
- During proliferative phase, endometrium becomes **3–4 mm** in thickness. Estrogen is derived from aromatization of testosterone in the granulosa cells of ovaries. Testosterone and 17-ketosteroids (DHEA and androstenedione) are synthesized in the theca interna of ovary under influence of LH.
- Estrogen has a negative feedback on FSH, the later resulting in the LH surge at mid-cycle induction of ovulation. The epithelium is cuboidal, growing taller as ovulation is approached. The glands are simple. The stromal cells are narrow spindles to begin with, become plump.
- Glands especially those of cervical region secrete a thin mucus which helps in guiding spermatozoa towards uterus. Histology of normal proliferative endometrium is shown in Fig. 24.43.

SECRETORY PHASE

Secretory phase starts on the 14th day of menstrual cycle till 21st day under the influence of progesterone hormone. Luteinizing hormone (LH) stimulates ovarian theca interna cells resulting in progesterone synthesis. Progesterone increases the body temperature, which is useful in documenting ovulation. Progesterone also increases salt and water retention and inhibits LH. Endometrium becomes 5–6 mm in thickness.

- During secretory phase, fertilization occurs in the ampullary portion of the fallopian tube. It requires 3 days for the embryo to move through the tube and another 2–3 days to implant in the endometrium (day 21).
- Syncytiotrophoblastic tissue of the developing placenta synthesizes β-hCG and LH analogue. Placenta maintains the production of progesterone by the corpus luteum for 8–10 weeks, after which the placenta takes over hormone production. If fertilization does not take place, the endometrium coupled

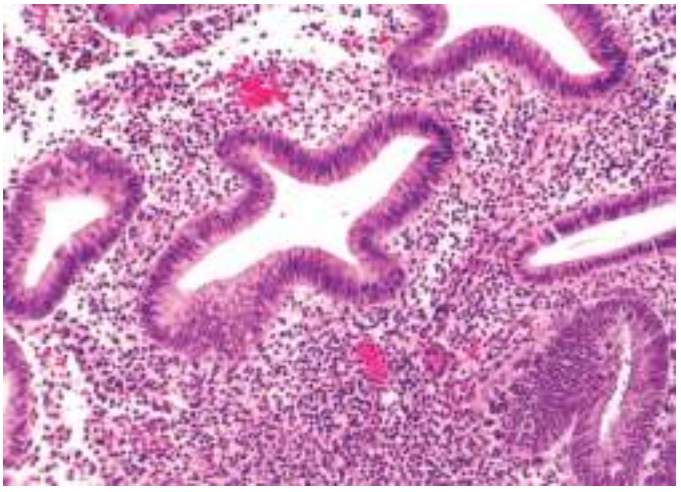


Fig. 24.43: Normal proliferative endometrium. This is the microscopic appearance of normal proliferative endometrium in the menstrual cycle. The proliferative phase is the variable part of the cycle. In this phase, tubular glands with columnar cells and surrounding dense stroma are proliferating to build up the endometrium following shedding with previous menstruation (400X).

with blood and mucus from the vagina and cervix make up the menstrual flow (menstruation) that leaves the body through vagina.

Surgical Pathology: Uterine Endometrium during Menstrual Cycle

Light Microscopy

Endometrial glands in secretory endometrium are dilated, tortuous, lined by tall epithelial cells associated with basal nuclei, clear cytoplasm and subnuclear vacuoles (Figs 24.44 and 24.45).

- **Early secretory endometrium (postovulatory days 2–5):** It occurs soon after ovulation. It is characterized by coiled endometrial glands in which >50% of glandular cells show cytoplasmic vacuoles, which constitute the marker of early secretory phase. The precise dating early secretory endometrium depends on location of cytoplasmic vacuoles (subnuclear or supranuclear) and the degree of mitotic activity.
- **Mid-secretory endometrium (postovulatory days 6–8):** It displays fully coiled endometrial glands lined by cells with round, vesicular nuclei and cytoplasm without vacuoles. Luminal secretion is present. The stroma does not demonstrate predeciduation. More precise dating mid-secretory endometrium depends on degree of stromal edema and extent of glandular luminal secretion.
- **Late secretory endometrium (postovulatory days 9–14):** It is characterized by prominent spiral arteries and appearance of predeciduation. The predeciduation is first observed around spiral arteries and later extends to the superficial stroma. At the ends of the secretory phase, decidual regions become confluent. Interstitial hemorrhage appears at the beginning of menstrual phase. Predeciduation is associated with increase in number of granulocytes in stroma.

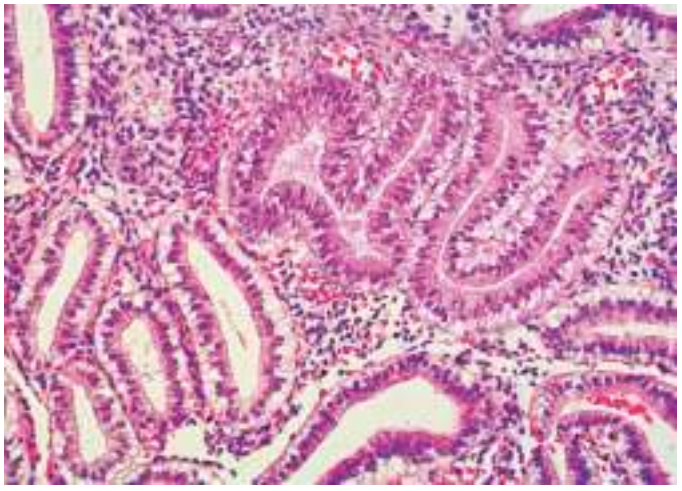


Fig. 24.44: Early secretory endometrium. The appearance with prominent subnuclear vacuoles in cells forming the glands is consistent with post-ovulatory day 2. The histologic changes following ovulation are quite constant over the 14 days to menstruation and can be utilized to date the endometrium (400X).

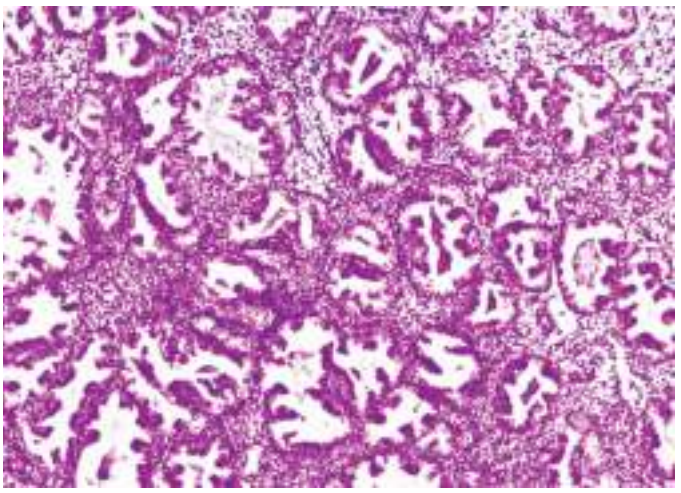


Fig. 24.45: Late secretory endometrium. This is normal secretory phase endometrium. Note the larger tortuous glands with secretions. The secretory phase starts at 14th day course leading to either implantation of a fertilized ovum or menstruation.

MENSTRUAL CYCLE DISORDERS

AMENORRHEA

Amenorrhea is the abnormal absence of menstruation. Amenorrhea occurs during normal pregnancy, lactation, before puberty and after menopause. Amenorrhea may be subdivided into primary or secondary. Primary amenorrhea is the absence of menarche in an adolescent girl after 16 years of age. Secondary amenorrhea is the absence of menstruation for at least 3 months after the normal onset of menarche.

- **Primary amenorrhea:** Imperforate hymen is the most important cause of primary amenorrhea. Dysfunctional hypothalamic-pituitary-ovarian axis causes primary amenorrhea. Turner's syndrome is the most common genetic cause of prepubertal ovarian failure. Anovulation occurs due to deficient synthesis of gonadotropins, estrogens, FSH and LH. Decreased estrogen levels may be secondary to a hypothalamic-pituitary disturbance (low FSH and LH). FSH and LH levels are increased in primary ovarian disorder.
- **Secondary amenorrhea:** Pregnancy and menopause are most common causes of secondary amenorrhea. Hypogonadotropic hypoestrogenic anovulation, endometrial adhesions (Asherman syndrome) and premature ovarian failure cause secondary amenorrhea. Patient presents with absence of menstruation, hirsutism, vasomotor flushes and vaginal atrophy. Acne is observed in secondary amenorrhea.

Laboratory Diagnosis

Radiograph establishes age of the patient. Blood pituitary gonadotropin level, thyroid profile, progesterone,

androgen and FSH levels are analyzed. Histologic examination of cervical mucus is performed to detect 'fern test' (estrogenic effect) to assess ovulation and to detect rupture of membranes. Vaginal secretions are inspected under a microscope for crystallization (ferning pattern), which occurs due to an increased concentration of protein and electrolytes within the fluid.

DYSMENORRHEA

Dysmenorrhea refers to the symptom of painful menstruation. It can be divided into two broad categories: (a) primary dysmenorrhea is defined as cramping pain in the lower abdomen occurring in the absence of inflammatory disease; and (b) secondary dysmenorrhea is resulting from identifiable organic disorders (e.g. endometriosis). Primary dysmenorrhea occurs just before or during menstruation in adolescence. Women with primary dysmenorrhea have increased production of prostaglandins resulting in increased uterine tone and stronger frequent uterine contraction. These cases with dysmenorrhea are treated by nonsteroidal anti-inflammatory drugs with the addition of oral contraceptive pills, when necessary.

DYSFUNCTIONAL UTERINE BLEEDING

Dysfunctional uterine bleeding (DUB) is defined as bleeding not associated with any structural abnormality in uterus and its appendages pelvis. The uterus and its appendages are structurally normal. DUB is presumably related to hormonal changes. The main causes of dysfunctional uterine bleeding include failure of ovulation (anovulatory cycle) and inadequate luteal phase. Causes of vaginal bleeding are given in Table 24.13.

- **Anovulatory cycle (failure of ovulation):** Dysfunctional uterine bleeding is presumably related to hormonal changes. Dysfunctional uterine bleeding may also be caused by neuroendocrine disturbances (e.g. anxiety and anorexia nervosa), severe malnutrition, and debilitating diseases.
- **Inadequate luteal phase:** If the corpus luteum does not secrete progesterone in adequate amounts, the level of progesterone may not be sufficient for full transition of proliferative endometrium to secretory endometrium. Such irregular maturation of the endometrium is usually associated with spotting, premature onset of menstrual bleeding or prolonged vaginal bleeding.

Table 24.13 Causes of vaginal bleeding

Age Group	Causes
Pre-puberty	Unexplained menstrual-like cyclic vaginal bleeding for many days without uterine and vaginal anomaly
Adolescence	Anovulatory cycles
Reproductive period	▪ Complication of pregnancy ▪ Organic lesion ▪ Anovulatory cycle ▪ Dysfunctional uterine bleeding
Perimenopausal women	Anovulatory cycle associated with irregular shedding
Postmenopausal women	▪ Atrophic endometrium ▪ Organic lesion

Pathology

If ovulation does not occur during secretory phase, the endometrium will continue to proliferate until it outgrows its own blood supply. At that point, the surface portion of the endometrium becomes ischemic and starts dying off. This leads to bleeding per vagina, which typically occurs 2 or 3 weeks after the date of the missed menstruation.

Clinical Features

Patient may present with menorrhagia (episodes of vaginal bleeding between menstrual cycles) or heavy or prolonged menstruation persisting for longer than 8 days, fatigue due to anemia and infertility as a result of anovulation.

Laboratory Diagnosis

Ovulatory body temperature monitoring, serum progesterone level and endometrial biopsy are performed in dysfunctional uterine bleeding cases. Patient should be investigated to rule out infections, polyps and cancers of the genital tract.

INFLAMMATORY DISORDERS OF UTERINE ENDOMETRIUM

ACUTE ENDOMETRITIS

Endometritis results from infection of the endometrium, which may be acute or chronic depending on underlying cause.

- Acute endometritis is common and symptomatic common often caused by *Staphylococcus aureus* or *Streptococcus* species, and sexually transmitted diseases (*Chlamydia trachomatis*, *Neisseria gonorrheae*). Acute endometritis is most often associated with intrauterine contraceptive devices, intrauterine trauma from instrumentation, and postpartum retention of placental fragments.
- Acute endometritis is characterized by microabscess formation and neutrophil invasion in the endometrial superficial epithelium, gland lumina, and uterine cavity. '**Cervical stenosis**' may also result in accumulation of pus in uterine cavity known as '**pyometra**'.

CHRONIC ENDOMETRITIS AND INFERTILITY

Chronic endometritis is rather silent without noticeable signs or mild symptoms involving the breakdown of the peaceful co-existence between microorganisms and the host immune system in the endometrium. It is diagnosed by endometrial biopsy.

- Chronic endometrial inflammation can alter endometrial cytokine production and damage endometrial function, resulting in the formation of abnormal patterns of lymphocyte subsets in the endometrium, and induction of altered secretion of paracrine factors, which ultimately may reduce the receptivity of embryos in the endometrium.
- Tuberculosis is most important cause of chronic endometritis in endemic regions. *Actinomyces israelii* infection is also responsible for chronic endometritis due to intrauterine contraceptive device.
- Patient presents with abnormal bleeding, pain, discharge, fever, and infertility.
- Fluid hysteroscopy is another powerful tool for diagnosing chronic endometritis, which demonstrates micropolyps, stromal edema, and focal or diffuse hyperemia.

Surgical Pathology: Chronic Endometritis

Light Microscopy

- Normal endometrium contains lymphocytes and lymphoid follicles but lack plasma cells.
- Presence of plasma cells in the endometrial stroma is diagnostic of chronic endometritis.
- Chronic endometritis is associated with infertility-related conditions such as repeated implantation failure and recurrent miscarriage. Histology of chronic endometritis is shown in Fig. 24.46.

Immunohistochemistry Technique

- Hematoxylin and eosin (H&E) staining in women with infertility and patients with a history of repeated spontaneous miscarriages had a low diagnostic rate.
- Recently, an immunohistochemical (IHC) stain capable of detecting CD38+ and CD138+ plasma cell specific surface antigens have been introduced for the confirmation of the presence of plasma cells inside the endometrium.
- Additional immunohistochemical staining for CD138 in H&E-stained tissue specimens have increased the diagnostic concordance between pathologists.

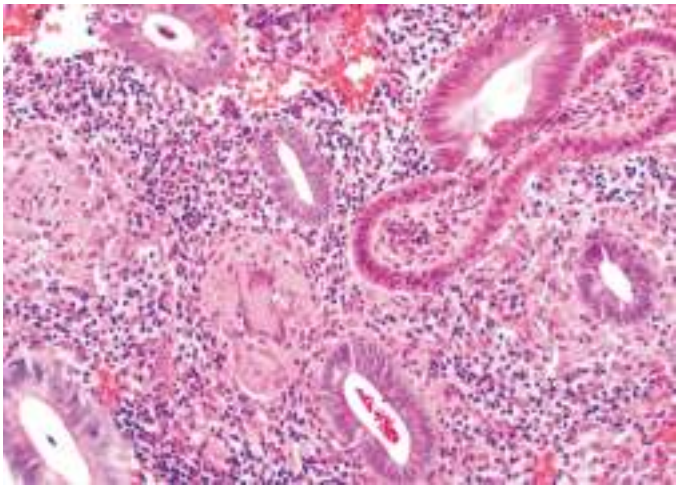


Fig. 24.46: Chronic endometritis. Chronic endometritis can occur in patients with chronic pelvic inflammatory disease, as a postpartum or post-abortion complication, in association with intrauterine devices (IUDs), or with tuberculosis. In a sixth of patients there is no definable cause for chronic endometritis (400X).

NON-NEOPLASTIC DISORDERS OF UTERINE ENDOMETRIUM

ENDOMETRIAL HYPERPLASIA

Endometrial hyperplasia refers to abnormal proliferation of endometrial glands. According to new 2014 WHO classification of endometrial hyperplasia, endometrial hyperplasia has been classified into two categories: (a) Nonatypical endometrial hyperplasia (benign hyperplasia) and (b) typical endometrial hyperplasia or endometrial intraepithelial neoplasia (EIN)/well differentiated carcinoma.

- The new scheme has improved the reproducibility and clearly distinguishes between clinicopathologic entities that are managed differently. Thereby, it should be considered for the diagnosis of endometrial biopsy/curettage specimens.
- Differential diagnosis between benign uterine lesions and atypical hyperplasia/EIN is based mainly on morphologic criteria but may be supported by additional immunohistochemical markers and molecular alterations in problematic cases.
- Endometrial hyperplasia is usually caused by estrogenic stimulation, which occurs in anovulatory menstrual cycles as a result of estrogenic effect not opposed by progesterone. Excess of estrogens also occurs in women with polycystic ovary disease, estrogen-secreting ovarian tumors, i.e. granulosa cell tumor, estrogen replacement therapy and ovarian cortical stromal hyperplasia.
- Endometrial hyperplasia may progress to atypical hyperplasia and ultimately endometrial carcinoma and manifest with postmenopausal bleeding. Young women manifest with menorrhagia and anovulation.

Surgical Pathology: Endometrial Hyperplasia

Gross Morphology

Uterine endometrium is diffusely thickened due to prolonged action of estrogens (Fig. 24.47).

Light Microscopy

Endometrial hyperplasia refers to a spectrum that ranges from simple glandular crowding to conspicuous proliferation of atypical glands, which is often difficult to distinguish from endometrial carcinoma. Estrogen exposure is thought to be a risk factor for both endometrial hyperplasia and endometrial carcinoma. Endometrial hyperplasia is of three types (e.g. simple, complex without atypia and complex with atypia) on histopathologic examination of endometrial biopsy described as under.

- **Simple (cystic) endometrial hyperplasia:** It demonstrates an increased number of dilated glands separated from each other by normal stroma giving '**Swiss cheese pattern**' (Fig. 24.48).
- **Complex (adenomatous) endometrial hyperplasia without atypia:** The irregularly shaped glands appear overcrowded and are surrounded by relatively scant stroma. The glandular epithelium is lined by uniform cells showing no atypia. Only 5% of patients with complex hyperplasia without atypia ultimately progress to endometrial carcinoma in a span of 10 years (Fig. 24.49).
- **Complex (adenomatous) endometrial hyperplasia with atypia:** It is characterized by cytologic atypia and marked glandular crowding with stratification of cells that often protrude into the lumen. Endometrial glands frequently show back-to-back arrangement. Most reliable diagnostic criteria are presence of round rather than oval cells with hyperchromatic nuclei, prominent nucleoli and high nuclear-to-cytoplasmic ratio. Approximately 25% of complex atypical endometrial hyperplasia may progress to endometrial carcinoma in a span of 4 years (Fig. 24.50).

ARIAS-STELLA REACTION

Arias-Stella reaction is an exaggerated hypersecretory response of the endometrium to hCG stimulation (endometrial hyperplasia), which may be observed in normal intrauterine pregnancy and ectopic pregnancy. It may provide initial information of ectopic pregnancy. Arias-Stella reaction can also be observed due to exogenous hormonal therapy, hydatidiform mole, choriocarcinoma and endometriosis.

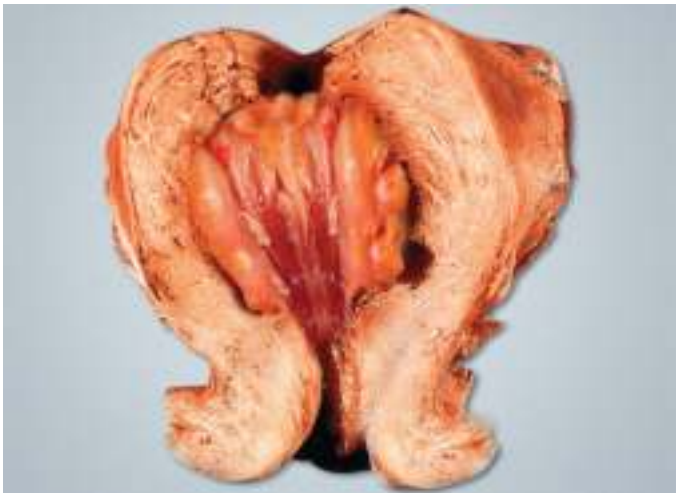


Fig. 24.47: Endometrial hyperplasia. The whole endometrium is involved, rather than there being a discrete polyp. It results with conditions of prolonged estrogen excess. The patient presented with metrorrhagia (uterine bleeding at irregular intervals), menorrhagia (excessive bleeding with menstrual periods), or menometrorrhagia.

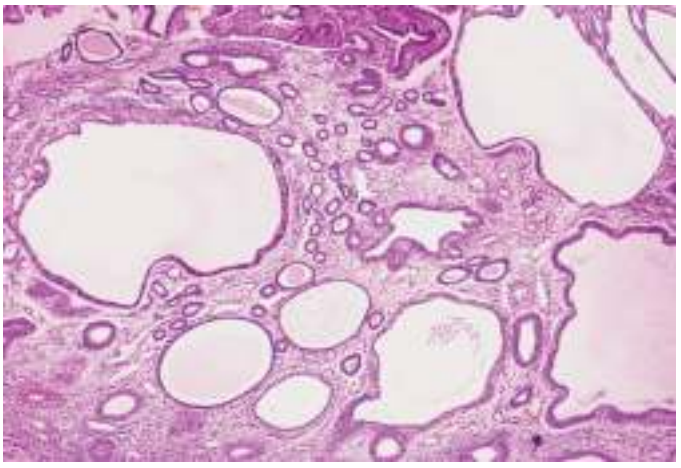


Fig. 24.48: Simple endometrial hyperplasia. This is endometrial cystic hyperplasia in which the amount of endometrium is abnormally increased and not cycling as it should. The glands are enlarged and irregular with columnar cells that lack atypia (100X).

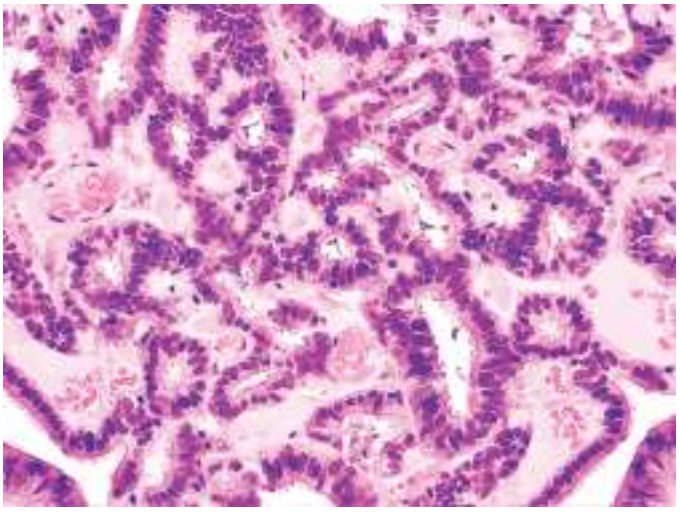


Fig. 24.49: Complex (adenomatous) hyperplasia without atypia. The irregularly shaped glands appear overcrowded and are surrounded by relatively scant stroma. The glandular epithelium is lined by uniform cells showing no atypia (400X).

Surgical Pathology: Arias-Stella Reaction

Light Microscopy

- Endometrial glands are hypersecretory with back-to-back arrangement. The cells of endometrial gland are large with abundant clear or eosinophilic cytoplasm, large hyperchromatic pleomorphic and smudged nuclei.
- There is presence of decidualized stroma. It may provide initial information of ectopic pregnancy (Fig. 24.51).

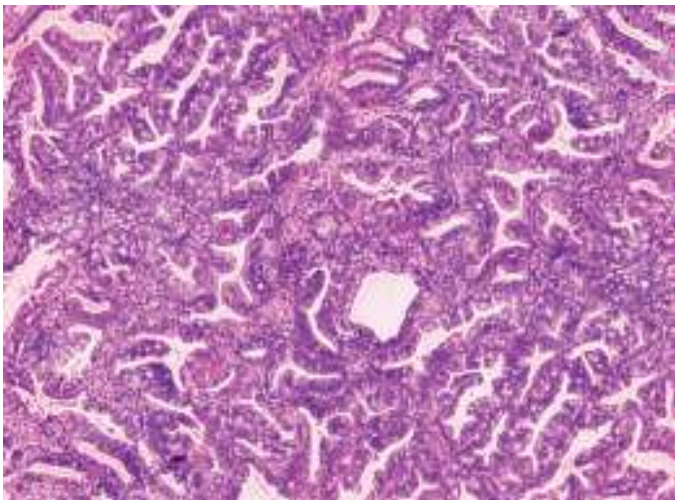


Fig. 24.50: Complex (adenomatous) endometrial hyperplasia with atypia. It shows marked glandular crowding with back-to-back arrangement and stratification of cells that often protrude into the lumen. Cells lining glands are round with hyperchromatic nuclei, prominent nucleoli and high nuclear-to-cytoplasmic ratio (400X).

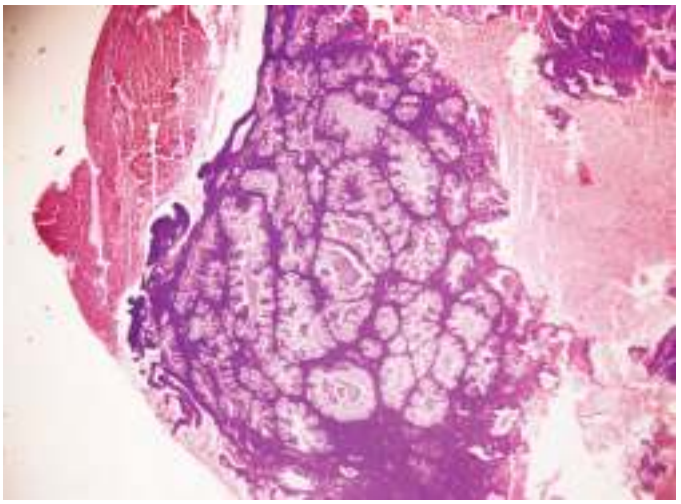


Fig. 24.51: Arias-Stella reaction. Endometrial glands are hypersecretory with back-to-back arrangement. The cells of endometrial gland are large with abundant clear or eosinophilic cytoplasm, large hyperchromatic pleomorphic and smudged nuclei. There is presence of decidualized stroma. It may provide initial information of ectopic pregnancy (400X).

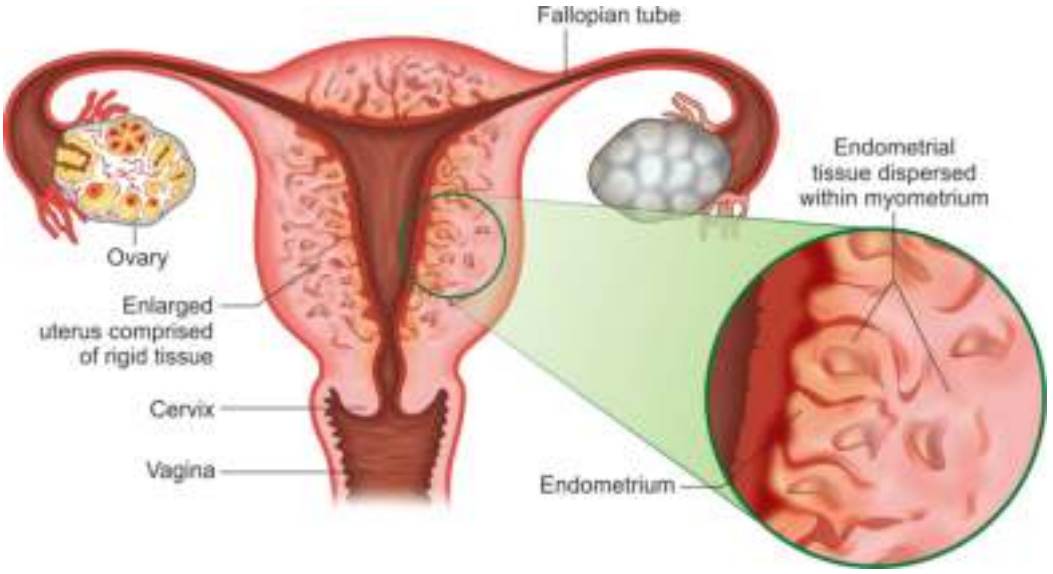


Fig. 24.52: Adenomyosis uterus. The thickened and spongy appearing myometrial wall of this sectioned uterus is typical of adenomyosis. This condition leads to uterine enlargement and irregular bleeding.

ADENOMYOSIS UTERI

Adenomyosis uteri occurs when the endometrial islands that normally lines the uterus grows into the muscular wall of the uterus. Approximately, 15–20% of all resected uteri show some evidence of adenomyosis. The endometrial islands continue to act normally by thickening, breaking down and painful prolonged bleeding during each menstrual cycle. Sometimes, patients remain asymptomatic. However, adenomyosis uteri can cause heavy painful menstrual bleeding during menstrual cycle, severe pelvic pain during menstrual cycle and painful intercourse (dyspareunia).

Surgical Pathology: Adenomyosis Uteri
Gross Morphology
Adenomyosis uteri reveals asymmetrical hyperplasia of uterus. Multiple small hemorrhagic cysts are seen in myometrium (Fig. 24.52).
Light Microscopy
Adenomyosis uteri shows presence of nests of endometrial glands lined by mild proliferative to inactive endometrium, surrounded by endometrial stroma with varying degrees of fibrosis in the myometrium (Fig. 24.53).

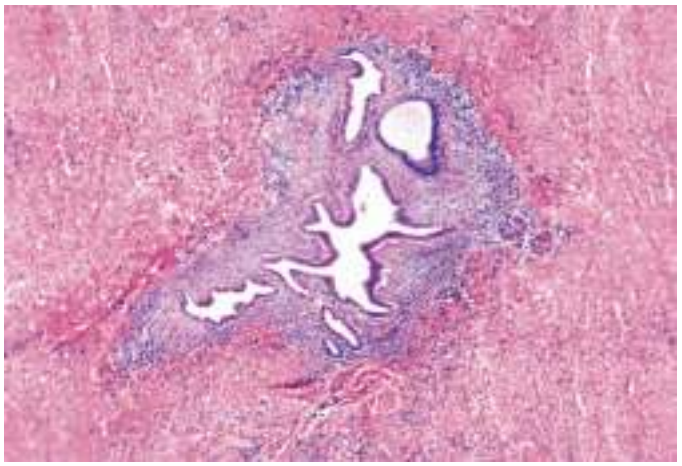


Fig. 24.53: Adenomyosis uterus. Adenomyosis occurs when endometrial glands and stroma are found in the myometrium, not just in the endometrium where they belong. This condition leads to uterine enlargement and irregular bleeding (100X).

ENDOMETRIOSIS

Endometriosis is presence of benign endometrial glands and stroma outside the uterus. It is an important cause of infertility. It affects 5–10% of women of reproductive age and regresses after menopause.

- Endometriosis occurs either by retrograde dissemination of endometrial fragments through fallopian tubes during menstruation, resulting in their implantation on the ovary, or lymphatic-hematogenous dissemination of endometrial fragments.
- Uterine as well as ectopic endometrial glands respond to estrogen and progesterone during each menstrual cycle resulting in sloughing and bleeding in ectopic endometrial glands forming large cysts. It leads to inflammation and fibrosis. Laparoscopy is the gold standard for screening, which shows red-blue to yellow-brown nodules measuring 1–5 mm. In occasional case, the ovarian chocolate cysts are formed measuring 20 cm in diameter.

Locations

The **ovaries** are the most common sites (80%), followed by the uterine ligaments, fallopian tubes, rectovaginal septum, rectosigmoid colon, and pelvic peritoneum (Table 24.14). Since the fimbriae openings of fallopian tubes are in the posterior area of pelvis, most ectopic endometrial glands are generally confined to the pelvic region. Other sites of endometriosis include urinary bladder, lung, pleura, kidneys, extremities and operated scars of abdomen (Fig. 24.54).

Clinical Features

Patient presents with severe menstrual-related pelvic pain due to inflammation and fibrosis in areas around ectopic endometrial glands.

Table 24.14 Locations of endometriosis

Ovaries (most common site in 80%)
Cul-de-sac (common site)
Broad ligament (common site)
Fallopian tubes (fimbriae openings)
Umbilical region
Urinary bladder
Ileum
Abdominal wall operated scar
Vulva
Perineum
Lymph nodes
Kidneys
Liver
Diaphragm

- Pain begins 5–7 days before onset of menstruation, peaks and lasts for 2–3 days. Patient may develop painful sexual intercourse. The inflammation causes fibrosis and adhesions often resulting in infertility.
- Endometriosis in the colon causes abdominal cramps, pelvic pain, painful defecation, constipation and bloody stools.
- Endometriosis in the urinary bladder causes suprapubic pain, dysuria and hematuria.

Surgical Pathology: Endometriosis

Gross Morphology

Menstrual-type bleeding occurs into the ectopic endometrial glands resulting in formation of blood-filled cysts up to 15 cm in diameter containing chocolate-colored material known as 'chocolate cysts' (Fig. 24.55).

Light Microscopy

The diagnosis of endometriosis is based on finding endometrial glands, stroma, and hemosiderin in the stroma on light microscopy of biopsy tissue. Foci of healed endometrium in the form of hemosiderin on the peritoneal surface are referred to as powder burns (Fig. 24.56).

NEOPLASTIC DISORDERS OF UTERINE ENDOMETRIUM

ENDOMETRIAL POLYP

Endometrial polyp most often occurs in the perimenopausal period. It is solitary finger-like benign lesion found in the uterine cavity. Most polyp is diagnosed

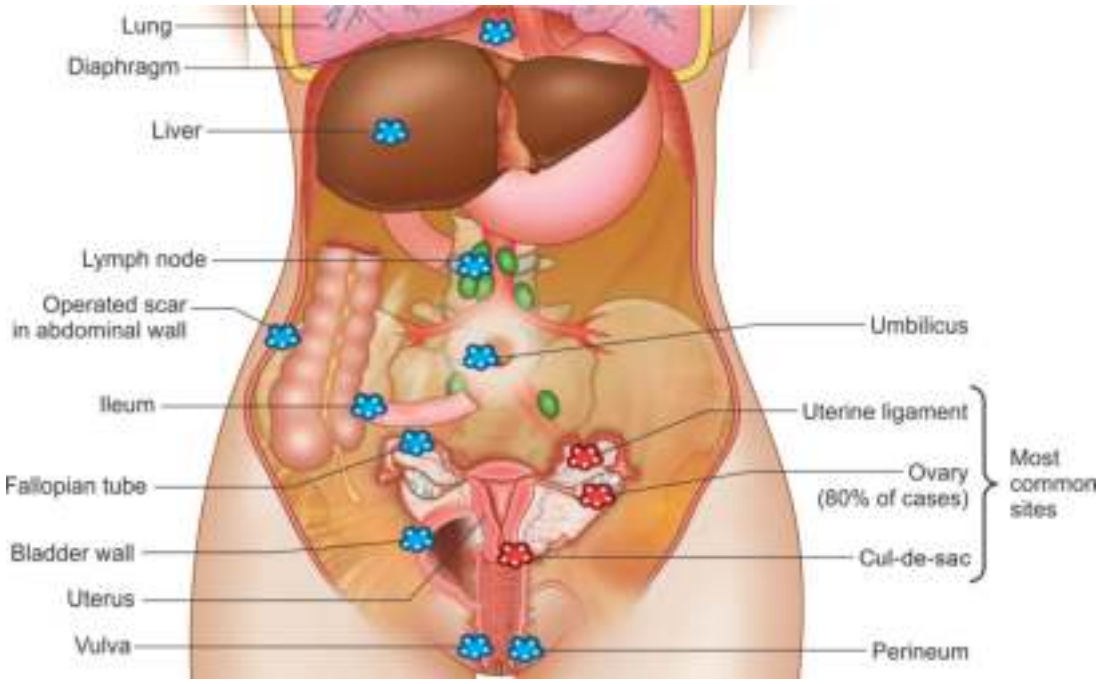


Fig. 24.54: Endometriosis shows some of the sites where endometrial deposits can occur. Red highlights the three most common sites; ovary, uterine ligaments and cul-de-sac.

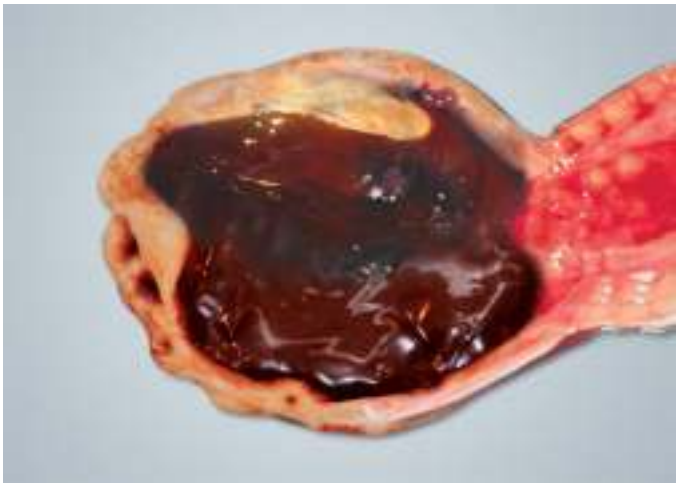


Fig. 24.55: Chocolate cyst in endometriosis. This is a section through an enlarged 12 cm ovary to demonstrate a cystic cavity filled with old blood typical for endometriosis with formation of an endometriotic, or chocolate cyst. The hemorrhage from endometriosis into the ovary may give rise to a large chocolate cyst so named because the old blood in the cystic space formed by the hemorrhage is broken down to produce much hemosiderin and a brown to black color.

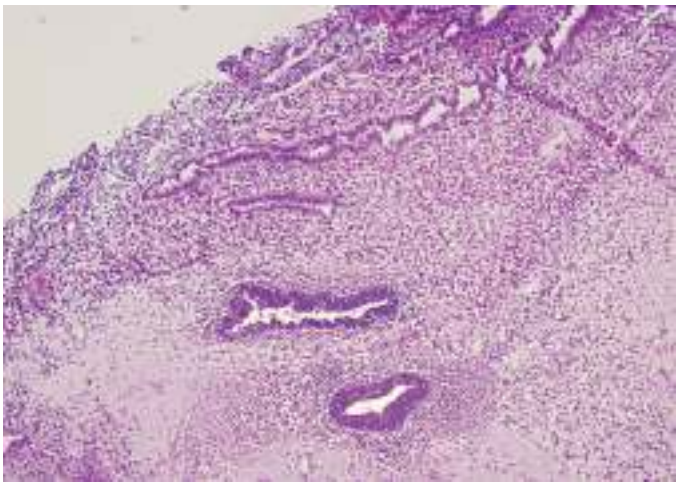


Fig. 24.56: Endometriosis. Endometriosis is derived from the word 'endometrium'. Endometrial glands and stroma that line the uterus grow outside the uterus and affects women during reproductive age. Women may present with infertility, difficulty to have pregnancy, excessive menstrual cramps, heavy menstrual flow, and pain during intercourse. Laparoscopy can be used to diagnose and treat endometriosis (100X).

in perimenopausal women complaining of spotting or irregular bleeding. Polyp is easily removed by curettage. Differential diagnoses of a polypoid lesion of the endometrial cavity include benign endometrial polyp, atypical polypoid adenomyoma, adenosarcoma, polyp with component of carcinoma and carcinosarcoma.

Pathogenesis

It is thought that endometrial foci are hypersensitive to estrogenic stimulation or unresponsive to progesterone. Hence, these endometrial foci do not slough during menstruation and continue to progress. Patient presents with spotting or irregular bleeding during menstrual cycel.

Surgical Pathology: Endometrial Polyp
Gross Morphology
Endometrial polyp protrudes into the uterine cavity. The lesion is believed to arise from endometrial foci that are hypersensitive to estrogen stimulation and that fails to slough with menstruation. Such benign polyp may cause uterine bleeding (Fig. 24.57).
Light Microscopy
Endometrial polyp consists of core covered by surface endometrium. Core of endometrial polyp comprises dilated and hyperplastic endometrial glands embedded in fibrous endometrial stroma containing thick-walled, coiled.

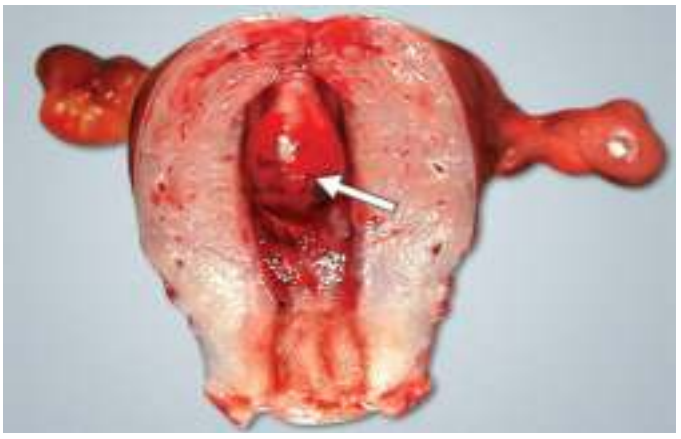


Fig. 24.57: Endometrial polyp protrudes into the uterine cavity. The lesions are believed to arise from endometrial foci that are hypersensitive to estrogen stimulation and that fail to slough with menstruation. Such benign polyps may cause uterine bleeding (arrow).

ENDOMETRIAL CARCINOMA

Endometrial carcinoma is most common gynecologic malignancy, which most frequently arises in the corpus uteri, and may also originate in the lower uterine segment. Well documented conditions known to be associated with an increased risk for endometrial carcinoma include obesity, nulliparity, early menarche, and late menopause. Obesity appears to be the greatest risk factor. Aromatization of androstenedione to estrone, which occurs in the peripheral adipose tissue, appears to be the source of the increased circulating levels of estrogen in obese patients. Diabetes mellitus, hypertension, family history and a high-fat diet and previous radiation have been implicated in the increased incidence of endometrial carcinoma. Tobacco smoking in conjunction with the use of exogenous estrogen significantly multiplies the risk for developing endometrial carcinoma, especially in thin women.

- In low volume disease, it is common to find no evidence of residual disease after diagnostic endometrial curettings. Localized disease (endometrial carcinoma) manifests as round, polypoid expansile masses that are friable and often hemorrhagic. Diffuse involvement of endometrium by endometrial carcinoma may show an indurated-appearing endometrial surface without a visible exophytic component. Foci of myometrial invasion by tumor usually appear grossly as well-demarcated gray-white areas that appears as lighter in color in the surrounding uninvolved myometrium.
- With the revised International Federation of Gynecologists and Obstetrics (FIGO) staging system, this important information may affect the remaining course of the surgical procedure. Intraoperative pathology consultation may be obtained to determine the depth of gross myometrial invasion by endometrial carcinoma or cervical extension. Ideally, the pathologist should measure both the width of the myometrium and depth of the tumor myometrial invasion.
- General principles in the macroscopic appearance, histologic classification, diagnosis and management of endometrial carcinoma are defined in the recent recommendations by the International Society of Gynecological Pathologists. Recent 2020 WHO classification of tumors of the uterine endometrium is given in Table 24.15.
- Incidence of endometrial carcinoma is higher in North America, whereas lower in developing countries and Japan. Incidence of endometrial carcinoma is twice high in white than black population.
- Based on clinicopathological features and molecular genetic features, endometrial carcinoma can be broadly classified into numerous histologic categories on the basis of clinicopathologic features, molecular features and immunohistochemistry: type 1 endometrioid carcinoma and type 2 nonendometrioid carcinoma.
  - **Type 1 endometrioid adenocarcinoma (85%):** It is the most common cell type also known as endometrial adenocarcinoma that occurs in the settings of endometrial hyperplasia, unopposed estrogen stimulation in obese women especially receiving exogenous estrogen in 85% of women. Many studies established that these patients have elevated serum estrogen level.
  - **Type 2 nonendometrioid carcinoma:** It originates in atrophic endometrium, and it is not related to hyperestrogenic state. Salient features of endometrial carcinoma are shown in Fig. 24.58. Comparison of type 1 endometrioid carcinoma and type 2 nonendometrioid carcinoma is given in Table 24.16.
- Histologic variants of type 1 endometrioid carcinoma include villonodular, secretory, ciliated cell, and adenocarcinoma with squamoid differentiation. Overall, the epidemiologic characteristics of the

Table 24.15 Recent 2020 WHO classification of tumors of the uterine endometrium

Endometrial Epithelial Tumors and Precursors
▪ Endometrial hyperplasia without atypia
▪ Atypical hyperplasia of the endometrium
▪ Endometrioid adenocarcinoma, NOS
• POLE-ultramutated endometrioid carcinoma
• Mismatch repair-deficient endometrioid carcinoma
• TP53-mutant endometrioid carcinoma
• No specific molecular profile (NSMP) endometrioid carcinoma
▪ Serous carcinoma (aggressive type)
▪ Clear cell adenocarcinoma, NOS (aggressive type)
▪ Carcinoma, undifferentiated, NOS
▪ Mixed cell adenocarcinoma
▪ Mesonephric adenocarcinoma
▪ Squamous cell carcinoma, NOS (aggressive type)
▪ Mucinous carcinoma, intestinal type (low-grade type)
▪ Mesonephric-like adenocarcinoma
▪ Carcinosarcoma, NOS
Tumor-like Lesions
▪ Endometrial polyp
▪ Endometrial metaplasia
▪ Arias-Stella reaction
Mixed Epithelial and Mesenchymal Tumors
▪ Adenomyoma, NOS
▪ Adenosarcoma
▪ Atypical polypoid adenomyoma
Miscellaneous Tumors
▪ Primitive neuroendocrine tumor, NOS
▪ Germ cell tumors, NOS
• Yolk sac tumor
• Mature teratoma, NOS
• Immature teratoma, NOS

1. Serous adenocarcinoma, mucinous adenocarcinoma, undifferentiated carcinoma and de-differentiated carcinoma are associated with poor prognosis.

2. Recent molecular genetic analysis supports that both components in these biphasic mixed type carcinomas are clonally derived from a transformed epithelial cell.

3. Clear cell adenocarcinomas share features with type 1 endometrioid carcinoma and serous endometrial carcinoma type 2.

histologic types are similar to typical type 1 endometrioid carcinomas. Histologic variants of type 2 nonendometrioid carcinoma include serous, clear cell, squamous and undifferentiated. There is no distinctive gross appearance to differentiate individual histologic subtypes of endometrial carcinoma. Some clear cell endometrial carcinomas share the features of type 1 endometrioid carcinoma and type 2 nonendometrioid carcinoma.

- Prognostic factors in endometrial carcinoma limited to the uterine corpus include: histologic type, tumor grade, lymphovascular invasion and lymph node involvement.
  - Depth of myometrial invasion by endometrial carcinoma has proven to be an independent predictor of prognosis in early-stage endometrial carcinoma. Increasing depth of myometrial invasion (inner third, middle third, inner third) is associated with a greater probability of lymph node metastases and mortality from tumor.
  - FIGO divides depth of myometrial invasion by endometrial carcinoma into halves. Ideally, total width of the myometrium should be measured and reported in addition to the measurement of the thickness of tumor invasion. Determining the depth of myometrial invasion by endometrial carcinoma may be complicated when foci of adenomyosis are involved.
  - Involvement of the cervix by endometrial carcinoma has adverse effects on prognosis. Vascular invasion is an important predictor of recurrence of the disease and its presence should always be mentioned in the report by pathologist. Metastatic tumors involving endometrium are uncommon. The most common primary sites are breast, stomach, colon and melanoma.
- Total abdominal hysterectomy with bilateral salpingo-oophorectomy is standard treatment of endometrial carcinoma. Regional lymph node dissection is performed in patients with type 1 endometrioid carcinoma, FIGO 2 or 3 high-grade histologic type, tumor >2 cm or with myometrial invasion on imaging or suspicious lymph nodes. Radiation therapy is indicated in patients with FIGO Ib or with local recurrence. Chemotherapy is indicated for high-risk early stage or advanced stage endometrial carcinoma, recurrences and distant metastases.

Type 1 Endometrioid Carcinoma

Type 1 endometrioid carcinoma is a glandular neoplasm displaying a glandular (acinar), papillary or a mixture of two, or partly solid configuration. Type 1 endometrioid carcinoma is considered to be estrogen dependent with a well-defined precursor lesion—atypical endometrial hyperplasia/endometrial intraepithelial neoplasia (AEH/EIN). Continuous unopposed estrogenic stimulation leads to progression of hyperplasia without cytologic atypia to AEH/EIN. Some studies revealed that AEH/EIN emerges as a clonal process that begins as a localized lesion usually in a background of endometrial hyperplasia without cytologic atypia. Risk for type I endometrioid carcinoma is increased associated with hereditary syndromes such as Cowden syndrome and Lynch syndrome (hereditary non-polyposis colon cancer).

Stage IV

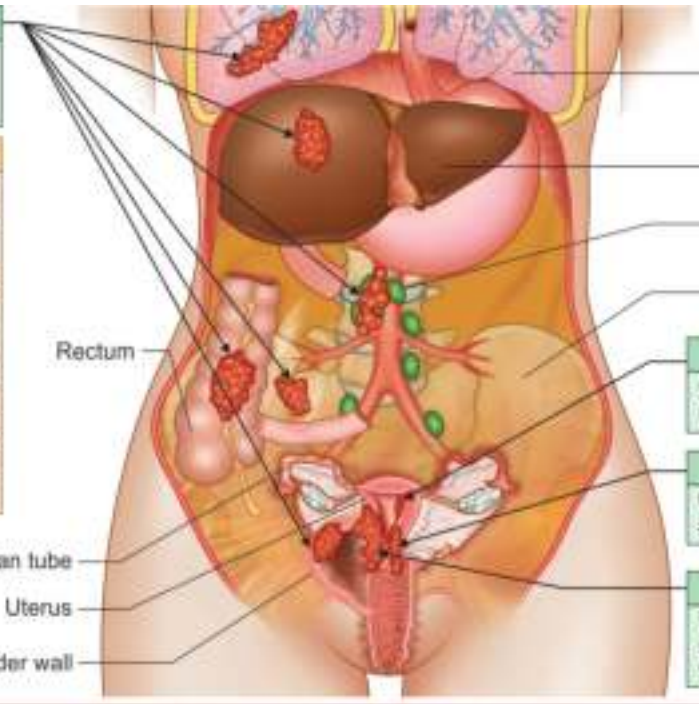
Invasion of bladder or rectum or metastasis to
- Bones
- Liver
- Lungs
- Brain (40%)
- Lymph nodes

Endometrial carcinoma: Work up

- **Clinical features:** Abnormal uterine bleeding, vaginal discharge, constitutional symptoms (weight loss, anemia).
- **Laboratory diagnosis:** Papanicolaou smear abnormalities, hysteroscopy, assessment of endometrial thickening, imaging techniques, biochemical and hematological tests. Endometrial tissue sampling, histologic examination (histologic type and tumor grade), immunohistochemistry and molecular testing (TP53 gene alteration is wild type in type 1 endometrioid carcinoma but mutant type in type 2 nonendometrioid carcinoma).



Endometrial carcinoma



Stage I
Confined to corpus (90%)

Stage II
Confined to corpus and cervix (50%)

Stage III
Extends outside uterus but confined to pelvis (30%)

Endometrial carcinoma: Histologic Variants

- **Type 1 endometrioid carcinoma:** It is linked to prolonged unopposed estrogen stimulation in women with prolonged reproductive period. Risk factors leading to hyperestrogenism include obesity, tamoxifen therapy in breast cancer, ovarian cortical hyperplasia, polycystic ovarian syndrome and hormone producing ovarian granulosa cell tumor. This histologic variant shows wild type alterations in TP53 gene. It is associated with good prognosis.
- **Type 2 nonendometrioid carcinoma:** It is not linked to estrogenic stimulation. It most often occurs in elderly women with atrophic endometrium. Histologic variants include papillary serous followed by serous, mucinous, clear cell and small cell. Serous carcinoma is characterized by early alterations mutant type in TP53 gene. Some type 2 nonendometrioid carcinoma behave more aggressively than type 1 endometrioid carcinoma. Clinical stage 1 disease often has extrauterine metastasis at the time of diagnosis.

Type 1 Endometrioid carcinoma: Biomarkers

- **Proteins:** Serum CA-125 and urinary gonadotropin levels.
- **Oncogenes:** K-RAS, HER2/neu, EGFR, PI3KCA and FGFR2.
- **Tumor suppressor genes:** PTEN, TP53 (wild type spontaneous mutation without first being affected by a mutagen), p21 and CDKN2A.
- **Mismatch repair genes:** hMLH1, hMSH2, hMSH6, PMS1 and PMS2.
- **Index of cell proliferation:** Ki-67.
- **Apoptosis related genes:** Proapoptotic BCL-2 associated X protein (BAX) and BCL-2 apoptosis suppressor gene.
- **Receptors status:** ER and PR.
- **Angiogenesis indices:** Microvascular density and VEGFR.
- **Invasion and metastasis:** E-cadherin, P-cadherin and β-catenin, and ploidy and aneuploidy of DNA.

Note: *Alteration in TP53 in type 1 endometrioid carcinoma is wild type spontaneous mutation without first being affected by a mutagen. On the contrary, alterations in TP53 gene in type 2 nonendometrioid carcinoma is mutant type.*

Mode of spread of endometrial carcinoma

Mode of spread	*Organs involved*
1. Local invasion	Fallopian tube, myometrium, cervix, vagina and urinary bladder
2. Tubal spread	Fallopian tube, ovaries and peritoneum
3. Lymphatic route	Pelvic lymph nodes (e.g. internal iliac and para-aortic lymph nodes), but some tumor cells may skip the pelvic lymph nodes; and involve peri-aortic lymph nodes.
4. Hematogenous route	Lungs, liver, bone and brain

Fig. 24.58: Salient features of endometrial carcinoma. It illustrates types of endometrial adenocarcinoma, mode of spread and staging (approximate five-year survival percentage is shown for various stages).

- The degree of cytologic atypia, the amount of nuclear stratification and the extent to which glands coalesce into foci of solid tumor growth increase as the grade increases. Type 1 endometrioid carcinoma can be graded: (a) grade 1 tumor grows in a glandular or papillary pattern with 5% of solid tumor growth, (b) grade 2 tumor contains 5–50% solid tumor growth, and (c) grade 3 tumor shows >50% solid

Table 24.16 Comparison of type 1 endometrioid carcinoma and type 2 nonendometrioid carcinoma

Parameters	Type 1 Endometrioid Carcinoma	Type 2 Nonendometrioid Carcinoma
Clinical settings		
Incidence	85%	15%
Unopposed action of estrogen	Present (elevated estrogen level)	Absent (low estrogen level)
Menopausal status	Premenopausal and perimenopausal obese, hypertensive and diabetic women (second to eighth decade with mean 60 years)	Postmenopausal (thin, normotensive and nondiabetic) women
Precursor lesion	Atypical endometrial hyperplasia	Atrophic endometrium
Histologic features		
Histological subtype	Endometrioid carcinoma resembles normal endometrium	Serous or clear cell carcinoma
Tumor grade	Low-grade tumor	High-grade tumor
Myometrial invasion	Variable, often minimal	Variable, often deep
Clinical course		
Behavior	Indolent behavior	Aggressive behavior
Prognosis	Good	Poor
Molecular genetic alterations		
ER/PR	Positive	Negative
TP53 gene mutation	Present (10%)	Present (90%)
Microsatellite instability (MSI) caused due to defects in DNA mismatch repair genes, mainly MLH1, MSH2, MSH6 and PMS2	Present (20–30%)	Present (11%)
PTEN mutation	Present (34–83%)	Present in subset of tumors
K-RAS mutation	Present	Absent
P13K mutation	Present	Present (subset of tumors)
FGF-2 mutation	Present	Absent
β-catenin mutation	Present	Absent
ARID1A (regulator of chromatin)	Present	Present
FBX W7 (regulator of Myc cyclin E)	Absent	Present
CHD4 (regulator of chromatin)	Absent	Present
Immunohistochemistry		
Markers	**Expression**	**Expression**
ER/PR (estrogen/progesterone receptors)	Positive	Positive (50%)
Ki-67 proliferative marker	Low expression	High expression
p53	Negative/positive expression (patchy/rare weakly cells)	Positive expression (strong diffuse staining)
p16	Negative/positive (staining patchy)	Positive (strong diffuse staining)
HNF-1β	Negative expression	Negative expression
WT1	Negative expression	Negative/positive expression

Both type 1 and type 2 endometrial carcinomas spread by local invasion, lymphatic route and hematogenous route. In addition, spreads by intraperitoneal spread occurs in type 2 endometrial carcinoma.

tumor growth. Areas of squamous or spindle cell differentiation are not counted in the determination of percentage of solid tumor growth.

- Well differentiated endometrioid carcinoma (grade 1) is composed of back-to-back glands in glandular or papillary patterns without intervening fibrovascular

stroma. The glands and papillae (with or without psammoma bodies) are lined by columnar cells with amphophilic to basophilic cytoplasm, and basally located round to oval nuclei with mild pleomorphism, which may contain nucleoli. Stromal foam cells are generally identified in well-differentiated endometrioid carcinoma, especially associated with endometrial hyperplasia. Although the endometrial glands within focus of complex endometrial hyperplasia are irregular and complex in configuration, intervening stroma is present between the glands. This histologic feature is important in distinguishing complex endometrial hyperplasia from well-differentiated endometrioid carcinoma.

- High-grade endometrioid carcinoma (grade 3) with significant solid component displays an increased amount of nuclear cytologic atypia, as demonstrated by pleomorphic, irregular chromatin clumping, and prominent nuclei. The degree of tumor necrosis is usually mild to moderate. Stroma adjacent to the invasive adenocarcinoma exhibits desmoplastic reaction, often with an accompanying lymphocytic infiltrate.
- Histologic variants of type 1 endometrioid carcinoma include villonodular (papillary), secretory, ciliated cell and adenocarcinoma with squamous differentiation.
  - **Villonodular carcinoma:** It is characterized by a papillary architecture with delicate fibrovascular stroma lined by cuboidal to columnar cells with minimal cellular stratification and mild nuclear pleomorphism with indistinct nucleoli. It is important to distinguish this well-differentiated papillary variant of endometrial carcinoma lacking cellular budding and high-grade cytologic nuclear atypia from serous carcinoma, which is often papillary but displays grade 3 nuclear cytologic atypia with vesicular nuclei, prominent nucleoli and necrosis.
  - **Secretory carcinoma:** It is composed of well-differentiated endometrial glands, which resemble secretory phase of endometrium with prominent vacuoles in subnuclear or supranuclear region of columnar cells and intraluminal secretions.
  - **Ciliated cell carcinoma:** It is composed of neoplastic glands lined by columnar cells showing mild to moderate nuclear cytologic atypia.
  - **Endometrioid carcinoma with squamoid differentiation:** It is composed of polygonal cells with abundant eosinophilic cytoplasm forming a nodular structure between neoplastic endometrial glands.
  - **Mucinous carcinoma:** It is generally well-differentiated low-grade tumor and characterized by columnar cells with basally located nuclei and mucin rich cytoplasm.

Etiopathogenesis

- **Hormonal imbalance:** Type 1 endometrioid carcinoma occurs due to prolonged unopposed estrogenic stimulation in women with prolonged reproductive period (early menarche and late menopause), infertile women, or estrogen hormonal replacement therapy in the setting of endometrial hyperplasia in obese and diabetic premenopausal or perimenopausal women in 85% of cases. Risk of type 1 endometrioid carcinoma is increased in women with estrogen-producing tumors (e.g. granulosa cell tumors of ovary) and familial endometrial carcinoma. Light microscopy shows well differentiated endometrial adenocarcinoma. It is associated with relatively good prognosis.
- **Constitutional factors:** Obesity, like estrogen replacement therapy is a well-defined risk factor for type 1 endometrioid carcinoma. In obese women, adipose tissue converts androgens into estrogens, fueling the proliferation of endometrial tissue. There is lower concentrations of sex hormone-binding globulins in obese women. Diabetes mellitus and hypertension have been reportedly associated with an increased risk of type 1 endometrioid carcinoma.
- **Dietary factors:** Endometrial carcinoma risk is correlated with sedentary lifestyle and total caloric intake, total protein intake, and frequency of consumption of oils, fats, milk and eggs. These factors are major determinants of obesity. A recent study suggests that physical activity decreases the risk of endometrial carcinoma independent of body weight.

Molecular Genetic Alterations: Type 1 Endometrioid Carcinoma

Atypical endometrial hyperplasia/endometrial intraepithelial neoplasia (AEH/EIN) contains many genetic alterations in type 1 endometrioid carcinoma, which include PTEN, TP53, microsatellite instability, K-RAS, CTNNB1 and other mutations.

- **PTEN gene mutation:** Recent studies have shown that most frequent altered gene implicated in the development of endometrioid carcinoma is the PTEN tumor suppressor gene located on chromosome 10q23.3 in 30–50% of cases. PTEN protein negatively regulates the PI3K-AKT signaling. PTEN encodes a dual-specificity phosphatase. The primary target is the lipid molecule phosphatidylinositol-3,4,5-triphosphate (PIP3) that regulates the cell cycle and survival through signal transduction pathway.
- **TP53 gene mutation:** TP53 gene provides instructions for making a DNA-binding phosphoprotein called tumor suppressor protein p53. This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing too fast or in an uncontrolled way. Mutation of TP53 is demonstrated in 10% of type 1 endometrioid carcinomas mostly in grade 3 tumors and

occasionally in grade 2 tumors and absent in grade 1 tumor. This finding suggests that TP53 gene is involved in progression of tumor rather than initiation of type 1 endometrioid carcinoma.

- **Microsatellite instability:** Another molecular genetic alteration in type 1 endometrioid carcinoma is the molecular phenotype called microsatellite instability.
  - Microsatellite instability refers to alterations in the length of short, repetitive DNA sequences called microsatellites, in tumors when compared to DNA prepared from the same patient's normal tissue.
  - Microsatellite instability has been demonstrated in hereditary nonpolyposis colorectal cancer (HNPCC), or Lynch syndrome. Lynch syndrome is characterized by development of adenocarcinoma of the endometrium, colon, and breast. Endometrial carcinoma may develop in these patients before gastrointestinal malignancies.
  - Strong family history in women >50 years of age should prompt investigation for mismatch repair defects.
  - Microsatellite instability is also demonstrated in 20% of sporadic endometrial carcinomas and complex atypical hyperplasia.
  - Unlike the familial cases, which occur due to germline mutations in one of the DNA mismatch repair genes (MSH2, MLH1, MSH6 or PMS2), microsatellite instability in the sporadic cases is most commonly due to inactivation of MLH1 gene by promoter hypermethylation.
- **K-RAS proto-oncogene mutation:** Normally, K-RAS proto-oncogene encodes a guanine nucleotide-binding protein, that plays a role in the regulation of cell growth and differentiation by transducing signals from activated transmembrane receptors. K-RAS proto-oncogene mutation has been demonstrated in all grades of type 1 endometrioid carcinoma and complex atypical complex hyperplasia of endometrium.
- **FGFR2 (fibroblast growth factor receptor 2) mutation:** Recently, mutation in FGFR2 has been demonstrated in type 1 endometrioid carcinoma.
- **ARIDIA (regulator of chromatin) gene mutation:** ARIDIA gene encodes ARIDIA, a member of the SW1/SNF chromatin remodeling complex. Mutation of ARIDIA gene induces changes in expression of multiple gene (CDKNIA, SMAD3, and PIK3IPI) via chromatin remodeling dysfunction in association with p13K/AKT pathway contributes in the pathogenesis of type 2 serous endometrial carcinoma as well as type 1 endometrioid carcinoma.
- **Amplification of other oncogenes:** Amplification of other genes such as EGFR, HER2/neu, c-Myc, BCL-2 and CFMS have been demonstrated in type 1 endometrioid carcinoma. Further studies on these genes are required to determine their role in the pathogenesis of endometrial carcinoma.

Clinical Features

Initially, patient with type 1 endometrioid carcinoma may be asymptomatic. Later, patient presents with abnormal dysfunctional or postmenopausal uterine bleeding per vagina, pelvic pain, symptoms related to compression effects of tumor on adjacent structures, abnormal bloating, dyspareunia, dysuria, anorexia, malaise and fatigue. The patient should be evaluated by direct endometrial sampling and cytologic screening.

Surgical Pathology: Type 1 Endometrioid Carcinoma

Gross Morphology

- Gross morphology of type 1 endometrioid carcinoma is similar to the various other types of endometrial carcinoma with the exception of serous endometrial carcinoma or malignant mesodermal mixed tumors.
- Uterine endometrial surface is shaggy in type 1 endometrioid carcinoma. Tumor is uniformly exophytic polypoid even when deeply invasive. Tumor may be focal or diffuse. At times, tumor may be composed of separate polypoid masses. Exophytic polypoid growth protrudes within the uterine cavity (Fig. 24.59).
- Necrosis is present in poorly differentiated or ulcerated tumor but absent in well differentiated tumor. Invasive type 1 endometrioid carcinoma extending through the myometrium into the serosa may result in uterus enlargement. Myometrial invasion usually appears as well-demarcated, gray-white, firm in consistency, with linear extension of the tumor beneath an exophytic mass.
- Extension of the tumor in lower uterine segment is common. Involvement of the uterine cervix occurs in 20% of cases. Type 1 endometrioid carcinoma may manifest different form of myometrial invasion. Tumor can invade myometrium diffusely as clusters, masses, cords or individual cells or deeply invasive involving serosa.

Light Microscopy

- Type 1 endometrioid carcinoma is determined by the histologic appearance of the tumor. It is based on the architectural pattern, FIGO nuclear grade or both. The architectural grade is determined by the extent of which the tumor is composed of solid masses of neoplastic cells as compared with well-formed glandular structures.
- Nuclear grade is determined by the variation in nuclear size and shape, chromatin distribution and the size of the nucleoli. Architectural and nuclear grading of type 1 endometrioid carcinoma is given in Table 24.17.
- Mitotic activity is an independent histologic variable. It is usually increased with increasing nuclear grade.
  - **Well-differentiated endometrioid carcinoma (FIGO grade 1):** Tumor is composed of well formed, closely packed glandular structures with cytologic atypia (nuclei oval, mildly enlarged with evenly dispersed chromatin) that bear resemblance to endometrial glands, hence known as endometrioid adenocarcinoma. Not more than 5% of the tumor is composed of solid masses (Fig. 24.60).
  - **Moderately-differentiated endometrioid carcinoma (FIGO grade 2):** Tumor is composed of nuclear features in between grade 1 and grade 3 with 6–50% of the tumor composed of solid masses.

- **Poorly-differentiated endometrioid carcinoma (FIGO grade 3):** Tumor is composed cells with pleomorphic and enlarged nuclei with irregular coarse chromatin and prominent eosinophilic nucleoli. More than 50% of the tumor is composed of solid masses.

Immunohistochemistry Technique

- Neoplastic cells of type 1 endometrioid carcinoma are immunoreactive to EMA, CA 125, Ber EP4, B72.3, CK7, ER/PR, BAX8, PTEN and p53.
- CA 125 is a cell surface glycoprotein originally identified in mucinous epithelial ovarian tumors. It is also expressed in adenocarcinoma of endometrium; cancers of gastrointestinal tract, thyroid and breast.

Markers	Expression
EMA	Positive
CA 125	Positive
Ber EP4	Positive
B72.3	Positive
CK7	Positive
ER/PR	Positive (>90%)
PAX8	Positive
PTEN	Positive
p53	Positive

Mode of Spread

Type 1 endometrioid carcinoma spreads by direct extension to contiguous organs and lymphovascular invasion.

- Lymphatic metastasis is more common than via hematogenous route.
- But the lung metastasis without mediastinal lymph nodes involvement suggests that the hematogenous spread may occur early in the course of the disease.
- Type 1 endometrioid carcinoma tends to spread to the pelvic lymph nodes before involvement of the para-aortic lymph nodes.

Treatment

Type 1 endometrioid carcinoma is treated by hysterectomy and bilateral salpingo-oophorectomy; radiotherapy and chemotherapy.

Type 2 Nonendometrioid Carcinoma

Aggressive type 2 nonendometrioid carcinoma accounts for 15% of overall cases, which affects multiparous and nonobese, thin and slim, normotensive women with

Table 24.17 Architectural and nuclear grading of type 1 endometrioid carcinoma

Grade of Tumor	Solid Component % in Type 1 Endometroid Carcinoma
Architectural grading of type 1 endometrioid carcinoma	
Grade 1	Not more than 5% of the tumor is composed of solid masses
Grade 2	6–50% of the tumor is composed of solid masses
Grade 3	More than 50% of the tumor is composed of solid masses
Nuclear grading of type 1 endometrioid carcinoma	
Grade 1	Nuclei are oval, mildly enlarged with evenly dispersed chromatin
Grade 2	Nuclei have features in between grade 1 and grade 3
Grade 3	Nuclei are pleomorphic and enlarged with irregular coarse chromatin and prominent eosinophilic nucleoli

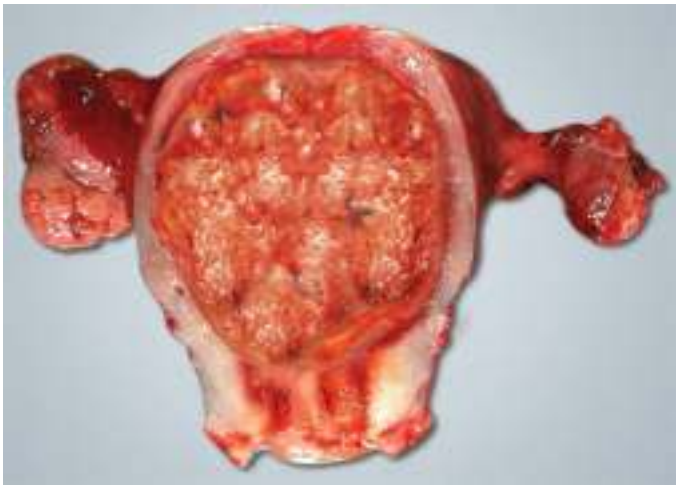


Fig. 24.59: Type 1 endometrioid carcinoma. The uterus is not enlarged, but there is an irregular mass in the upper fundus that proved to be endometrial adenocarcinoma on biopsy. Such carcinomas are more likely to occur in postmenopausal women. Thus, any postmenopausal bleeding should make you suspect that this lesion may be present.

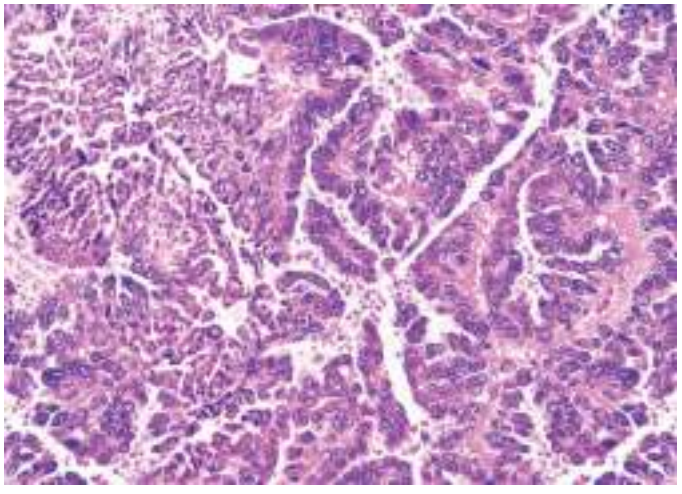


Fig. 24.60: Type 1 endometrioid carcinoma. It is invading into the smooth muscle bundles of the myometrial wall of the uterus. This neoplasm has a higher stage than a neoplasm that is just confined to the endometrium or is superficially invasive (400X).

precursor atrophic endometrium and associated with poor prognosis of five years survival. Tumor occurs disproportionately African-American women. TP53 gene mutation has been identified in the patients. Histologic variants of aggressive type 2 nonendometrioid carcinoma include serous, clear cell, squamous and undifferentiated. There is no distinctive gross appearance to differentiate individual histologic subtypes of endometrial carcinoma.

- **Serous endometrial carcinoma:** It is composed of fibrovascular papillae lined by vesicular nuclei with prominent eosinophilic nucleoli. Small clusters of cells that have separated from the papillae with fibrovascular core are referred as cellular budding. Serous endometrial carcinoma can also demonstrate psammoma bodies in 33% of cases, which may also be found in the villonodular variant of endometrioid carcinoma.
- **Clear cell endometrial carcinoma:** It occurs almost exclusively in postmenopausal women associated with poor prognosis. Although there are no macroscopic features, there are four histologic growth patterns in decreasing frequency: papillary, tubulocystic, glandular and solid. Most tumors display two or more patterns, as well as a mixture of cell types. Papillary pattern of clear cell endometrial carcinoma demonstrates complex papillae with fibrovascular cores lined by one to multiple layers of clear cells. Hobnail cells protrude into a cystic space. Clear cell endometrial carcinoma may exhibit a solid pattern consisting of sheets of clear cells separated focally by thin fibrous bands.
- **Squamous cell endometrial carcinoma:** It is composed of cells with eosinophilic cytoplasm and marked nuclear atypia, intercellular bridges and keratin pearls.
- **Undifferentiated endometrial carcinoma:** It is grade 3 tumor, which lacks glandular or squamous differentiation.

Molecular Genetic Alterations: Type 2 Nonendometrioid Carcinoma

- Compared to type 1 endometrioid carcinoma, relatively little is known about type 2 nonendometrioid carcinoma of endometrium at the molecular level, because of its relative infrequency.
- TP53 gene is a tumor suppressor gene, which encodes p53 protein, that regulates cell division by keeping cells from growing and proliferating too fast or in an uncontrolled way. Mutation in TP53 gene is demonstrated in type 2 nonendometrioid carcinoma in 90% of cases. It has been observed that intense, diffuse immunohistochemical staining for p53 correlates well with TP53 gene mutation. These findings indicate that in type 2 nonendometrioid carcinoma. TP53 gene mutations occur relatively early and its central role in the pathogenesis in this tumor type. It is associated with aggressive behavior. It is worth mentioning that mutation in p53 gene is also demonstrated in grade 3 of type 1 endometrioid carcinoma.
- Recent studies have suggested that mutations in P13K/PTEN/AKT pathway participate in pathogenesis of a subset of type 2 nonendometrioid carcinoma.
- ARIDIA (regulator of chromatin) gene encodes ARIDIA, a member of the SW1/SNF chromatin remodeling complex. Mutation of AIDIA gene induces changes in expression of multiple gene (CDKNIA, SMAD3, and PIK3IPI) via chromatin remodeling dysfunction in association with p13K/AKT pathway contributes in the pathogenesis of type 2 nonendometrioid carcinoma as well as type 1 endometrioid carcinoma.

Clinical Features

Patient presents with abnormal vaginal bleeding. It resembles ovarian serous carcinoma. It spreads throughout abdomen in a similar manner. It metastasizes to urinary bladder simulating a urinary bladder neoplasm.

Surgical Pathology: Type 2 Nonendometrioid Carcinoma

Gross Morphology

Uterus is small and atrophic for high-grade tumor serous carcinoma of endometrium. Generally, the tumor is exophytic and has a papillary appearance. The depth of the tumor invasion is difficult to assess. Tumor may be benign-appearing polyp in hysterectomy specimen. Endocervical involvement can be demonstrated in 25% of cases. Drop metastasis to vagina is common. Transtubal spread is common, that may create ovarian implants and omental tumor nodules.

Light Microscopy

- The cytologic features of type 2 nonendometrioid carcinoma of endometrium are quite varied. Tumor exhibits well-formed thick or thin papillary pattern predominantly; however, glandular and solid pattern may occur.
- Type 2 nonendometrioid carcinoma originally was considered as having thick, short papillae, but subsequent studies revealed that thin papillae may be present in 50% of cases. Tumor cells covering the papillae and lining the glands form small papillary tufts, which may be detached and float freely in the spaces between the papillae and in glandular lumens.
- Tumor is composed of polyhedral cells with clear or eosinophilic cytoplasm, but hobnail cells are among these polyhedral cells are frequently observed.
- In histopathology, hobnail cell has a bulbous nucleus and nuclear projections into the cytoplasm.
- Marked nuclear atypia is always present and required for a tumor to qualify for type 2 nonendometrioid carcinoma. Thus, type 2 nonendometrioid carcinoma of endometrium is characterized by the discordance between its architecture, which appears well-differentiated (papillary or glandular pattern) and its nuclear morphology, which is high-grade 3

nuclei. Many tumor cells are highly pleomorphic with marked nuclear atypia, hyperchromatic nuclei and macronucleoli (Fig. 24.61).
- Multinucleated giant cells, giant nuclei and bizarre forms are demonstrated in the 50% of cases.
- Lobulated nuclei with smudged chromatin, numerous abnormal mitoses and psammoma bodies are encountered. Nests of tumor cells within vascular spaces are usually demonstrated.
- Tumor is considered high-grade and is not graded using FIGO. Tumor has prominent myometrial invasion. Abrupt transition from normal to type 2 nonendometrioid carcinoma is common.
- The adjacent endometrium in type 2 nonendometrioid carcinoma is atrophic in almost all cases.

Immunohistochemistry Technique

Neoplastic cells of type 2 nonendometrioid carcinoma are immunoreactive with p53, CK7, CA 125, Ki-67, vimentin, PAX8, HER2/neu, EGFR, ER/PR, CEA and CK20.

Markers	Expression
p53	Positive
CK7	Positive
CA 125	Positive
Ki-67	Positive
Vimentin	Positive
PAX8	Positive
HER2/neu	Positive
EGFR	Positive
ER/PR	Positive/ Negative
CEA	Negative
CK20	Negative

Mode of Spread

Type 2 nonendometrioid carcinoma has a propensity for myometrial and lymphatic invasion. The hysterectomy specimens often demonstrate neoplastic cells in lymphatics within the myometrium, cervix, fallopian tube, broad ligament and ovarian hilus in the absence of gross tumor in these sites. Serous carcinoma involves peritoneal surfaces in the abdomen and pelvis in the early course of disease. In addition to intraperitoneal spread, serous carcinoma can metastasize to the liver, brain and skin.

Prognosis

- Current approach of management of type 2 nonendometrioid carcinoma is hysterectomy and bilateral salpingo-oophorectomy along with omentectomy.
- Patients receiving chemotherapy and radiotherapy are associated with favorable prognosis.
- Poor prognostic factors include age more than 60 years, lymphovascular invasion, myometrial invasion, stage III and stage IV.
- In summary, it is essential for patients with serous carcinoma to be carefully staged as there may be presence of extrauterine disease even if there is no or minimal invasion of the myometrium.
- Prognosis is excellent in the absence of extrauterine disease. International Federation of Gynecology and Obstetrics Staging of Endometrial Carcinoma (2019) is given in Table 24.18.

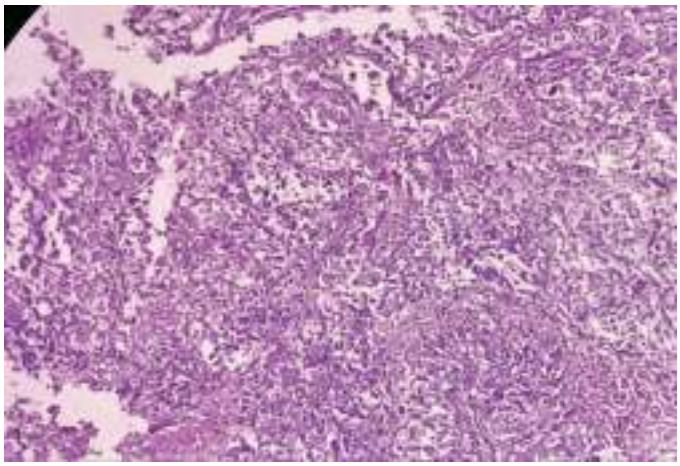


Fig. 24.61: Serous variant of type 2 nonendometrioid carcinoma. Tumor is composed of polyhedral cells with clear or eosinophilic cytoplasm, but hobnail cells are among these polyhedral cells are frequently observed. In histopathology, hobnail cell has a bulbous nucleus and nuclear projections into the cytoplasm. Marked nuclear atypia is always present and required for a tumor to qualify for serous carcinoma. Thus, serous variant of type 2 nonendometrioid carcinoma is characterized by the discordance between its architecture, which appears well-differentiated (papillary or glandular pattern) and its nuclear morphology, which is high-grade 3 nuclei. Many tumor cells are highly pleomorphic with marked nuclear atypia, hyperchromatic nuclei and macronucleoli (400X).

Clinical Pearls: Protocols for Reporting Endometrial Carcinoma on Resection Specimens

- Specimen (endometrial biopsy or endometrial curettings or tumor resection with no residual tumor or hysterectomy-specimen)
- Lymph node sampling
- Specimen integrity
- Tumor site and size
- Histology type: Type 1 endometrioid carcinoma, type 2 nonendometrioid carcinoma
- Histologic FIGO grading system on percentage of solid component in type 1 endometrioid carcinoma (grade 1: ≤5% tumor growth, grade 2:5–50% tumor growth, grade 3: >50% tumor growth)
- Myometrial invasion
- Serosal involvement
- Adnexal involvement
- Involvement of the cervix
- Extent of involvement of other organs
- Lymphovascular invasion
- Optional protocols include peritoneal fluid, additional pathologic findings, ancillary studies (ER/PR, HER2/neu, ploidy) and clinical history.

Table 24.18 International Federation of Gynecology and Obstetrics Staging of Endometrial Carcinoma (2019)

Stage	Endometrial Carcinoma
Stage I	Confined to the uterine corpus
Stage IA	Limited to endometrium or involves less than half of the myometrium
Stage IB	Invasion of half or more of the myometrium
Stage II	Invasion of the cervical stroma but no extension outside the uterus
Stage III	Local and/or regional spread of the tumor
Stage IIIA	Invasion of serosa, adnexa, or both (direct extension or metastasis)
Stage IIIB	Metastases or direct spread to the vagina and/or spread to the parametria
Stage IIIC	Metastases to pelvic or para-aortic lymph nodes or to both
Stage IIIC1	Metastases to pelvic lymph nodes
Stage IIIC2	Metastases to para-aortic lymph nodes, with or without metastases to pelvic lymph nodes
Stage IV	Involvement of the bladder and/or intestinal mucosa and/or distant metastases
Stage IVA	Invasion of the bladder, intestinal mucosa, or both
Stage IVB	Distant metastases, including metastases to the inguinal lymph nodes, lungs, liver, or bone and intraperitoneal disease

1. Endometrial cancer is usually surgically staged. Ideally, the thickness of the myometrium should be measured along with depth of tumor invasion.

2. For all but stage IVB, grade (G) indicates percentage of tumor with a nonsquamous or nonmorular solid growth pattern:

- *G1: ≤ 5%*
- *G2: 6–50%*
- *G3: > 50%*

3. Nuclear atypia excessive for the grade raises the grade of a G1 or G2 tumor by 1. In serous adenocarcinomas, clear cell adenocarcinomas, and squamous cell carcinomas, nuclear grading takes precedence. Adenocarcinomas with squamous differentiation are graded according to the nuclear grade of the glandular component.

4. Adapted from International Federation of Gynecology and Obstetrics (FIGO) 2019.

ENDOMETRIAL STROMAL TUMORS

Endometrial stromal tumor is rare low- to high-grade tumors. Low-grade endometrial stromal tumor invades extensively through the lymphatics of the myometrium. The tumor cells are bland and showing few mitoses. High-grade endometrial stromal sarcoma shows a vascular supporting framework with spindle-shaped neoplastic cells concentrically arranged around blood vessels. These are much rarer than leiomyosarcomas. It difficult to separate these tumors from uterine leiomyosarcoma. Classification of endometrial stromal tumors is given in Table 24.19.

Table 24.19 Classification of endometrial stromal tumors

Features	Endometrial Stromal Nodule	Endometrial Stromal Sarcoma	Undifferentiated Endometrial Sarcoma (Monomorphic)	Undifferentiated Endometrial Sarcoma (Polymorphic)
Category	Benign tumor	Malignant tumor low-grade	Malignant tumor high-grade	Malignant tumor high-grade
Tumor margin	Well circumscribed tumor	Invasive tumor	Invasive tumor	Invasive tumor
Nuclear atypia	Minimal	Minimal	Moderate to marked	Marked
Nuclear pleomorphism	Minimal	Minimal	Minimal to moderate	Marked
Mitotic activity	<3 mitoses/10 HPF	<3 mitoses/10 HPF	>10 mitoses/10 HPF	>10 mitoses/10 HPF
Immunohistochemistry	▪ CD10 +/+++ ▪ Desmin –/+ ▪ Smooth muscle actin –/++	▪ CD10 +/+++ ▪ Desmin –/+ ▪ Smooth muscle actin –/++	▪ CD10 +/+++ ▪ Desmin –/+ ▪ Smooth muscle actin –/++	▪ CD10 +/+++ ▪ Desmin –/+ ▪ Smooth muscle actin –/++

Pathology Pearls: Endometrial Stromal Tumors	
Immunohistochemistry Technique	
Endometrial stromal tumors of the uterus (endometrial stromal nodule and endometrial stromal sarcoma) are mentioned below.	
Markers	**Expression**
▪ CD10	▪ Positive
▪ ER/PR	▪ Positive
▪ JAZFI-JJAZ1 gene fusion	▪ Positive (50%)
▪ Smooth muscle cell actin (SMA) also known as α-actin	▪ Negative/positive
▪ Desmin	▪ Negative/positive
▪ h-caldesmon (encoded by CALD-1 gene) contractile apparatus associated protein	▪ Negative

MIXED MÜLLERIAN TUMOR

Mixed Müllerian tumor (carcinosarcoma) is a highly malignant mixed tumor comprising of malignant epithelial and stromal components. It affects post-menopausal women. Mixed Müllerian tumor is derived from multipotential stromal cells. Patient presents with bleeding per vagina.

Clinical Features

Patient presents with bleeding per vagina. On per vaginal examination, tumor is soft, bulky and fleshy protruding through the cervix into the vagina.

Surgical Pathology: Mixed Müllerian Tumor
Gross Morphology
Mixed Müllerian tumor is soft and fleshy. Cut surface shows areas of hemorrhage and necrosis.
Light Microscopy
Mixed Müllerian tumor is composed of malignant glandular and stromal components. Sarcomatous element can be homogenous nonspecific malignant stroma or heterologous malignant elements of a different tissues element particularly cartilage.

Prognosis

Tumor usually metastasizes as adenocarcinoma associated with highly aggressive course. Prognosis depends on the stage of the disease. Five-year disease-free is demonstrated in 55% of cases. In stage IV, patient develops extrapelvic disease associated with fatal outcome.

DISORDERS OF UTERINE MYOMETRIUM

NEOPLASTIC DISORDERS OF UTERINE MYOMETRIUM

Smooth muscle cell tumors of the uterus are leiomyomas (most common) and leiomyosarcomas. Recent 2020 WHO classification of mesenchymal tumors specific to uterus is given in Table 24.20.

LEIOMYOMA (FIBROID/FIBROMYOMA/MYOMA)

Leiomyomas are most common benign tumors of smooth muscle origin, also known as fibroids, which usually occur in the uterine corpus (intramural, submucous and subserous regions). These may also arise in cervix or uterine ligaments. Leiomyomas most often occur during reproductive period and most tumors

Table 24.20 Recent 2020 WHO classification of mesenchymal tumors specific to uterus

Mesenchymal Tumors Specific to Uterus		
Leiomyoma, NOS		
▪ Lipoleiomyoma	▪ Cellular leiomyoma	▪ Leiomyomatosis, NOS
▪ Leiomyoma, apoplectic type	▪ Myxoid leiomyoma	▪ Intravenous leiomyomatosis
▪ Leiomyoma, hydropic type	▪ Epithelioid leiomyoma	▪ Metastatizing leiomyoma
▪ Dissecting leiomyoma	▪ Symplastic leiomyoma	
Smooth muscle tumor of uncertain malignant potential		
▪ Epithelioid smooth muscle tumor of uncertain malignant potential	▪ Myxoid smooth muscle tumor of uncertain malignant potential	▪ Spindle smooth muscle tumor of uncertain malignant potential
Leiomyosarcoma, NOS		
▪ Spindle leiomyosarcoma	▪ Epithelioid leiomyosarcoma	▪ Myxoid leiomyosarcoma
Endometrial stomal nodule		
▪ Endometrial stomal nodule, low-grade	▪ Endometrial stomal nodule, high-grade	
Undifferentiated sarcoma		
Uterine tumor resembling ovarian sex cord tumor		

Contd...

Table 24.20 Recent 2020 WHO classification of mesenchymal tumors specific to uterus (*Contd...*)

▪ Perivascular epithelioid tumor, benign	▪ Perivascular epithelioid tumor, malignant	
Inflammatory myofibroblastic tumor		
Epithelioid myofibroblastic tumor		
Mixed Epithelial and Mesenchymal Tumors		
Adenomyoma, NOS		
Atypical polypoid adenomyoma		
Adenosarcoma		
Miscellaneous Tumors		
Primitive neuroendocrine tumor, NOS		
Germ cell tumors, NOS		
▪ Yolk sac tumor	▪ Mature teratoma, NOS	▪ Immature teratoma, NOS

regress after the menopause. Leiomyomas can cause painful menstruation and contribute to infertility. The tumors are estrogen-sensitive. Estrogen promotes the growth of leiomyomas, although it does not initiate them. Both estrogen and progestin receptors are present in leiomyomas. Elevated estrogen levels may cause leiomyoma's enlargement during first trimester of pregnancy.

Clinical Features

In most instances, leiomyomas are asymptomatic and discovered by gynecologists during routine pelvic examination. About 25% of patients are symptomatic. Symptoms depend on size and location of leiomyoma. Menorrhagia and pelvic pain are observed in 33% of patients. Intramural large leiomyoma may compress the urinary bladder and rectum; resulting in feeling of heaviness, pain, incontinence of urine and urgency of defecation. Subserosal leiomyomas undergo torsion due to twisting of pedicle, and detachment resulting in pain and signs of peritonitis. Submucosal leiomyoma is pedunculated tumor, which increases the endometrial surface area and the patient presents with excessive uterine bleeding per vagina.

Surgical Pathology: Leiomyoma

Gross Morphology

- Leiomyomas are discrete, firm, pale gray, sharply circumscribed and varying in size without encapsulation. Cut section reveals whorled pattern reflecting the fact that these are composed of smooth muscle bundles.
- Most leiomyomas are present within wall (intramural), but some are pedunculated and present in submucosal and subserosal region (Fig. 24.62).
- Secondary changes in large leiomyomas are yellow-brown to red softening (cystic degeneration), atrophy, hyaline degeneration, cystic degeneration, fatty change, dystrophic calcification, red degeneration, and chondroid/osteoid metaplasia (Fig. 24.63).

Light Microscopy

Histologic variants of uterine leiomyoma are given in Table 24.21. Leiomyoma composed of whorled bundles of smooth muscle cells that resemble the uninvolved myometrium. Individual smooth muscle cells are uniform in size and shape containing oval nucleus and long, slender bipolar cytoplasmic processes. Mitotic figures are sparse. Tumor cells are arranged in whorled fashion (Fig. 24.64).

- **Cellular leiomyoma:** It is also known as apoleptic leiomyoma shows areas of hemorrhage. It may rupture resulting in hemoperitoneum. Cellular leiomyoma occurs in pregnant women or young females on oral contraceptives.
- **Symplastic (bizarre) leiomyoma:** It is also known as bizarre leiomyoma. It is composed of scattered pleomorphic cells with irregular hyperchromatic nuclei, tumor giant cells with a low mitotic index.
- **Lipoleiomyoma:** It refers to classic leiomyoma which contains islands of mature adipocytes.
- **Epithelioid leiomyoma:** It is composed of predominantly epithelioid cells with clear to eosinophilic cytoplasm and nuclei with fine chromatin.
- **Benign metastasizing leiomyoma:** It is invading blood vessels and metastasizing to lungs.
- **Disseminated peritoneal leiomyomatosis:** It leads to formation of small nodules on the peritoneum. The tumor is considered benign despite its unusual behavior.

Immunohistochemistry Technique

Neoplastic cells of leiomyoma are immunoreactive SMA, desmin, MSA, h-caldesmon and SMMHC, calponin, ER/PR.

Markers	Expression
▪ Smooth muscle cell actin (SMA)	▪ Positive
▪ Desmin (intermediate filament)	▪ Positive
▪ Muscle-specific actin (MSA)	▪ Positive
▪ h-caldesmon (encoded by CALD-1 gene) contractile apparatus associated protein	▪ Positive

▪ Smooth muscle myosin heavy chain (SMMHC) contractile apparatus associated protein	▪ Positive
▪ Calponin (contractile apparatus associated protein)	▪ Positive
▪ Estrogen/progesterone (ER/PR) receptors	▪ Positive

Molecular Genetic Alterations

Leiomyomas show clonal abnormalities of 12q/7q and 6p resulting in rearrangement of genes.



Fig. 24.62: Leiomyomas of uterus. Leiomyoma is a benign mesenchymal tumor derived from smooth muscle and located in the uterus: intramural, submucosal and subserosal. These are most often multiple, well circumscribed and nonencapsulated. Cut surface of leiomyomas in uterus reveals white or tan-white whorled, firm and bulging above surface.

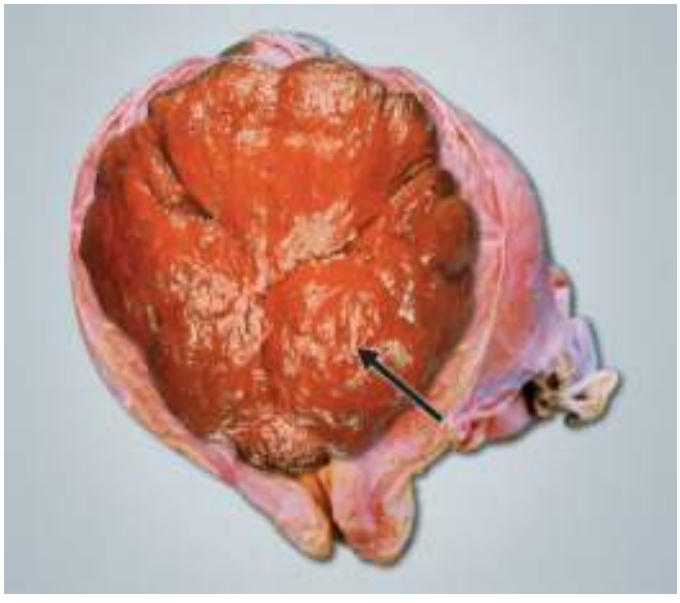


Fig. 24.63: Red degeneration in leiomyoma. Tumor shows red degeneration giving red meat appearance (arrow).

Diagnosis

Leiomyoma can be established by resection of whole uterus (hysterectomy), or resection of the leiomyoma if accessible or curetting of submucosal leiomyoma or resection of subserosal leiomyoma.

Prognostic Factors

Uterine leiomyoma may recur especially after myomectomy. Fumarate hydratase deficient leiomyoma recurs more frequently.

SMOOTH MUSCLE TUMOR OF UNCERTAIN MALIGNANT POTENTIAL (STUMP)

According to World Health Organization (WHO) classification of smooth muscle tumors, uterine smooth muscle tumor of uncertain malignant potential (STUMP) is a rare tumor and regarded as sub-classification in

Table 24.21 Histologic variants of uterine leiomyoma

Cellular leiomyoma
Leiomyoma with bizarre nuclei
Mitotically active leiomyoma
Hydropic leiomyoma
Apoplectic leiomyoma
Lipomatous leiomyoma (lipoleiomyoma)
Epithelioid leiomyoma
Myxoid leiomyoma
Dissecting (cotyledon-like) leiomyoma
Diffuse leiomyomatosis leiomyoma
Intravenous leiomyoma
Metastasizing leiomyoma

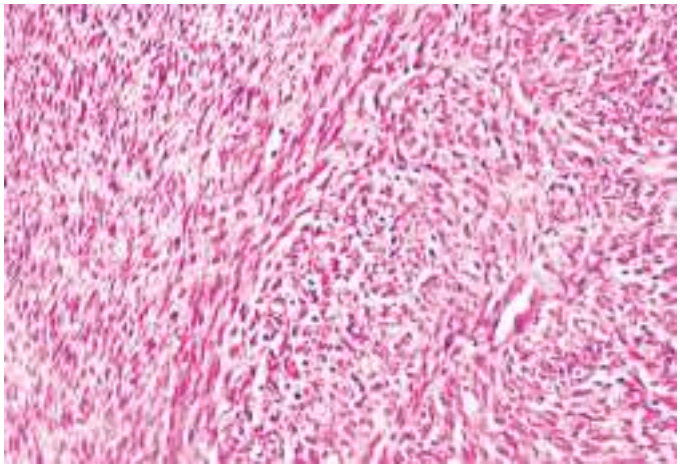


Fig. 24.64: Leiomyoma. The microscopic appearance of a benign leiomyoma. Normal myometrium is at the left, and the neoplasm is well-differentiated so that the leiomyoma at the right hardly appears different. Bundles of smooth muscle are interlacing in the tumor mass (400X).

Table 24.22 Diagnostic criteria for smooth muscle tumor of uncertain malignant potential (STUMP)

Tumor Cell Necrosis	Cellular Atypia (++/+++)	Mitosis	Recurrence Rate
Absent	Focal/diffuse	<10/10 HPF	13%
Absent	Diffuse	<10/10 HPF	11%
Present	Absent	<10/10 HPF	27%
Absent	Absent	>10/10 HPF	Zero

uterine smooth muscle tumors between benign and malignant criteria. Diagnostic criteria of smooth muscle tumor of uncertain malignant potential (STUMP) are given in Table 24.22.

- STUMPs are morphologically heterogenous and diagnostically requiring generous sampling of myometrial growth for histologic examination.
- STUMPs usually occur in women of reproductive age or postmenopausal women in the myometrium.
- Recurrence rate is 7–30% after surgery. Epithelioid and myxoid STUMPs may be more likely to recur after surgical resection. Median survival of patients is 5.5 years for recurrent STUMPs.
- Surveillance of STUMPs is warranted every six months for five years and then yearly.

LEIOMYOSARCOMA

Leiomyosarcoma is a highly malignant tumor of smooth muscle cell origin *de novo*, and unrelated to pre-existing leiomyoma. Single best criterion for predicting the behavior of smooth muscle tumors of uterus is mitotic activity.

Surgical Pathology: Uterine Leiomyosarcoma

Gross Morphology

- One should suspect uterine conventional leiomyosarcoma, if an apparent smooth muscle tumor is soft with irregular borders and areas of necrosis on gross examination.
- Tumor is bulky and fleshy invading into myometrial wall or polypoid tumor projecting into lumen.
- On cut section, tumor is not bulging above the surface. Tumor is invasive with infiltrative growth. Areas of hemorrhage necrosis are present (Fig. 24.65).

Light Microscopy

- Conventional leiomyosarcoma is composed of spindled/fascicular cells resembling smooth muscle with moderate to marked pleomorphism.
- Diagnostic criteria of leiomyosarcoma include following features on light microscopy: (a) 10 or more mitoses per 10 high power fields (HPF); (b) 5 or more mitoses per 10 HPF, with nuclear atypia and necrosis; and (c) myxoid and epithelioid smooth muscle tumors with 5 or more mitoses per 10 HPF (Fig. 24.66A and B).
- Some tumors that meet some, but not all, of the diagnostic criteria for leiomyosarcoma are considered 'STUMP' (smooth muscle tumors of uncertain malignant potential).
- Comparison of leiomyoma and leiomyosarcoma is given in Table 24.23. Histologic criteria for the diagnosis of uterine smooth muscle tumors are given in Table 24.24. Histologic features for the differential diagnosis between ischemic necrosis and tumor cell necrosis in leiomyosarcoma are given in Table 24.25.

Immunohistochemistry Technique

Neoplastic cells of leiomyosarcoma are immunoreactive with SMA, desmin, MSA, h-caldesmon, SMMHC, calponin and ER/PR.

Markers	Expression
Smooth muscle actin (SMA)	Positive
Desmin (intermediate filament)	Positive
Muscle-specific actin (MSA)	Positive
h-caldesmon (encoded by CALD-1 gene) contractile apparatus associated protein	Positive
Smooth muscle myosin heavy chain (SMMHC) contractile apparatus associated protein	Positive
Calponin (contractile apparatus associated protein)	Positive
Estrogen/progesterone (ER/PR) receptors	Positive

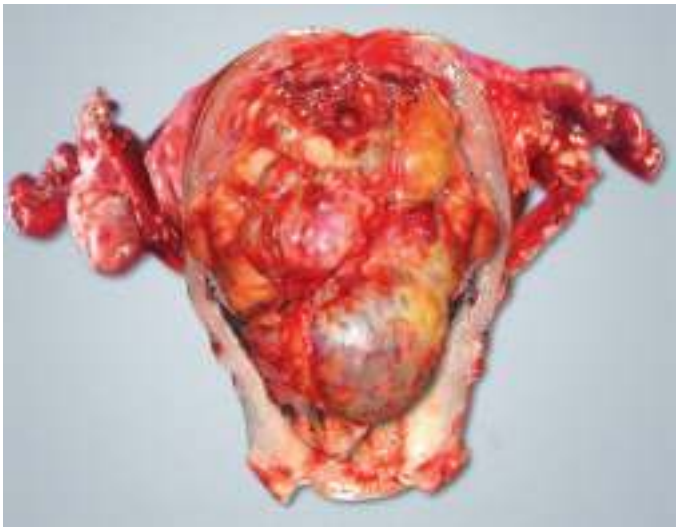


Fig. 24.65: Leiomyosarcoma of the uterus. The uterus is expanded by a massive, fleshy, partly necrotic and hemorrhagic-looking malignant tumor.

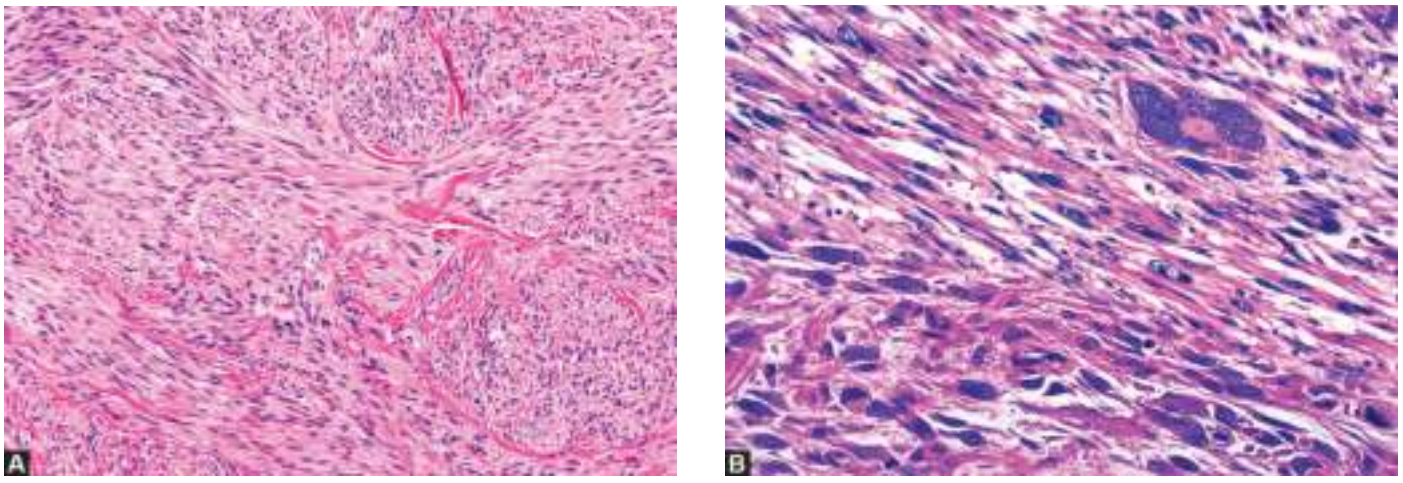


Fig. 24.66A and B: Leiomyosarcoma of uterus. As with sarcomas in general, leiomyosarcomas have spindle cells. Several mitoses are seen here, just in this one high power field (400X).

Table 24.23 Comparison of uterine leiomyoma and leiomyosarcoma

Feature	Leiomyoma	Leiomyosarcoma
Gross morphology		
Number	Usually multiple	Usually solitary (50–70%)
Size	Large tumor 3–5 cm size	Large tumor 5–10 cm size or larger size
Consistency	Firm tumor	Soft tumor
Cut surface	White color with whirling appearance	Yellow to tan with fleshy appearance
Hemorrhage and necrosis	Infrequent	Frequent
Histologic examination		
Hemorrhage and necrosis	Infrequent	Frequent
Mitoses	Mitoses <5/10 HPF	Mitoses ≥10/10 HPF
Immunohistochemistry technique		
SMA (smooth muscle actin)	Positive	Positive
Desmin	Positive	Positive
Caldesmon	Positive	Positive/negative
p16	Negative	Positive
PR	Positive	Negative
MIB-1	Negative	Positive
PHH3 (phosphohistone H3)	Negative	Positive

Mitotically active leiomyoma mitosis ≥5/10 HPF.
Atypical leiomyoma with low risk of recurrence shows diffuse moderate to severe atypia mitoses <5/10 HPF.
STUMP shows tumor necrosis diffuse severe atypia and mitoses 5–9/10 HPF.

Molecular Genetic Alterations

Complex numerical and structural chromosomal aberrations suggesting that chromosomal instability is the hallmark of uterine smooth muscle tumors. Mutations of TP53 (33%), TRRX (26%), and MED12 (21%) genes have been demonstrated in uterine leiomyosarcoma.

Mode of Spread

Leiomyosarcoma of uterus spreads by local invasion and via hematogenous route to most commonly to lungs, liver, brain and bone. Other rare metastatic sites have been reported including pancreas, skull, adrenal glands, heart, bile duct, bowel, breast, orbit and gingiva. It rarely spreads via lymphatic route to the lymph nodes.

Table 24.24 Histologic criteria for the diagnosis of uterine smooth muscle tumors

Uterine Smooth Muscle Tumor	Tumor Necrosis	Cellular Atypia	Mitotic Figures/10 HPF
Leiomyoma	Absent	None to mild	<5/10 HPF
Leiomyosarcoma	Present	Present	≥10/HPF
Atypical leiomyoma	Absent	Focal moderate to severe atypia	<5/10 HPF
Mitotically active leiomyoma with low risk of recurrence	Absent	Focal moderate to severe atypia	<5/10 HPF
STUMP (smooth muscle tumors of uncertain malignant potential)	▪ Absent ▪ Absent ▪ Absent	▪ None to mild atypia (rule out infarcted leiomyoma) ▪ Focal moderate to severe atypia ▪ Diffuse moderate to severe atypia	▪ <10/10 HPF ▪ 5/10 HPF ▪ 5–9/10 HPF

Table 24.25 Histologic features for the differential diagnosis between ischemic necrosis and tumor cell necrosis in leiomyosarcoma

Gross Morphologic and Histologic Features	Ischemic Necrosis	Tumor Necrosis
Necrotic tumor	No cell shadow (cellular outlines and details lost)	Shadows of necrotic cells
Transition between vital and necrotic area	Zone of granulation tissue in older necrosis	Abrupt
Hemorrhage and inflammation	Usual	Unusual

DISORDERS OF FALLOPIAN TUBES

FALLOPIAN TUBES: ANATOMY

Fallopian tube consists of interstitial portion (uterine end), isthmus, ampulla (wider portion) and infundibulum and opens as finger-like projections (towards ovary). Salpingitis can predispose to ectopic pregnancy.

INFLAMMATORY DISORDERS OF FALLOPIAN TUBES

ACUTE SALPINGITIS

Acute salpingitis is most often associated with inflammation of endometrium, ovaries and pelvic inflammatory disease in young- to middle-aged women. In some cases, acute salpingitis occurs due to abortion, puerperal sepsis, trauma and surgical manipulation.

- Acute salpingitis is caused by *Escherichia coli*, *Neisseria gonorrhoeae* (gram-negative diplococci), *Chlamydia trachomatis*, and Mycoplasma, Staphylococcus and streptococci organisms resulting in suppuration.
- Damage to the fallopian tube resulting from acute salpingitis may lead to infertility. Antibiotic therapy resolves inflammation of acute cervicitis. If resolution of the acute inflammation does not occur, chronic salpingitis follows with formation of tubo-ovarian mass.

Surgical Pathology: Acute Salpingitis

Gross Morphology

Bilateral fallopian tubes are congested, edematous and lumen filled with purulent exudates (pyosalpinx).

Light Microscopy

Fallopian tube epithelium shows marked infiltration by numerous neutrophils with congestion and edema (Fig. 24.67).

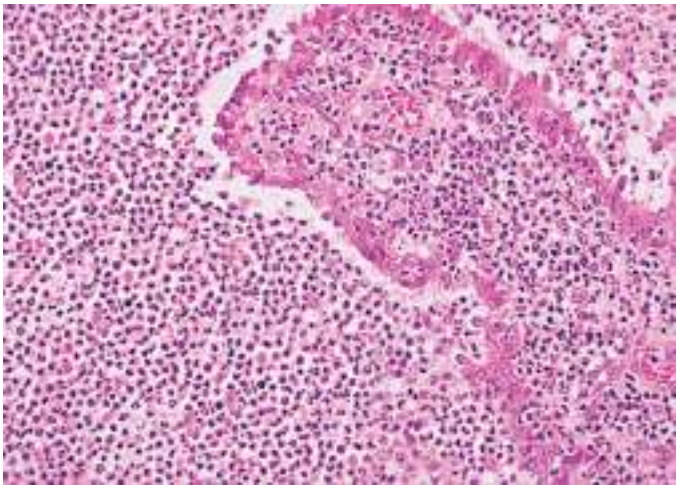


Fig. 24.67: Acute salpingitis. A remnant of tubal epithelium is seen here surrounded and infiltrated by numerous neutrophils. This is acute salpingitis. *Neisseria gonorrhoeae* was cultured (400X).

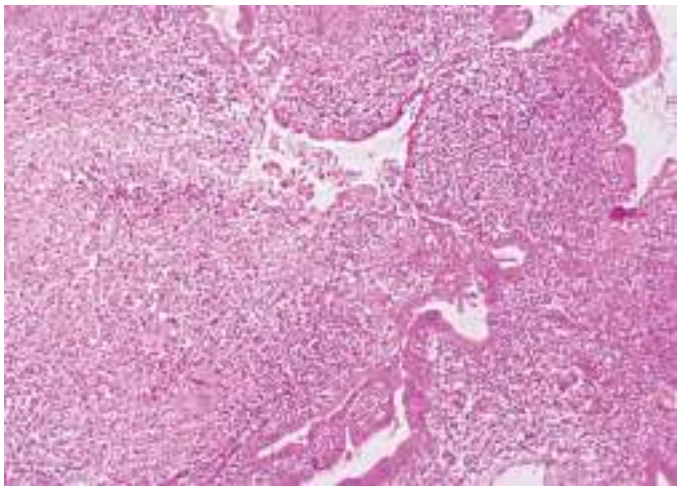


Fig. 24.68: Tubercular salpingitis. It shows large number of epithelioid cell granulomas, Langhans giant cells and caseous material. In the background, tubal wall is infiltrated by lymphocytes, macrophages and plasma cells (100X).

CHRONIC SALPINGITIS

Chronic salpingitis is characterized by presence of plasma cells and lymphocytes in the submucosa with or without foamy histiocytes and granulomas. Plicae are fibrotic, fused, short and thick.

- Repeated attacks by organisms cause chronic salpingitis with formation of violin string adhesion. Hydrosalpinx may be end stage of chronic salpingitis characterized by thin wall with a few plicae and lumen filled with clear fluid. Numerous adhesions occur on the serosal surface.
- Granulomatous salpingitis may be caused in the settings of tuberculosis, sarcoidosis, Crohn's disease and fungal infections. Tubercular salpingitis occurs as a result of extension of tubercular infection from endometrium. Spread of tubercular infection through intraluminal route causes adhesions of lining epithelium resulting in infertility. Tuberculous salpingitis shows epithelioid cell granulomas, Langhans' giant cells and caseous necrosis (Fig. 24.68).

Surgical Pathology: Chronic Salpingitis
Gross Morphology
Fallopian tube is most often enlarged, fibrotic, disordered and adhered to the ovary and surrounding structures.
Light Microscopy
Plicae of fallopian tube show infiltration by lymphocytes and plasma cells. Fusion of plicae may lead to formation of follicle-like spaces known as salpingitis follicularis.

SALPINGITIS ISTHMICA NODOSA

Salpingitis isthmica nodosa is characterized by outpouching of follopian tube epithelium into tubal wall. In severe cases, lumen may be completely obstructed. It ocurs in African Americans and Caucasians, and involves both tubes in 85% of cases. Salpingitis isthmica nodosa is most often associated with ectopic pregnancy and infertility in young women. It may lead to ectopic pregnancy. Its pathogenesis is not clear.

Surgical Pathology: Salpingitis Isthmica Nodosa
Gross Morphology
Fallopian tubal wall shows a yellow-white nodule in the isthmus region measuring 1–2 cm in diameter. Bilateral fallopian tubes are involved in 85% of cases.
Light Microscopy
▪ Fallopian tubal lesion consists of outpouchings of tubal epithelium surrounded by a thickened wall of smooth muscle. ▪ Glands may be cystically dilated and are true diverticula that communicate with tubal lumen.

NON-NEOPLASTIC DISORDERS OF FALLOPIAN TUBES

TUBAL ENDOMETRIOSIS

Presence of normal endometrial glands and stroma in fallopian tubes outside uterine cavity is known as endometriosis. Fallopian tubes are most frequently involved due to endometriosis in 1–2% women during reproductive period. It is an important cause of infertility. Endometriosis regresses after menopause. It is described in details in placental and pregnancy associated disorders in this chapter.

Surgical Pathology: Tubal Endometriosis
Gross Morphology
Fallopian tube endometriosis consists of dark brown chocolate-colored serosal nodules.
Light Microscopy
Fallopian tube shows presence of endometrial glands surrounded by endometrial stroma associated with hemosiderin-laden macrophages and chronic inflammatory cells.

TUBAL ECTOPIC PREGNANCY

Ampulla of the fallopian tube is the most common site of ectopic pregnancy. It is described in details in placental and pregnancy associated disorders. Risk factors for tubal ectopic pregnancy include salpingitis, prior ectopic pregnancy, salpingitis isthmica nodosa, congenital tubal anomalies and endometriosis.

- The classic clinical triad is pain abdomen, amenorrhea and vaginal bleeding in 50% of cases. On clinical examination, palpable adnexal mass and abdominal tenderness are demonstrated in many patients.

- Rupture of tubal ectopic pregnancy products lead to hypotensive shock. Fallopian tube shows dilatation, hemorrhagic, chorionic villi with or without embryo.
- Fallopian tubal mucosa or wall shows products of gestation (chorionic villi or trophoblast). Tubal pregnancy is given in details under pregnancy associated disorders in this chapter.

NEOPLASTIC DISORDERS OF FALLOPIAN TUBES

ADENOMATOID TUMOR OF FALLOPIAN TUBES

Adenomatoid tumor is the most common benign tumor of the fallopian tubes. It is derived from mesothelium.

Surgical Pathology: Adenomatoid Tumor of Fallopian Tube
Gross Morphology
Tumor is well circumscribed and white tan-colored seen in tubal wall.
Light Microscopy
Tumor shows slit-like spaces lined by single layer of flattened cuboidal cells (Fig. 24.69).
Immunohistochemistry Technique
Tumor cells are positive for expression of cytokeratin, calretinin, EMA and vimentin. These cells are negative for factor VIII-related antigen and CD31, a profile indicating a mesothelial origin rather from vascular endothelium.

Markers	Expression
Cytokeratin	Positive
Calretinin	Positive
Epithelial membrane antigen (EMA)	Positive
Vimentin	Positive
Factor VIII-related antigen and CD31	Positive

ADENOCARCINOMA OF FALLOPIAN TUBES

Primary adenocarcinoma of fallopian tube is extremely rare, and associated with BRCA gene mutation. In the vast majority of cases, bulky tumor extending from the ovary or endometrium involves fallopian tube. There may be a watery secretion resulting in serous discharge per vagina.

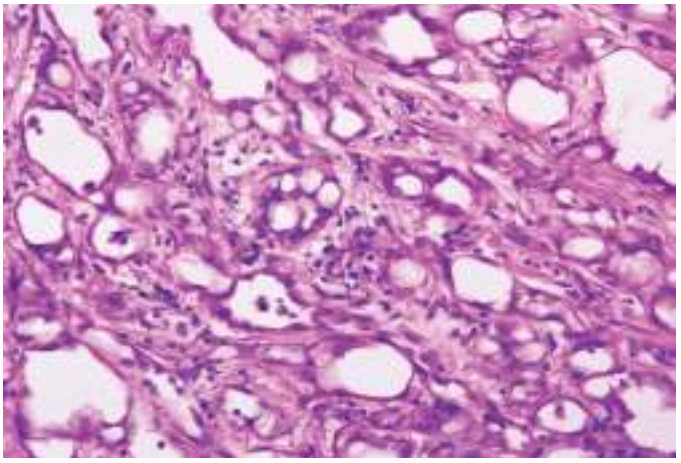


Fig. 24.69: Adenomatoid tumor of the fallopian tube. It shows slit-like spaces lined by single layer of flattened cuboidal cells (400X).

DISORDERS OF OVARIES

OVARIES: OVERVIEW

The ovaries are paired intraperitoneal gonads in the abdomen. These play a fundamental role in reproduction as well as the production of hormones.

- Granulosa cells and theca cells present in the ovary synthesize estrogen and progesterone.
- Graafian follicles mature during the proliferative phase of menstrual cycle. Once the dominant graafian follicle matures and luteinizing hormone surges, the oocyte will be expelled and begin its journey through the fallopian tubes and into the uterus.
- The corpus luteum functions as a temporary endocrine organ, that synthesizes progesterone along with smaller amounts of inhibin A and estradiol, which protects the oocyte and allows time for union of spermatozoa and ovum and implantation before menstruation.
- If fertilization takes place, the newly formed blastocyst synthesizes human chorionic gonadotropin hormone, which transmits signals to the corpus luteum to continue synthesizing progesterone. This function is now taken over by the placenta once it has matured.
- If fertilization does not occur, then the corpus luteum will degenerate and become corpus albicans, and the withdrawal of progesterone will initiate the process of menstruation.

INFLAMMATORY DISORDERS OF OVARIES

Inflammatory disorders of ovaries include acute oophoritis, autoimmune oophoritis and noninfectious granulomatous oophoritis (Table 24.26).

Table 24.26 Inflammatory disorders of ovaries

Characteristics	Acute Oophoritis	Autoimmune Oophoritis	Noninfectious Granulomatous Oophoritis
Underlying causes	▪ Salpingitis ▪ Systemic infections ▪ Tubo-ovarian abscess ▪ Ascending pelvic inflammatory disease	▪ Oligomenorrhea ▪ Infertility	▪ Sarcoidosis ▪ Foreign body granulomas following pelvic or abdominal surgery
Histologic features	Polymorphonuclear infiltration in ovaries	Lymphocytes and plasma cells infiltration in developing follicles (ovaries normal size)	Granulomatous inflammation
Clinical course	Subsequent fibrosis, scarring from pelvic inflammatory disease leading to infertility	Premature ovarian failure and premature menopause	Incidental finding

ACUTE OOPHORITIS

In most cases, inflammation of ovaries is caused by a bacterial infection spreading from the vagina or cervix and fallopian tubes. Pelvic inflammatory disease is often caused by more than one type of bacterium, which involves internal reproductive organs. Patient presents with pain in lower abdomen and pelvis and discharge per vagina. Acute oophoritis is characterized by infiltration by polymorphonuclear cells. Subsequent fibrosis, and scarring from pelvic inflammatory disease leading to infertility.

AUTOIMMUNE OOPHORITIS

Autoimmune oophoritis is associated with oligomenorrhea and infertility. Ovaries are of normal size, which show lymphocytes and plasma cells infiltration in developing graafian follicles. Women develop premature ovarian failure and premature menopause.

NONINFECTIOUS GRANULOMATOUS OOPHORITIS

Noninfectious granulomatous oophoritis is seen as incidental finding secondary to sarcoidosis and following pelvic or abdominal surgery. Ovaries show granulomas. Women with noninfectious granulomatous oophoritis are asymptomatic.

OVARIAN CYSTS

During the proliferative phase of the menstrual cycle, many graafian follicles are released, but only one reaches maturity progressing to development of corpus luteum. It eventually undergoes degeneration during the menstrual cycle. There are two types of functional ovarian cysts (follicular and luteal cysts): small non-neoplastic sacs containing fluid or semisolid material. These cysts require thorough investigation as possible sites of malignant change. Salient features of ovarian cysts are given in Table 24.27.

FOLLICULAR CYST

Follicular cyst occurs in the first 2 weeks of the menstrual cycle during prepubescent or reproductive age group. When the graafian follicle fails to rupture, it continues to grow and resulting in follicular cyst. It is sometimes associated with hyperestrinism and endometrial hyperplasia.

Clinical Features

Patient presents with abdominal mass, rupture. Follicular cysts may be associated with polycystic ovarian syndrome or McCune-Albright syndrome.

Surgical Pathology: Follicular Cyst in Ovary
Gross Morphology
Follicular cyst is unilateral measuring 2.5–10 cm in diameter. Normal follicle measures up to 1 cm. Follicular cyst contains serosanguineous fluid.
Light Microscopy
Follicular cyst is lined by inner granulosa cells and outer theca cells.

CORPUS LUTEAL CYST

Corpus luteal cyst occurs in the second half of the menstrual cycle during reproductive age group. Hemorrhage into a persistent mature corpus luteum results in formation of corpus luteal cyst. Woman with corpus luteal cyst is usually associated with menstrual irregularity, occasionally with intraperitoneal hemorrhage. Corpus luteal cyst measures >3 cm in diameter and contains hemorrhagic fluid. Corpus luteal cyst shows granulosa cells interspersed with peripheral theca cells similar to normal corpus luteum.

POLYCYSTIC OVARY SYNDROME (STEIN-LEVENTHAL SYNDROME)

Polycystic ovary syndrome (Stein-Leventhal syndrome) is a disorder in which ovaries produce an abnormal

Table 24.27 Salient features of ovarian cysts

Ovarian Cyst	Age Group	Gross Morphology and Histologic Features	Clinical Features
Follicular cysts	Prepubertal and reproductive period	Unilateral cyst 2.5–10 cm with sanguineous fluid; and lined by inner granulosa cells and outer theca cells	Abdominal mass, rupture, may be associated with polycystic ovarian syndrome or McCune-Albright cyst
Corpus luteal cysts	Reproductive period	Cyst >3 cm size with hemorrhagic fluid, composed of granulosa cells interspersed with peripheral theca cells similar to corpus luteum	Menstrual cycle abnormalities, occasional intraperitoneal hemorrhage
Polycystic ovarian syndrome	20–30 years	Ovaries enlarged, thickened, collagenized cortical surface containing multiple uniform graafian follicles, absence of corpus luteum indicating infrequent ovulation	Anovulation, infertility, hirsutism, increased incidence of endometrial hyperplasia and endometrioid carcinoma
Surface epithelial inclusion cysts	Reproductive period	Surface epithelial inclusion cysts 1 cm size formed due to invagination of ovarian surface epithelium secondary to rupture with ovulation; lined by single layer of cuboidal or columnar cells, psammoma bodies may be present	Surface inclusion cyst may be precursor of benign, borderline and low-grade epithelial neoplasms
Paraovarian or paratubal cysts	Reproductive period	Cysts present in hilar region of ovary derived from Wolffian duct lined by simple or stratified epithelium and Müllerian ducts lined by columnar epithelium and nonciliated cells similar to surface inclusion cyst	Asymptomatic
Endometriosis (endometrial glands and stroma outside uterus most often in ovary)	Reproductive period	Chocolate cysts in ovarian cortex filled with thick brown fluid resembling chocolate syrup; presence of endometrial glands, hemosiderin-laden macrophages, fibroblasts and inflammation simulating malignancy	Menstrual cycle associated with pain and infertility
Theca luteal cysts (formed due to gonadotropin stimulation)	Cysts formed in hydatidiform mole and choriocarcinoma cases	Theca luteal cysts small size, lined by luteinized theca cells	Women with hydatidiform mole and choriocarcinoma develop theca luteal cysts

amount of androgens, male sex hormones that are usually present in women in small amounts. Polycystic ovaries, hormonal imbalance and irregular menstrual periods are signs and symptoms of polycystic ovary syndrome. Clinical manifestations of polycystic ovary syndrome (Stein-Leventhal syndrome) are related to excess synthesis of androgenic hormones (amenorrhea or oligomenorrhea), persistent anovulation (important cause of infertility), and small subcapsular cysts in ovaries, obesity, and hirsutism in young women of 20–30 years of age group.

Pathogenesis

Patient synthesizes excess of luteinizing hormone (LH) and androgens. Increased synthesis of androgens occurs by two mechanisms: LH may stimulate follicular theca-lutein cells, with consequent increased synthesis of androgens. Hyperinsulinemia in insulin resistance diabetes mellitus may lead to increased ovarian androgen production, which then may cause increased LH synthesis.

Complications

Women develop anovulation, irregular menstrual cycles, infertility and hirsutism. Unopposed acyclic estrogen synthesis in women with polycystic ovary syndrome (Stein-Leventhal syndrome) leads to increased incidence of endometrial hyperplasia and/or adenocarcinoma.

Surgical Pathology: Polycystic Ovary Syndrome (Stein-Leventhal Syndrome)

Gross Morphology

- Both ovaries are enlarged. Cut section reveals thickened collagenized cortex surface.
- Numerous cysts in ovaries measuring 2–8 mm in diameter are arranged peripherally around a dense core of stroma.

Light Microscopy

- Ovaries show multiple uniform graafian follicles lined by granulosa cells and absence of corpus lutea (indicating infrequent ovulation).
- Ovaries show marked thickened ovarian capsule and cortical stromal fibrosis with islands of focal luteinization.

SURFACE EPITHELIAL INCLUSION CYSTS

Surface epithelial inclusion cysts in ovaries are seen during reproductive period. These are formed as a result of repeated invaginations of ovarian epithelium secondary to rupture with ovulation. These surface epithelial inclusion cysts may be precursor of benign, borderline and low-grade serous epithelial neoplasms.

Surgical Pathology: Surface Epithelial Inclusion Cysts in Ovaries

Gross Morphology

Surface epithelial inclusion cysts are less than 1 cm in diameter.

Light Microscopy

- Surface epithelial inclusion cysts are lined by single layer of cuboidal or columnar cells.
- Psammoma bodies may be present. Paramesonephric cysts are lined by columnar epithelium with mixture of ciliated and nonciliated cells similar to epithelial inclusion cysts.

PARAOVARIAN OR PARATUBAL CYSTS

Paraovarian or paratubal cysts are formed during reproductive age group. Cysts are derived from mesonephric (Wolffian) or paramesonephric (Müllerian) ducts and found in the hilar region of the ovary. Women are asymptomatic.

Surgical Pathology: Paraovarian or Paratubal Cysts

Gross Morphology

Paraovarian or paratubal cysts are of small size.

Light Microscopy

Mesonephric cysts are lined by simple or stratified epithelium with prominent muscular walls. Paramesonephric cysts are lined by columnar epithelium with mixture of ciliated and nonciliated cells similar to epithelial inclusion cysts.

ENDOMETRIOSIS (CHOCOLATE CYSTS)

Endometriosis represents endometrial glands and stroma outside uterus during reproductive period. The ovary is the most frequent site of endometriosis. Hemorrhage into endometriosis focus results in formation of blood-filled cysts on the ovary termed chocolate cysts. Endometriosis is associated with pain during menstrual cycle. It is frequent cause of infertility.

Surgical Pathology: Paraovarian or Paratubal Cysts in Ovaries

Gross Morphology

Ovaries show thickened cortex, surface adhesion. Cysts are filled with thick brown fluid resembling chocolate syrup, the so-called chocolate cysts.

Light Microscopy

Endometrial glands and stroma associated with hemosiderin-laden macrophages and fibroblasts. These cysts may contain atypical cytologic features and acute inflammation raising suspicious of malignancy.

THECA LUTEAL CYSTS

Theca luteal cysts in ovaries are most often multiple and bilateral. These are lined by luteinized theca cells. Theca luteal cysts are formed due to gonadotropin stimulation in patients associated with choriocarcinoma and hydatidiform mole. Ovaries contain multiple small cysts of variable diameter. Theca luteal cysts are lined by luteinized theca cells.

NEOPLASTIC DISORDERS OF OVARY

Ovarian cancer is the second most common malignancy of the genital tract and most common cause of death due to late diagnosis.

- Most of the ovarian tumors are of epithelial origin, which occur after the age of 35 years with increasing incidence as age advances.
- Approximately 3% of ovarian tumors occur before 35 years of age, which are mostly nonepithelial in origin as germ cell tumors.
- Primary ovarian tumors are classified according to recent 2020 World Health Organization (WHO) classification, which divides primary tumors into three main groups based on line of differentiation—surface epithelial-stromal tumors (Müllerian epithelium), germ cell tumors (ovum), and sex cord-stromal tumors (Fig. 24.70). Recent 2020 WHO classification of tumors of the ovary is given in Table 24.28. Most common histologic subtypes of surface

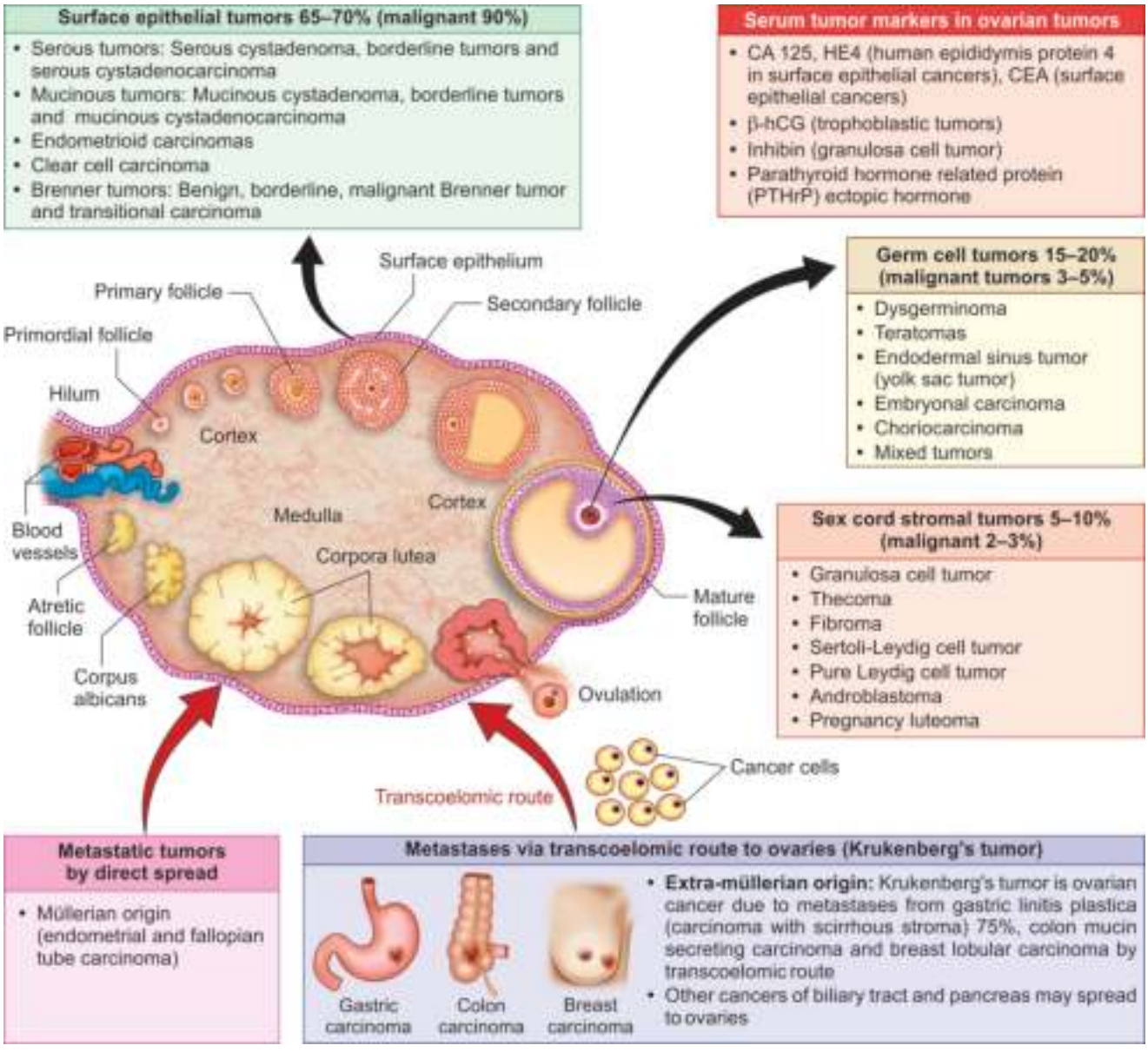


Fig. 24.70: Classification of ovarian tumors.

epithelial carcinomas of ovary are given in Table 24.29. Differentiating features of benign and malignant ovarian tumors are given in Table 24.30. Salient features of ovarian masses are given in Table 24.31.

KRUKENBERG TUMOR (METASTATIC TUMOR)

Krukenberg tumor is the metastatic carcinoma of ovaries. The gastric mucin secreting adenocarcinoma is the primary site in 75% of cases, followed by colon mucin secreting adenocarcinoma and lobular carcinoma of breast. Metastases to ovary(ies) occur by transcoelomic spread. One should suspect metastatic carcinoma involving bilateral ovaries with multiple nodules.

Surgical Pathology: Krukenberg Tumor

Gross Morphology

Tumor shows multiple solid nodules. Cut surface shows gray white, gelatinous, areas of extensive necrosis and cystic spaces (Fig. 24.71).

Light Microscopy

Krukenberg tumor is characterized by ovarian metastases, in which the tumor shows nests of mucin-filled signet ring epithelial cells with eccentric nuclei within a cellular stroma derived from the ovary (Fig. 24.72).

Immunohistochemistry Technique

- Metastatic gastric mucin secreting carcinoma to ovary is immunoreactive with cytokeratin, epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA).

- Metastatic colon mucin secreting carcinoma to ovary is immunoactive with CK20, CDX2, MUC1, CEA, β-catenin, SAT2 and p53.
- Breast lobular carcinoma metastasizing to ovary is immunoreactive with estrogen receptor/progesterone receptor (ER/PR). It is negative for E-cadherin and HER2/neu.

Markers	Expression
Metastatic gastric carcinoma to ovaries	
▪ Cytokeratin	▪ Positive
▪ CEA	▪ Positive
▪ EMA	▪ Positive
Metastatic colon carcinoma to ovaries	
▪ CK20	▪ Positive
▪ CDX2	▪ Positive
▪ MUC1	▪ Positive
▪ CEA	▪ Positive
▪ β-catenin	▪ Positive
▪ SAT2	▪ Positive
▪ p53	▪ Positive
Metastatic lobular carcinoma of breast to ovaries	
▪ ER/PR	▪ Positive
▪ E-cadherin	▪ Negative/decreased expression
▪ HER2/neu	▪ Negative/decreased expression

Table 24.28 Recent 2020 WHO classification of tumors of the ovary

Surface Epithelial Tumors	
Serous tumors	▪ Serous cystadenoma, NOS • Serous surface papilloma • Serous adenofibroma, NOS • Serous cystadenofibroma, NOS ▪ Serous borderline tumor, NOS ▪ Serous borderline tumor, micropapillary variant ▪ Serous cystadenocarcinoma • Low-grade serous cystadenocarcinoma • High-grade serous cystadenocarcinoma
Mucinous tumors	▪ Mucinous cystadenoma, NOS ▪ Mucinous adenofibroma, NOS ▪ Mucinous borderline tumor ▪ Mucinous adenocarcinoma
Endometrioid tumors	▪ Endometrioid cystadenoma, NOS ▪ Endometrioid adenofibroma, NOS ▪ Endometrioid tumor, borderline ▪ Endometrioid adenocarcinoma, NOS ▪ Seromucinous carcinoma
Clear cell carcinoma	▪ Clear cell cystadenoma ▪ Clear cell cystadenofibroma ▪ Clear cell borderline tumor ▪ Clear cell adenocarcinoma, NOS
Seromucinous tumors	▪ Seromucinous cystadenoma ▪ Seromucinous adenofibroma ▪ Seromucinous borderline tumor
Brenner tumors	▪ Brenner tumor, NOS ▪ Brenner tumor, borderline malignant ▪ Brenner tumor, malignant
Other carcinomas	▪ Mesonephric-like adenocarcinoma ▪ Carcinoma, undifferentiated, NOS ▪ Dedifferentiated carcinoma ▪ Carcinosarcoma, NOS ▪ Mixed cell adenocarcinoma
Sex Cord-stromal Tumors	
Pure stromal tumors	▪ Fibroma, NOS ▪ Cellular fibroma
	▪ Thecoma, NOS ▪ Thecoma luteinized ▪ Sclerosing stromal tumor ▪ Microcystic stromal tumor ▪ Signet-ring stromal tumor ▪ Leydig cell tumor of the ovary, NOS ▪ Steroid cell tumor, malignant ▪ Fibrosarcoma
Pure sex cord tumors	▪ Adult granulosa cell tumor ▪ Granulosa cell tumor, juvenile ▪ Sertoli cell tumor, NOS ▪ Sex cord tumor with annular tubules
Mixed sex cord-stromal tumors	▪ Sertoli-Leydig cell tumor, NOS ▪ Sertoli-Leydig cell tumor, well-differentiated ▪ Sertoli-Leydig cell tumor, moderately differentiated ▪ Sertoli-Leydig cell tumor, poorly-differentiated ▪ Sertoli-Leydig cell tumor, retiform ▪ Sex cord tumor, NOS ▪ Gynandroblastoma
Germ Cell Tumors	
Germ cell tumors examples	▪ Teratoma ▪ Immature teratoma, NOS ▪ Dysgerminoma ▪ Yolk sac tumor, NOS ▪ Embryonal carcinoma, NOS ▪ Choriocarcinoma, NOS ▪ Mixed germ cell tumor
Monodermal teratomas and somatic-type tumors arising from a dermoid cyst	▪ Struma ovarii, NOS ▪ Struma ovarii, malignant ▪ Struma carcinoid tumor ▪ Teratoma with malignant transformation ▪ Mature cystic teratoma, NOS ▪ Immature teratoma, NOS
Germ cell-sex cord-stromal tumors	▪ Gonadoblastoma • Dissecting gonadoblastoma • Undifferentiated gonadal tissue ▪ Mixed germ cell-sex cord-stromal tumor, NOS

Contd...

Contd...

Table 24.28 Recent 2020 WHO classification of tumors of the ovary (*Contd...*)

Mesenchymal Tumors	
Mesenchymal tumors examples	▪ Endometrioid stromal sarcoma, low-grade ▪ Endometrioid stromal sarcoma, high-grade ▪ Leiomyoma, NOS ▪ Leiomyosarcoma, NOS ▪ Smooth muscle tumor of uncertain malignant potential ▪ Myxoma, NOS
Mixed epithelial and mesenchymal tumors	▪ Adenosarcoma

Miscellaneous Tumors	
Miscellaneous tumors examples	▪ Adenoma of rete ovarii ▪ Adenocarcinoma of rete ovarii ▪ Wolffian tumor ▪ Solid pseudopapillary tumor of ovary ▪ Small cell carcinoma, hypercalcemia type ▪ Small cell carcinoma and large cell carcinoma ▪ Wilm's tumor

Contd...

Table 24.29 Most common histologic subtypes of surface epithelial carcinomas of ovary

Ovarian Carcinoma Subtype	Frequency (%)	Morphologic Features of Well Differentiated Ovarian Carcinoma
Serous cystadenocarcinoma	50%	Tumor is composed of columnar cells with a prominent ciliated border that often form finger-like projections around fibrovascular core (papillae) within the inner lining of cysts filled with serous fluid
Endometrioid carcinoma	25–30%	Tumor is composed of glandular structures lined by low columnar cells that may be filled with bloody material
Mucinous cystadenocarcinoma	10–15%	Tumor is composed of columnar cells with clear mucus pushing cell nucleus toward basal pole, typically forming complex cystic structures
Clear cell carcinoma	5%	Tumor is composed of low columnar to cuboidal cells with clear cytoplasm and hobnail appearance, often forms small glandular structure or solid nests
Other tumors	<5%	Heterogenous group that includes ovarian tumor so poorly differentiated that their exact lineage cannot be determined as well as rare subtypes such as tumors showing features of transitional epithelium (malignant Brenner tumor) and others

Table 24.30 Differentiating features of benign and malignant ovarian tumors

Characteristics	Benign Ovarian Tumors	Malignant Ovarian Tumors
Clinical examination		
Mobility of tumor	Mobile	Immobile and fixed
Consistency	Cystic tumor	Solid and cystic tumor
Laterality of tumor	Unilateral tumor	Bilateral tumors
Vaginal examination	Smooth tumor on per vagina examination	Nodular tumor on per vagina examination
Intraoperative findings		
Imaging technique findings	Unilateral cyst with intact capsule	Ovarian capsule breached
Size	Most often, 10 cm size	Size varies
Septate	Septate <3 mm in thickness	Multiple septate >3 mm in thickness
Calcification	Present in benign cystic teratoma	Absent
Omental involvement	Absent	Present
Ascites	Absent (present in fibroma presenting as Meig's syndrome)	Present

Table 24.31 Salient features of ovarian masses

Ovarian Tumors	Clinical Manifestations	Tumor Markers	Facts
Germ cell tumors	▪ Young women ▪ Pain in adnexa ▪ Complex cystic mass	▪ LDH ▪ β-hCG ▪ AFP (α-fetoproteins)	Dysgerminoma most common malignant tumor
Surface epithelial tumors	▪ Postmenopausal women ▪ Distended abdomen ▪ Adnexal mass/pain ▪ Weight loss	▪ CA 125 ▪ CEA (carcinoembryonic antigen)	▪ Most common ovarian cancer ▪ Most common subtype (serous cystadenocarcinoma)
Granulosa-theca cell tumors	▪ Postmenopausal women ▪ Postmenopausal bleeding	Estrogen	Synthesizes estrogen resulting in endometrial hyperplasia
Sertoli-Leydig cell tumors	Women with masculinization features (deepening of voice and more hair on body)	Testosterone	Synthesizes testosterone
Krukenberg tumors	▪ History of gastric ulcer with worsening epigastric pain ▪ Breast lump	CEA (carcinoembryonic antigen)	Mucin secreting adenocarcinoma from stomach but with metastasis to ovaries

Serum CA 125 is elevated in ovarian cancer and also elevated in cirrhosis, endometriosis, peritonitis and pancreatitis. Ultrasonography is preformed to confirm ovarian mass. Serum tumor markers and histologic examination of biopsy are needed to establish diagnosis. Salpingo-oophorectomy is performed during childbearing women. In postmenopausal women, abdominal hysterectomy with bilateral salpingo-oophorectomy is done.



Fig. 24.71: Krukenberg's tumor: Both ovaries show involvement as a result of metastases from primary carcinomas of stomach or colon or breast (arrows).

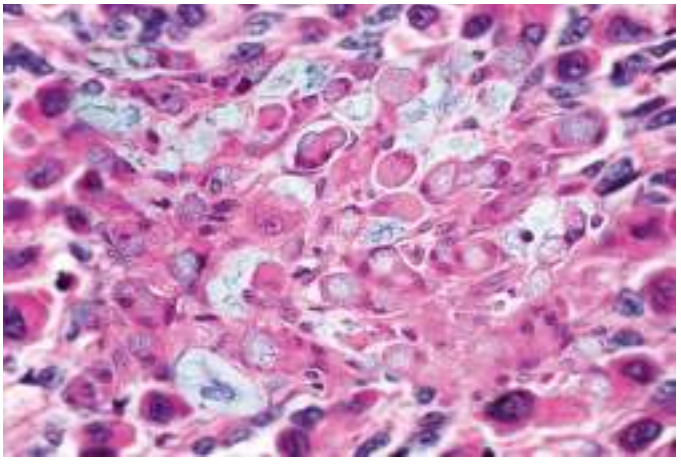


Fig. 24.72: Krukenberg's tumor. It shows nests of mucin-filled signet ring epithelial cells with eccentric nuclei within a cellular stroma (400X).

SURFACE EPITHELIAL TUMORS OF OVARY

Primary surface epithelial tumors account for 65–70% of all ovarian tumors, which include serous, mucinous, endometrioid, clear cell, and transitional (Brenner) tumors.

- **Histogenesis:** During embryonic life, the coelomic cavity is lined by a mesothelium. This mesothelium gives rise to Müllerian ducts, from which uterus, fallopian tubes, and vagina originate. Pluripotency of the ovarian surface epithelium accounts for the spectrum of histologic tumor variants seen in this location.
- **Differentiation of surface epithelium:** Surface epithelium has potential to differentiate to ciliated columnar epithelium resembling tubal epithelium, tall mucus-secreting nonciliated epithelium resembling endocervix, uterine endometrial glands and transitional epithelium resembling urinary bladder. Surface epithelium of ovary has potential to differentiate into various surface epithelial tumors (Table 24.32). Classification of ovarian surface epithelial tumors based on their malignant potential is given in Table 24.33. Risk factors for surface epithelial tumors of ovary are given in Table 24.34. Risk factors for familial ovarian carcinoma are given in Table 24.35. Molecular genetic alterations associated with sporadic (nonfamilial) ovarian carcinomas are given in Table 24.36.

Table 24.32 Surface epithelium of ovary has potential to differentiate into various surface epithelial tumors

Lining Epithelium	Similar to Epithelial Lining and Examples of Ovarian Tumors	Examples of Ovarian Tumors
Ciliated columnar epithelium	Fallopian tube	Serous tumors
Tall mucus-secreting nonciliated epithelium	Endocervix	Mucinous tumors
Epithelium similar to uterine endometrial glands	▪ Uterine endometrial glands ▪ Uterine endometrial glands during pregnancy	▪ Endometrioid tumors ▪ Clear cell tumors (glycogen rich)
Transitional epithelium	Urinary bladder	Brenner tumors

Table 24.33 Classification of ovarian surface epithelial tumors based on their malignant potential

Nature	Features
Benign surface epithelial tumors	▪ Surface epithelial benign tumors include serous cystadenoma, mucinous cystadenoma and Brenner tumors. Serous tumors account for approximately 20% of all ovarian tumors. Serous epithelial tumors are most often bilateral and are more common than mucinous tumors ▪ Fluid-filled cysts lined by single layer of neoplastic epithelium supported by a fibrovascular wall; without invasion or metastatic ability
Borderline surface epithelial tumor or low malignant potential	Approximately 10% of ovarian surface epithelial tumors are borderline malignant, which show varying degree of nuclear atypia and increased mitotic activity. Neoplastic cells form complex structures such as '**papillae**' compared to carcinomas, without or reduced invasive ability. These remain confined to the ovary. Rarely, these may metastasize to peritoneum ▪ Serous borderline tumors most often occur in 20–50 years of age group. Cells may shed from the surface and implant on peritoneum, possibly reflecting multifocal origin. S-100 protein is positive in particularly borderline tumors ▪ Mucinous borderline tumors most often occur in younger women. Hart Norris criteria of borderline mucinous tumor include atypical epithelial cells >4 layers thick, are showing pleomorphism and arranged in cribriform pattern. There is lack of fibrovascular cores in these tumors ▪ Borderline endometrioid tumors exhibit well differentiated adenocarcinoma with lack of stromal invasion ▪ Borderline Brenner tumor is composed of cysts and papillae. These papillae are projecting in cystic spaces and lined by stratified lining. Stromal invasion is absent
Invasive surface epithelial carcinomas	Well differentiated malignant surface epithelial tumors tend to be associated with early stage disease. Mucinous and endometrial tumors have better prognosis than serous cystadenocarcinomas. Serous epithelial tumors are most often bilateral with malignant potential in 30% of patients ▪ Low-grade invasive surface epithelial carcinomas of ovary retain the ability to form organized structures such as gonads or papillae; neoplastic cells are abnormal, but similarities with their normal counterparts are readily apparent, mild nuclear polymorphism ▪ High-grade invasive surface epithelial carcinomas of ovary tend to form solid sheets with no organization; cellular features seen in the benign counterparts of these tumors, such as secretions and others, are not readily apparent, marked nuclear polymorphism

Table 24.34 Risk factors for surface epithelial tumors of ovary

Risk Factors	Characteristics
Age group	As a group, younger patients have a better outcome due to occurrence of higher percentage of borderline, well differentiated, and stage I tumors in this age group
Nulliparity	It is dangerous because repeated disruption and repair of the epithelial surface. It occurs during normal cyclic ovulation in nulliparous. Conversely, suppression of ovulation in multiparous women is protective due to repeated pregnancy, lactation and oral contraceptive pills
Long fertile period	Early menarche (>12 years age), late menopause and long-term estrogen replacement therapy increase risk of ovarian cancer
Family history	BRCA1 is implicated in cancers of breast and ovary. If mother had ovarian cancer then daughter is at increased risk of developing cancer of breast and ovary

Contd...

Table 24.34 Risk factors for surface epithelial tumors of ovary (*Contd...*)

Risk Factors	Characteristics
Gonadal dysgenesis	Patient develops ovarian childhood malignancies and rarely gonadoblastoma
Superovulation therapy for infertility treatment	It may also be a predisposing factor (controversial)
BRCA1 tumor suppressor gene	Mutation of BRCA1 has been implicated in familial cancers of ovary (50%) and breast. These patients tend to develop ovarian cancer considerably earlier than women who have sporadic ovarian cancer
ERBB2 (HER2/neu)	Gene amplification of ERBB2 (HER2/neu) is demonstrated by immunohistochemistry technique (monoclonal antibody) on the cell membrane or using fluorescent *in situ* hybridization. Mutation of ERBB2 (HER2/neu) is observed in 30% of ovarian cancers. Herceptin is administered to treat ovarian cancers
SMADA4 tumor suppressor gene	Mutation of DPC4 (deletion) has been demonstrated in 55% of pancreatic carcinoma and rarely seen in cancers of breast and ovaries
DNA mismatch repair genes inactivation	There is increased risk for development of carcinoma of endometrium (first most common cancer), right colon and ovary in patients with hereditary nonpolyposis colon cancer (Lynch syndrome)

Definite risk factors can be identified only in minority of cases and include ovarian dysgenesis, family cancer syndrome, and BRCA1 and BRCA2 gene mutations linked with an increased incidence of breast cancer

Table 24.35 Risk factors for familial ovarian carinoma

Family History of Carcinoma	Additional Risk Factor	Gene Mutation
First-degree relative with ovarian carcinoma	History of infertility	BRCA2 tumor suppressor gene
Second-degree relative with ovarian carcinoma	Nulliparity (no history of pregnancies)	BRCA1 tumor suppressor gene
Two or more relatives with ovarian carcinoma	Long fertile period (early menarche and late menopause)	SMADA4 tumor suppressor gene
Hereditary breast-ovarian carcinoma (HBOC) syndrome	North American/European women	ERBB2 (HER2/neu)
Hereditary nonpolyposis colorectal carcinoma (HNPCC)	▪ Gonadal dysgenesis ▪ Superovulation therapy for infertility treatment ▪ Exposure to talc	DNA mismatch repair genes inactivation

Table 24.36 Molecular genetic alterations associated with sporadic (nonfamilial) ovarian carcinomas

Category	Gene and Chromosome Alterations	Comment
Oncogenes	▪ HER2/neu	▪ Receptor tyrosine kinase; overexpression is associated with poor prognosis and therapeutic response
	▪ K-RAS, H-RAS, BRAF	▪ Mutations in K-RAS, H-RAS and BRAF genes are frequent in serous and mucinous ovarian low-grade carcinomas and tumors of low malignant potential
	▪ AKT2	▪ Member of a subfamily of protein-serine/threonine kinases is linked to ovarian cancer
	▪ Cyclin E	▪ Overexpression in ovarian carcinoma has been associated with poor prognosis
Tumor suppressor genes	▪ TP53	▪ Regulator of cell cycle progression and apoptosis. TP53 gene mutation is linked to ovarian cancer
	▪ PTEN	▪ PTEN gene results in part, in inhibition of cell death; it is mutated in a significant proportion of endometrioid ovarian carcinomas
	▪ BAF250α	▪ Component of the SW1-SNF chromatic remodeling complex. BAF250α gene mutation is linked to ovarian carcinoma

Contd...

Table 24.36 Molecular genetic alterations associated with sporadic (nonfamilial) ovarian carcinomas (*Contd...*)

Category	Gene and Chromosome Alterations	Comment
	▪ NOEY2	▪ Induces p21 and downregulates cyclin D. NOEY2 gene mutation is linked to ovarian cancer
	▪ SPARC2	▪ Encodes a calcium binding matrix protein that contributes to cell adhesion. SPARC2 gene mutation is linked to ovarian cancer
	▪ DOC-2	▪ Binds GRB-2 upstream of RAS. DOC-2 gene mutation is linked to ovarian cancer
Chromosomes with frequent losses of heterozygosity	Chromosomes: 3p, 6p, 6q, 9p, 1p, 11q, 13q, 17p, 17q, 19q, 22q, Xp, Xq	Multiple candidate tumor suppressor genes have been reported on these chromosomes, but their role in ovarian carcinogenesis is unclear
Altered signal transduction pathways	▪ PIK3CA	▪ Amplification and overexpression of PIK3CA has been demonstrated in ovarian sporadic cancer
	▪ Wnt/β-catenin	▪ The Wnt/β-catenin pathway plays critical roles in regulating embryonic development and physiological processes. In the absence of Wnt/β-catenin ligands, β-catenin is degraded by a destruction complex. Aberrant activation of Wnt pathway has been reported in ovarian sporadic cancer. Aberrant activation of Wnt pathway leads to hyperactivity of β-catenin, which interacts with TCF/LEF transcription factors to regulate target gene expression
	▪ EGFR (tyrosine kinase activity)	▪ EGFR plays a major role in the etiology and progression of many carcinomas, including epithelial ovarian cancer

Mode of Spread of Surface Epithelial Carcinomas of Ovary

Surface epithelial ovarian carcinomas initially spread by direct seeding of the peritoneal surfaces, and is found on the underside of the diaphragm, paracolic gutters, urinary bladder, cul-de-sac, surface of liver, mesentery and serosa of large and small bowel, omentum, uterus and para-aortic and pelvic lymph nodes. Mode of spread of surface epithelial carcinomas of ovary is shown in Fig. 24.73 and Table 24.37.

Clinical Course of Surface Epithelial Tumors of Ovary

- **Benign surface epithelial tumors:** These constitute 80% of ovarian tumors during reproductive period. Most ovarian tumors are benign and nonfunctional and do not produce clinical effects until they have grown to a large size and present with increasing mass or girth.
- **Malignant surface epithelial tumors:** These tumors affect elderly women (40–65 years), which rapidly spread outside the ovary by local extension or surface seeding and occasionally through lymphatic route and hematogenous route involving extra-abdominal organs such as lung at the time of diagnosis. Patient presents with abdominal mass, ascites, cachexia, and pleural effusion, signs of increased pressure on neighboring organs such as urinary frequency, constipation, torsion, feminizing or masculinizing effects. These tumors often have poor prognosis.

Role of Immunohistochemistry Technique in Primary Surface Epithelial Tumors of Ovary and Krukenberg Tumor of Ovary

Immunohistochemistry aids in differentiating primary surface epithelial tumors and metastatic tumors to ovary (Krukenberg tumor). Most common panel of immunohistochemical markers is used to differentiate primary surface epithelial tumors from metastatic tumors to ovary. These markers are CK7, CK20, CEA, CA 125, ER, WT1 and S-100 protein.

- **CK7 marker:** It is the most helpful in differentiating primary surface epithelial cystadenocarcinoma and metastatic colorectal carcinoma to ovary. CK7 is positive in primary adenocarcinoma of ovary in 96% of cases.
- **CK7, CK20 and CEA:** These aid in differentiating primary surface serous cystadenocarcinoma from primary mucinous carcinoma of ovary. These immunohistochemical markers are less helpful in differentiating primary surface adenocarcinoma form colorectal adenocarcinoma metastasizing to ovaries.
- **S-100 protein:** It is positive in borderline serous epithelial tumors of ovary.
- **WT1 marker:** It stains diffusely in most primary serous epithelial adenocarcinomas within the female genital tract.
- **PAX2 marker:** It is positive in primary serous cystadenocarcinomas of ovary.
- **SMO47 marker:** It is used to establish origin of cystadenocarcinoma from ovary. It is strongly expressed in most primary serous cystadenocarcinomas of ovary. SMO47 is not expressed in primary mucinous cystadenocarcinomas.

- **Anti-MUC16 (OC125):** Anti-MUC16 (OC125) monoclonal antibody recognizes an epitope on a molecule called cancer antigen 125 (CA 125). The human cancer antigen 125 (CA 125) plays an important role in the pathogenesis of ovarian surface epithelial carcinoma. Hence, it shows overexpression in the primary ovarian surface epithelial cancer cells.

Markers	Expression	Ovarian Surface Epithelial Tumor
▪ CA 125 (anti-MUC16 (OC125)	▪ Positive	▪ Mucinous cystadenocarcinoma ▪ Cervical carcinoma ▪ Endometrial carcinoma ▪ Breast carcinoma ▪ Thyroid carcinoma ▪ GIT carcinoma
▪ ER/PR	▪ Positive	▪ Surface epithelial tumors
▪ S-100 protein	▪ Positive	▪ Borderline serous surface epithelial tumors
▪ CK7	▪ Positive	▪ Serous cystadenocarcinoma
▪ CK20	▪ Positive	▪ Serous cystadenocarcinoma
▪ CEA	▪ Positive	▪ Serous cystadenocarcinoma
▪ WT1	▪ Positive (diffuse staining)	▪ Serous cystadenocarcinoma
▪ PAX2	▪ Positive (strong staining in most tumors)	▪ Serous cystadenocarcinoma
▪ SMO47	▪ Positive (useful marker in ascertaining the female genital tract tumor)	▪ Serous cystadenocarcinoma
▪ CK7, CK20, CEA	▪ Negative	▪ Mucinous cystadenocarcinoma

CA 125 is also positive in cervical carcinoma, endometrial carcinoma, breast carcinoma, thyroid carcinoma, gastric carcinoma and colon carcinoma. CEA: Carcinoembryonic antigen; ER: Estrogen receptor; PR: Progesterone receptor.

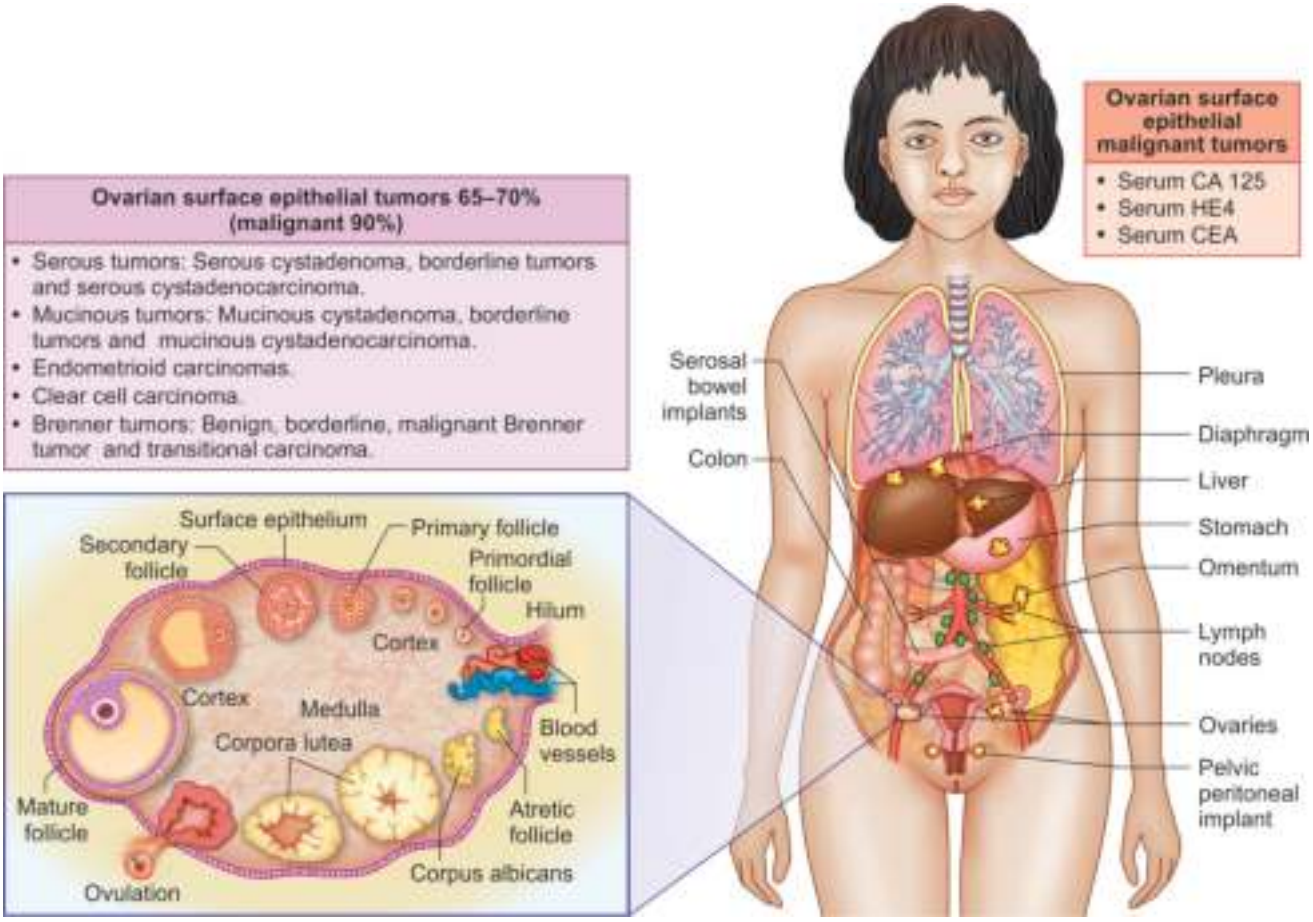


Fig. 24.73: Metastases of surface epithelial ovarian carcinoma to various organs.

Table 24.37 Mode of spread of surface epithelial carcinomas of ovary

Surface Epithelial Carcinoma of Ovary	Metastases to Organs
Direct extension of tumor	
Serous cystadenocarcinoma or mucinous cystadenocarcinoma or endometrioid carcinoma	▪ Omentum or pelvic peritoneum ▪ Serosa of the uterine corpus ▪ Fallopian tube ▪ Ovary (autoimplants) and opposite ovary ▪ GIT (serosa of stomach, sigmoid colon, cecum) ▪ Liver, spleen due to spread from peritoneum ▪ Widespread peritoneal cavity covered by tumor implants
Borderline serous tumors	▪ Peritoneal implants (16–47%) ▪ Psammoma bodies more numerous in noninvasive carcinoma than invasive peritoneal implants
Peritoneal dissemination	
Serous cystadenocarcinoma	Peritoneal involvement most common resulting in ascites
Mucinous cystadenocarcinoma	Peritoneal involvement less common
Endometrioid carcinoma	Peritoneal involvement less common
Lymphatic route	
Papillary serous cystadenocarcinoma	▪ Most common spread by lymphatic route ▪ Contralateral ovary ▪ Lymph nodes (para-aortic, pelvic, hepatic groups) ▪ Omentum peritoneal cavity ▪ An umbilical metastasis ('Sister Joseph's nodule') may be the first manifestation of the disease
Mucinous cystadenocarcinoma	Rarely spreads by lymphatic route
Hematogenous route	
Papillary serous cystadenocarcinoma	▪ Lungs ▪ Pleural cavity results in pleural effusion

Serous and mucinous cystadenocarcinoma and endometrioid carcinoma: These metastasize to pleura, diaphragm, lymph nodes, liver, stomach, omentum, serosa of bowel, pelvic peritoneal implant and opposite ovary. Mucinous and endometrioid ovarian carcinomas: These have less tendency to early and widespread peritoneal involvement in comparison to serous cystadenocarcinoma. Serous cystadenocarcinoma: It most often spreads by lymphatic route. Mucinous cystadenocarcinoma: It rarely spreads by lymphatic route.

SEROUS SURFACE EPITHELIAL TUMORS

Serous surface epithelial tumors account for approximately 20% of all ovarian tumors. Serous surface epithelial tumors of ovary are more common than mucinous tumors. These are most often bilateral with malignant potential in 30% of patients. These occur in women during reproductive age group (20–50 years). On the other hand, mucinous surface epithelial tumors are most often unilateral, therefore, mucinous surface epithelial tumors have better prognosis than serous surface epithelial tumors with bilateral involvement.

- Histologic types include serous cystadenoma, borderline tumor and serous cystadenocarcinoma. The tumor cells from serous borderline tumor may shed from the surface and implant on peritoneum, possibly reflecting multifocal origins, which show positivity for **S-100**. On electron microscopy, cilia are often found in ovarian benign and borderline tumors, but usually absent in serous cystadenocarcinomas.
- The neoplastic cells of serous surface epithelial tumors are always reactive to cytokeratins (CK7+/CK20), also express **EMA** and **CK18**. **S-100 protein** is positive in particularly borderline tumors. **WT1** stains diffusely most ovarian serous carcinomas ascertaining the site of origin of serous cystadenocarcinomas, within the female genital tract. **PAX8** is commonly positive in serous surface epithelial tumors of the ovary. Various monoclonal antibodies have been produced against surface epithelial (mainly serous) ovarian adenocarcinomas such as **CA 125** and **SMO47**.

Serous Cystadenoma of Ovary

Serous cystadenomas are most common ovarian tumors, and account for 66% of benign ovarian epithelial tumors, which occur in adult women in all age groups with mean of 40–60 years of age. Tumor measuring 1–3 cm is usually incidental finding. Large tumor produces nonspecific pelvic pain, discomfort and symptomatic pelvic mass. Patient is treated by unilateral salpingo-oophorectomy or ovarian cystectomy.

Surgical Pathology: Serous Cystadenoma of Ovary

Gross Morphology

- Serous cystadenomas of ovary are unilateral in majority of women and bilateral in 10–20% of cases.
- Tumor may be unilocular or multilocular cysts measuring 10–15 cm and filled with clear serous fluid.
- Cyst is either covered by smooth glistening capsule or partly by papillary excrescences (Fig. 24.74A and B).

Light Microscopy

- Serous cystadenoma of ovary is composed of fibrovascular core lined by pseudostratified single layer ciliated tall columnar epithelium similar to seen in fallopian tube epithelium, with round nuclei without atypia and mitoses. The columnar cells lining cyst contain mucin.
- There is presence of psammoma bodies with concentric layers of calcification (calcification of necrotic cells) in the fibrovascular core in 30% of cases. It has been suggested that a serous cystadenoma can be diagnosed only if the tumor measures more than one centimeter in diameter.
- A recent study indicates that the cells lining the cysts in most serous cystadenomas are polyclonal and therefore, non-neoplastic (Fig. 24.74C).

Immunohistochemistry Technique

Serous cystadenoma of ovary shows positivity for p16 epithelial marker.

Borderline Serous Tumor of Ovary

Approximately 10% of ovarian surface epithelial tumors are borderline low malignant potential. These tumors are predominantly cystic and seen in perimenopausal women. Borderline serous tumor remains confined to the ovary. Rarely, tumor cells may shed from the ovarian surface and implant on peritoneum, possibly reflecting multifocal origin.

Surgical Pathology: Borderline Serous Tumor of Ovary

Gross Morphology

- Borderline serous tumor of ovary tends to have fine, friable, and exuberant papillary projections on the internal and external surfaces of the cyst in 70% of tumors in contrast to the smooth-walled cysts of serous cystadenoma.
- Noninvasive desmoplastic implants are firm in consistency and are covered by an inflammatory exudate in 20% of cases.
- Thorough sampling of the primary tumor for histologic examination is essential to rule out tumor invasion.
- Recent data suggests that at least two sections per centimeter are required to confidently exclude tumor invasion.

Light Microscopy

- Borderline serous tumor is of ovary composed of extensive epithelial stratification, tufting (budding), and detachment of individual tumor cells and cell clusters in addition to small or large papillary branching.
- Stratification and tufting in at least 10% of the tumor warrant the diagnosis of borderline serous tumor.
- The cells in borderline serous tumor show features of epithelial and occasionally mesothelial differentiation.
- Ciliated cells resembling fallopian tubes are demonstrated in 33% of cases. The nuclei resemble those present in serous cystadenoma with slight nuclear atypia. The nuclei are ovoid or round with fine chromatin and nucleoli and basal located. Mitoses rarely exceed 4/10 high power fields.
- Psammoma bodies are demonstrated in ≤50% of cases.
- Autoimplants are demonstrated on the ovarian surface and inbetween exophytic surface papillae. Autoimplants range from 0.1 to 2.5 cm in size in 66% of cases (Fig. 24.75).

Immunohistochemistry Technique

S-100 protein is positive in borderline serous tumors.

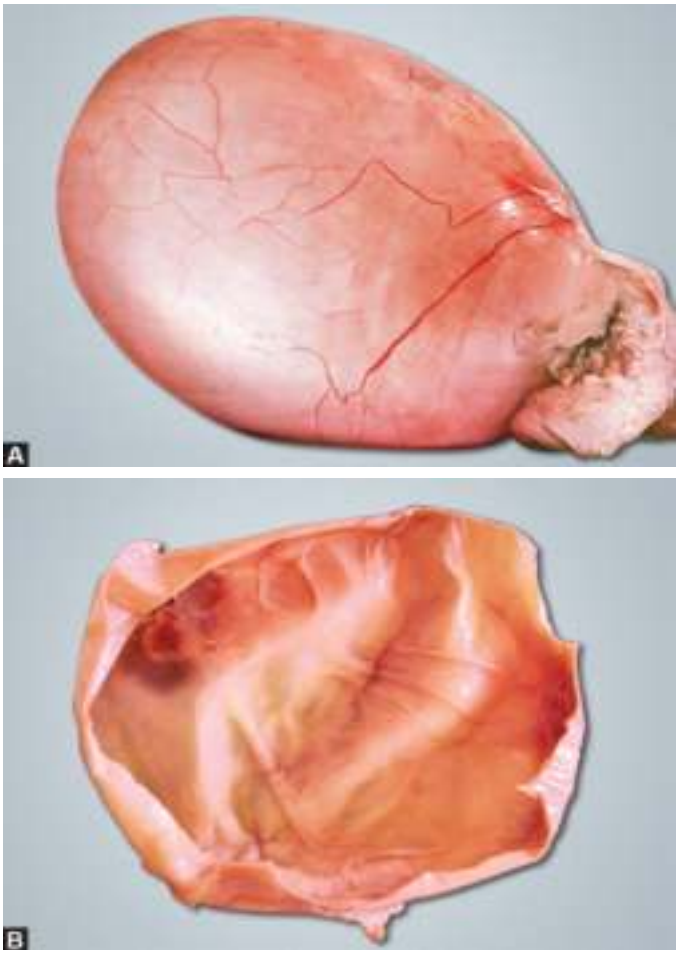


Fig. 24.74: (A) Serous cystadenoma, (B) cut opened unilocular, thin-walled cyst was containing clear fluid.

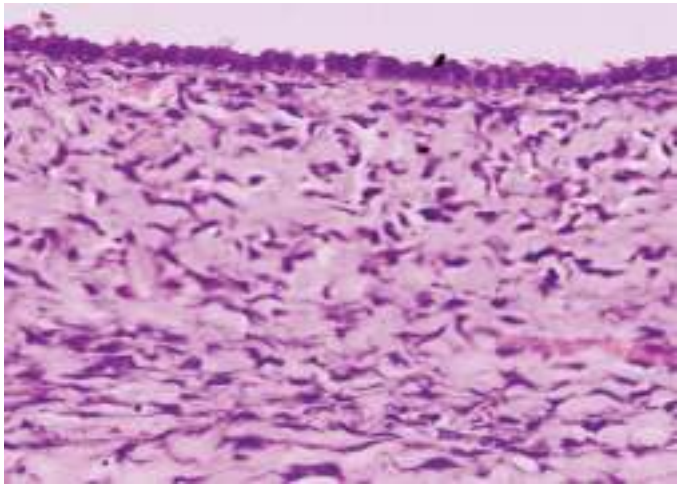


Fig. 24.74C: Serous cystadenoma of ovary. Tumor is composed of fibrovascular core lined by single layer ciliated tall columnar epithelium similar to fallopian tube epithelium, without atypia (100X).

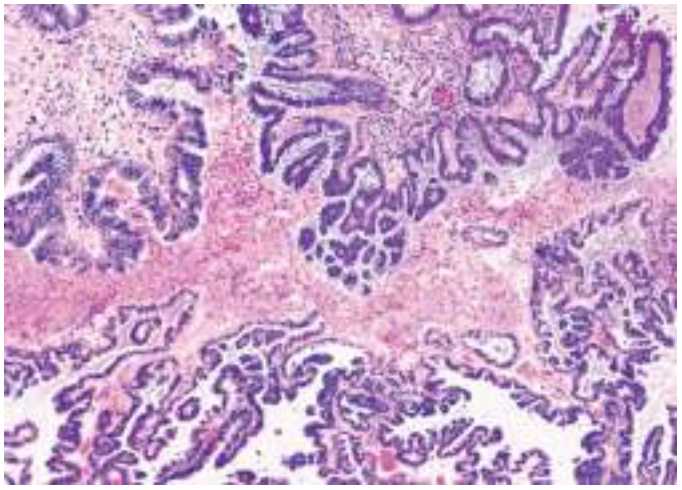


Fig. 24.75: Serous borderline tumor. Tumor shows nuclear atypia and increased mitotic activity. It is confined to be ovary (100X).

Serous Cystadenocarcinoma of Ovary

Serous cystadenocarcinoma accounts for approximately 50% of ovarian carcinomas, which is most often bilateral, solid and cystic ovarian masses. The diagnostic criteria of malignancy are stromal invasion.

- Presence of psammoma bodies in a patient with ascites is most compatible with the diagnosis of papillary serous cystadenocarcinoma of the ovary.
- Tumor most often occurs in the sixth and seventh decades of life. Patient undergoes rapid deterioration associated with fatal outcome.
- Patient presents with abdominal pain and distension due to ascites or bulky abdominal tumor, dysuria and bleeding per vagina.
- Peritoneal carcinomatosis is most demonstrated due to serous cystadenocarcinoma of ovary.
- During surgery, one can find advanced stage disease (stage II or III) of serous cystadenocarcinoma with seeding of surfaces of pelvic and abdominal peritoneum and bowel in majority of cases.

Surgical Pathology: Serous Cystadenocarcinoma of Ovary

Gross Morphology

- Serous cystadenocarcinomas of ovary range from microscopic to 20 cm in diameter. Tumor is typically multilocular with both solid and cystic elements. Tumor has soft, friable papillary projections filling the cavity.
- Stromal invasion by tumor and foci of necrosis are present.
- The multilocular cycts contain serous, turbid or hemorrhagic fluid. Solid areas of the tumor are pink to gray and soft to firm in consistency. Hemorrhage and necrosis are present.
- The outgrowth of tumor cells through the capsule of the tumor is an ominous sign.
- Omental metastases are characterized by firm nodules of variable size with gray-white surfaces. On gross examination, omentum contains microscopic tumor in 20% of cases (Fig. 24.76A).

Light Microscopy

- Serous cystadenocarcinoma of ovary displays complex multilayered papillary and solid patterns, marked cytologic atypia and psammoma bodies.
- Frequently, tumor displays lace-like pattern is characterized by extensive bridging and coalescence of papillae resulting in slit-like spaces. Areas of solid growth, glandular and cribriform patterns are commonly demonstrated.
- Neoplastic cells may be small and uniform focally contain pleomorphic nuclei with obvious malignant features, numerous mitoses and abnormal mitoses invading the stroma.
- There is presence of focal areas with papillary or glandular architecture that establish the diagnosis.
- The presence of tumor nodules on the peritoneum and ascites fluid that contains tumor cells is other sign of malignancy.
- Glandular tissue may be present. Tumor could be demonstrated at any stage of differentiation (Figs 24.76B and 24.77).

Immunohistochemistry Technique

Panel of immunohistochemical markers applied to paraffin-embedded sections of serous cystadenocarcinoma of ovary includes CA 125, CK7, CK20, SMO47, B72.3, CEA, Ber-EP4, PAX8, placental alkaline phosphatase (PLAP), hormone receptors (ER/PR) and WT1 (*see* Table 24.20).

Markers	Expression
CA 125	Positive
CK7	Positive
CK20	Positive
SMO47	Positive
B72.3 (anti-TAG72)	Positive
CEA	Positive
Ber-EP4	Positive
PAX8	Positive
PLAP	Positive
ER/PR	Positive
WT1	Positive
BRCA1	Positive (high-grade tumor)
p53	Positive (high-grade tumor) and negative (low-grade tumor)
p16	Positive (diffuse staining)

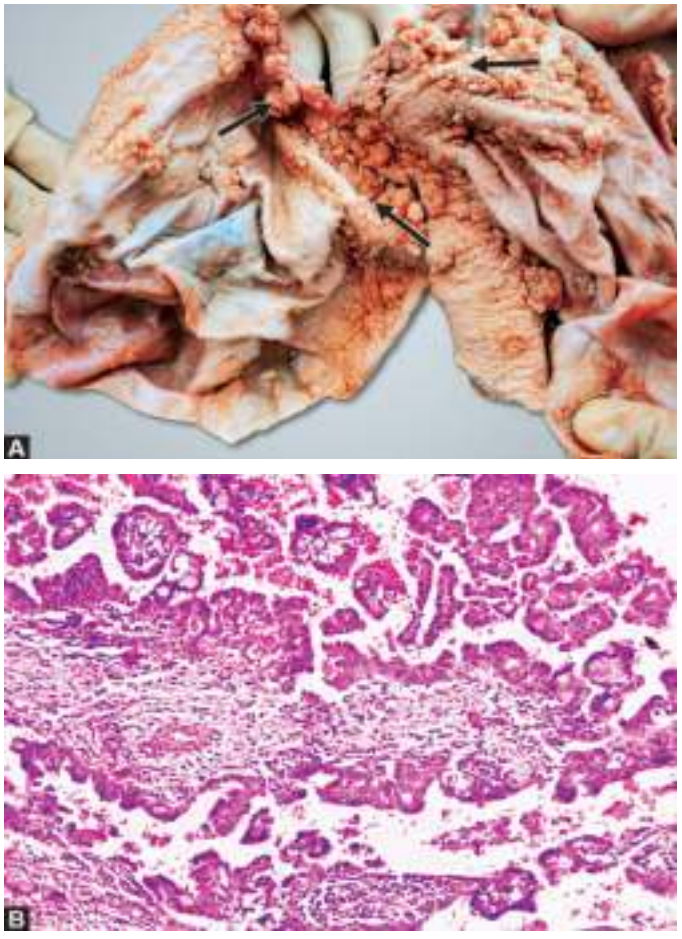


Fig. 24.76: (A) Papillary serous cystadenocarcinoma of the ovary. Note the papillary projections in the lumen of the cyst, and also on its surface. In a benign papillary cystadenoma, the papillae are present only on the inner surface of the cyst (arrows). (B) Papillary serous cystadenocarcinoma of the ovary. The tumor is composed of multilayered cells with solid papillae formation, cytologic atypia and psammoma bodies. Stromal invasion by tumor cells is evident (100X).

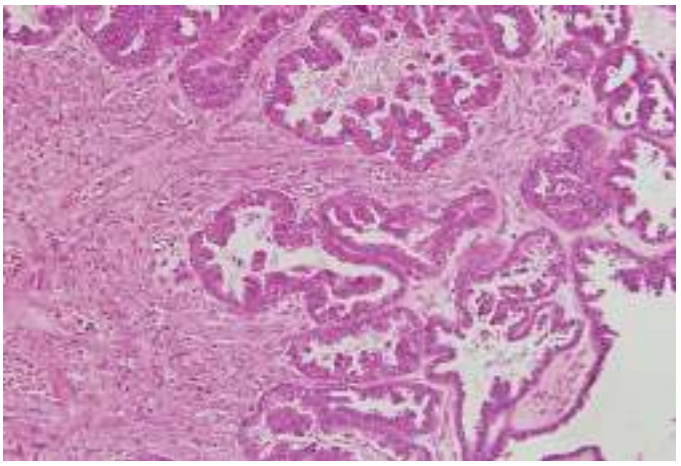


Fig. 24.77: Papillary serous cystadenocarcinoma of the ovary. The tumor is composed of multilayered cells with solid papillae formation, cytologic atypia and psammoma bodies. Stromal invasion by tumor cells is evident (400X).

MUCINOUS SURFACE EPITHELIAL TUMORS

Mucinous surface epithelial tumors of ovary are less common than serous tumors, which constitute 25% of all ovarian tumors. These are more often unilateral in 80% and bilateral in 20% of cases. Mucinous surface epithelial tumors are larger and usually multilocular filled with viscous mucus, which occur in middle-aged women. Like serous tumors, mucinous surface epithelial tumors can be classified as benign mucinous cystadenoma (80%), borderline mucinous tumor (10–15%) and mucinous cystadenocarcinoma (5–10%), and may be lined by endocervical or intestinal type of epithelium. Mucinous cystadenoma and borderline mucinous tumor are noninvasive; and mucinous cystadenocarcinoma is invasive tumor of ovary.

Mucinous Cystadenoma of Ovary

Mucinous cystadenoma of ovary can reach a very large size. Over 80% of mucinous cystadenomas are of intestinal type. Tumor is multilocular cystic tumor ranging from a few cm to 30 cm with a mean of 10 cm filled with viscous gelatinous material. It is most often unilateral in 75%, but bilateral in 25% of cases. It may undergo torsion, rupture, and transformation to borderline mucinous tumor or mucinous cystadenocarcinoma. Surgical excision of mucinous cystadenoma of ovary cures the patient.

Surgical Pathology: Mucinous Cystadenoma of Ovary

Gross Morphology

- Mucinous cystadenoma of ovary has a thick white capsule with smooth surface. Cut surface reveals large multilocular cyst.
- Cut surface shows multiple cystic spaces filled with viscous mucus. In a few cases, it may reach truly massive proportion, exceeding 50 cm in diameter (Fig. 24.78).

Light Microscopy

- Majority of mucinous cystadenomas of ovary are composed of glands and cysts lined by simple nonstratified mucinous epithelium resembling intestinal epithelium containing goblet cells or gastric foveolar-type epithelium with apical vacuolation.
- Tumors predominantly composed of mucinous cystadenoma with <10% of borderline features is diagnosed as mucinous cystadenoma with focal proliferation or focal atypia.
- The majority of tumors contain calcification. Muciphages, luteinized stromal cells and pseudoxanthoma cells are present in 40–50% of cases.
- Dissection of acellular mucin in the stroma of tumor is referred to as '**pseudomyxoma ovarii**' in 10% of cases.
- Multinucleated giant cells may be present in 10% of cases.
- Some mucinous cystadenomas can be admixed with Brenner tumor component. Histology of mucinous cystadenoma of ovary is shown in Fig. 24.79.

Borderline Mucinous Tumor of Ovary

Borderline mucinous tumor of ovary is analogous to borderline serous tumor. Most recent studies established 100% survival in these patients. It is important to rule out metastatic ovarian carcinomas arising from biliary tract, pancreas and cervix.

Surgical Pathology: Borderline Mucinous Tumor of Ovary

Gross Morphology

- Borderline mucinous tumor of ovary is large multicystic tumor with a smooth capsule and usually unilateral.
- Tumor measures 20–22 cm in diameter. Cut surface reveals multilocular tumor filled with mucinous material.

Light Microscopy

Mucinous borderline tumor of ovary is lined by stratified, proliferative gastrointestinal-type mucinous epithelium exhibiting tufted and villonodular or papillary intraglandular growth with mild to moderate nuclear atypia with mitotic activity confined to epithelium; and by definition, stromal invasion by tumor cells absent (Fig. 24.80).

Mucinous Cystadenocarcinoma of Ovary

Mucinous cystadenocarcinoma of ovary accounts for 10% of malignant ovarian tumors. It occurs most often unilateral, and multilocular cysts.

- Largest tumor measures 25 cm. Thin-walled tumor is containing mucinous fluid. Tumor most often occurs in perimenopausal women.
- Approximately 5% of women with mucinous cystadenocarcinomas of ovary present with pseudomyxoma peritonei, known as 'jelly belly', is a condition characterized by filling of peritoneal cavity with pool of mucin due to peritoneal tumor implants.



Fig. 24.78: Mucinous cystadenoma of the ovary. Tumor shows multiple cystic spaces filled with viscous mucus.

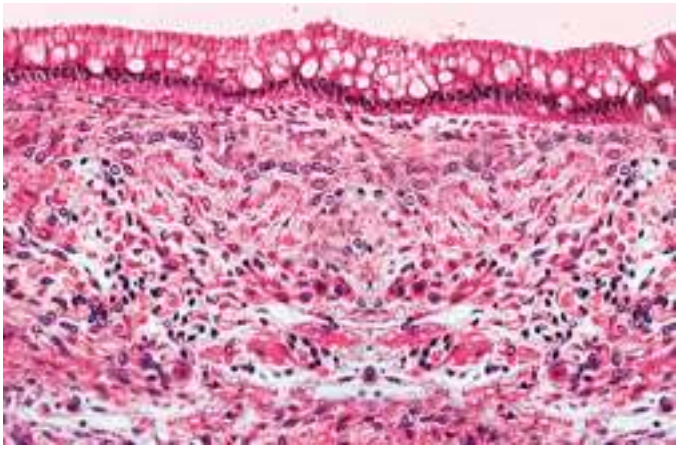


Fig. 24.79: Mucinous cystadenoma of the ovary. Tumor is composed of glands and cysts lined by simple nonstratified mucinous epithelium resembling intestinal epithelium containing goblet cells or gastric foveolar-type epithelium with apical vacuolation. Tumor composed predominantly of mucinous cystadenoma with <10% of borderline features is diagnosed as mucinous cystadenoma with focal proliferation or focal atypia (100X).

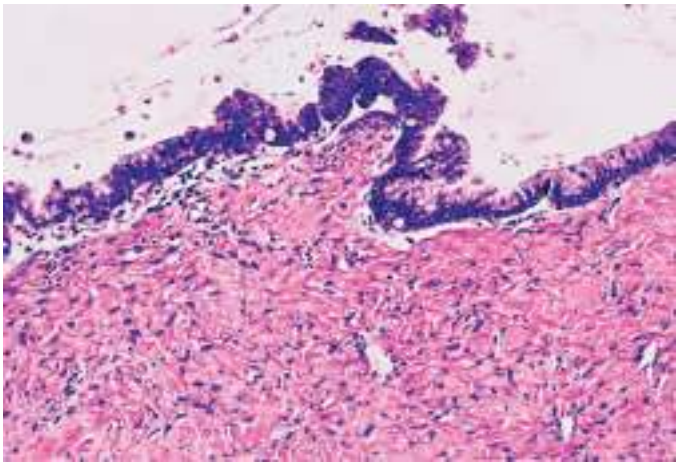


Fig. 24.80: Mucinous borderline tumor of ovary. Tumor is showing varying degree of nuclear atypia and increased mitotic activity confined to epithelium. There is no invasion of stroma by tumor cells (100X).

- Most mucinous cystadenocarcinomas associated with pseudomyxoma peritonei are metastatic in origin. It is worth mentioning that pseudomyxoma peritonei may also occur in mucocele of appendix. Accumulated mucinous contents may cause intestinal adhesions resulting in fatal outcome.
- Mucinous cystadenocarcinomas rarely spread via lymphatic route. These are clinically silent; hence the majority of patients have advanced metastatic disease at diagnosis.

Molecular Genetic Alterations: Mucinous Cystadenocarcinoma of Ovary

- K-RAS mutations have been detected in mucinous cystadenocarcinomas, adjacent mucinous cystadenoma and borderline mucinous tumor of ovary.
- Ovarian mucinous cystadenomas express several mucin genes (MUC2, MUC3 and MUC17), these are characteristic of mucinous carcinomas irrespective of their tissues of origin.
- Because primary ovarian mucinous cyst adenocarcinomas often display intestinal-type of differentiation, these express homeobox transcription factors CDX1, CDX2 and LGALS4 (galectin 4), which encode an intestinal cell surface adhesion molecule that is overexpressed in intestinal carcinomas.
- Galectin 4 (LGALS4) is not expressed by normal ovarian surface epithelium, but it is overexpressed in mucinous cystadenoma, borderline mucinous tumor and mucinous cystadenocarcinoma.

Surgical Pathology: Mucinous Cystadenocarcinoma of Ovary

Gross Morphology

Mucinous cystadenocarcinoma of ovary is large unilateral multilocular tumor with smooth white capsule. Cut surface shows multiple cystic spaces filled with mucinous fluid. Tumor may contain solid areas and foci of necrosis and hemorrhage (Fig. 24.81).

Light Microscopy

Frank stromal invasion by tumor cells of mucinous cystadenocarcinoma is demonstrated in histological sections. Two patterns of invasion are recognized—expansile and destructive.

- **Expansile pattern of invasion:** Tumor shows multilayered atypical epithelial cells arranged in crowded glandular fashion, a little stroma and sometimes a cribriform architecture.
- **Destructive pattern of invasion:** Tumor shows single gland or individual atypical cells invading >3 mm in two linear dimensions or >10 mm in 2 areas (Fig. 24.82A and B). Mucinous cystadenocarcinoma of ovary should be differentiated from metastatic colorectal adenocarcinoma metastasizing to ovaries.

Immunohistochemistry Technique

Ovarian mucinous cystadenocarcinomas show positivity with CA 125, CK7 and CEA. Metastatic colorectal adenocarcinomas show positivity with CK20 and CEA.

Markers	Expression
▪ CA 125	▪ Positive
▪ CK7	▪ Positive
▪ Carcinoembryonic antigen (CEA)	▪ Positive
▪ CD29	▪ Negative/positive (focal staining)
▪ CDX2	▪ Negative/positive (focal staining)

Clinical Course and Treatment

Patients with stage I mucinous cystadenocarcinoma displays expansile invasive growth have survival in 90%



Fig. 24.81: Mucinous cystadenocarcinoma of ovary. Cut surface of tumor shows multilocular cysts containing mucinous material. Solid areas are malignant.

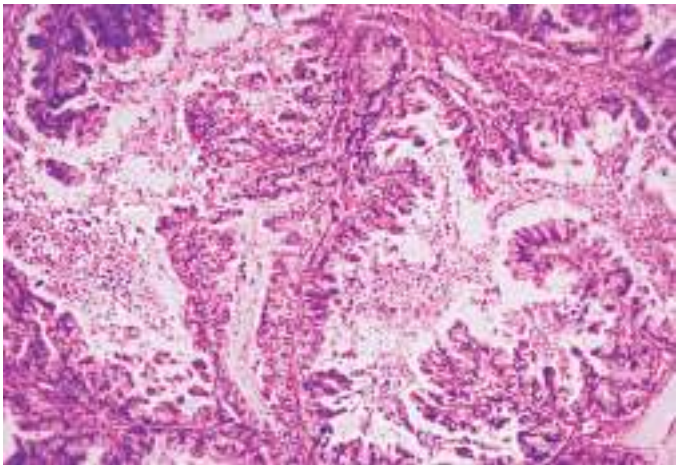


Fig. 24.82A: Mucinous cystadenocarcinoma of ovary. Tumor is multilayered atypical epithelial cells arranged in crowded glandular fashion, a little stroma and sometimes a cribriform architecture (400X).

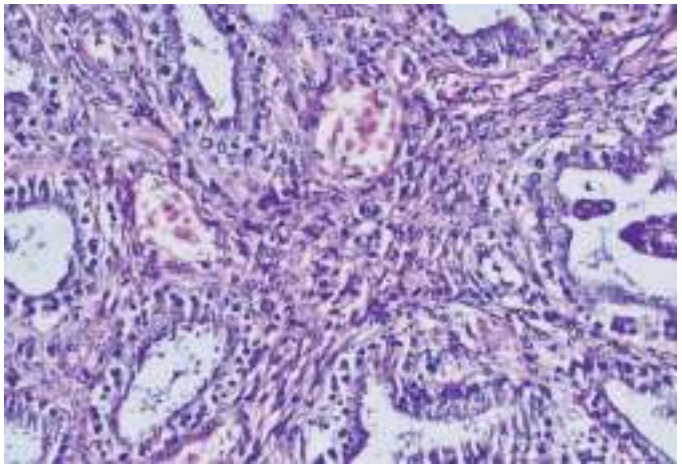


Fig. 24.82B: Mucinous cystadenocarcinoma of ovary. Tumor is composed of cystic spaces lined by atypical tall columnar cells with abundant intracytoplasmic mucin. The tumor cells are arranged in round or crowded glandular fashin with little stroma. Tumor cells exhibit stratification. Tufting and papillary formation with variable cytologic atypia. Tumor demonstrates both intracellular and extracellular mucin (400X).

of cases. Patients are treated with platinum-paclitaxel. Advanced disease is associated with fatal outcome.

Pathology Pearls: Metastatic Mucinous Carcinoma to Ovaries

- Metastatic mucinous carcinomas most often involve bilateral ovaries.
- Metastatic deposits are demonstrated on the ovarian surface and superficial cortex in a nodular or infiltrative haphazard pattern of stromal invasion.
- Some metastatic mucinous carcinomas are derived from the colorectum, pancreatobiliary tract, appendix and endocervix.
- Sometimes, metastatic mucinous carcinomas present in the ovary may resemble occult primary.
- Immunohistochemistry technique plays an important role in diagnosing primary and metastatic mucinous carcinomas.
- Distinction between primary and secondary colorectal mucinous carcinomas in the ovary is given in Table 24.38.

ENDOMETRIOID ADENOCARCINOMA

Endometrioid adenocarcinoma of ovary histologically resembles well differentiated carcinoma of endometrium. Majority of endometrioid tumors of the ovary are carcinomas. Squamous differentiation may be demonstrated in all types of endometrioid tumors.

Table 24.38 Distinction between primary and secondary colorectal mucinous carcinomas in the ovary

Feature	Primary Mucinous Carcinoma of Ovary	Metastatic Mucinous Colorectal Carcinoma in Ovary
Extraovarian disease	Usually absent (stage 1)	Often present (implants on peritoneum and omentum)
Gross morphology		
Ovary involvement	Unilateral (95%)	Bilateral (65%)
▪ Size of tumor ▪ Capsule ▪ Cut surface ▪ Presence of tumor	▪ Large tumor (mean 21 cm) ▪ Smooth capsule ▪ Multicystic tumor with solid foci ▪ Within ovarian stroma	▪ Small tumor (mean 2–18 cm) ▪ Smooth capsule ▪ Nodular to multicystic tumor ▪ On surface, superficial cortex and within ovarian stroma
Histologic features		
Tumor cells arrangement	▪ Well differentiated mucinous carcinoma forming crowded glandular/ cribriform pattern with little stroma ▪ Destructive pattern shows single gland or individual atypical cells	▪ Mucinous epithelium forming glandular pattern with nuclear atypia ▪ Garland pattern of glands lining cystic spaces, which contain dirty necrosis (abundant karyorrhectic debris)
Immunohistochemistry		
Immunohistochemistry is a diagnostic tool to establish diagnosis		
Markers	**Expression**	**Markers** / **Expression**
Primary mucinous cystadenocarcinoma of ovary		
▪ CK7 ▪ CK20 ▪ CA 125	▪ Positive ▪ Negative ▪ Positive	▪ CEA ▪ CD29 ▪ CDX2	▪ Negative ▪ Negative/positive (focal staining) ▪ Negative/positive (focal staining)

Contd...

Table 24.38 Distinction between primary and secondary colorectal mucinous carcinomas in the ovary (*Contd...*)

Pancreatic adenocarcinoma			
▪ CK7 ▪ CK20 ▪ CA19-9 ▪ DPC4 (SMAD4, MADH4) tumor suppressor gene	▪ Positive ▪ Positive ▪ Positive ▪ Allelic loss	▪ CEA ▪ MUC1 ▪ MUC5 ▪ EMA	▪ Positive ▪ Positive ▪ Positive ▪ Negative
Colorectal carcinoma			
▪ CEA ▪ CK7 ▪ CK20 ▪ CDX2 (transcription factor)	▪ Positive ▪ Positive ▪ Positive ▪ Positive (specific marker associated with poor prognosis)	▪ SAT (transcription factor) ▪ β-Catenin ▪ p53 ▪ Villin	▪ Positive ▪ Positive ▪ Positive ▪ Positive

- Endometrioid adenocarcinoma of ovary is a surface epithelial tumor arising from the coelomic epithelium, which constitutes 10% of the malignant ovarian tumors affecting perimenopausal age group. Approximately 10–20% of cases are associated with endometriosis and endometrial carcinoma. Benign endometrioid tumors (adenofibromas/cystadenofibromas) are rare.
- Endometrioid adenocarcinoma of ovary is composed of endometrial glands arranged in fibrous stroma, which is distinguished from endometriosis by lack of associated endometrial stroma and hemosiderin-laden macrophages.

Molecular Genetic Alterations

Molecular genetic studies have revealed that endometrioid carcinoma of ovary have similarities with, and differences from their uterine endometrioid carcinomas. Both ovarian and uterine endometrioid carcinomas demonstrate mutations in PTEN, PIK3CA and CTNNB1 genes. Primary ovarian endometrioid carcinoma demonstrates mutations in PTEN, PIK3CA, CTNNB1, TP53 and mismatch repair genes.

Molecular Genetic Alterations: Endometrioid Carcinoma of Ovary

- PTEN gene mutation
- PIK3CA gene mutation
- Mismatch repair gene mutation
- TP53 gene mutation (high-grade tumor)
- CTNNB-1 gene mutation (low-grade tumor showing squamous differentiation)

Clinical Features

Ovarian endometrioid adenocarcinoma affects women in fifth to sixth decades of life. Patient presents with abdominal distension and pain in pelvic and abdomen; and abnormal vaginal bleeding.

Surgical Pathology: Endometrioid Adenocarcinoma of Ovary

Gross Morphology

Endometrioid adenocarcinoma of ovary has an outer smooth surface. Cut surface reveals solid and cystic areas in contrast to serous and mucinous tumors. Cystic areas are filled with friable soft masses and hemorrhagic greenish turbid fluid. Less, commonly, the tumor is solid with extensive hemorrhage and necrosis. Tumors arising in endometriotic cyst may display cysts with chocolate-colored fluid. Visible papillary formations are usually absent or inconspicuous. It is mostly unilocular cystic tumor containing turbid brown fluid.

Light Microscopy

- Endometrioid adenocarcinoma of ovary resembles endometrial adenocarcinoma.
- Endometrioid adenocarcinoma of ovary has destructive infiltrative growth pattern. Most tumors are well differentiated, with or without papillary formations.
- Tumor is composed of angulated glands with and infiltrative pattern, jagged irregularly spaced and unevenly shaped nests and solid sheets.
- Presence of confluent glandular proliferation exceeding 5 mm (the limit of microinvasion) is termed 'expansile invasion'.
- Some tumors may show squamous cell differentiation in 50% of cases. Mitotic figures are commonly demonstrated. Histology of ovarian endometrioid adenocarcinoma is shown in Fig. 24.83. FIGO grading system for endometrioid adenocarcinoma of ovary is given in Table 24.39. Similarly, some tumors have foci resembling serous or mucinous carcinomas. Comparison of serous and mucinous and endometrioid carcinomas is given in Table 24.40.

Histochemistry

Mucin present within lumen or surface epithelium is demonstrated by mucicarmine.

Immunohistochemistry Technique	
Neoplastic cells of endometrioid carcinoma show positivity for hormone receptors (ER/PR).	
Marker	**Expression**
Estrogen/progesterone (ER/PR) receptors	Positive (>90%)

CLEAR CELL CARCINOMA OF OVARY

Clear cell carcinoma of ovary (mesonephroid tumor) is the least common tumor. The mean age of patients is between 50 and 55 years. These have strong association of clear cell carcinoma of ovary to ovarian endometriosis (70%) and endometrioid adenocarcinomas. Majority of clear cell tumors of ovary are frank high-grade adenocarcinomas associated with venous thromboembolism (15–45%) and paraneoplastic hypercalcemia.

Prognostic Factors

Staging is the only single prognostic factor for clear cell carcinoma of ovary. Tumor is confined to the pelvis and associated with better prognosis than high-grade serous cystadenocarcinoma of ovary. Advanced stage disease is associated with worse prognosis. Clear cell carcinoma associated with mutation in GPC3 gene located on Xp26 encoding glypican 3 is associated with poor prognosis. Approximately 50% cases have 5-year survival rate, when the tumor is confined to the ovaries.

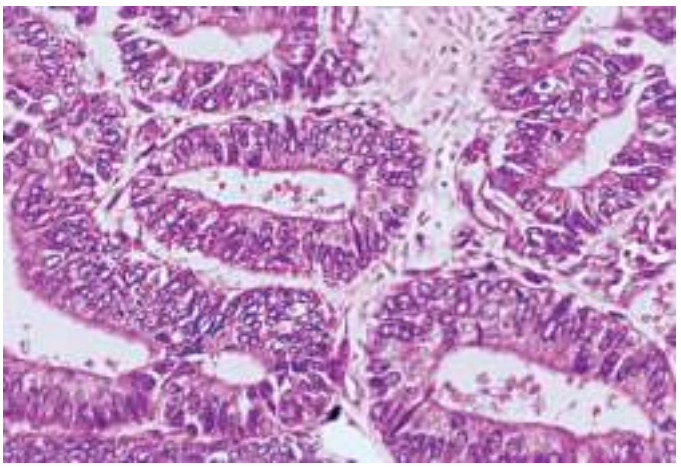


Fig. 24.83: Endometrioid adenocarcinoma of ovary. Tumor is composed of angulated glands with and infiltrative pattern, jagged irregularly spaced and unevenly shaped nests and solid sheets. Presence of confluent glandular proliferation exceeding 5 mm (the limit of microinvasion) is termed 'expansile invasion' (400X).

Table 24.39 FIGO grading system for endometrioid adenocarcinoma of ovary

Grade	Differentiation	Growth Pattern	Histologic Features
Grade 1	Well differentiated tumor	<5% solid growth pattern	Well formed glandular structures
Grade 2	Moderately differentiated tumor	6–50% solid growth	More complex glandular architecture with increased stratification
Grade 3	Poorly differentiated tumor	>50% sold growth	Poorly formed glands with large sheets of cells

Table 24.40 Comparison of primary endometrioid adenocarcinoma, mucinous cystadenocarcinoma and serous cystadenocarcinoma of ovary

Characteristics	Endometrioid Adenocarcinoma	Mucinous Cystadenocarcinoma	Serous Cystadenocarcinoma
Age group	Perimenopausal women	Middle adult age	Reproductive age
Relative frequency	10–25%	5–15% (less common)	60–80% (more common)
Ovary involvement	Bilateral 15–30%	Often unilateral (10–20%)	Often bilateral (30–50%)
Tumor	Solid or cystic	Multilocular cysts	Unilocular cyst
Size	Moderate	Often large size	Moderate size
Fluid content	Hemorrhagic fluid	Mucinous fluid	Serous fluid
Coexistent endometrial hyperplasia, or carcinoma	15–30% of cases associated with endometriosis	Exceptional case	Exceptional case
Torsion	Absent	Absent	Common
Pseudomyxoma peritonei	Absent	Most often present	Absent
Lining epithelium	Columnar, with centrally located nucleus	Columnar, with basally located nucleus	Cuboidal
Mucin	Mucin present in luminal border	Abundant mucin present in cytoplasm	Present only in luminal border
Squamous metaplasia	50% of cases	Exceptional case	Exceptional case
Cilia	Present in rare case	Absent	Frequent
Psammoma bodies	Exceptional case	Exceptional case	30%



Fig. 24.84: Clear cell carcinoma of ovary. Cut surface of tumor shows solid and cystic spongy areas giving honeycomb appearance.

Clinical Features

Patient presents with pelvic or abdominal mass. Clear cell carcinoma of ovary is frequently associated with ovarian or pelvic endometriosis, paraneoplastic hypercalcemia and venous thromboembolism.

Surgical Pathology: Clear Cell Carcinoma of Ovary

Gross Morphology

- Clear cell carcinoma of ovary is usually unilateral with size up to 30 cm (mean 13–15 cm).
- On cut surface, tumor exhibits a combination of solid white-tan to yellowish and thick-walled cystic spongy areas giving honeycomb appearance.
- Multiple yellow fleshy nodules are protruding in the lumen of the cyst reflecting focus of malignant transformation.
- These nodules may be lined by an endometriotic cyst, which typically contains chocolate brown fluid (Fig. 24.84).

Light Microscopy

- Clear cell carcinoma of ovary displays different histologic patterns: tubulocystic, papillary and solid.
- Cystic tumors exhibit small, regular and frequently hyalinized papillae, whereas tubulocystic pattern tumor tends to dominate in the adenofibromatous tumors.
- Tubulocystic pattern of tumor is characterized by varying size tubules and cysts. Cystic variant is more frequently associated with endometriosis. These two variants arise along different pathways.
- Solid pattern of tumor is composed of polygonal to cuboidal and flattened cells with clear slightly granular cytoplasm containing glycogen, mucin, and fat giving signet ring appearance. Some of the nuclei protrude into the lumina, resulting in hobnail configuration. These cells are arranged in solid sheets, tubular and papillary fashion separated by delicate fibrovascular septae or dense fibrotic stroma. The cores of the papillae often exhibit prominent eosinophilic hyalinized bodies (PAS positive) and lack epithelial stratification. In cystic areas, it shows neoplastic cells lining the spaces. Nuclear grade is nearly always regardless of the grading system. Mitotic activity tends to be low exceeding 10/10 HPF (Fig. 24.85).

Histochemistry

Cells may exhibit positivity with periodic acid–Schiff (PAS), diastase-resistant hyaline globules.

Immunohistochemistry Technique

Neoplastic cells of clear cell carcinoma show positivity for HNF-1β, CA 125, CEA, EMA, PAX8, CK7, CAM 5.2, CD15 (LeuM1), vimentin, BCL-2, OC125 and hormone receptors (ER/PR).

Markers	Expression	Markers	Expression
▪ HNF-1β	▪ Positive	▪ CD15 (LeuM1)	▪ Positive
▪ CA 125	▪ Positive	▪ B72.3	▪ Positive
▪ CEA	▪ Positive	▪ Vimentin	▪ Positive
▪ EMA	▪ Positive	▪ BCL-2	▪ Positive
▪ PAX8	▪ Positive	▪ OC125	▪ Positive
▪ CK7	▪ Positive	▪ ER/PR	▪ Negative
▪ CAM 5.2	▪ Positive		

Clinical Course and Treatment

Comprehensive surgical staging is essential in the early stage of clear cell carcinoma. Patient is treated by bilateral salpingo-oophorectomy, total hysterectomy, and lymph node dissection. Advanced disease is treated with paclitaxel plus carboplatin chemotherapy. Clear cell carcinoma is resistant to platinum-based chemotherapy, making metastatic disease difficult to treat.

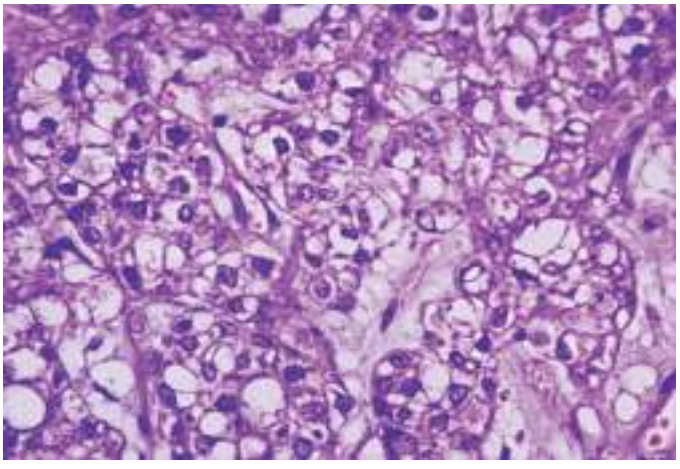


Fig. 24.85: Clear cell carcinoma of ovary. It shows large tumor cells with clear cytoplasm arranged in solid sheets, tubular and papillary fashion (400X).

BRENNER TUMOR OF OVARY

Brenner tumors account 10% of ovarian surface epithelial neoplasms and 1–2% of all ovarian tumors. Nearly all Brenner tumors are benign and uncommon include borderline and malignant forms. Brenner tumor occurs in premenopausal (70%) and postmenopausal (0–1%) women. Some cases are accompanied by signs of hyperestrinism, such as uterine bleeding from endometrial hyperplasia in the postmenopausal women.

Surgical Pathology: Brenner Tumor of Ovary

Gross Morphology

Brenner tumor of ovary is encapsulated with solid consistency variable in size usually unilateral, firm, white or yellowish resembling fibroma but contain small cystic areas filled with opaque, viscous, yellowish-brown fluid (Fig. 24.86A and B).

Light Microscopy

- **Benign Brenner tumor:** Tumor is composed of uniform population of stratified transitional cells with sharp outlines, ovoid nuclei displaying longitudinal groove (**coffee bean appearance**) arranged in well-formed solid nests, resembling transitional epithelium of urinary bladder. Tumor cells are surrounded by abundant dense, fibrous stroma. Most often, mucinous glands are present in the center and scattered throughout the dense fibrous stroma, resembling that of an ovary. Such neoplasm may have hormonal activity.
- **Borderline Brenner tumor:** This variant has low malignant potential. Tumor is composed of cysts and papillae. These papillae are projecting in cystic spaces and lined by stratified lining. Stromal invasion by tumor cells is absent.
- **Malignant Brenner tumor:** Tumor is composed of atypical large pleomorphic cells arranged in large nests with irregular borders. Stromal invasion by tumor cells is present.
- **Transitional cell carcinoma:** Tumor closely resembles transitional cell carcinoma of urinary bladder with same grading (Fig. 24.87).

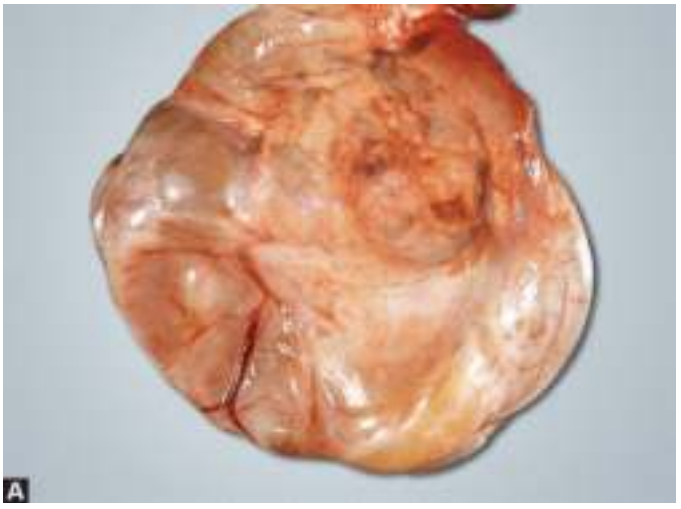
Histochemistry

The tumor cells of Brenner tumor of ovary may contain glycogen, mucin, and lipid. Lipid is present in larger amounts in the stromal cells in the cases accompanied by hyperestrinism.

Immunohistochemistry Technique

The tumor cells of Brenner tumor of ovary are reactive to cytokeratin, EMA, CEA (the latter also present in the lumen of the cysts), CA 19-9, uroplakin III and thrombomodulin.

Markers	Expression
▪ Cytokeratin	▪ Positive
▪ EMA	▪ Positive
▪ CEA	▪ Positive
▪ CA 19-9	▪ Positive
▪ Uroplakin III	▪ Positive
▪ Thrombomodulin	▪ Positive



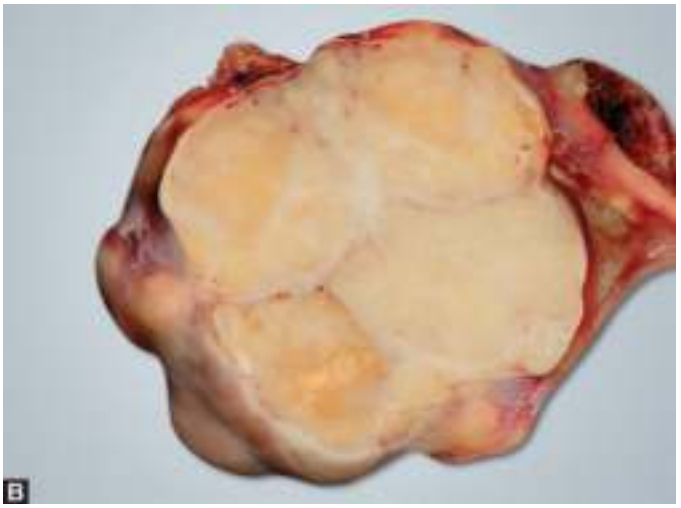


Fig. 24.86A and B: Brenner tumor of ovary. (A) Tumor is encapsulated solid tumor, (B) cut surface is white or yellow with firm consistency.

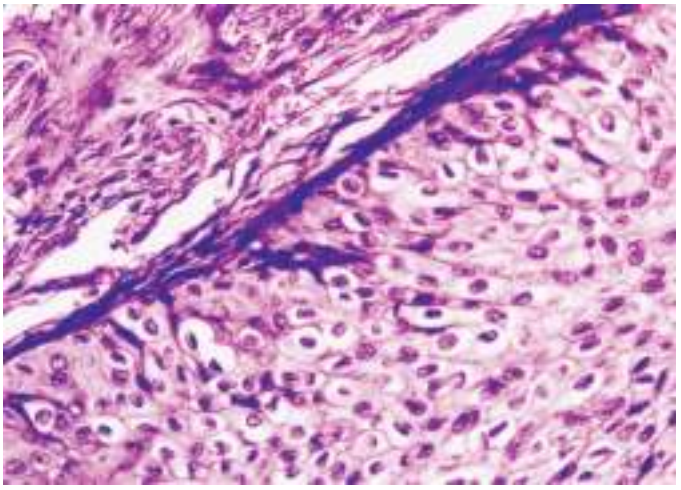


Fig. 24.87: Benign Brenner tumor of ovary. It shows solid and cystic nests of epithelial cells with oval nuclei, small nucleoli in a fibrous stroma. Many of these cells exhibit longitudinal grooves (400X).

Table 24.41 Recent 2020 World Health Organization of germ cell tumors of the ovary

Germ Cell Tumors
▪ Dysgerminoma • Classic dysgerminoma • Dysgerminoma with syncytiotrophoblastic cells • Dysgerminoma with yolk sac element • Anaplastic dysgerminoma
▪ Yolk sac tumor (endodermal sinus tumor) (extraembryonic differentiation) • Polyvesicular vitelline yolk sac tumor • Hepatoid yolk sac tumor • Glandular yolk sac tumor
▪ Choriocarcinoma (extraembryonic differentiation)
▪ Embryonal carcinoma (embryonic differentiation)
▪ Teratoma • Teratoma (embryonic differentiation) • Mature cystic teratoma (dermoid cyst) • Mature solid teratoma with malignant transformation • Immature teratoma • Monodermal and highly specialized types ◆ Struma ovarii (with thyroid tissue) ◆ Carcinoid tumor (e.g. insular, trabecular, stromal carcinoid, mucinous carcinoid)
▪ Polyembryoma
▪ Neuroectodermal tumors
Germ Cell-Sex Cord-Stromal Derivatives
▪ Gonadoblastoma with dysgerminoma and other germ cell tumors
▪ Germ cell-sex cord-stromal tumor with dysgerminoma and other germ cell tumors

Contd...

Clinical Course and Treatment

Benign and borderline Brenner tumors of ovary have a benign clinical course. Complete excision (unilateral oophorectomy, bilateral oophorectomy or total hysterectomy) is curative. About 80% of malignant Brenner tumors are in stage I and have an excellent prognosis after surgery.

GERM CELL TUMORS OF OVARY

Germ cell tumors constitute approximately 25% of all ovarian tumors, which are the most common tumors in children, accounting for 60–70% of all ovarian tumors in these age groups especially mature cystic teratomas.

- Germ cell tumors of ovary include dysgerminoma, germ cell tumors with embryonic differentiation (embryonal carcinoma, teratoma) and extraembryonic differentiation (yolk sac tumor, choriocarcinoma).
- There may be mixed germ cell tumor (dysgerminoma + benign cystic teratoma). Except mature cystic teratomas, the proportion of malignant germ cell tumors (especially immature teratomas, yolk sac tumors, and dysgerminomas) is much greater than in adults. Recent 2020 WHO of germ cell tumors of the ovary is given in Table 24.41.

DYSGERMINOMA OF OVARY

Dysgerminoma of ovary is composed of germ cells that exhibit morphologic, structural and histochemical similarity to primordial germ cell. The germ cells are considered to be arrested at an early and sexually stage of differentiation. Tumor is analogous to testicular seminoma. Dysgerminoma accounts for 50% of germ cell tumors and 2% of all ovarian tumors and affects 10–30 years of age group especially second and third decades. Dysgerminoma is extremely radiosensitive, and most often unilateral in 80–90% of cases. Incidence is more in Turner's syndrome, gonadal dysgenesis and pseudohermaphroditism.

Clinical Features

Patient presents with abdominal/pelvic pain followed by an abdominal mass, fever, vaginal bleeding and occasionally ascites. Dysgerminoma is usually unilateral, and may be bilateral in 10–20% of cases.

Surgical Pathology: Dysgerminoma of Ovary

Gross Morphology

- Dysgerminoma affects right ovary (50%), left ovary (35%) and bilateral (15%). There is increased prevalence of bilateral dysgerminomas associated with gonadoblastoma.
- Pure dysgerminoma is a solid tumor, round, oval or lobulated with a smooth glistening fibrous capsule ranging from a few centimeters in diameter to large size measuring 50 cm, which fills the pelvic and abdominal cavities. Tumors weighing 5 kg have been documented. The capsule is usually intact, but may be ruptured in case of large tumor.
- The consistency of dysgerminoma varies from firm and rubbery in small tumors and soft in large tumor. On cut surface, tumor is solid and varying from gray-pink to light tan.
- Cut surface of large tumor shows red brown or yellow discoloration due to hemorrhage and necrosis.
- The presence of cystic areas on cut surface suggests the possibility of teratoma component (mixed germ cell tumor). It is important to do extensive sampling of the tumor (Fig. 24.88).

Light Microscopy

Like testicular seminoma, ovarian dysgerminoma may exhibit following histologic variants described as under.

- **Classic dysgerminoma:** Tumor is composed of large nests of monotonously uniform round or oval cells with centrally located deeply staining nuclei with finely granular chromatin and prominent eosinophilic 1–2 nucleoli, and abundant clear glycogen-filled cytoplasm. Tumor cells are separated by fibrous septa ranging from fine, delicate fibrovascular network to densely hyalinized stroma containing mostly T cells and granulomas (Fig. 24.89).
- **Dysgerminoma with syncytiotrophoblastic element:** Tumor is composed of scattered hCG positive syncytiotrophoblastic cells around proximity to blood vessels or to hemorrhagic foci in 6–8% of tumors. It may be accompanied by serum elevation of hCG and tissue immunoreactivity for this marker. The syncytiotrophoblastic giant cells may form large syncytial mass resembling the syncytiotrophoblast element of choriocarcinoma, but may differ from choriocarcinoma because there is no cytotrophoblast. Presence of choriocarcinoma in association with dysgerminoma is not frequent. There is no evidence that dysgerminoma with syncytiotrophoblastic element is associated with worse prognosis.
- **Dysgerminoma with yolk sac tumor:** Pure dysgerminoma is not associated with elevated levels of serum α-fetoprotein (AFT). The presence of elevated levels of AFT indicates that dysgerminoma contains primary yolk sac tumor or its metastases.
- **Anaplastic dysgerminoma:** Tumor shows increased mitotic activity over 30 mitoses/10 high power fields. This variant is reactive for keratin.

Histochemistry

- The cytoplasm of the tumor cells contains glycogen, which can be demonstrated with the periodic acid–Schiff (PAS) reaction and is removed by diastase digestion. The amount of glycogen in tumor cells is variable, and the glycogen is lost during prolonged fixation with formalin. Hence, PAS reaction may be vary from strong to weak.
- Presence of lipid in tumor cells can be demonstrated in the cytoplasm of tumor cells in frozen tissue stained by Sudan III, Sudan IV, Sudan black and Oil red O stains.

Immunohistochemistry Technique

Neoplastic cells of dysgerminoma are positive for placental alkaline phosphatase (PLAP), CD117, SALL4, D2–40, PAS and OCT4 (POU5F1). SALL4 is highly sensitive and specific for primitive germ cell tumors, including dysgerminoma.

Markers	Expression
PLAP	Positive
CD117(c-KIT)	Positive
SALL4	Positive (nuclear staining)
D2–40 (podoplanin)	Positive (membrane staining)
PAS	Positive
OCT4 (POU5F1)	Positive (nuclear staining)
CAM 5.2	Negative
AE1/3	Negative
EMA	Negative

Molecular Genetic Alterations

Neoplastic cells of dysgerminoma of ovary contain double the amount of DNA in the nuclei as compared to normal somatic cells. Isochromosome 12 has been demonstrated in dysgerminoma as well as seminoma.

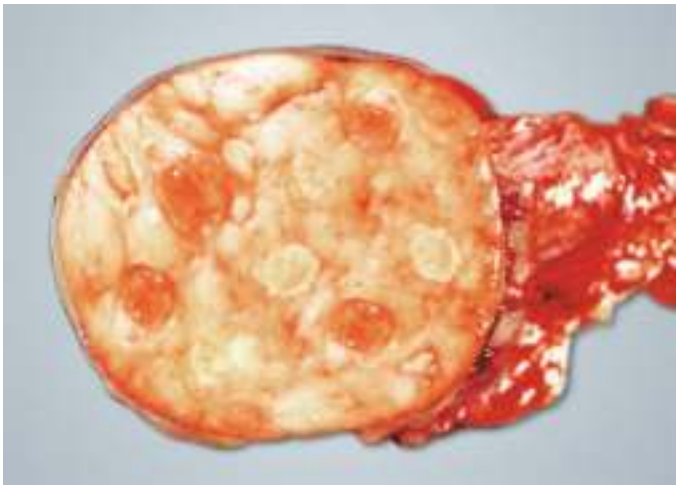


Fig. 24.88: Dysgerminoma of ovary. The tumor has a cream colored, fleshy appearance on its cut surface, with some areas of hemorrhage. This tumor has the same microscopic appearances as a seminoma of the testis.

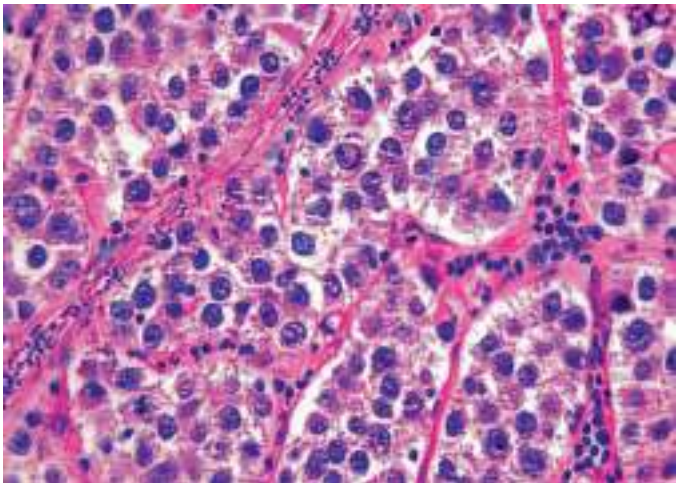


Fig. 24.89: Dysgerminoma of ovary. Tumor is composed of large nests of monotonously uniform round or oval cells with centrally located deeply staining nuclei with finely granular chromatin and prominent eosinophilic 1–2 nucleoli, and abundant clear glycogen-filled cytoplasm. These tumor cells are separated by fibrous septa ranging from fine, delicate fibrovascular network to densely hyalinized stroma containing mostly T cells (400X).

Clinical Course

Dysgerminoma is a rapidly growing malignant tumor capable of local invasion and late metastases. But metastases do not occur in the early course of disease.

- Dysgerminoma usually metastasizes more commonly in the contralateral ovary, retroperitoneal nodes, and peritoneal cavity.
  - Small tumor is freely mobile, and its capsule is usually intact.
  - Large tumor is adherent to surrounding structures. Such tumor may rupture either spontaneously or at operation resulting in spillage of the tumor contents on peritoneum. Involvement of peritoneum is associated with poor survival rate.
- Dysgerminoma can spread via lymphatic route to lymph nodes in the vicinity of the iliac arteries and terminal part of the abdominal aorta, and then to mediastinal and supraclavicular region.
- Dysgerminoma can spread via hematogenous route to liver, lungs and bones. Metastatic tumor showing marked atypia, high mitotic activity and small amounts of stroma with scant lymphocytic infiltration tend to be more aggressive. Primary tumor and its metastases can be demonstrated by CT scan and MRI. Elevated LDH (isoenzyme 1) can be used as serum tumor marker in 95% of cases.

Treatment

Dysgerminoma is extremely responsive to radiotherapy and chemotherapy (cisplatin, etoposide and bleomycin). Prognosis is excellent in 95% of stage I cases, if tumor is unilateral and salpingo-oophorectomy is done. Unfavorable prognostic factors include adhesion of large tumor and presence of metastases.

YOLK SAC TUMOR OF OVARY

Yolk sac tumor of ovary is highly malignant germ cell tumor with extraembryonic differentiation. It is also known as endodermal sinus tumor analogous to endodermal sinus tumor of the testis.

- It is thought to arise from the undifferentiated and multipotential embryonal carcinoma by selective differentiation toward yolk sac or villetine structures, in the same way as nongestational choriocarcinoma differentiates into trophoblastic structures.
- Tumor histologically resembles mesenchyme of the primitive yolk sac. When examined under electronic microscope, the cell of the yolk sac tumor resembles those of the normal human yolk sac.
- Yolk sac tumor of the ovary most often affects children and young women under 30 years of age. It is most commonly encountered in the second and third decades followed by first and fourth decades. It has also been reported in the elderly women.
- Tumor produces α-fetoprotein (AFP). Its detection in the blood is useful both for diagnosis of yolk sac tumor and for monitoring the effectiveness of therapy. Disorders associated with increased AFP levels are given in Table 24.42.

Table 24.42 Disorders associated with increased α-fetoprotein (AFP) levels

Yolk sac tumors of gonads
Pregnancy
Hepatocellular carcinoma
Hepatoblastoma
Cirrhosis
Massive hepatocellular necrosis with regeneration
Chronic hepatitis

Clinical Features

Patient presents with abdominal enlargement and pain abdomen due to rapidly growing pelvic mass. Occasionally, patient may present with acute severe pain abdomen, that simulates ruptured ectopic pregnancy and acute appendicitis. On clinical examination, a tumor mass is usually palpable of considerable size. Untreated cases have poor prognosis with two years survival.

Surgical Pathology: Yolk Sac Tumor of Ovary

Gross Morphology

- Yolk sac tumor of ovary is almost always unilateral. Bilateral involvement of ovaries is a manifestation of metastatic spread of tumor. The tumor is large partly cystic varying in size from 3 to 30 cm in diameter. Most tumors measure >10 cm in diameter and weighs 500 g–5 kg. External surface of tumor is smooth and glistening.
- Tumor is usually encapsulated, round, oval or globular; firm, smooth, or somewhat lobulated.
- Cut surface of tumor shows variegated appearance, partially cystic areas containing gelatinous material, and solid area often containing large foci of hemorrhage and necrosis (grayish brown).

Light Microscopy

- Yolk sac tumor of ovary shows a network of embryonal cells (large cells with prominent nuclei and mitotic activity) and connective tissue structures arranged around blood vessels as the endodermal sinus of rat placenta 'Schiller-Düval bodies' (resembling glomerulus-like structures), which are hallmark of yolk sac tumor.

- Schiller-Düval bodies contain central tissue core containing a longitudinal vessel surrounded by epithelial-like cells into the surrounding capsular sinusoid space. Intracellular and extracellular hyaline globules/droplets (PAS positive) are present in all tumors.
- All the histologic patterns of yolk sac tumor can be observed in the same yolk sac tumor or one or two predominate, which include: (a) microcystic or reticular lined by flat, pleomorphic hyperchromatic nuclei, (b) endodermal sinus, (c) solid, (d) alveolar-glandular, (e) polyvesicular vitelline, (f) myxomatous, (g) papillary, (h) macrocystic, (i) hepatoid, and (j) glandular or primitive endodermal (intestinal). Histology of yolk sac tumor is shown in Fig. 24.90A and B.

Immunohistochemistry Technique

Neoplastic cells of yolk sac tumor of ovary show positive for placental alkaline phosphatase (PLAP), SALL4, CAM 5.2, AE1/AE3, α-fetoproteins (AFP), α_1-antitrypsin, and negative for CD117 (c-KIT) and D2–40 (podoplanin).

Markers	Expression
PLAP	Positive
SALL4	Positive
CAM 5.2	Positive
AE1/AE3	Positive
α-Fetoproteins	Positive (not specific marker)
α_1-Antitrypsin	Positive
CD117	Negative
D2–40	Negative

Clinical Course

Yolk sac tumor is a highly malignant tumor. It metastasizes early and invades the surrounding structures.

- Local invasion and intra-coelomic spread of tumor frequently results in extensive involvement of the abdominal cavity.
- Tumor metastasizes first via lymphatic route to para-aortic lymph nodes and then mediastinal and supraclavicular lymph nodes.
- Tumor later spreads via hematogenous route to lungs, liver and other organs.

Treatment

Patients are treated with conservative surgery (unilateral salpingo-oophorectomy) and adjuvant multiagent combination chemotherapy. Recurrence is common even after surgical excision of the tumor.

- Serum AFP analysis is useful in diagnosing and monitoring of yolk sac tumor to therapeutic response.
- Serum α_1-antitrypsin can be used to monitor therapeutic response of tumor, but it is inferior to serum AFP.

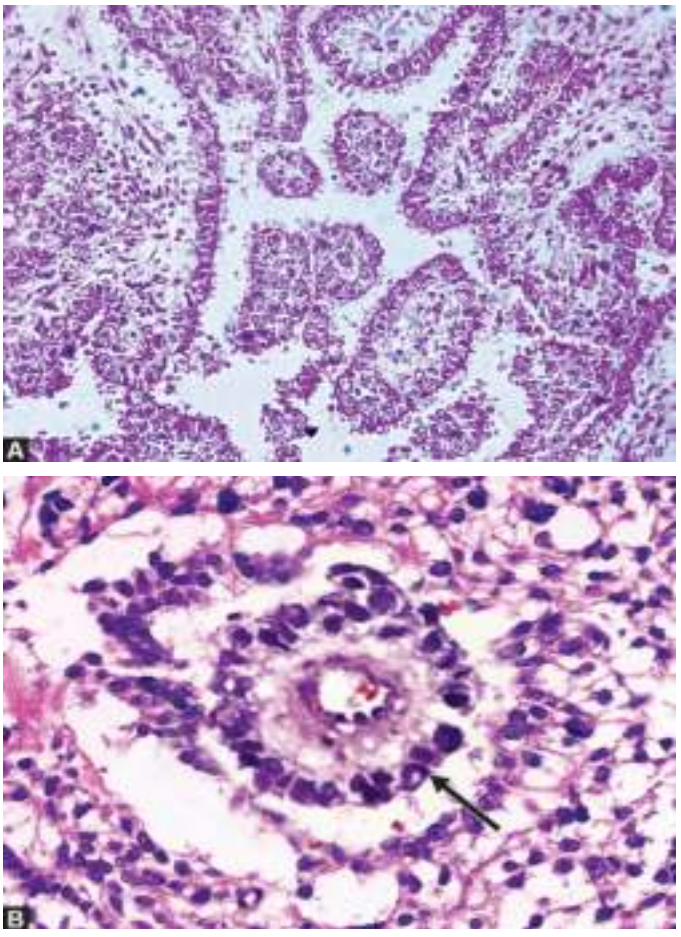


Fig. 24.90: Yolk sac tumor of ovary. (A) The endodermal sinus pattern of yolk sac tumor is characterized by the presence of irregular interconnecting labyrinthine-like spaces and Schiller-Düval bodies, several of which are nicely illustrated (200X), (B) tumor is showing Schiller-Düval bodies (arrow) (400X).

EMBRYONAL CARCINOMA OF OVARY

Embryonal carcinoma of ovary is highly malignant and least differentiated germ cell tumor. Embryonal carcinoma usually affects children and young women (mean age 15 years).

- Tumor may differentiate either to somatic (teratomatous tumors of various degrees of differentiation) or extraembryonal structures, forming yolk sac or vitelline (yolk sac tumor) or trophoblastic elements (choriocarcinoma).
- Tumor is considered to be homologous with its testicular counterpart. It is usually combined with other germ cell tumor component, most frequently yolk sac tumor and forms a part of mixed germ cell tumor. Tumor has been observed in association with gonadoblastoma.
- At surgery, there is extension of embryonal carcinoma of ovary beyond the ovary in 40% of cases. Tumor is generally large with a median diameter of 17 cm.

Clinical Features

Patient presents with ovarian mass (average 17 cm), precocious puberty (47%), vaginal bleeding (33%), amenorrhea (7%) and hirsutism. Embryonal carcinoma of ovary may synthesize AFP, even when it is not combined with yolk sac tumor, but the serum AFP levels are slightly raised. When embryonal carcinoma contains syncytiotrophoblastic element, it is usually combined with choriocarcinoma, which synthesizes hCG and associated with precocious puberty. A positive pregnancy test is present in almost all such patients due to high serum hCG and its excretion in urine.

Surgical Pathology: Embryonal Carcinoma of Ovary

Gross Morphology

- Because embryonal carcinoma is most often a component of mixed germ cell tumor.
- The gross appearance of the tumor varies to the type and amount of the different components present in the tumor.
- Median diameter of tumor is 17 cm. External surface is smooth and glistening.
- Cut surface of tumor is predominantly solid gray-white, granular and variegated, with extensive areas of necrosis and hemorrhage in large tumor.
- The tumor is variably demarcated from surrounding ovary and structures (Fig. 24.91).

Light Microscopy

- **Primitive and undifferentiated embryonal carcinoma:** Embryonal carcinoma has a similar morphology to the embryonal carcinoma of the adult testis.
  - In its most primitive and undifferentiated form, tumor is composed of solid aggregates of epithelial-like, medium to large polygonal or ovoid cells with prominent vesicular centrally located hyperchromatic nuclei and numerous nucleoli; and pale eosinophilic granular cytoplasm, frequently forming syncytial arrangement and occasionally forming glandular and papillary structures.
  - Mitotic activity is usually brisk. Abnormal mitoses are frequently demonstrated. Pleomorphism is usually marked. Scattered giant cells and multinucleated giant cells are present.
- **Moderately differentiated embryonal carcinoma:** In moderately differentiated embryonal carcinoma, the tumor cells are arranged in solid sheets.
  - Tumor cells tend to line clefts and spaces and form papillary structures. Tumor cells appear more like epithelial cell-like than those of more primitive undifferentiated type. These papillae must be differentiated from perivascular formation demonstrated in yolk sac tumor. Very primitive mesenchymal tissue may be present in conjunction with the epithelial-like component.
  - Tumor cells are cuboidal or columnar in shape tend to form glandular differentiation, but lacking true gland formation.
  - Syncytiotrophoblastic element immediately adjacent to aggregates of embryonal carcinoma cells or lying isolated in the stroma are frequently demonstrated.
  - Areas of hemorrhage and necrosis are usually seen. Tumor cells are immunoreactive for hCG. Embryonal carcinomas largely composed of embryoid bodies are referred to as 'polyembryomas' (Fig. 24.92).

Immunohistochemistry Technique

Neoplastic cells of embryonic cell carcinoma are positive for placental alkaline phosphatase (PLAP), OCT4 (POU5F1), SALL4 and CD30. These demonstrate variable staining for D2–40 (podoplanin) and negative staining for CD117 (c-KIT).

Markers	Expression
▪ PLAP	▪ Positive
▪ OCT4 (POU5F1)	▪ Positive (consistent staining)
▪ SALL4	▪ Positive (nuclear staining)
▪ CD30	▪ Positive
▪ D2–40 (podoplanin)	▪ Positive/negative
▪ CD117 (c-KIT)	▪ Negative

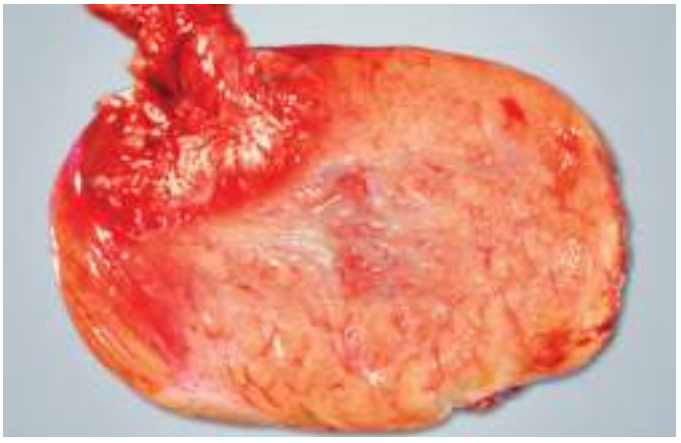


Fig. 24.91: Embryonal carcinoma of ovary. Cut surface shows variegated appearance with extensive areas of hemorrhage and necrosis.

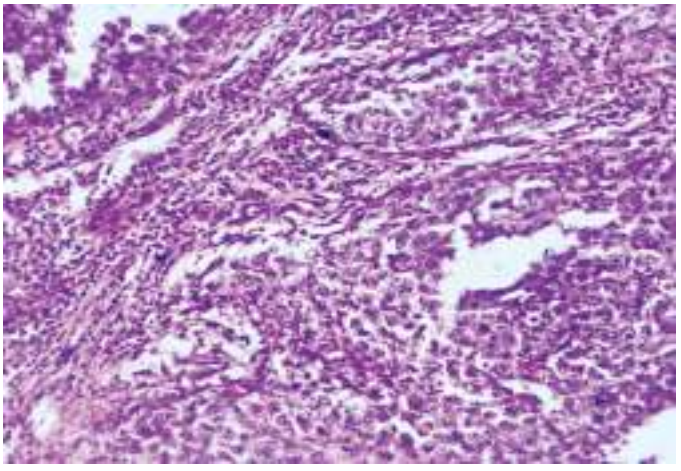


Fig. 24.92: Embryonal carcinoma of ovary. Tumor is composed of solid sheets of large anaplastic cells with hyperchromatic nuclei (400X).

Serum Tumor Markers

Serum α-fetoprotein level is often elevated as a result of its association with yolk sac tumor. Serum human chorionic gonadotropin (hCG) level is invariably high.

Clinical Course

Embryonal carcinoma of ovary is highly malignant tumor, which spreads by direct invasion into the peritoneal cavity, via lymphatic route to lymph nodes in the vicinity of the common iliac arteries and terminal part of the abdominal aorta, and then to mediastinal and supraclavicular region. Tumor can spread via hematogenous route to liver, lungs and bones.

Treatment

Patient with embryonal carcinoma of ovary is treated by unilateral salpingo-oophorectomy and combination chemotherapy (bleomycin, vinblastine and cisplatin).

CHORIOCARCINOMA OF OVARY

Choriocarcinoma of ovary is a rare malignant germ cell tumor with extraembryonic differentiation, which may be divided into two groups: (a) gestational choriocarcinoma and (b) nongestational choriocarcinoma, *de novo* (98%). Tumor is differentiating in the direction of trophoblastic elements, usually admixed with other neoplastic germ cell elements, which mimics the epithelial covering of placental villi (cytotrophoblast and syncytiotrophoblast). Most choriocarcinomas occur in combination with other germ cell tumors. Pure ovarian choriocarcinomas are rare.

Histogenesis

Choriocarcinoma of the ovary may arise in the three different ways: (a) germ cell tumor is differentiating in the direction of trophoblastic elements, and usually admixed with other neoplastic germ cell elements, (b) primary gestational choriocarcinoma is associated with ovarian pregnancy, and (c) as metastatic choriocarcinoma is derived from a primary gestational choriocarcinoma in other parts of female genital tract mainly in the uterus.

Clinical Features

Clinical findings in patient with ovarian nongestational choriocarcinoma are similar to those present in other malignant ovarian germ cell tumors, except that clinical manifestations are modified by the endocrine activity of the tumor, which synthesizes hCG.

- The endocrine effect is observed in prepubertal girls, who present with precocious sexual development, rapid breast enlargement growth of pubic and axillary hair, and menstrual irregularities. Occasionally, patient presents with symptoms of thyrotoxicosis.

Table 24.43 Elevated human choriogonadotropin (β-hCG) in various disorders

Choriocarcinoma of gonads and extragonadal regions
Embryonal carcinoma of the ovary
Hydatidiform mole (molar pregnancy)
Seminoma with syncytiotrophoblastic element
Dysgerminoma with syncytiotrophoblastic element

- Women of reproductive age may present with metastasis in lung, liver and brain.
- In some cases, choriocarcinoma of ovary becomes evident 10 or more years after the last pregnancy.

Serum and Urine Marker

Choriocarcinoma of ovary synthesizes human chorionic gonadotropin (hCG). There is increased hCG in serum and urine in these women. Elevated human choriogonadotropin (β-hCG) in various disorders is given in Table 24.43.

Surgical Pathology: Choriocarcinoma of Ovary

Gross Morphology

Ovarian pure choriocarcinoma is large, unilateral, solid, gray-white and hemorrhagic. Necrosis may be evident. Because most of the tumors are composed of combination of neoplastic cytotrophoblastic and syncytiotrophoblastic elements, the gross appearance tend to be vary according to the neoplastic elements present in the tumor (Fig. 24.93).

Light Microscopy

- Choriocarcinoma of ovary shows an admixture of malignant cytotrophoblastic and syncytiotrophoblastic elements.
- Cytotrophoblastic component is composed of medium-sized polygonal, round or oval cells with clear cytoplasm and sharp borders. Some tumor cells contain centrally placed nuclei, whereas other cells have large vesicular nuclei with nucleoli showing brisk mitotic activity. Cytotrophoblastic cells are usually dispersed centrally in tumor mass, and surrounded by syncytiotrophoblastic element.
- Syncytiotrophoblastic element is composed of large basophilic, vacuolated cells with irregular outlines and multiple hyperchromatic nuclei varying in size and shape.
- Cells of syncytiotrophoblastic element of choriocarcinoma are nondividing, which synthesize hCG, which accounts for the frequent finding of a positive pregnancy test result (Fig. 24.94).

Immunohistochemistry Technique

Tumor cells of choriocarcinoma show positivity for CAM 5.2, β-hCG (most specific marker), inhibin, human placental lactogen (hPL) and cytokeratin 7 (CK7). These cells show variable staining for SALL4 and placental alkaline phosphatase (PLAP).

Markers	Expression
▪ CAM 5.2	▪ Positive
▪ β-hCG	▪ Positive
▪ Inhibin	▪ Positive
▪ Human placental lactogen (hPL)	▪ Positive
▪ CK7	▪ Positive
▪ LK26 (folate binding protein)	▪ Positive
▪ SALL4	▪ Positive/negative (nuclear staining)
▪ PLAP	▪ Positive/negative
▪ CD117 (c-KIT)	▪ Negative
▪ OCT4 (POU5F1)	▪ Negative
▪ D2–40 (podoplanin)	▪ Negative

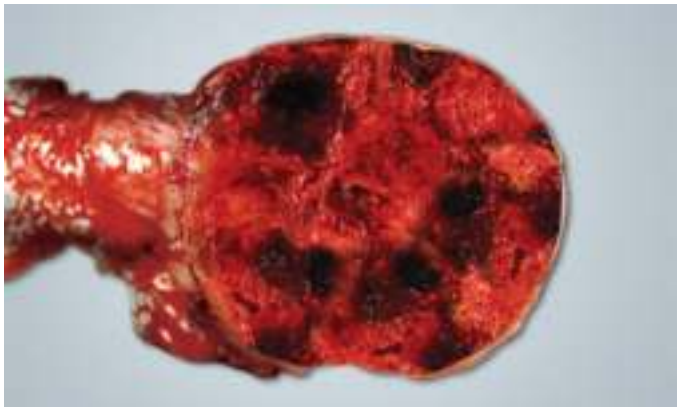


Fig. 24.93: Choriocarcinoma. The testis is replaced by a bloody bulging mass. More typically, choriocarcinoma tends to be somewhat smaller, and may not significantly distort the ovary.

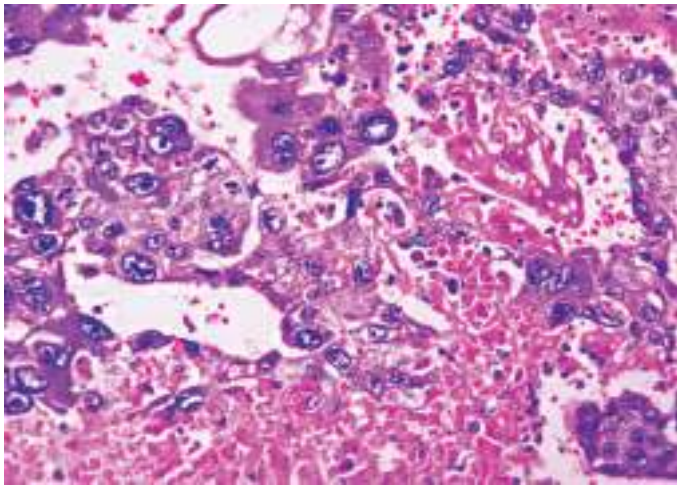


Fig. 24.94: Choriocarcinoma of the ovary. Tumor is composed of multinucleated syncytiotrophoblastic and cytotrophoblastic cells. The multinucleated syncytiotrophoblasts have voluminous eosinophilic cytoplasm and large darkly staining irregular nuclei with prominent nucleoli. The smaller mononuclear cytotrophoblasts are fairly uniform in size with amphophilic cytoplasm and distinct cellular outlines resembling to the tumor cells of solid yolk sac tumor (400X).

Clinical Course

Nongestational ovarian choriocarcinoma is a highly malignant germ cell tumor. It spreads by direct invasion in the surrounding structures throughout abdominal cavity, via lymphatic route to lymph nodes and via hematogenous route to distant organs. Sometimes, hematogenous spread of tumor is less evident.

Treatment

Treatment of choriocarcinoma of ovary has been revolutionized by the administration of combined chemotherapy containing cisplatin, etoposide and bleomycin with marked improvement in prognosis and survival. On the other hand, gestational choriocarcinoma is responsive to chemotherapy with better prognosis and survival.

TERATOMA OF OVARY

Teratomas of ovary demonstrate tissue elements derived from two or three embryonic layers. These tumors are observed in distinct forms: mature cystic teratoma, mature solid teratoma, immature teratoma and monodermal specialized teratomas (struma ovarii, carcinoid tumor, and teratoma with malignant transformation). Most teratomas (95%) are mature cystic teratomas also known as dermoid cysts.

Mature Cystic Teratoma of Ovary

Mature cystic teratoma of ovary is also known as dermoid cyst. Mature cystic teratomas constitute 95% of germ cell tumors and 20% of all ovarian neoplasms, which most often occur in young women during reproductive period. Dermoid cyst is usually unilateral in 75% and bilateral in 25% of cases.

- **Origin:** Mature cystic teratoma arises from haploid fertilized germ cell (? ovum after 1st meiotic division). It is considered that haploid (post-meiotic) germ cells autofertilize, yielding diploid tumor cells that are genetically female (46XX). The radiographic calcifications are highly suggestive of mature cystic teratoma of ovary, i.e. dermoid cyst.
- **Malignant transformation:** An element of mature cystic teratoma may undergo malignant transformation in about 1% of cases, usually squamous cell carcinoma (most common), followed by carcinoid tumor, adenocarcinoma, thyroid carcinoma, melanoma, osteosarcoma, carcinosarcoma, and glioblastoma multiforme. Malignant transformation in mature cystic teratoma is accompanied by complex chromosomal aberrations. Teratomas are graded I, II and III based on the presence of immature neuroepithelium in the tumor.

- **Karyotyping by banding technique:** Mature cystic teratomas of ovary show 46XX chromosomal patterns. Some believe that these tumors arise from an ovum after first meiotic division.
- **Clinical features:** Patient presents with constant slight to moderate abdominal pain (50%), abdominal freely mobile mass and abnormal uterine bleeding. Dermoid cysts most often undergo torsion and subsequent hemorrhagic infarction resulting in abdominal pain.

Surgical Pathology: Mature Cystic Teratoma of Ovary

Gross Morphology

- Mature cystic teratoma of ovary (i.e. dermoid cyst) is usually unilateral, however, bilateral tumors occur in 15% of cases. Tumor varies to several kilograms, which is usually freely mobile, but occasionally form adhesions to surrounding structures due to leakage of the tumor contents. Tumor is round, oval or globular with a smooth, gray-white and glistening surface. Tumor is soft and fluctuant with hard and soft areas.
- Cut surface shows a cavity filled with hair and fatty material similar to sebum surrounded by a firm capsule of normal varying thickness.
  - Tumor is usually unilocular but may be multilocular. Several dermoid cysts may be present in the same ovary.
  - Originating from the cyst wall and projecting into the tumor cavity from a small to large solid and cystic nodule termed Rokitansky or dermoid protuberance. Hair present in the tumor cavity originates from this dermoid protuberance, when bone or teeth are present.
  - Well-formed teeth are present in 31% of mature cystic teratomas.
  - Sometimes, phalanges, long bones or other bones may be occasionally demonstrated in these tumors, and these tumors are termed '**fetiform teratomas**'. Gross morphology of mature cystic teratoma of ovary is shown in Fig. 24.95.

Light Microscopy

- Outer surface of wall of mature cystic teratoma is composed of ovarian hyalinized stroma.
- The cavity of the dermoid cyst wall is lined by skin, and cutaneous structures. The skin is composed of keratinized squamous epithelium and contains sebaceous and eccrine glands associated with adipose tissue. Cyst contains hair and other dermal appendages.
- Some tumors may demonstrate lipogranulomatous, fat necrosis-like, sieve-like or pneumatosis cystoides-like pattern.
- Occasional dermoid cyst wall may be lined by bronchial or gastrointestinal cuboidal or columnar epithelium. Squamous epithelium may be present in the region of Rokitansky or dermoid protuberance.
- Due to desquamation of lining, foreign body giant cell reaction may be demonstrated. Foreign body giant cell reaction may occur when the contents of the dermoid cysts are spilled resulting in formation of adhesions.
- Area around Rokitansky or dermoid protuberance may contain tissues derived from the three germ layers: ectodermal structures (100%), mesodermal structures (90%) and endodermal structures (70%).
  - Structures derived from ectoderm are squamous epithelium, other skin derivatives, brain tissue, neural tissue, retina, choroid plexus and ganglia.
  - Mesodermal tissue is represented cartilage, bone, smooth muscle, adipose tissue and fibrous tissue.
  - Endoderm derived structures include gastrointestinal and bronchial epithelial lining and glands, thyroid tissue, salivary gland tissue.
  - These structures may be scattered diffusely, and these do not show the orderly haphazard arrangement that is seen in immature teratoma. Histology of mature cystic teratoma of ovary is shown in Fig. 24.96.



Fig. 24.95: Mature cystic teratoma of ovary. Cut surface of cavity shows hair and teeth surrounded by a firm capsule.

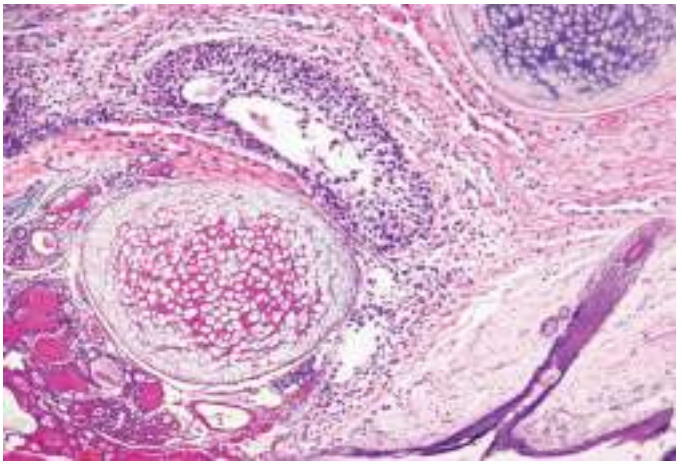


Fig. 24.96: Mature cystic teratoma of ovary. Outer surface of wall of mature cystic teratoma is composed of ovarian hyalinized stroma. The cavity of the dermoid cyst wall is lined by skin, and cutaneous structures. The skin is composed of keratinized squamous epithelium and contains sebaceous and eccrine glands associated with adipose tissue. Some tumors may demonstrate lipogranulomatous, fat necrosis-like, sieve-like or pneumatosis cystoides-like pattern. Cyst contains hair and other dermal appendages (400X).

Clinical Course: Mature Cystic Teratoma of Ovary

Treatment of choice for uncomplicated mature cystic teratoma in young women is excision of the tumor with conservative of the ovary is possible. Local recurrence after conservative treatment for mature cystic teratoma is uncommon and occurs in less than 1% of cases. Mature cystic teratoma of the ovary may be associated many complications: (a) torsion, (b) rupture, (c) infection, (d) autoimmune hemolytic anemia, and (e) development of cancer.

- **Torsion:** Torsion is the most frequent complication of mature cystic teratoma of ovary especially during pregnancy (20–40%) and puerperium (1–10%). Torsion of dermoid cyst during pregnancy has considerable clinical significance. Torsion of dermoid cyst tends to rupture. Patient presents with severe acute abdominal pain, and the condition is an acute emergency. Excision of the affected ovary or salpingo-oophorectomy is treatment of choice.
- **Rupture:** Immediate result of the rupture of dermoid cyst is hemorrhage and shock. Rupture of the tumor into the peritoneal cavity leads to chemical peritonitis as a result of spillage of dermoid cyst contents, which leads to granulomatous reaction and formation of dense adhesions throughout the peritoneum. Glial implants may be demonstrated on the peritoneum as a result of spillage of neuroglial tissue. Rupture of the tumor may involve urinary bladder and rectum.
- **Infections:** Patient with mature cystic teratoma is prone to infections due to coliform organisms and *Salmonella typhi*.

Mature Solid Teratoma of Ovary

Mature solid teratoma of ovary is a rare ovarian neoplasm affecting young women, predominantly in the second decade.

- Structures in mature solid teratoma are derived from all germ cell layers unlike dermoid cyst that mainly composed of structures derived from ectoderm.
- Mature solid teratoma is difficult to differentiate from malignant immature teratoma. The prognosis is excellent, even if peritoneal implants are present.
- The tumor shows 46XX chromosomal patterns by karyotyping banding technique.
- Some believe that mature solid teratoma arises from an ovum after first meiotic division.

Surgical Pathology: Mature Solid Teratoma of Ovary

Gross Morphology

Mature solid teratoma of ovary is usually large solid tumor, but multiple small cystic areas also are present. Tumor is usually unilateral in all cases (Fig. 24.97).

Light Microscopy

- Mature solid teratoma is composed entirely of mature tissues derived from all three germ layers arranged in an orderly manner resembling mature cystic teratoma except that the tumor is solid.
- Mature tissues derived from three germ cell layers such as skin, hair, sebaceous gland, teeth, neural and retinal tissues, cartilage, bone, muscle, hemopoietic tissue, bronchial glands, gastrointestinal tract mucosa, salivary tissue, thyroid tissue and pancreatic tissue.
- Occasionally, mature solid teratoma may be associated with peritoneal implants composed of mature glial tissue.

Immunohistochemistry Technique

Staining for glial fibrillary acidic protein (GFAP) is helpful in the identification of mature and immature glial tissues. Drawback is that GFAP is also detectable in chondrocytes. Immature and mature neural tissues are reactive for long-chain polysialic acid moiety of the neural cell adhesion molecule (NCAM).

Markers	Expression
▪ GFAP	▪ Positive
▪ NCAM	▪ Positive

Clinical Course and Treatment

Because mature solid teratoma is unilateral tumor, oophorectomy or unilateral salpingo-oophorectomy is the treatment of choice associated with complete cure.

Immature Teratoma of Ovary

Immature teratoma of ovary is an aggressive rapidly growing malignant tumor. Tumor is composed of tissues derived from the three germ cell layers: ectoderm, mesoderm and endoderm in contrast to the mature cystic teratoma of ovary.

- Mature tissues are frequently present in immature teratoma of ovary. In these cases, immature teratoma should be differentiated from a mature cystic teratoma with malignant transformation.
- Tumor most often occurs in children and adolescents age group under 20 years of age (average 18 years).



Fig. 24.97: Mature solid teratoma of ovary. Cut surface shows variegated appearance with solid and cystic areas.

Clinical Features

Patient is usually asymptomatic until immature teratoma of ovary attains a considerable size. Patient presents with pelvic or abdominal mass as a result of pressure on surrounding tissues, abdominal heaviness and dull pain abdomen. Tumor may undergo torsion and cause acute abdominal pain.

Surgical Pathology: Immature Teratoma of Ovary

Gross Morphology

- Immature teratoma of ovary is composed of immature and mature tissues derived from all three germ cell layers: ectoderm, mesoderm and endoderm.
- Ectoderm-derived tissues are ciliated epithelium and occasionally gastrointestinal or renal epithelium.
- All the tissues derived from three germ cell layers in immature teratoma of ovary are varying from embryonic to mature tissue, arranged haphazardly throughout the tumor so differ from the orderly organized arrangement of the tissues in mature cystic teratoma.
- Immature teratoma may be combined with other germ cell tumor elements such as dysgerminoma, yolk sac tumor, embryonal carcinoma, choriocarcinoma and polyembryoma.
- Therefore, it is important to do thorough sampling of the tumor. Immature teratoma is solid bulky with smooth external surface.
- Capsular penetration by the tumor is usual. Cut surface is solid with multiple minute cysts, or predominantly cystic. Hair, bone, cartilage and calcification may be demonstrated.

Light Microscopy

- Immature teratoma is composed of a mixture of embryonal and adult tissues derived from all three germ cell layers, regardless of its gross appearance, which are most often primitive neural elements, but mesodermal elements are also common.
- Some tumors are predominantly composed of endodermal derivatives, including esophagus, liver, and intestinal structures.
- Embryonal component of the tumor resembles tissues from an embryo or fetus than an adult. Immature cartilage, glands, bone, muscle, neural rosettes are present (Fig. 24.98).

Immunohistochemistry Technique

Staining for GFAP is helpful in the identification of mature and immature glial tissues. Drawback suggests that GFAP is also detectable in chondrocytes. Immature and mature neural tissues are reactive for long-chain polysialic acid moiety of the NCAM.

Markers	Expression
▪ GFAP	▪ Positive
▪ NCAM	▪ Positive

Clinical Course

Immature teratoma of ovary is malignant tumor, that tends to grow rapidly and penetrates capsule and invades surrounding structures resulting in formation of adhesions. It spreads throughout the peritoneal cavity.

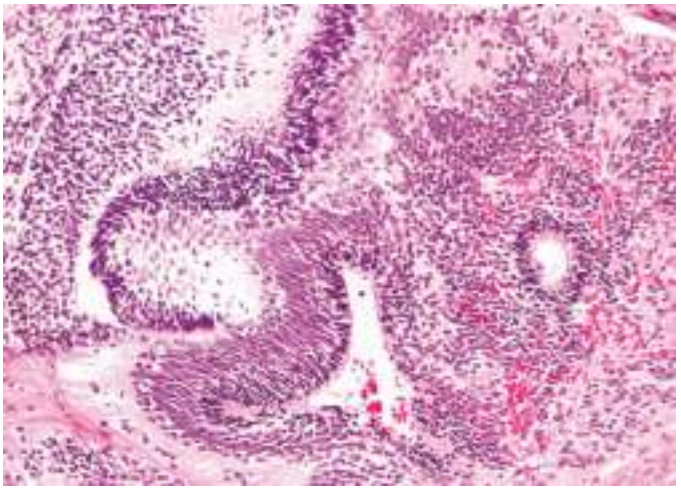


Fig. 24.98: Immature teratoma of ovary. Tumor is composed of a mixture of embryonal and adult tissues derived from all three germ layers, regardless of its gross appearance, which are most often primitive neural elements, but mesodermal elements are also common (400X).

- Tumor spreads via lymphatic route to retroperitoneal lymph nodes, para-aortic lymph nodes and more distant lymph nodes; and via hematogenous route to lungs, liver and other organs.
- Prognosis depends on grade of immature teratoma of ovary. Histologic features of the tumor affect prognosis. Very immature and poorly differentiated tumors are associated with poor prognosis. Immature teratoma consisting of mature and better differentiated components is associated with more favorable prognosis. Prognosis is poor except for stage I disease.
- Recurrence occurs within two years of excision. Chemotherapy in low-grade tumor may induce maturation of the immature elements. The risk for extraovarian spread depends on the amount of immature tissue present.

Treatment

Treatment for immature teratoma of ovary includes surgery and chemotherapy. Grade I immature teratoma limited to ovary or fallopian tube is treated by surgery. Pure ovarian immature teratoma is a potentially curable disease.

Struma Ovarii (Monodermal Teratoma)

Thyroid tissue is a relatively frequent constituent of mature cystic teratoma demonstrated in 5–20% of cases. Struma ovarii is a monodermal (specialized) teratoma composed of functional ectopic thyroid tissue. Tumor is most often unilateral tumors and bilateral in a few cases. The term 'struma ovarii' should be reserved for tumors composed of entirely or predominantly

thyroid tissue. Struma ovarii accounts for 3% of ovarian teratomas during reproductive period.

Clinical Features

Patient may present with features of hyperthyroidism due to thyroxine production by the tumor.

- Tumor has a benign course (95%), but may transform to papillary thyroid carcinoma in 5% of cases.
- BRAF gene mutations and RET/PTC rearrangements have been demonstrated in papillary carcinoma.
- Papillary thyroid carcinoma arising in struma ovarii invades peritoneum and metastasize via lymphatic route to para-aortic lymph nodes and other lymph nodes; and via hematogenous route to lungs and bones.
- These patients are treated by oophorectomy and thyroidectomy with administration of radioactive iodine (^{131}I).
- Long-term follow-up with serum thyroglobulin measurements are essential in these cases. Another complication of struma ovarii is the presence of ascites associated with pleural effusion producing 'Meig syndrome' even in benign tumor.

Treatment

Most cases are treated by excision of ovary or unilateral salpingo-oophorectomy.

Surgical Pathology: Struma Ovarii

Gross Morphology

- Struma ovarii tumor varies in size and measures less than 10% cm. The surface is usually smooth.
- Cut surface resembles a mature cystic teratoma. Cut surface of the tumor may be composed of light tan, glistening thyroid tissue. Hemorrhage, necrosis and foci of fibrosis may be present.
- Solid tumors with small amounts of colloid appear less glistening and more fleshy appearance. Some tumors may be cystic.

Light Microscopy

- Struma ovarii is composed of mature thyroid follicles of variable size lined by a single layer of columnar or flattened epithelium.
- The follicular acini contain eosinophilic, PAS positive colloid material. The intensity of the PAS staining may vary. Follicular acini are of variable size and containing colloid material.
- Sometimes, tumor may demonstrate papillary projections and resemble adenomatous goiter.
- Presence of marked crowded thyroid follicles without features of malignancy is designated as 'proliferative struma ovarii'.
- Struma ovarii showing features of Hashimoto's thyroiditis has also been reported (Fig. 24.99).

Immunohistochemistry Technique

Immunohistochemical studies for TTF-1 and thyroid hormones have established thyroid tissue in nature of this lesion.

Markers	Expression
Thyroid transcription factor 1 (TTF-1)	Positive
Thyroglobulin	Positive
Epithelial membrane antigen (EMA)	Positive

Carcinoid Tumor of Ovary

Carcinoid tumor of ovary is monodermal specialized functional monodermal teratoma, which may be primary or metastatic. Primary carcinoid tumors are divided into four categories in decreased frequency: insular (most common), trabecular, stromal and mucinous. Insular carcinoid tumor is considered to be of midgut derivation, which originates in association with gastrointestinal or respiratory epithelium in a mature cystic teratoma.

- About 40% of carcinoid tumor occur in pure form and remaining 60% are combined. Most patients are perimenopausal or menopausal women in the age group ranging from 30 to 80 years.
- About 33% of patients are associated with carcinoid syndrome due to synthesis of 5-HT (5-hydroxytryptamine). This is in contrast to intestinal carcinoids, which are associated with carcinoid syndrome only when there is metastatic spread to the liver. The reason for this difference is that the blood flow from the ovary is directly drained into the systemic circulation without reaching through the liver, which inactivates the serotonin synthesized by the tumor.

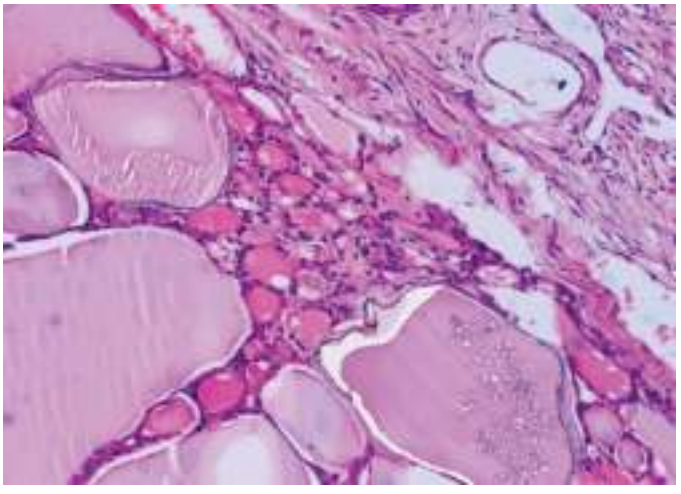


Fig. 24.99: Struma ovarii. Struma ovarii is composed of mature thyroifollicles of variable size lined by a single layer of columnar or flattened epithelium. The follicular acini contain eosinophilic colloid material (400X).

- The presence or absence of symptoms of carcinoid syndrome is also dependent on the number of tumor cells synthesizing 5-HT (5-hydroxytryptamine).
- Excision of carcinoid tumor is associated with rapid complete remission of the clinical symptoms of carcinoid syndrome, disappearance of 5-hydroxy-indoleacetic acid (5-HIAA) from the urine and marked decrease of serum serotonin.
- Analysis of serum serotonin and urinary 5-hydroxy-indoleacetic acid (**5-HIAA**) may be used to monitor the activity of the tumor and response to therapy.

Surgical Pathology: Carcinoid Tumor of Ovary

Gross Morphology

- Carcinoid tumor is solid and smooth on external surface. It varies in size from microscopic to 20 cm with mean 10 cm.
- Primary carcinoid is always unilateral, and may be associated with mature cystic teratoma in contralateral ovary.
- But metastatic intestinal tumor involves both ovaries. Cut surface is predominantly solid, firm, tan to yellow, homogeneous with a few cystic spaces.

Light Microscopy

- Carcinoid tumor of ovary is composed of numerous small acini and solid nests of uniform polygonal cells with abundant basophilic or amphophilic cytoplasm and round to oval hyperchromatic nuclei.
- Cytoplasm of tumor cells may contain red, brown, or orange argentaffin or argyrophil granules, which have property to reduce silver salts.
- Mitotic activity is low.
- Rarely, mixed tumor may occur, that is composed of struma ovarii and carcinoid tumor. Histology of carcinoid tumor of ovary is shown in Fig. 24.100.

Electron Microscopy

Tumor cells of insular carcinoid tumor contain abundant neurosecretory granules. Tumor cells exhibit marked variation in size and shape, being round, oval or elongated.

Immunohistochemistry Technique

Neoplastic cells of carcinoid tumor of ovary are immunoreactive to chromogranin, synaptophysin, neuron-specific enolase and 5-HT.

Markers	Expression
Chromogranin	Positive (diffuse and strong staining)
Synaptophysin	Positive (diffuse and strong staining)
Neuron-specific enolase (NSE)	Positive
Serotonin (5-hydroxytryptamine, i.e. 5-HT)	Positive

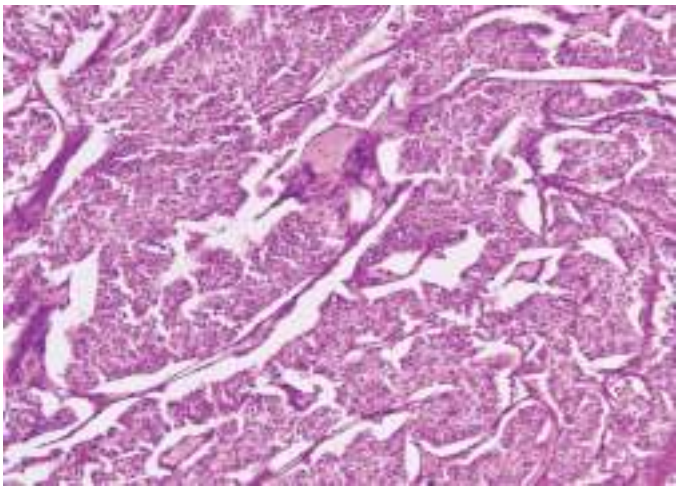


Fig. 24.100: Carcinoid of the ovary. Tumor is composed of numerous small acini and solid nests of uniform polygonal cells with abundant basophilic or amphophilic cytoplasm with round to oval hyperchromatic nuclei. Mitotic activity is low (400X).

POLYEMBRYOMA OF OVARY

Polyembryoma is rare but highly malignant ovarian neoplasm. Tumor is composed of numerous embryoid bodies resembling morphologically normal presomite embryo. It is most often a component of mixed germ cell tumor of ovary.

Surgical Pathology: Polyembryoma of Ovary

Gross Morphology

- Tumor is usually unilateral and solid areas of hemorrhage and necrosis. Gross morphology is similar to those seen in malignant germ cell tumors.
- Tumor varies in size from 9/5 cm to large tumor almost filling whole abdominal cavity and invading surrounding tissue.

Light Microscopy

- Tumor is composed of embryoid bodies and better-differentiated.
- Embryoid bodies are composed of embryonic discs lined by endoderm on one side, ectoderm on opposite side and associated with yolk sac and amniotic cavities.

Clinical Course

Polyembryoma of ovary is a highly malignant germ cell tumor. It spreads by direct invasion in surrounding structures, via lymphatic route to various lymph nodes and via hematogenous route to distant organs.

Treatment

Patient with polyembryoma of ovary is treated by surgical excision and combination chemotherapy.

MIXED GERM CELL TUMORS OF OVARY

Mixed germ cell tumors of ovary constitute 10% of germ cell tumors. Most common combination is dysgerminoma and yolk sac tumor, although any combination may occur.

SEX CORD-STROMAL TUMORS OF OVARY

Sex cord-stromal tumors of ovary constitute 10–25% of cases, which are derived from ovarian sex cords of embryonic gonads. Recent 2020 WHO classification of sex cord-stromal tumors of ovary is given in Table 24.44.

- Sex cord-stromal tumors of ovary may be divided into two broad groups: (a) estrogen producing granulosa cell tumor and the coma and (b) androgen producing Sertoli-Leydig cell tumors, hilus cell tumors and lipid cell tumors. Except fibromas, all these tumors are endocrinologically functional.
- Most sex cord-stromal tumors of ovary are benign. Approximately 25% of all granulosa cell tumors are prone to recur or metastasize during 10-year period after diagnosis and are considered low-grade malignant. Highly malignant tumors are extremely rare.

Table 24.44 Recent 2020 WHO classification of sex cord-stromal tumors of ovary

Granulosa-Stromal Tumors
- Granulosa cell tumor
  - Granulosa cell tumor (adult type)
  - Granulosa cell tumor (juvenile type)
- Tumors of thecoma-fibroma group
  - Typical thecoma
  - Luteinized thecoma
  - Thecoma associated with sclerosing peritonitis
  - Unclassified thecoma
  - Fibroma
  - Cellular fibroma
  - Fibrosarcoma
  - Sclerosing stromal tumor
  - Signet cell-stromal tumor
  - Microcystic stromal tumor

Sertoli-Stromal Tumors
- Sertoli cell tumor
- Leydig cell tumor
- Sertoli-Leydig cell tumors
  - Sertoli-Leydig cell tumor well differentiated
  - Sertoli-Leydig cell tumor intermediate differentiation
  - Sertoli-Leydig cell tumor poorly differentiated
  - Sertoli-Leydig cell tumor with heterologous elements
  - Retiform Sertoli-Leydig cell tumor

Gynandroblastoma (Specify Components)

Sex Cord Tumor with Annular Tubules

Unclassified Tumors

GRANULOSA CELL TUMOR OF OVARY

Granulosa cell tumor is potentially malignant ovarian tumor associated with estrogen production, which is derived from sex cord-stromal cells.

- Approximately 75% of granulosa cell tumors of ovary secrete estrogens, which also synthesize weak androgens.
- Excess of estrogen causes endometrial hyperplasia (most common finding) that may progress to endometrial adenocarcinoma if the functioning granulosa cell tumor remains undetected in adult women.
- Most adult granulosa cell tumors of ovary occur in postmenopausal women and associated with endometrial hyperplasia or endometrial carcinoma or fibrocystic disease of breast.
- Juvenile granulosa cell tumor of ovary affecting children and young women results in excess estrogens and isosexual precocious puberty. Flow cytometry reveals greater percentage of aneuploidy in juvenile than adult granulosa cell tumor variants, but without prognostic significance.

Chromosomal Aberrations

Granulosa cell tumor of ovary shows consistent trisomy of chromosome 12. Trisomy of chromosomes 14 and 22 has also been demonstrated. All adult granulosa cell tumors show characteristic somatic mutation in the FOXL2 gene. Flow cytometry reveals DNA polyploidy.

Clinical Features

Adult woman with granulosa cell tumor of ovary presents with abnormal uterine bleeding or postmenopausal bleeding increased abdominal size with abdominal pain and increase in abdominal girth.

Surgical Pathology: Granulosa Cell Tumors of Ovary

Gross Morphology

Granulosa cell tumor of ovary is unilateral solid tumor, microscopic size to large solid, cystic encapsulated masses, usually not firm as fibromas and thecomas (Fig. 24.101).

Light Microscopy

- Granulosa cell tumor of ovary is composed of small cuboidal to polygonal, deeply staining granulosa cells arranged in sheets and strands.
- 'Call-Exner bodies' are formed by tumor cells forming rosette-like structures (small follicles) filled with eosinophilic secretion arranged in a circular pattern resembling an ovarian immature follicle.
- Another variant is luteinized granulosa cell tumor comprised of plump granulosa theca cells with ample cytoplasm.

- Granulosa cell tumor chiefly composed of theca cell is almost never malignant (Fig. 24.102). Differentiating features of adult and juvenile granulosa cell tumors are given in Table 24.45.
  - **Adult granulosa cell tumor of ovary:** Tumor is composed of uniform cells with scant cytoplasm and pale grooved nuclei; low mitotic count, occasional **Call-Exner bodies** and **mature follicles**.
  - **Juvenile granulosa cell tumor of ovary:** Tumor is composed of pleomorphic cells with hyperchromatic ungrooved nuclei, high mitotic count and immature follicles. Call-Exner bodies are rarely demonstrated.

Differential Diagnosis

Adult granulosa cell tumor should be differentiated from endometrioid adenocarcinoma, poorly differentiated ovarian carcinoma, thecoma, cellular fibroma, small cell carcinoma of ovary, endometrioid stromal sarcoma, stromal tumor with minor-sex cord elements and yolk sac tumor.

Immunohistochemistry Technique

Neoplastic cells of granulosa cell tumor of ovary show positivity for CD99, inhibin, calretinin, melan-A (MART-1, clone A103), ER/PR, vimentin, desmoplakin, follicle regulatory proteins and S-100 protein.

Markers	Expression
▪ CD99	▪ Positive
▪ Inhibin	▪ Positive (except fibroma)
▪ Calretinin	▪ Positive (most sensitive marker than inhibin)
▪ Melan-A (MART-1, clone A103)	▪ Positive
▪ Estrogen/progesterone (ER/PR) receptors	▪ Positive
▪ Vimentin	▪ Positive (50%)
▪ Desmoplakin	▪ Positive (50%)
▪ Follicle regulatory proteins	▪ Positive (50%)
▪ S-100 protein	▪ Positive (50%)



Fig. 24.101: Granulosa cell tumor of ovary. These tumors are usually yellow. Positive diagnosis depends on the microscopic appearances. They sometimes secrete estrogen.

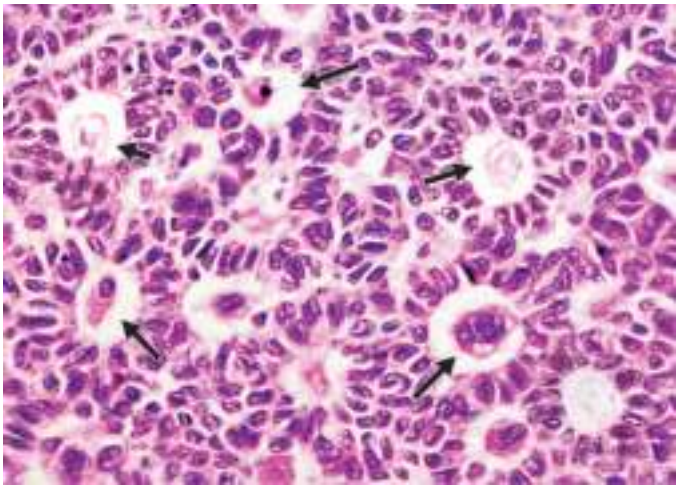


Fig. 24.102: Granulosa cell tumor of ovary. Tumor is composed of small cuboidal to polygonal, deeply staining granulosa cells arranged in sheets and strands. Call-Exner bodies are formed by tumor cells forming rossette-like structures (small follicles) filled with eosinophilic secretion arranged in a circular pattern resembling an ovarian immature follicle (arrows) (400X).

Table 24.45 Differentiating features of adult and juvenile granulosa cell tumors of ovary

Feature	Adult Granulosa Cell Tumor of Ovary	Juvenile Granulosa Cell Tumor of Ovary
Age group	<30 years	Mostly postmenopausal (all ages)
Behavior	Occasionally malignant often with protracted course	Rarely malignant
Estrogenic	Never estrogenic	Usually estrogenic
Luteinization	Infrequent	Frequent
Serum calcium	Present (common)	Absent
Thecoma component	Fibrothecoma component common	Thecomatous component common
Mucinous epithelium	Present but rare	Absent
Cell and nuclear morphology	Nuclei pale and often grooved	Pleomorphic cells with hyperchromatic ungrooved nuclei
Mitoses	Variable	Numerous
Follicles	Mature follicles	Immature follicles
Call-Exner bodies	Occasional	Rare
Cytoplasm	Scanty	Scanty to abundant

Clinical Course

It is difficult to predict biologic behavior on histologic evaluation of granulosa cell tumors. Granulosa cell tumors of ovary are potentially malignant, which may recur or metastasize in 25% of cases during 10-year period after diagnosis. 10-year survival is in 85% of cases.

THECOMA OF OVARY

Thecoma is a benign functional ovarian tumor, which occasionally synthesizes estrogens. It most often affects 30–50 years of women. In most cases, tumor produces signs of estrogen synthesis. The tumor is unilateral in 90% and bilateral in 10% of cases. Thecomas are almost always benign. Some tumors in young women are heavily calcified.

Surgical Pathology: Thecoma of Ovary

Gross Morphology

- Thecomas are solid tumors of 5–10 cm in diameter. These tumors are spherical, gray-white encapsulated tumors, covered by glistening intact ovarian serosa.
- Cut surface appears yellow due to presence many lipid-laden theca cells. Pure thecomas are rare.
- Many tumors contain a mixture of fibroma and thecoma components, hence called fibroma–thecoma (Fig. 24.103).

Light Microscopy

- Thecoma is composed of round lipid-containing theca cells in addition to fibroblasts.
- Theca cells contain fat and synthesize estrogens.
- Bands of hyalinized collagen separate nests of theca cells.

Histochemistry

- Lipids in theca cells may be demonstrated by frozen section technique. Tissue sections are stained with fat stains such as Sudan III, Sudan IV, Sudan black and Oil red O.
- Silver stains demonstrate reticulin fibers surrounding individual cells (as opposed to granulosa cell tumor, in which the reticulin surrounds clusters of cells).

FIBROMA OF OVARY

Fibroma is most common benign ovarian stromal tumor, which occurs at all ages, with a peak in the perimenopausal period, and is virtually always benign. Cytogenetic analysis demonstrates trisomy of chromosome 12 in fibroma of ovary.

Clinical Features

Fibroma may be associated with **Meigs' syndrome**, a triad of ovarian fibroma >8.0 cm in size, ascites, and hydrothorax in 40% of cases. Rarely, it may undergo malignant change and present as fibrosarcoma.



Fig. 24.103: Thecoma of ovary. This is distinguished from fibroma by the presence of lipid, which gives it a yellow color.



Fig. 24.104: Fibroma of ovary. The cut surface of the tumor shows a homogeneous white appearance with hemorrhage and necrosis.

Surgical Pathology: Fibroma of Ovary

Gross Morphology

The tumor is solid, firm, and gray-white. There is absence of hemorrhage and necrosis (Fig. 24.104).

Light Microscopy

- Tumor cells of fibroma resemble the stroma of the normal ovarian cortex.
- Fibroma is composed of well-differentiated bundles of spindle-shaped fibroblasts and variable amounts of collagen (Fig. 24.105). Distinction between thecoma and fibroma of ovary is given in Table 24.46.

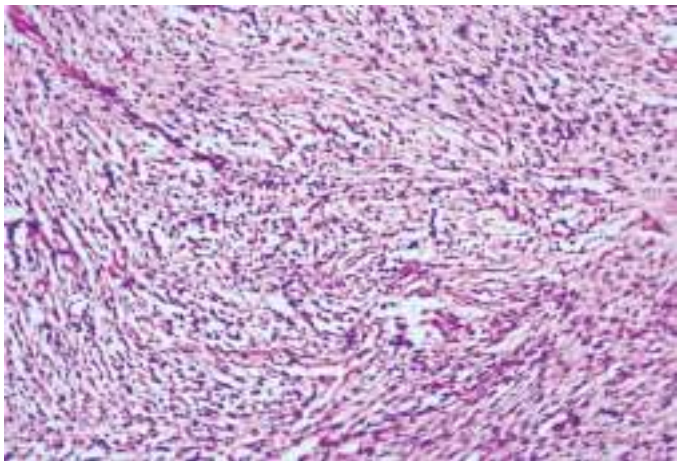


Fig. 24.105: Fibroma of ovary. Tumor is composed of well-differentiated bundles of spindle-shaped fibroblasts and variable amounts of collagen (400X).

SERTOLI-LEYDIG CELL TUMORS OF OVARY

Sertoli-Leydig cell tumor is a rare mesenchymal neoplasm of the ovary of low malignant potential, which resembles the embryonic testis.

- Tumor usually occurs in young women of childbearing age. However, it can affect all ages.
- Tumor is androgen (dehydroepiandrosterone) secreting tumor associated with virilism (masculinization). Due to excess of androgens, approximately 50% of patients initially present with breast atrophy, amenorrhea, and loss of hip fat followed by hirsutism, male escutcheon, enlarged clitoris, and deepened voice.

PURE LEYDIG CELL TUMOR OF OVARY

Pure Leydig cell tumor is a benign tumor present in the hilus or the ovary. It may synthesize androgens. Due to excess androgens, patient presents with masculinization, hirsutism, voice changes, and clitoris enlargement. There is increased excretion of 17-ketosteroid unresponsive to cortisone suppression. Surgical excision of tumor cures the patient.

Surgical Pathology: Pure Leydig Cell Tumor of Ovary

Gross Morphology

Pure Leydig cell tumor is small yellow-colored, unilateral less than 3 cm in diameter.

Light Microscopy

Pure Leydig cell tumor is composed large lipid-laden cells with distinct borders. A typical cytoplasmic structure characteristic of Leydig cells (Reinke crystalloid) is usually present.

ANDROBLASTOMA OF OVARY

Androblastoma is unilateral ovarian tumor, which affects all ages but peak incidence in second and third decades, which has masculinization effect or defeminization effect. This is biphasic tumor, which contains cells resembling testicular Sertoli and Leydig cells interspersed with stroma. Tumor constitutes <5% of cases.

Clinical Features

Androblastoma of ovary synthesizes androgens, which causes atrophy of breasts and external genitalia, amenorrhea, infertility, deepening of voice, male distribution of hair on face and chest; and hypertrophy of clitoris.

Surgical Pathology: Androblastoma of Ovary

Gross Morphology

Androblastoma is unilateral small tumor and resembles granulosa theca cell tumor. Cut surface is gray to golden brown.

Light Microscopy

- Tumor is a well-differentiated tumor composed of Sertoli cells or Leydig cells interspersed with stroma. Intermediate differentiated tumor is composed of immature tubules and large eosinophilic Leydig cells.
- Poorly differentiated tumor shows sarcomatous pattern, disorderly disposition of epithelial cell cords and absence of Leydig cells.
- Heterologous elements like mucinous glands, bone and cartilage are present.

Table 24.46 Distinction between thecoma and fibroma of ovary

Feature	Thecoma of Ovary	Fibroma of Ovary
Age group	Mean age 63 years	<30 years
Function	Typically, estrogenic	Absent
Gross morphology variegation	No	No
Pseudolobulation	Rare	Rare
Prominent ectatic blood vessels	Rare	Rare
Two-cell type	Only in luteinized form	Absent
Hyaline plaques	Common	Common
Clinical behavior	Almost always benign	Almost always benign

GONADOBLASTOMA OF OVARY

Gonadoblastoma is an uncommon ovarian tumor, which occurs in persons with abnormal sexual development and in gonads of indeterminate nature. Approximately 80% of patients are phenotypic females and 20% are phenotypic males with undescended testicles and female internal secondary organs.

Surgical Pathology: Gonadoblastoma of Ovary
Light Microscopy
Tumor is composed of mixture of germ cells and sex cord derivatives resemble immature Sertoli and granulosa cells. A co-existent dysgerminoma occurs in 50% of cases.

PREGNANCY LUTEOMA OF OVARY

Ovary during pregnancy may exhibit nodular proliferation of theca cells, in response to gonadotrophins. Rarely a frank tumor may develop termed pregnancy luteoma that resembles corpus luteum of pregnancy.

- Pregnancy luteoma has been associated with virilization in pregnancy patients and in their respective female infants.
- Pregnancy luteoma should be differentiated from functional luteal cysts (regress in 4–6 weeks), follicular cyst, corpus luteal cysts and theca lutein cyst, subserous leiomyoma, extrauterine pregnancy, hydrosalpinx, appendicular mass, tubercular abdomen, hydatid cyst, and ascites.

LABORATORY DIAGNOSIS OF OVARIAN TUMORS

No high risk groups of ovarian tumors can be identified for whom screening might be appropriate with the rare exception of families with hereditary ovarian cancer. No screening methods have proven to be effective at this time-trials examining screening based upon physical examination, ultrasound imaging, and biomarker serum CA 125 analysis had been unreliable, and productive of a high rate of false positives. The routine Papanicolaou smear is not effective for ovarian cancer screening.

CLINICAL FEATURES

Ovarian tumors are mainly asymptomatic. About 25% of ovarian neoplasms are malignant. Adnexal tumor over 6 cm; if persisting/increasing in size in a postmenopausal woman, ovarian tumor be suspected.

- Benign ovarian tumor is usually slow-growing, unilateral, cystic-free mobile. A solid/cystic, bilateral, rapidly growing tumor associated with pressure symptoms especially in postmenopausal woman is very likely to be malignant.

Table 24.47 Serological markers in ovarian malignant tumors in clinical practice

Serum CA 125: It is a glycoprotein often produced by surface epithelial ovarian tumors. It may help characterize ovarian masses and monitor the extent of disease
Estrogen levels: These are increased in thecomas and granulosa cell tumors
Androgen levels: These are increased in Sertoli-Leydig and hilar tumors
α-Fetoprotein levels: These are demonstrated in yolk sac tumors
Human chorionic gonadotropin β (hCG-β) levels: These are increased in choriocarcinomas

- In hormones producing tumors; clinical history, vaginal cytology, endometrial histology, estimation of hormone levels in blood and urine are significant. Physical examination and clinical examination of the vagina and rectum can detect most important ovarian masses.
- Radiograph has diagnostic utility to demonstrate calcification in cases of dermoid cyst and serous cystadenoma of ovary.

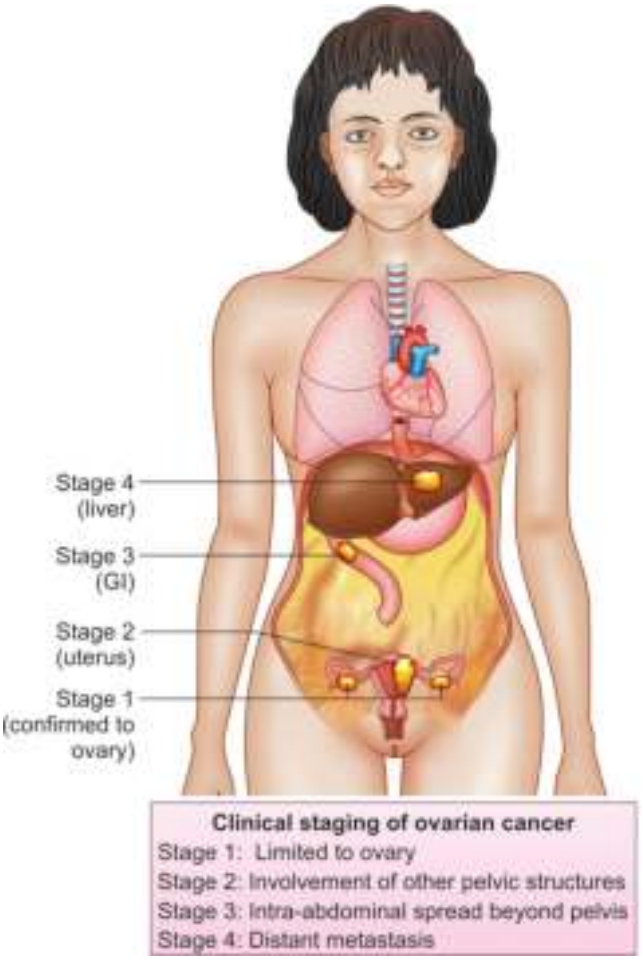


Fig. 24.106: Staging of ovarian cancers.

- Pelvic ultrasound can be very valuable in determining the characteristics of ovarian and other pelvic masses. Ascitic fluid cytology is done to demonstrate malignant cells of ovarian malignant tumors.
- Serological markers in ovarian malignant tumors in clinical practice are given in Table 24.47.

HISTOLOGIC EXAMINATION

A tissue sample is necessary to make an unequivocal diagnosis, this may be obtained by exploratory laparotomy (surgical incision of the abdominal wall to examine the contents of the peritoneal cavity) or laparoscopy. Surgeons take care to avoid rupture of ovarian masses when a diagnosis of cancer is suspected, so as not to spread the disease.

STAGING SYSTEM

Staging is a clinical exercise that classifies tumors according to their size, invasiveness and spread. Tumors are staged according to the TNM system (T: Tumor size, N: Lymph node involvement, M: Metastases to distant organs). Schemes for TNM classification vary according to tumor type and organ involved. Grading classifies tumor according to their light microscopic characteristics (Fig. 24.106 and Table 24.48).

Table 24.48 Stages of ovarian cancers

Stage	Extent of Ovarian Growth
Stage 1	Growth limited to ovaries
Stage 2	Growth in one or both ovaries with pelvic extension
Stage 3	Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes or superficial liver metastases
Stage 4	Growth involving one or both ovaries with distant metastases, if a pleural effusion is present there must be positive cytology; parenchymal (internal) liver metastases—stage 4

GESTATIONAL TROPHOBLASTIC NEOPLASTIC DISORDERS AND RELATED TUMOR-LIKE LESIONS

GESTATIONAL TROPHOBLASTIC DISEASE

Gestational trophoblastic disease with abnormal chorionic villi (hydatidiform mole) is characterized by a spectrum of villous populations ranging from normal size to substantial enlargement, irregular contours and mild, focal trophoblastic hyperplasia.

- Gestational trophoblastic disease includes hydatidiform mole (complete, partial and invasive), benign tumor-like diseases of the trophoblast (placental site nodule) and choriocarcinoma, latter being the only malignant tumor, but responsive to chemotherapy. Revised 2020 WHO of gestational trophoblastic disease is given in Table 24.49.
- Immunohistochemical analysis of placental site nodule, placental site trophoblastic tumor, epithelioid trophoblastic tumor and choriocarcinoma is given in Table 24.50.
- Hydatidiform mole represents abnormal placenta during pregnancies, which results from faulty fertilization. It is characterized by cystic swelling of the chorionic villi and accompanied by variable trophoblastic proliferation. It occurs in two variants: complete, partial and invasive hydatidiform moles. Invasion of hydatidiform mole into myometrium is termed invasive hydatidiform mole.
- Complete and partial hydatidiform moles are shown in Fig. 24.107. Comparison of complete and partial hydatidiform moles is given in Table 24.51.

Table 24.49 Revised 2020 WHO of gestational trophoblastic disease

- **Hydatidiform Moles (Abnormally Formed Placentas during Pregnancy)**
  - Complete mole
  - Partial mole
  - Invasive mole
- **Gestational Trophoblastic Neoplastic Disorders**
  - Choriocarcinoma
  - Placental site trophoblastic tumor (PSTT)
  - Epithelioid trophoblastic tumor (ETT)
- **Trophoblastic Tumor-like Benign Lesions**
  - Exaggerated placental site/reaction
  - Placental site nodule
- **Abnormal (Nonmolar) Villous Lesions**

HYDATIDIFORM MOLE

Hydatidiform mole is characterized by proliferation of villous trophoblast with cystic 'swollen' villi. Macroscopically, these can be visible during second trimester of pregnancy, as clusters of vesicles similar to small grapes developed from the transformation of chorionic villi.

- Complete hydatidiform mole differs from partial hydatidiform mole in cytogenetic and histologic appearance. Important is a complete lack of embryonic/fetal tissue in complete hydatidiform

Table 24.50 Immunohistochemical markers of placental site nodule, placental site trophoblastic tumor, epithelioid trophoblastic tumor and choriocarcinoma

Immunohistochemical Markers	Placental Site Nodule	Placental Site Trophoblastic Tumor	Epithelioid Trophoblastic Tumor	Choriocarcinoma
Mel-Cam (CD146) membranous staining	Positive (0–2%)	Positive (75–100%)	Positive (0–2%)	Positive (6–75%)
HPL (human placental lactogen)	Positive (0–2%)	Positive (25–75%)	Positive (0–2%)	Positive staining in intermediate trophoblastic and syncytiotrophoblastic elements
β-hCG	Positive (0–25%)	Positive (0–25%)	Positive (0–25%)	Positive staining in syncytiotrophoblastic element
p53	Positive (>50–75%)	Negative	Positive (<25–75%)	Positive (<25%)
Ki-67 (MIB-1)	Positive (3–10%)	Positive (>10%)	Positive (>10%)	Positive (49–89%)
Cyclin E	Focal staining	Negative	>50%	Negative

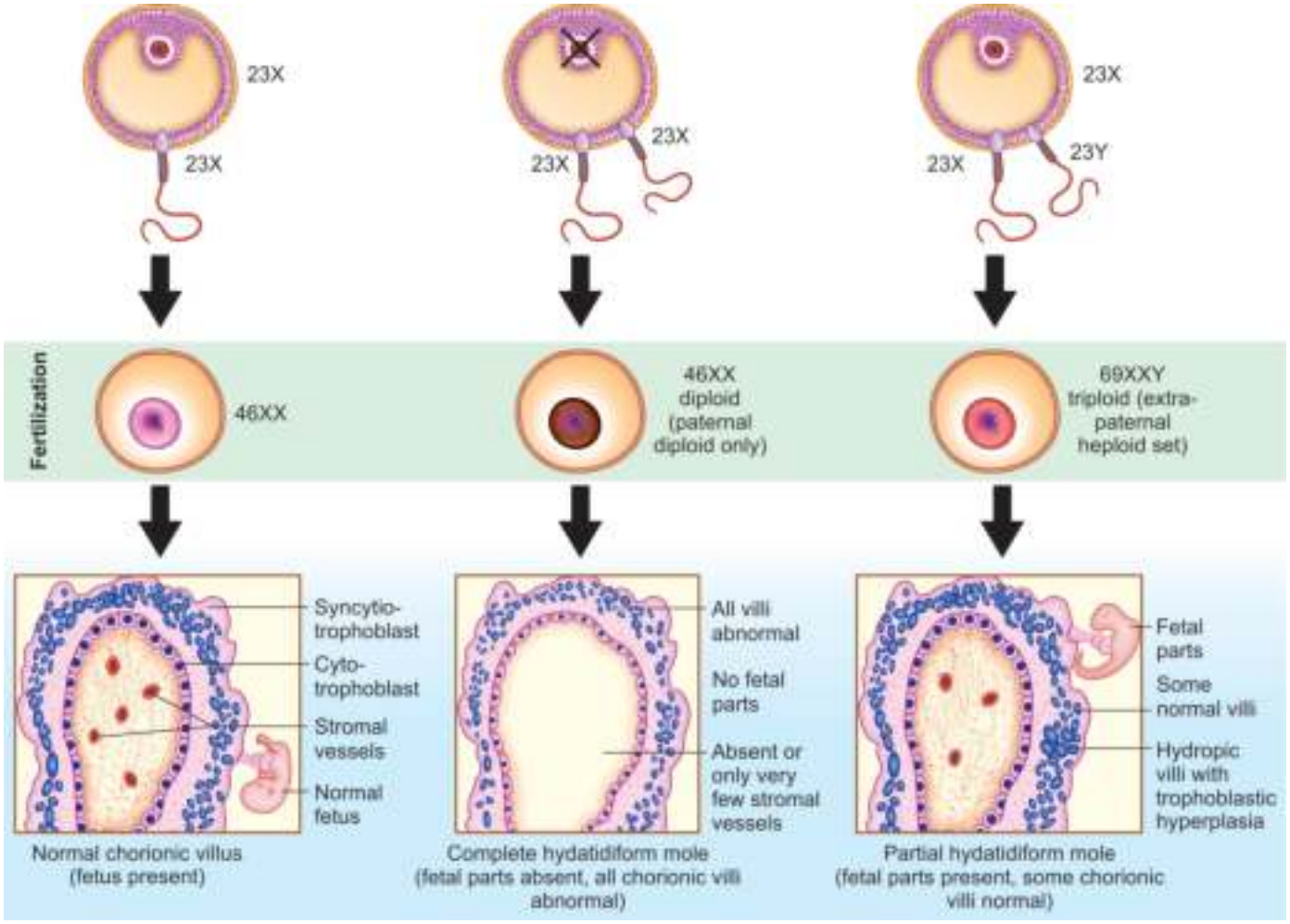


Fig. 24.107: Complete and partial hydatidiform moles. Complete hydatidiform moles result from androgenesis due to fertilization of an empty ovum (lacking functional maternal chromosomes) by one spermatozoon that goes duplication thus resulting in a 46XX karyotype in 90% of cases. Therefore, all the chromosomes are of paternal origin (paternal androgenesis). Fetal parts are absent. All chorionic villi are abnormal. Partial moles result from fertilization of a normal ovum (23X) by two normal spermatozoa each carrying 23 chromosomes—a process called dispermy triploidy. The zygote is triploid (69XXY or 69XYY) with two sets of paternal chromosomes and one set of maternal chromosome or rarely tetraploidy. Ratio of paternal to maternal chromosomes is 2:1. Fetal parts and some chorionic villi are present.

Table 24.51 Comparison of complete and partial hydatidiform moles

Features	Complete Hydatidiform Mole	Partial Hydatidiform Mole
Normal chorionic villi	Absent	May be present
Edema in chorionic villi	All chorionic villi edematous	Some chorionic villi edematous
Capillaries in the chorionic villi	Few or no fetal RBCs in chorionic villi	Many fetal RBCs in chorionic villi
Trophoblastic hyperplasia	Diffuse and marked	Focal (minimal to moderate)
Embryo (fetal parts)	No cord, amnion or fetus	Abnormal fetus (may be present) is viable for a few weeks
Gestational age	8–16 weeks	10–26 weeks
Cytogenetics	46XX/46XY (diploid)	Triploid 69XXX, tetraploid 92XXXY
Chromosomal pattern	Paternal chromosome only	Maternal and paternal chromosomes
Serum β-hCG	Markedly increased	Mild to moderately increased
Tissue β-hCG	Strong positivity	Mild positivity
Pathogenesis	Fertilization of empty egg by one or two sperms: No maternal DNA	Fertilization of ovum by one or two sperms: Paternal and maternal DNA
Ultrasonography finding	Snow storm sign, uterus large for duration of pregnancy	Uterus small for fetus during pregnancy
p57 staining in cytotrophoblastic and villus stromal cells	Low or absent staining	Diffuse staining
Risk of development of gestational trophoblastic disease	≤30%	5%
Malignant transformation	Choriocarcinoma (2%)	Choriocarcinoma minimal risk

mole and presence of embryonic tissue in partial hydatidiform mole.

- An important marker that aids in diagnosis of the complete hydatidiform mole is the absence of p57 in immunohistochemistry. This p57 marker is a paternal imprint inhibitor gene, so its expression implies the maternal contribution. In brief, the absence of p57 expression supports the diagnosis of complete hydatidiform mole.
- Invasive hydatidiform mole is defined as molar gestation invading myometrium or uterine blood vessels.
- Patient presents with vaginal bleeding and presistent elevation of human choriogonadotropin hormone (hCG). Extrauterine spread to lungs, vagina, vulva and broad ligament is observed in 20–40% cases.

COMPLETE HYDATIDIFORM MOLE

Complete hydatidiform mole is a proliferative disorder of the placenta resulting in hydropic change and trophoblastic hyperplasia without development of embryo. Ultrasonography demonstrates characteristic 'snowstorm appearance'. Patient typically presents with vaginal bleeding or a missed abortion.

- In complete hydatidiform mole, molar changes occur as early as in 4th week but most cases present in 4–5th months of pregnancy. Since the embryo dies at a very early stage, fetal parts are absent. Chorionic villi show florid trophoblastic proliferation and hydropic change.
- Complete hydatidiform mole resembles bunches of grapes, exhibits **diploidy (46XX karyotype)** and shows only paternal chromosomes. Ratio of paternal to maternal chromosomes is 2:0. It most often affects young females 40 years of low socioeconomic group.
- Most molar pregnancies are diagnosed by ultrasound in the early stages of pregnancy. Molar pregnancies can be completely evacuated by curettage. Complete hydatidiform mole becomes locally invasive in 15–20% of cases. Malignant transformation (choriocarcinoma) develops in about 2% of cases.

Pathogenesis

Complete hydatidiform mole results from androgenesis due to fertilization of an empty ovum (lacking functional maternal chromosomes) by one spermatozoon that goes duplication thus resulting in a 46XX karyotype in 90% of cases. Fertilization of the empty ovum occurs by two spermatozoa (dispermy) results in a 46XY phenotype in 10% of cases. Therefore, all the chromosomes are of paternal origin (paternal androgenesis).

Clinical Features

Patient presents with history of amenorrhea of less than 24 weeks of gestation, passage of grape-like structures (vesicles) per vagina, abdominal pain, and hyperemesis.

- Complete hydatidiform mole can be mistaken for a normal pregnancy, but the uterus is often too large for the supposed state of gestation.
- There is increase in serum hCG levels after 14th week, as opposed to the drop typically seen in the course of normal gestation.
- Hyperstimulation of ovaries by human chorionic gonadotropin (hCG) synthesis is responsible for bilateral ovarian luteal cysts associated with trophoblastic tumors.
- Normal trophoblastic tissue synthesizes β-hCG, estrogen, progesterone and TSH-like hormones. Excess of TSH production causes hyperthyroidism in 2% of cases.

Complications

Complications of complete hydatidiform mole include hemorrhage, anemia, uterine sepsis, uterine perforation during suction curettage, choriocarcinoma, and disseminated intravascular coagulation (DIC).

- Perforation of the uterus during suction curettage sometimes occurs due to large and boggy uterus. If uterine perforation is observed, the suction procedure should be completed under laparoscopic guidance.
- Hemorrhage is a frequent complication during the evacuation of a molar pregnancy.
- For this reason, intravenous oxytocin should be started at the initiation of the suctioning. If a molar pregnancy is not completely treated, it can cause a range of serious complications such as gestational trophoblastic disease (GTD) including persistent gestational trophoblastic disease such as persistent growth of the placental tissue and invasive mole invading uterine wall.

Surgical Pathology: Complete Hydatidiform Mole

Gross Morphology

- Grossly bulky tissue with hydropic changes in all chorionic villi resemble 'bunch of grapes' pearly white, semitransparent vesicles of variable size measuring a few mm to 2–3 cm. The total weight of bunch of grapes is usually over 200 g in complete hydatidiform mole.
- Normal placental structures or fetal parts are absent. Early complete hydatidiform mole may not demonstrate evidence of abnormal chorionic villi (Fig. 24.108).

Light Microscopy

- In complete hydatidiform mole, chronic villi show diffuse enlargement with marked hydropic changes and irregular scalloped borders.
- Hydropic change in chorionic villi occurs due to abnormal angiogenesis and decreased blood vessels in the chorionic villi resulting in lack of vascular drainage and accumulation of fluid, which show variable degrees of trophoblastic hyperplasia usually in a circumferential pattern.
- Remarkable cytologic atypia is almost always present in chorionic villi. Mitoses are common.
- The blood vessels are absent or inadequate in loose edematous myxomatous stroma.
- Stromal mucin and stromal nuclear debris (apoptosis), appear very early and represent a diagnostic clue. Histology of complete hydatidiform mole is shown in Fig. 24.109.

Molecular Genetic Alterations

Ploidy analysis is a useful adjunct in diagnosis of complete hydatidiform mole. Novel missense NLRP7 gene mutation located on chromosome 19q has been identified in cases of familial clusters of biparental complete hydatidiform moles.

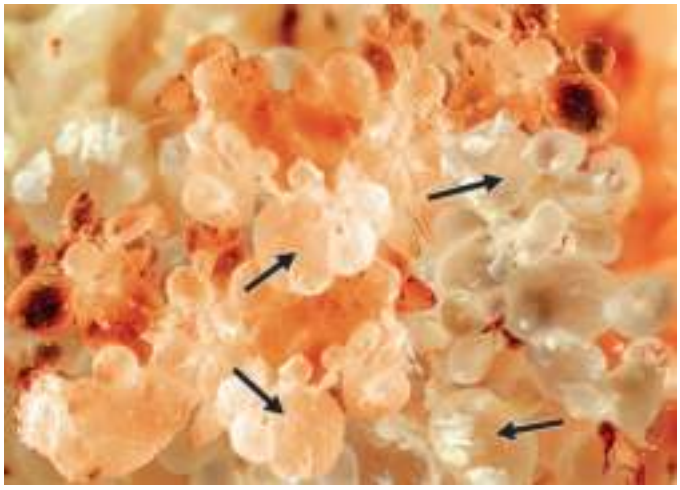


Fig. 24.108: Complete hydatidiform mole. Uterine cavity is occupied by a mass of abnormal chorionic villi resembling a bunch of grapes. Each villus is distended by fluid to reach a diameter of about 1–3 mm. No fetus is present (arrows).

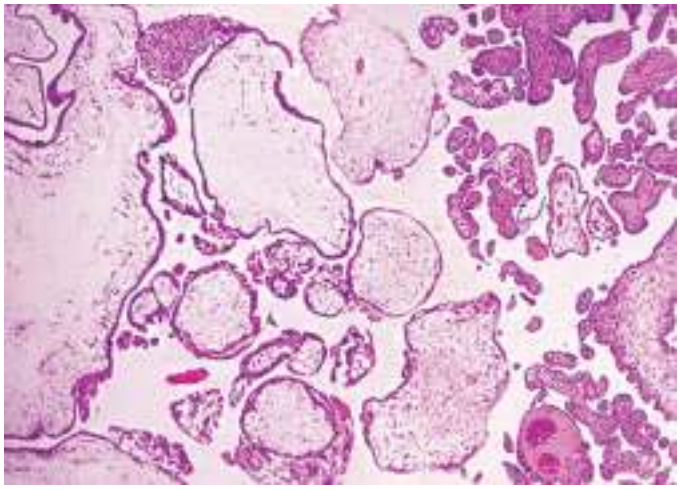


Fig. 24.109: Complete hydatidiform mole. Histologic examination shows fluid-filled, avascular villi with overlying hyperplastic trophoblast that shows cellular atypia (100X).

Laboratory Diagnosis

In complete hydatidiform mole, ultrasonography reveals snowstorm/speckled appearance. Doppler study reveals absent fetal heart sounds. X-ray chest is done to rule out metastatic choriocarcinoma. An hCG level of less than 5 mIU/ml is considered negative for pregnancy, and anything above 25 mIU/ml is considered positive for pregnancy.

- Patient with complete hydatidiform mole shows urinary hCG excretion >100,000 IU/24 hours; and serum hCG of 100,000 mIU/ml is demonstrated in 50% of cases.
- Post-evacuation serum quantitative hCG measurements should be performed every 1–2 weeks until 3 consecutive tests are normal, followed by hCG measurement at 3-month intervals for 6 months. The patient should be followed up by estimation of serum human chorionic gonadotropin up to 2 years to rule out development of choriocarcinoma.

Treatment

Suction and evacuation of complete hydatidiform mole is done. Chemotherapy should be given to the patients, who have persistent uterine bleeding despite curettage, elevated serum hCG levels remain even after 12 weeks of post-evacuation; and choriocarcinoma on endometrial biopsy.

PARTIAL HYDATIDIFORM MOLE

Partial hydatidiform mole exhibits partially formed fetal parts, normal and abnormal hydropic chorionic villi with irregular scalloped borders and variable degree of proliferation. Fetus associated with partial hydatidiform mole usually dies after 10 weeks of gestation followed by abortion of the partial hydatidiform mole shortly thereafter. Partial hydatidiform moles generally do not predispose to choriocarcinoma.

Pathogenesis

Partial hydatidiform mole results from fertilization of a normal ovum (23X) by two normal spermatozoa; each carrying 23 chromosomes—a process called dispermy triploidy. By alternate mechanism, it results from fertilization of a normal ovum (23X) by single spermatozoon that has not undergone meiotic reduction and bears 46 chromosomes. The zygote is triploid (69XXY or 69XYY) with two sets of paternal chromosomes and one set of maternal chromosome or rarely tetraploidy. Ratio of paternal to maternal chromosomes is 2:1.

Surgical Pathology: Partial Hydatidiform Mole

Gross Morphology

In partial hydatidiform mole, immature placental tissue is admixed with vesicles that tend to be smaller and less numerous than those of a complete hydatidiform mole. Fetal parts including nucleated red blood cells in villous capillaries, as well as a gestational sac may be present.

Light Microscopy

- Partial hydatidiform mole is composed of heterogenicity in villus size with discrete populations of chorionic villi ranging from large hydropic villi and small fibrotic villi.
- The enlarged chorionic villi are of irregular shape with scalloped borders and secondary inclusions.
- Chorionic villi show circumferential trophoblastic hyperplasia.
- Presence of at least 3 of these histologic features of partial hydatidiform mole correlates with triploidy on FISH and flow cytometry analysis.

Flow Cytometry

- Flow cytometry can be performed on paraffin-embedded samples to determine ploidy in partial hydatidiform mole.
- Flow cytometry cannot distinguish between maternal or paternal origin of genetic material.
- A triploid karyotype by flow cytometry is consistent with partial hydatidiform mole in conjunction with morphological features.

Molecular Genetic Alterations

In partial hydatidiform mole, testing of both maternal decidua and villous tissue can identify triploid genotype (69XXY or 69XYY) by polymerase chain reaction (PCR). Fluorescence *in situ* hybridization (FISH) uses fluorescently labeled DNA probes targeting regions of various chromosomes, including X and Y. FISH can be used to determine sex chromosome and copy number. It cannot differentiate between maternal and paternal origins of genetic material.

Treatment

Patient of partial hydatidiform mole is treated by evacuation and curettage with follow-up with serum human chorionic gonadotropin (hCG) levels and contraception until undetectable levels. Some patients may require chemotherapy in 1% of cases compared with 14% for complete hydatidiform mole.

INVASIVE HYDATIDIFORM MOLE

Invasive hydatidiform mole is the most common form of persistent or metastatic gestational trophoblastic disease (GTD) after complete or partial hydatidiform mole. It is also termed chorioadenoma destruens. It occurs more frequently than choriocarcinoma.

- Invasive hydatidiform mole is defined as molar gestation invading underlying myometrium. It can spread to extrauterine structures such as parametrium, broad ligament, vagina, vulva and lungs in 20–40% of cases. It may rarely invade paraspinal tissue.
- Patient usually presents with vaginal bleeding and persistent elevation of β-hCG.
- Invasive hydatidiform mole is commonly diagnosed after primary evacuation of complete or partial hydatidiform mole.
- Chemotherapy is highly effective in treating these cases associated with 80% cure rate.

Clinical Features

Invasive hydatidiform mole may cause uterine perforation and embolize to lung, brain, but does not grow there. The hCG-β levels are persistently increased in invasive hydatidiform mole and choriocarcinoma. It responds well to chemotherapy. Normally, serum hCG-β level is 120–160 IU/ml in normal pregnancy after 70–90 days. But in invasive hydatidiform mole, serum hCG-β level is 1000 IU/ml in pregnancy after 70–90 days.

Surgical Pathology: Invasive Hydatidiform Mole

Gross Morphology

In invasive hydatidiform mole, hydropic chorionic villi are observed extending from endometrium to myometrium. Invasive hydatidiform mole in the uterus is commonly seen as an erosive hemorrhagic lesion, that may perforate the uterus (Fig. 24.110).

Light Microscopy

- Invasive hydatidiform mole shows dysmorphic, less hydropic chorionic villi and extravillous trophoblast invading the myometrium and blood vessels.
- Tissue exhibits morphologic characteristics of hydatidiform complete mole (most common) or partial mole are retained in the invasive hydatidiform mole.
- Chorionic villi are less hydropic than complete or partial mole.
- Trophoblastic proliferation with mild to severe dysplasia is usually demonstrated. Metastatic invasive hydatidiform mole is diagnosed by demonstration of dysmorphic chorionic villi in the blood vessels without tissue invasion.
- Since the diagnosis requires the presence of myometrium and blood vessels invaded by chorionic villi, it can be confirmed on histopathologic examination of resected tissue.

Treatment and Prognosis

Patient with invasive hydatidiform mole has a good prognosis even when it has spread to extrauterine sites. Patient should be treated with eight cycles of two weekly methotrexate and followed up for one year.

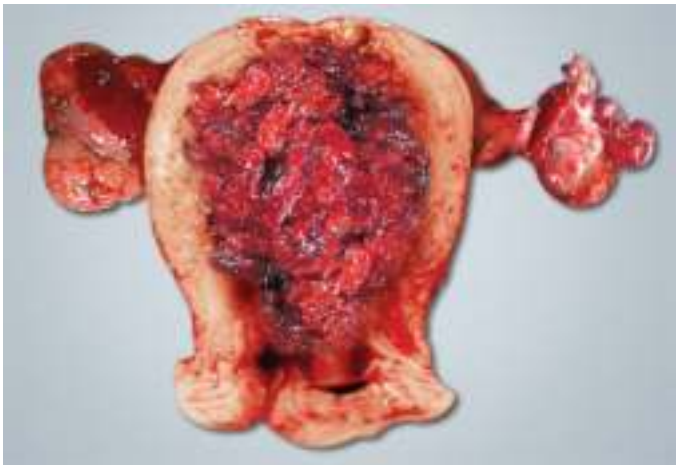


Fig. 24.110: Invasive hydatidiform mole. It is invading myometrium.

GESTATIONAL TROPHOBLASTIC NEOPLASTIC DISORDERS

GESTATIONAL CHORIOCARCINOMA

Gestational choriocarcinoma lacks chorionic villi. It is highly invasive epithelial malignant tumor of trophoblast, which shows widespread metastases.

- Tumor is derived from hydatidiform mole (50% of cases), previous abortion (25%), normal pregnancy (22%), and ectopic pregnancy in 1 of 30,000 pregnancies.
- Tumor occurs more often in Asian women especially younger than 20 and older than 40 years. Very young and older women (<20 and >40 years) are most commonly affected.

Clinical Features

Patient presents with irregular bloody, foul smelling discharge per vagina, fever, cachexia with raised serum hCG level. Rapidly growing tumor causes ischemic necrosis, hemorrhage and secondary infection. Presence of infection reveals foul smell discharge per vagina.

Surgical Pathology: Gestational Choriocarcinoma

Gross Morphology

Gestational choriocarcinoma is soft, fleshy, yellow white, large pale areas, ischemia, necrosis, foci of cystic softening with extensive hemorrhage. It does not classically produce a large bulky mass (Fig. 24.111).

Light Microscopy

Gestational choriocarcinoma is composed of abnormal proliferation of syncytiotrophoblast and cytotrophoblast. It does not form chorionic villi. Areas of hemorrhage and necrosis are present (Fig. 24.112).

Immunohistochemistry Technique	
Neoplastic cells of gestational choriocarcinoma are immunoreactive to β-hCG, CD10, inhibin, CD146, p63, Ki-67 (MIB-1), HPL, Mel-Cam (CD146), p53.	
Markers	**Expression**
▪ β-hCG	▪ Positive (100%)
▪ CD10	▪ Positive (100%)
▪ Inhibin	▪ Positive (85%)
▪ CD146	▪ Positive (70%)
▪ p63	▪ Positive (70%)
▪ Ki-67 (MIB-1)	▪ Positive (49–89%)
▪ HPL (human placental lactogen)	▪ Positive (45%)
▪ Mel-Cam (CD146)	▪ Positive (6–75%)
▪ p53	▪ Positive (<25%)
▪ p16	▪ Negative
▪ Pan cytokeratin (CK5/CK6) AE1/AE3	▪ Negative
▪ CEA	▪ Negative

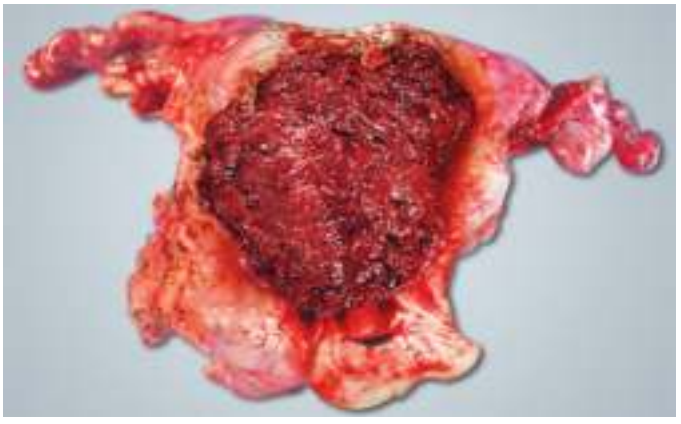


Fig. 24.111: Gestational choriocarcinoma. It shows soft and fleshy tumor with extensive hemorrhage and necrosis.

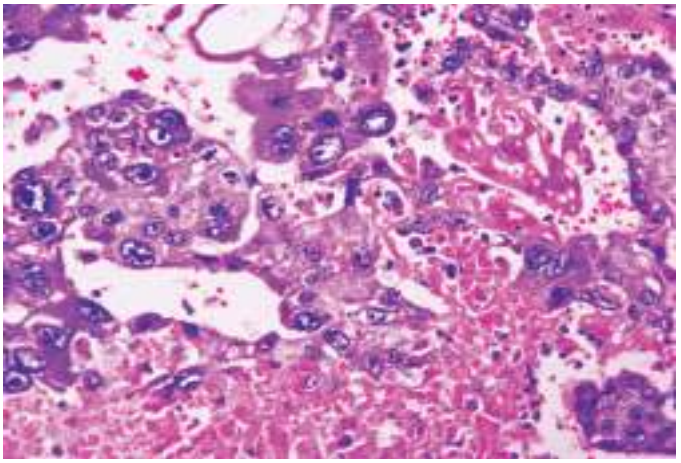


Fig. 24.112: Gestational choriocarcinoma. Tumor is composed of multinucleated syncytiotrophoblastic and cytotrophoblastic cells. The multinucleated syncytiotrophoblasts have voluminous eosinophilic cytoplasm and large darkly staining irregular nuclei with prominent nucleoli. The smaller mononuclear cytotrophoblasts are fairly uniform in size with amphophilic cytoplasm and distinct cellular outlines resembling to the tumor cells of solid yolk sac tumor (400X).

Mode of Spread

The gestational choriocarcinoma invades the underlying myometrium, extends uterine to serosa, lymphatic channels and blood vessels. It is associated with extensive hemorrhage and necrosis. In fatal cases, metastases are seen in lung (50%), vagina (30%) brain, bone marrow, liver, kidney and other organs. In occasional case, primary tumor has undergone total necrosis, while the patient has clinical features due to metastases.

Prognosis and Treatment

Gestational choriocarcinoma is treated by evacuation of uterine contents, surgery and chemotherapy. Tumor is highly responsive to chemotherapy regimens, such as EMA/CO (methotrexate, actinomycin D, cyclophosphamide, vincristine and etoposide).

- Tumor arising from the placenta carries paternal gene that are foreign to the host.
- In contrast, tumor arising from the host's own germ cells has the same genes as the host. This accounts for the fact that gestational choriocarcinomas are readily curable with chemotherapy. It probably stimulates the host immune system in the defense against the choriocarcinoma cancer stem cells (CSCs).
- On the other hand, germ cell choriocarcinomas are incurable. Prognosis is very good with 100% cure rate/remission.

PLACENTAL SITE TROPHOBLASTIC TUMOR

Placental site trophoblastic tumor (PSTT) originates from the intermediate trophoblastic cells of the implantation site. It usually occurs in multiparous women, and sometimes long after the last known pregnancy.

- Usual location of PSTT is in the uterine fundus, where it invades the myometrium and serous coat. It is preceded by normal pregnancy in 50% of cases. Spontaneous abortion can occur with increased serum hCG level. Serum levels of hCG are generally low.
- PSTT is composed of intermediate trophoblastic cells that display a marked propensity to invade blood vessels. Vascular invasion is associated with fibrin deposition.
- PSTT is cured by curettage alone. PSTT may undergo malignant transformation in 10–15% of cases.
- The diagnosis of placental site trophoblastic tumors, particularly the distinction between placental site tumor from an exaggerated placental site can be difficult. Mel-Cam (also known as CD146 and MUC18) is recently recognized cell adhesion molecule belonging to the immunoglobulin gene superfamily that specifically identifies intermediate trophoblast. A double-staining technique using MIB-1 antibody determines the Ki-67 proliferative index in Mel-Cam

defined intermediate trophoblast is a useful technique in the diagnosis of exaggerated PSTT.

Clinical Features

Patient with placental site trophoblastic tumor can present with either amenorrhea or abnormal bleeding per vagina, often accompanied by uterine enlargement. When uterine enlargement ceases the diagnosis of missed abortion is sometimes made. About 66% of cases follow a full term pregnancy and are in stage I. Recurrence or metastases occurs in 25–30% of cases.

Surgical Pathology: Placental Site Trophoblastic Tumor

Gross Morphology

- Placental site trophoblastic tumor (PSTT) is well circumscribed nodular mass up to 10 cm projecting into uterine cavity or predominantly involving the myometrium.
- Cut surface is usually solid, fleshy and white-tan to light yellow, containing only focal areas of hemorrhage or necrosis if present.
- Deep myometrial invasion is observed in 50% of cases, which frequently extends into the uterine serosa (18%) and rarely adnexal structures and broad ligament.

Light Microscopy

- PSTT has infiltrative growth pattern, which consists of sheets of large, polyhedral to round intermediate trophoblastic mononuclear cells and scattered multinucleated cells.
- At the periphery, trophoblast cells invade singly or in cords and nests in the myometrium by separating individual muscle fibers or groups of muscle fibers. Vascular invasion is commonly seen.
- Most PSTTs have a low mitotic count 2–4 mitoses per high power fields.
- Decidua or Arias-Stella reaction my be present in the adjacent uninvolved endometrium. Chorionic villi are never identified.

Immunohistochemistry Technique

PSTT is immunoreactive to Mel-Cam (CD146), human placental lactogen (HPL), β-hCG, Ki-67 (MIB-1), p63.

Markers	Expression
▪ Mel-Cam (CD146)	▪ Positive membranous staining (75–100%)
▪ Human placental lactogen (HPL)	▪ Positive (25–75%)
▪ β-hCG	▪ Positive (>10%)
▪ Ki-67 (MIB-1)	▪ Positive
▪ p63	▪ Negative

Prognosis

Prognosis is poor, if the tumor develops after 4 years interval from prior pregnancy. In 10% of cases, widespread metastases are seen. ETT behaves in a benign fashion in two-thirds of cases. It may recur and metastasize in one-third of cases.

EPITHELIOID TROPHOBLASTIC TUMOR

Epithelioid trophoblastic tumor (ETT) is a rare trophoblastic tumor composed of chorionic-type intermediate trophoblast. ETT originates in the uterine corpus, lower uterine segment, or endocervix and grows in a nodular expansive manner.

Surgical Pathology: Epithelioid Trophoblastic Tumor

Light Microscopy

- Histologically, epithelioid trophoblastic tumor (ETT) resembles a carcinoma. It is composed of cells with distinct borders and abundant eosinophilic to vacuolated cytoplasm.
- Histologic hallmark of ETT is the presence of multifocal necrosis with accumulation of a fibrinoid eosinophilic material.
- Another key feature is that the neoplastic cells may displace the endometrial and endocervical epithelium.

Immunohistochemistry Technique

Neoplastic cells of epithelioid trophoblastic tumor are immunoreactive to p53, cyclin E, Ki-67 (MIB-1), HPL (human placental lactogen) and β-hCG.

Markers	Expression
▪ p53	▪ Positive (<25–75%)
▪ Cyclin E	▪ >50%
▪ Ki-67 (MIB-1)	▪ Positive (>10%)
▪ Mel-Cam (CD146)	▪ Positive (0–2%) membranous staining
▪ Human placental lactogen (HPL)	▪ Positive (0–2%)
▪ β-hCG	▪ Positive (0–25%)

PREGNANCY ASSOCIATED NON-NEOPLASTIC DISORDERS

TOXEMIA OF PREGNANCY

Toxemia of pregnancy most often occurs during third trimester in women with first pregnancy. It most often affects kidneys, liver and brain (hemorrhages) in acute toxemia of pregnancy. Patient develops puffiness on face, pitting edema and convulsions in true eclampsia. It usually begins insidiously after the 20th week of pregnancy. Toxemia of pregnancy exists in two forms: pre-eclampsia and eclampsia.

- Pre-eclampsia is defined as a previously normotensive pregnant woman whose blood pressure reaches 140/90 mm Hg or greater after 20 weeks gestation on at least two occasions (pregnancy-induced

hypertension). The addition of proteinuria (≥1+ on urine dipstick confirmed by a 24-hour collection ≥300 mg of protein) indicates that pre-eclampsia has occurred.

- Eclampsia of pregnancy indicates one or more of the following: blood pressure systolic >160 or diastolic >100 mm Hg, proteinuria >5 g/24 hours or ≥3+ proteinuria or two dipstick evaluations at least 4-hour apart; headache; visual disturbances, epigastric or right upper quadrant pain; oliguria, thrombocytopenia, and/or elevation of hepatocellular enzymes, fetal growth restriction, and/or pulmonary edema.
  - Hemolysis elevated liver enzymes, low platelet count syndrome is a form of severe eclampsia before the 37th week of pregnancy with a specific triad finding including hemolysis, elevated hepatocellular enzymes, and thrombocytopenia. Convulsions, signify the onset of eclampsia of pregnancy.
  - Eclampsia of pregnancy adversely affects kidneys, liver (subcapsular hemorrhages and periportal necrosis), and brain (hemorrhages). Patient may also develop placental infarcts.
  - Delivery of the platient is the only effective medical intervention. The definitive therapy is the removal of the placenta, hopefully by normal delivery (Table 24.52 and Fig. 24.113).

Table 24.52 Differences between pre-eclampsia (mild) and eclampsia (severe) of pregnancy

Parameters	Pre-eclampsia of Pregnancy	Eclampsia of Pregnancy
Blood pressure	140/90	>160/110 mm Hg
Proteinuria	Traces to mild	Moderate to marked proteinuria
Urine output	Normal	Oliguria
Headache	Absent	Present
Cerebral and visual disturbances	Absent	Present
Upper abdominal pain	Absent	Present
Convulsions	Absent	Present
Serum creatinine	Normal range	Elevated
Thrombocytopenia	Absent	Present
Pulmonary edema	Absent	Present
Fetal growth retardation	Absent	Present

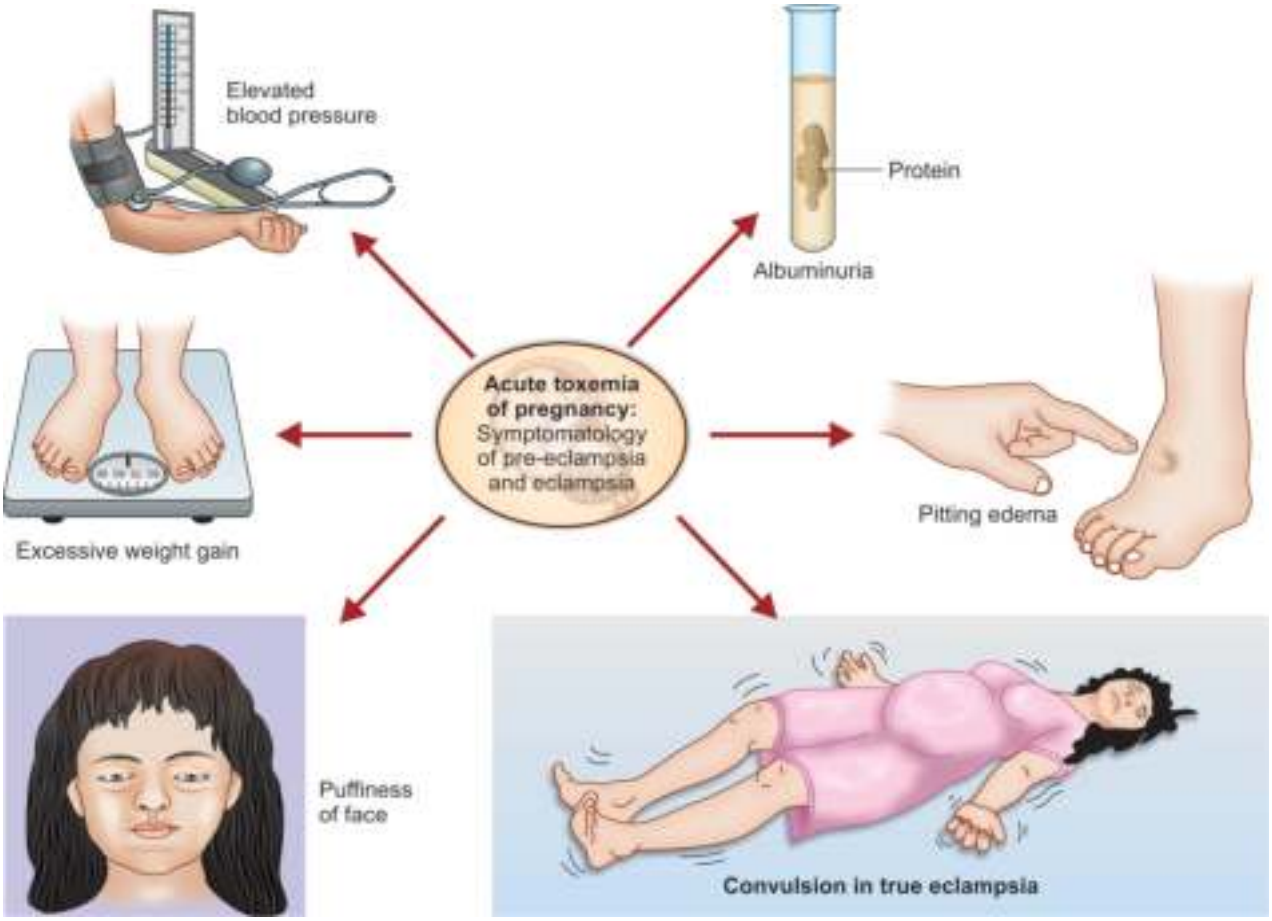


Fig. 24.113: Toxemia of pregnancy: Clinical manifestations in pre-eclampsia and eclampsia of pregnancy.

ETIOPATHOGENESIS

The spiral arteries that supply maternal blood flow to the placenta normally expand to accommodate the growing placentofetal unit, which usually occurs due to trophoblastic remodeling of spiral arteries. Failure to expand spiral arteries results in decidual vasculopathy, reduced maternal blood flow, placental ischemia, and toxemia of pregnancy.

- Ischemic placenta synthesizes soluble factors resulting in generalized maternal dysfunction. The soluble factors synthesized by ischemic placenta act by neutralizing the angiogenic and vasodilatory effects of vascular endothelial growth factor and placental growth factor produced by placenta.
- Two types of abnormal placentas are associated with pre-eclampsia of pregnancy.
- Small placenta with decidual vasculopathy and thin umbilical cord is most common cause of maternal vascular perfusion. Less common is the large placenta associated with diabetes mellitus, placental hydrops, multiple gestation and hydatidiform mole.

CLINICAL FEATURES

As the disease progresses the diastolic pressure persistently exceeds 110 mm Hg. Proteinuria is ≥3 g per day, and renal function declines. It is complicated by convulsions, and disseminated intravascular coagulation the term eclampsia is applied, that can be fatal. Disseminated intravascular coagulation (DIC) is characterized by formation of fibrin thrombi in the liver, brain, and kidneys. Patient may develop subcapsular hemorrhage in the liver.

ECTOPIC PREGNANCY

Ectopic pregnancy is defined as implantation (gestation) of the fertilized ovum outside the normal uterine cavity. Ectopic pregnancy is usually discovered early in a suspected pregnancy when ultrasound examination reveals the uterus to be empty. Ampulla of the fallopian tube is the most common site of ectopic pregnancy. The classic clinical triad of ectopic pregnany is pain abdomen, amenorrhea and vaginal bleeding in 50% of cases. On clinical examination, palpable adnexal mass and abdominal tenderness are demonstrated in many patients. Rupture of tubal ectopic pregnancy products leads to hypotensive shock.

- **Predisposing factors:** Predisposing factors include pelvic inflammatory disease (15–50%) with chronic salpingitis often gonorrheal infection leading to partial blockage, peritubal adhesions. There has been increased incidence of ectopic pregnancy in women fitted with IUCD (50%) with normal tubes, prior ectopic pregnancy, congenital tubal anomalies and endometriosis.
- **Locations:** Over 95% of ectopic pregnancies occur in the fallopian tube. Ectopic pregnancy in ampullary portion of the fallopian tube is commonest followed by distil/middle-third of tube and isthmus. Other uncommon sites of implantation are ovary, cervix and abdominal cavity.
- **Clinical features:** Patient presents with severe abdominal pain about 6 weeks after previous normal menstrual period. Tubal rupture causes bleeding in tube (hematosalpinx) and pelvis resulting in hypotensive shock. Tubal pregnancy is suspected when a tubal hematoma is present.
- **Tubal changes:** The fertilized ovum implants in tubal wall with minimal decidual reaction. By 12th week of gestation, the trophoblast easily penetrates the mucosa and thin tubal wall resulting in rupture and intratubal as well as intraperitoneal hemorrhage. Less commonly tubal pregnancy may regress spontaneously with resorption of the entire gestation.
- **Ultrasonography:** USG, estimation of blood hCG levels and laparoscopy aid in diagnosis of ectopic tubal pregnancy.

Surgical Pathology: Ectopic Pregnancy in Fallopian Tube
Gross Morphology
Fallopian tube shows dilatation, hemorrhagic, chorionic villi with or without embryo (Fig. 24.114).
Light Microscopy
Fallopian tubal mucosa or wall demonstrates gestation products (chorionic villi or trophoblast) (Fig. 24.115).



Fig. 24.114: Tubal ectopic pregnancy. Cut opened tube demonstrates gestation products.

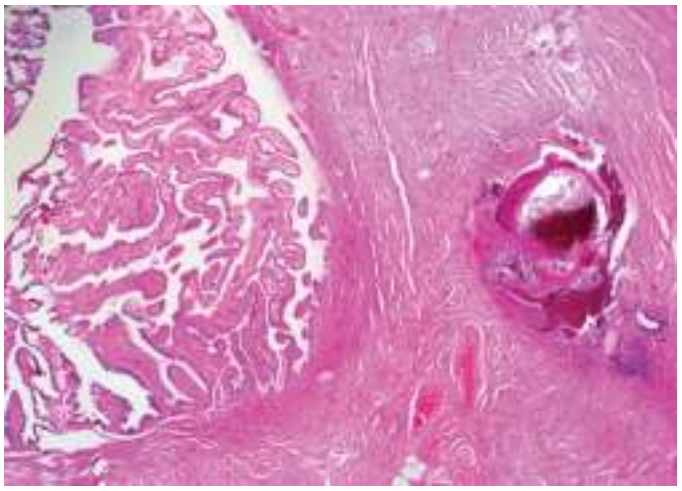


Fig. 24.115: Ectopic tubal pregnancy. A positive pregnancy test (presence of human chorionic gonadotropin), ultrasound, and culdocentesis with presence of blood are helpful in making the diagnosis of ectopic pregnancy. Seen here is tubal epithelium at the right, with rupture site and chorionic villi at the lower left (100X).

THROMBOEMBOLISM

There is increased risk for deep vein thrombosis resulting in pulmonary embolism during pregnancy and postpartum period. The weight of the fetus on femoral vessels slows blood venous return. Hormonal changes and stress precipitates thrombi formation. Thromboplastin released from amniotic fluid results in activation of coagulation system.

- Venous thrombi are present in deep veins in the lower extremities below knee joint (most common in 90%), superior saphenous, ovarian or uterine veins.
- Patient with deep vein thrombosis results in pain and distal edema (Homar's sign). Patient may develop migratory thrombophlebitis and pulmonary embolization. Iliofemoral venous thrombus is formed during puerperium.
- Venous thrombi formed in the lower extremities propagate (extend) toward the heart may cause pulmonary artery embolization.
- Deep vein thrombosis is entirely asymptomatic in 50% of affected patients. It is recognized only after thrombus is embolized.

AMNIOTIC FLUID EMBOLISM

The cause of amniotic fluid embolism is a tear in the placental membranes and rupture of maternal veins. Amniotic fluid embolism is characterized by sudden respiratory difficulty, progressing to shock and often to death. Amniotic fluid embolism and abruptio placentae are well-known causes of disseminated intravascular coagulation (DIC). It is marked by masses of debris and epithelial squamous cells in the maternal pulmonary microcirculation. Amniotic fluid embolism should not be confused with the amniotic fluid aspiration syndrome seen in neonates.

Amniotic Fluid Aspiration Syndrome in Newborn

Amniotic fluid aspiration syndrome in newborn characterized by squamous epithelial cells of amniotic fluid origin in fetal terminal air spaces and major bronchi.

GESTATIONAL DIABETES MELLITUS

Pregnancy associated transient diabetes mellitus is known as gestational diabetes mellitus. Gestational diabetes mellitus is usually identified in the 24–28 weeks of gestation. A woman, who develops gestational diabetes mellitus, has high risk for development of type 2 diabetes mellitus later in life.

- During pregnancy, anti-insulin hormones are synthesized by placenta such as estrogen, prolactin, human chorionic somatomammotropin, cortisol, and progesterone. Human chorionic somatomammotropin regulates carbohydrate and protein metabolism of the mother to ensure delivery to the fetus of glucose for energy and protein for fetal growth.
- Gestational diabetes mellitus is diagnosed with a glucose tolerance test, in which the woman drinks 50 glucose. Blood glucose level after an hour >140 mg/dl is considered positive. The confirmatory test is a 3-hour, 100 glucose tolerance test in which the blood glucose values are set at highest sensitivity. Urine analysis is done for sugar.
- Due to increased fetal birth weight in gestational diabetes mellitus, there is increased indication of cesarean section. There is increased fetal birth weight, brachial plexus injuries, and neonatal respiratory distress syndrome (hyaline membrane disease).
- During intrauterine life in diabetic mothers, fetus develops pancreatic β cell hyperplasia in response to hyperglycemia and increased demand of insulin during early gestation. Pancreatic β cells, which may secrete insulin autonomously and cause hypoglycemia at birth.

SHEEHAN'S SYNDROME

During postpartum period, woman may develop postpartum pituitary gland necrosis, which is characterized by hypopituitarism caused by obstetric blood loss, hypovolemic shock with resultant ischemia of anterior pituitary during delivery and over weeks and months after childbirth (Fig. 24.116).

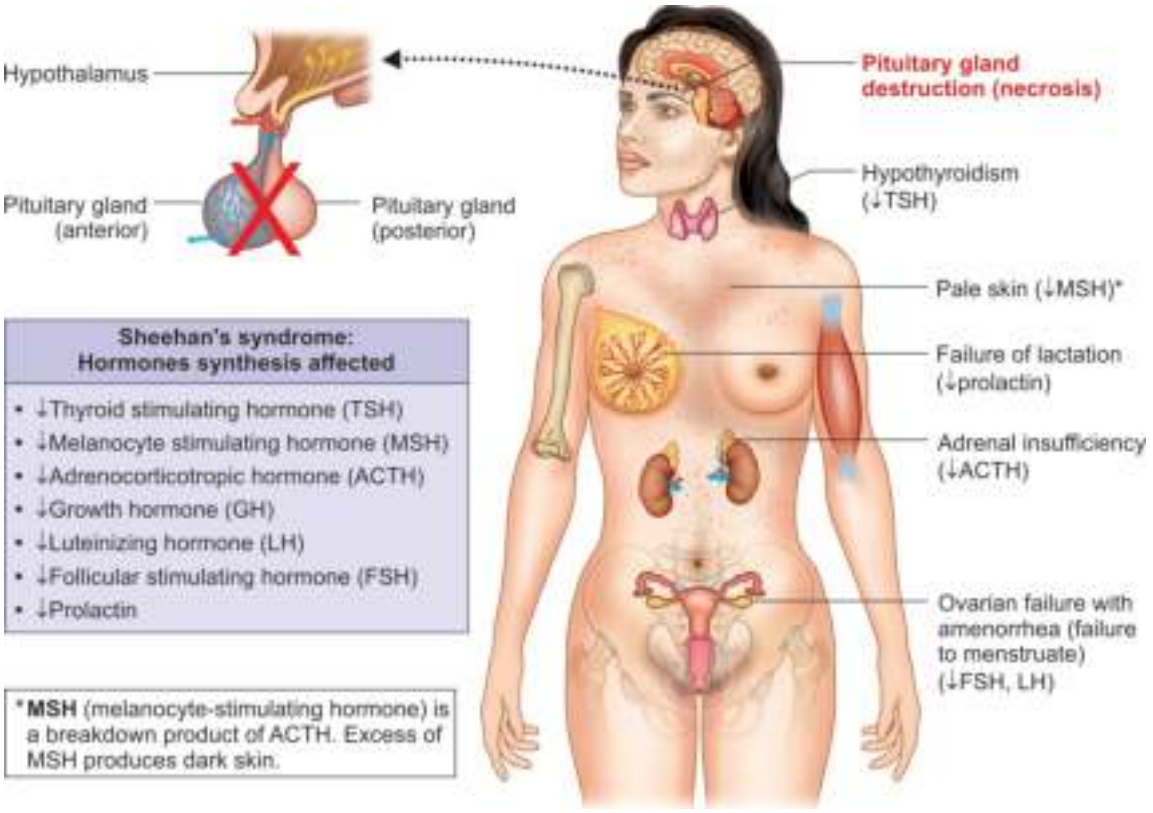


Fig. 24.116: Sheehan's syndrome. It is caused by hemorrhagic destruction of the pituitary in pregnancy.

PLACENTAL DISORDERS

PLACENTAL MALPERFUSION

Placental malperfusion refers to chronic perfusion ischemia resulting from maternal vascular diseases such as pre-eclampsia, eclampsia, chronic hypertension, diabetes mellitus, systemic lupus erythematosus, antiphospholipid syndrome, thrombophilia and cocaine drug. Histologic examination of placenta shows infarcts of usual type.

LONG OR SHORT UMBILICAL CORD

Length of the umbilical cord should be documented in pathology reports. The mean length of umbilical cord is 50–60 cm. A long umbilical cord more than 70 cm is prone to knots, torsion, prolapse, enlargement and compression, which my result in decreased blood flow and increased morbidity and mortality of the fetus. Conversely, a short umbilical cord is defined as measuring <32 cm, which is associated with restricted fetal movements.

VELAMENTOUS INSERTION AND VASA PREVIA

Normally, the umbilical cord insertion is located centrally or near the center of the placental disc. Marginal insertion refers to an umbilical cord that inserts within <2 cm from the disc margin. A velamentous umbilical cord inserts into the fetal membranes, so that the blood vessels travelling through the membranes are susceptible to compression, thrombosis, and rupture.

- Velamentous umbilical cord insertion can cause vasa previa, in which the umbilical cord blood vessels within the membrane present before the fetus during delivery and may undergo rupture resulting in catastrophic fetal hemorrhage.
- It is important to measure the sample of any membranous blood vessels to check for thrombosis, inflammation or necrosis.

OLIGOHYDRAMNIOS

Oligohydramnios is defined as an abnormally reduced volume of amniotic fluid. The reduced available space results in fetal deformities as well as compression of the umbilical cord.

CHORIOAMNIONITIS

Chorioamnionitis results from infectious organisms travelling through the birth canal (vagina or cervix) to the amniotic cavity, which often follows premature rupture of membranes. It can have devastating consequences for both the mother and the fetus. Maternal

neutrophils migrate from the decidua to the chorion and later into the amniotic membrane. Fetal neutrophils migrate from chorionic blood vessels to amnion and from the umbilical cord and blood vessels as well (funisitis).

- Staging and grading of chorioamnionitis is based on assessment of both maternal and fetal responses.
- In full term pregnancy, chorioamnionitis usually follows rupture of the membranes. In preterm pregnancies, chorioamnionitis predisposes to premature rupture of the membranes and various fetal complications. Group B streptococci are the most frequent etiological organisms of severe fetal/neonatal sepsis.
- Clinical diagnostic criteria for chorioamnionitis include maternal fever during labor, leukocytosis, uterine tenderness, and maternal or fetal tachycardia (Fig. 24.117).

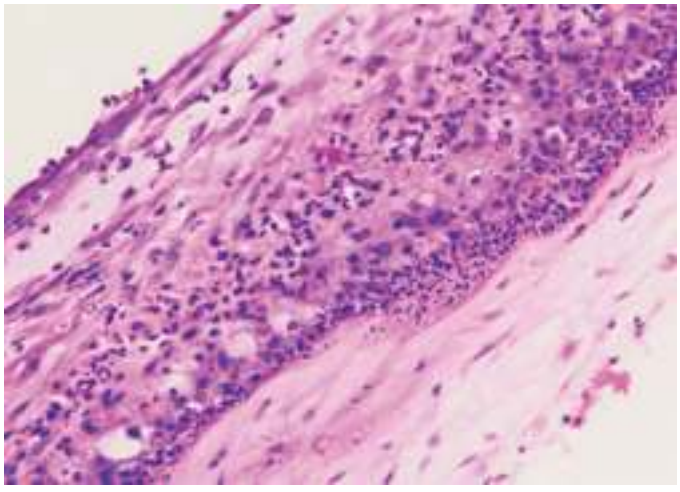


Fig. 24.117: Chorioamnionitis (400X).

ABNORMALITIES OF PLACENTAL ATTACHMENT

Abnormal adherence of the placenta to the uterine wall is categorized according to the depth of chorionic villous invasion into the myometrium.

ABRUPTIO PLACENTAE

Abruptio placentae is premature separation of the placenta and an important cause of antepartum bleeding and fetal death. It is often associated with ectopic tubal pregnancy and disseminated intravascular coagulation.

PLACENTA ACCRETA

Placenta accreta is an abnormal adherence of the placenta to the underlying uterine wall without further invasion; the decidual layer is defective. It most often occurs in inflamed endometrium and old scars from prior cesarean sections or other surgery. Most patients have a normal pregnancy and delivery. Impaired placental separation leads to severe bleeding in the third trimester before delivery.

PLACENTA INCRETA

Placenta increta defines chorionic villi invading the underlying myometrium.

PLACENTA PERCRETA

Placenta percreta refers to penetration of placental villi into full thickness of the uterine wall.

PLACENTA PREVIA

Placenta previa is an attachment of the placenta to the lower uterine segment, partially or completely covering the cervical os. This may coexist with placenta accreta. It is often manifested by bleeding.

CHAPTER

25

Mammary Glands

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

FEMALE BREASTS

- Female breasts: overview
  - Embryology of female breasts
  - Anatomy and physiology of female breasts
- Breast lumps and their anatomical correlation
  - Clinical presentation of breast lumps
  - Clinical examination of breast lumps

INFLAMMATORY DISORDERS OF BREAST

- Inflammatory breast diseases
  - Acute mastitis
  - Periductal mastitis
  - Mammary duct ectasia
  - Fat necrosis of breast
  - Granulomatous mastitis
  - Silicone breast implants
  - Sclerosing lymphocytic lobulitis
  - Diabetic mastopathy

FIBROCYSTIC DISEASE OF BREAST

- Fibrocystic disease of breast: overview
- Fibrocystic disease of breast: histopathological changes
  - Non-proliferative changes in breasts
  - Proliferative changes in breasts
- Fibrocystic disease of breast: diagnosis

REVISED 2019 WHO CLASSIFICATION OF BREAST TUMORS

- Stromal tumors of breast
  - Fibroadenoma of breast
  - Giant juvenile fibroadenoma of breast
  - Phyllodes tumor of breast
  - Juvenile papillomatosis of breast
  - Pseudoangiomatous stromal hyperplasia
- Benign epithelial tumors of breast
  - Intraductal papilloma of breast
  - Tubular adenoma of breast
  - Lactating adenoma of breast
  - Complex fibroadenoma of breast
  - Adenoma of the nipple
- Malignant epithelial tumors of breast
  - Breast carcinoma: overview
  - Breast carcinoma: risk factors
  - Molecular genetic alterations
- Major molecular pathways: evolution of breast carcinoma
  - Estrogen positive molecular pathway (luminal A and luminal B)
  - HER2/neu enriched molecular pathway
  - ER, PR and HER2/neu triple negative basal-like molecular pathway
  - Normal breast-like molecular pathway
- Breast carcinoma: histologic variants
  - Ductal carcinoma: basic concept
  - Lobular carcinoma: basic concept
  - Invasive ductal carcinoma (NOS) of breast
  - Paget's disease of breast
  - Inflammatory carcinoma of breast
  - Medullary carcinoma of breast
  - Colloid (mucinous) carcinoma of breast
  - Tubular carcinoma of breast
  - Papillary carcinoma of breast
  - Invasive micropapillary carcinoma of breast
  - Metaplastic carcinoma of breast
  - Apocrine carcinoma of breast
  - Secretory carcinoma of breast
  - Cribriform carcinoma of breast
  - Cutaneous angiosarcoma of breast
- Breast carcinoma: mode of spread, prognostic and predictive factors
  - Breast carcinoma: routes of metastasis
  - Breast carcinoma: prognostic and predictive factors
- Breast lumps: laboratory diagnosis
  - Clinical history and examination
  - Imaging diagnostic techniques
  - Triple assessment of breast lumps
  - Serum tumor markers
  - Fine needle aspiration cytology
  - Breast lump biopsy
  - Frozen/cryostat section technique
  - Histologic examination
  - Immunohistochemistry technique
  - Recent advances: molecular genetic techniques in breast cancer
    - Oncotype DX assay
    - MammaPrint gene expression profile test
    - Prosigna Kit test (PAM50) risk of recurrence score
    - Breast carcinoma index (BCI) test
    - EndoPredict breast cancer prognostic test
    - Next-generation sequencing in breast cancer
    - Microarray studies in breast cancer
    - cDNA microarray in breast cancer
    - Oligonucleotide microarray in breast cancer
- Breast carcinoma: Management
  - Breast lumpectomy
  - Modified radical mastectomy
  - Radiation therapy
  - Chemotherapy
  - Hormonal therapy
  - Aromatase inhibitors therapy
  - Biological therapy
- Miscellaneous disorders of female breasts
  - Congenital hypoplasia of breast
  - Juvenile hypertrophy of breast
  - Accessory nipple

MALE BREASTS

- Non-neoplastic disorders of male breasts
  - Gynecomastia
- Neoplastic disorders of male breasts
  - Invasive ductal carcinoma

FEMALE BREASTS

FEMALE BREASTS: OVERVIEW

EMBRYOLOGY OF FEMALE BREASTS

Each breast is a group of large glands derived from epidermis, which lies in a network of fascia derived from the dermis and the superficial fascia at the ventral surface of the thorax.

- Breast development begins in the 6th week of fetus development.
- Mammary gland originates from milk streaks, bilateral ectodermal thickenings that extend from the axilla to the groin.
- The ectoderm invaginates into the surrounding mesenchyme.

ANATOMY AND PHYSIOLOGY OF FEMALE BREASTS

The female breasts are modified sweat glands, which develop from the milk line. At birth there is no difference between male and female breasts. Pubertal growth of breasts occurs due to glandular and fibrofatty proliferation. The amount of fibroglandular tissue decreases with age. Multiple hormonal stimulation significantly increases the volume of the breast tissue during pregnancy (Fig. 25.1).

Structure of Female Breasts

Each female breast is composed of 15–20 lobes interspersed with adipose tissue and connective tissue arranged in radial fashion extending from the nipple. The nipple is situated in the center of a darker area of skin called the areola. The areola contains small glands, called Montgomery glands, which lubricate the nipple during breastfeeding. There are no muscles in the breasts, but the pectoral muscles lie under each breast and cover the ribs. Structure of female breast is shown in Fig. 25.2.

Terminal Duct Lobular Unit

The lobules of each female breast consist of acini or glands lined by an outer myoepithelial cells and inner secretory cells, also known as luminal cells. Each lobe of the breast has one unique functional terminal duct lobular unit (TDLU), which drains via a branching duct system, i.e. intralobular ducts, interlobular ducts and lactiferous sinuses outside through the nipple. Duct system carries milk to the nipples. Each duct acinus has a lining epithelium surrounded by a thin myoepithelial cell layer responsive to oxytocin, the hormone that stimulates lactation.

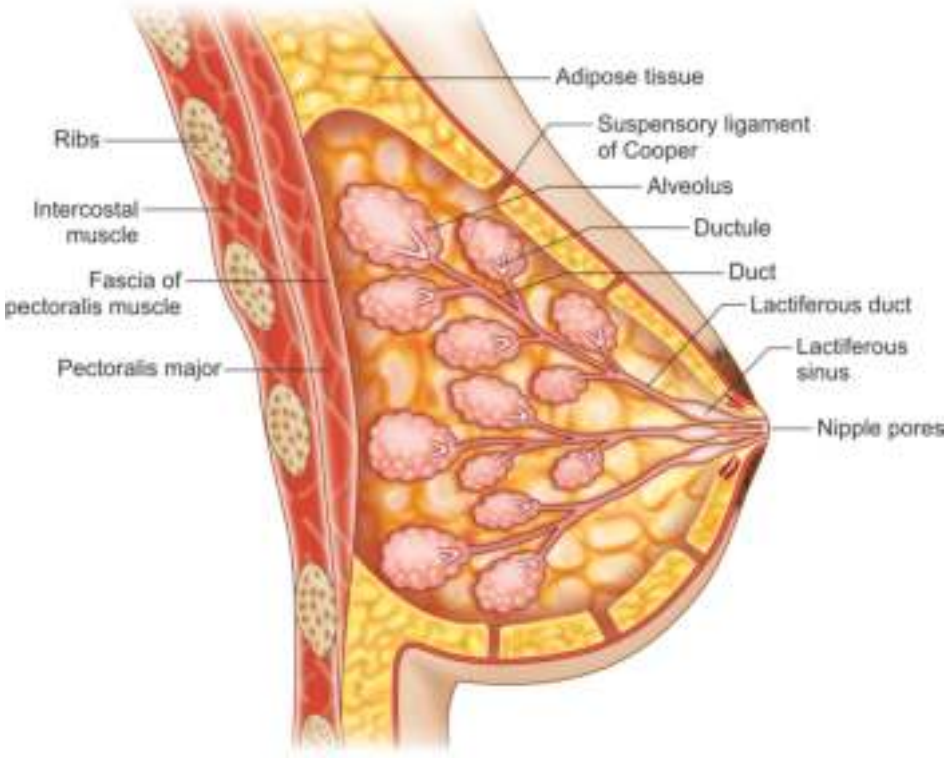


Fig. 25.1: Structure of the adult female breast showing major components and their functions.

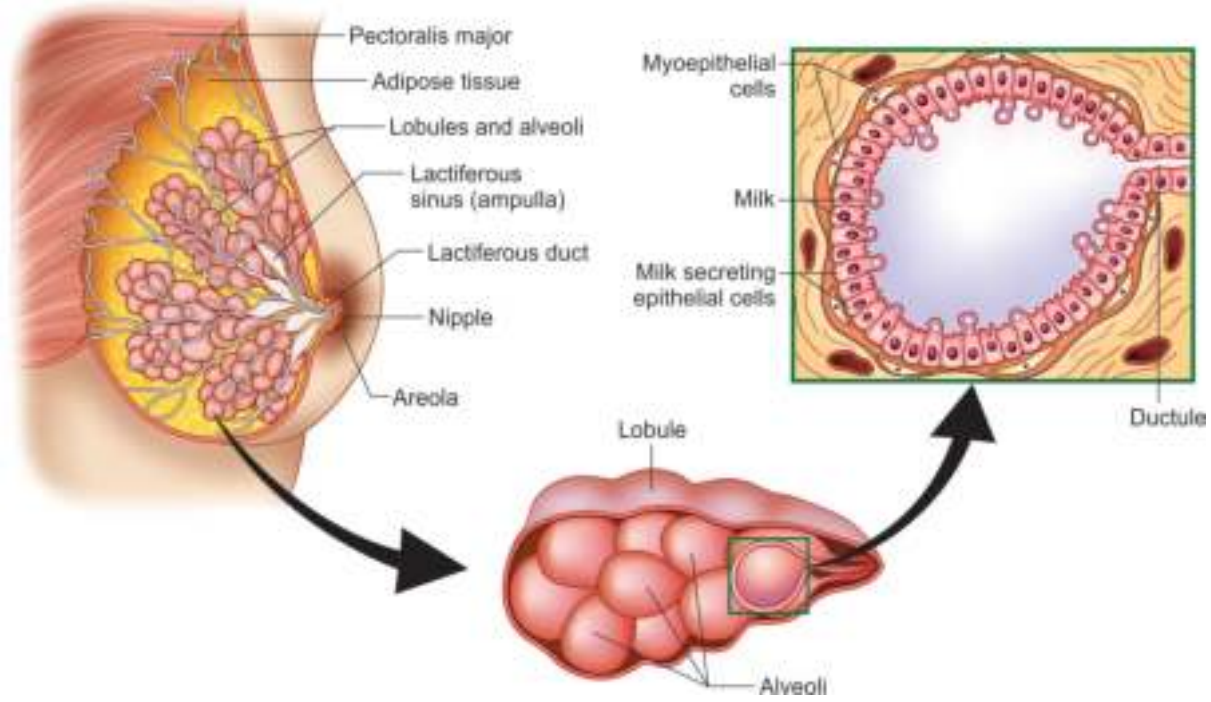


Fig. 25.2: Structure of female breast. Each lobe of the breast has one unique terminal duct lobular unit (functional unit), which drains via a branching duct system, i.e. intralobular ducts, interlobular ducts and lactiferous sinuses outside through the nipple. Duct system carries milk to the nipples. Each duct has a lining epithelium surrounded by a thin myoepithelial cell layer responsive to oxytocin, the hormone that stimulates lactation.

- During 30 weeks of pregnancy, each breast shows controlled proliferation of lobular acini lined by cells containing secretory vacuoles and secretions in their lumina. Breast milk comprises casein, α-lactalbumin and milk fat globule derived from the luminal surface of ductal cells.
- The acinus is lined by epithelial cells surrounded by myoepithelial cells and the basement membrane. Myoepithelial cells may function as a guardian of breast tissue integrity by maintaining its intergrity. Myoepithelial cells suppress stromal invasion by breast ductal/lobular carcinoma tumor cells by the synthesis of various anti-angiogenic and anti-invasive factors.
- Benign breast diseases and carcinomas arise in the terminal duct lobular unit (TDLU).
- Myoepithelial cells are smooth muscle cell-like cells present in the glandular epithelium as a thin layer above the basement membrane and beneath the luminal cells of breast. Myoepithelial cell layer is intact in normal breast and ductal carcinoma *in situ* (DCIS); but absent in invasive ductal carcinoma. Microglandular adenosis lacks myoepithelial cells. Panel of immunohistochemical markers is used on paraffin-embedded tissue section of breast lesions to demonstrate myoepithelial cells in breast.
  - Myoepithelial cells contain microfilaments and smooth muscle-specific cytoskeletal proteins such as α-actin and myosin that are responsible for the contractile phenotype mediated by oxytocin during lactation. Smooth muscle actin (α-actin), calponin, smooth muscle myosin heavy chain (SMMHC) and p63 are usual immunomarkers for myoepithelial cells.
  - Other immunomarkers are CK14 (HMWCK), S-100 protein, glial fibrillary acidic protein (GFAP) and CD10. Panel of immunohistochemical markers is used to demonstrate myoepithelial cells is given in Table 25.1.

Table 25.1 Panel of immunohistochemical markers is used to demonstrate myoepithelial cells

Markers	Expression
Smooth muscle actin (α-actin)	Positive
Calponin	Positive
Smooth muscle myosin heavy chain (SMMHC)	Positive
p53	Positive
CK14 (HMWCK)	Positive
S-100 protein	Positive
Glial fibrillary acidic protein (GFAP)	Positive
CD10	Positive

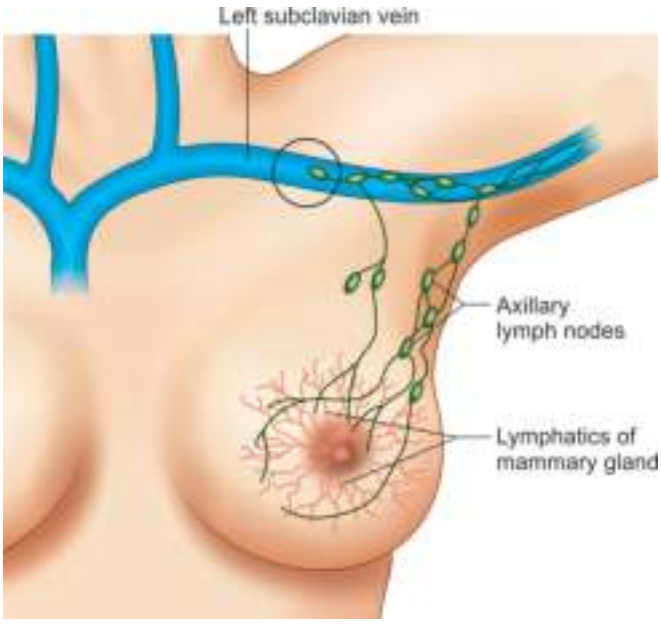


Fig. 25.3: Indicate a chain of the lymph nodes near the axilla and breast. Another point of contact between the two circulations (lymphatic and venous circulation).

Blood Supply of Female Breasts

The vascular supply of the breast is from internal mammary and lateral thoracic artery. There is communication between lymphatic and venous drainage in subclavicular region (Fig. 25.3).

Lymphatic Drainage of Female Breasts

The breast has extensive lymphatic drainage and lymph node groups: axillary nodes and internal thoracic lymph nodes. Approximately 75% of the lymphatic drainage occurs to the axillary lymph nodes. So, there is greater frequency of breast carcinoma metastases to axillary lymph nodes. Approximately 25% of lymphatic drainage from inner quadrants of breast is drained to internal mammary (parasternal) lymph nodes and skin lymphatic channels. To a lesser extent, lymph is also drained to lymph nodes adjacent to the vertebra.

There are five groups of breast lymph nodes:

1. **Central axillary lymph nodes:** These lymph nodes are located higher up in the middle of the axilla, over the ribs and serratus anterior muscle. These lymph nodes receive lymph from the other three groups of pectoral, subscapular and lateral lymph nodes.
2. **Pectoral (anterior) lymph nodes:** These lymph nodes are located along the lateral edge of the pectoralis major muscle, just inside the anterior axillary fold.
3. **Subscapular (posterior) lymph nodes:** These lymph nodes are situated along the lateral edge of the scapula, deep in the posterior axillary fold.
4. **Lateral lymph nodes:** These lymph nodes are situated along the humerus inside upper arm.
5. **Apical lymph nodes:** These lymph nodes are situated in the apical region of axilla (Fig. 25.4).

Role of Hormones in Breasts Development

The female breast development depends on a variety of hormones for its normal activity, which exhibits structural and functional variation throughout life, especially during puberty, pregnancy, lactation, normal menstrual cycle, and postmenopausal state.

- **Hormones and breast development:** Breast development and function depend on the ovarian hormones estrogen and progesterone. Hormones reaching breast via blood stream either interact with membrane receptors (prolactin) or nuclear receptors (estrogen). The hormone receptor interaction activates DNA synthesis of transcription factors responsible for proliferation and differentiation of terminal duct lobular unit. Estrogen hormone elongates the ducts and causes them to create side branches. Progesterone hormone increases the number and size of the lobules in order to prepare the breasts for nourishing a baby. Growth hormone, insulin and glucocorticoids also participate in proliferation of breast lobules.
- **Breast changes during pregnancy:** After ovulation, progesterone hormone makes the breasts to grow and blood vessels become filled with blood. At this time, the breasts often become engorged with fluid. Breasts may become tender and swollen. The female breasts during pregnancy undergo lobular hypertrophy; so that lactation can occur due to action of prolactin. Breast histology from a pregnant woman (30 weeks of gestation) shows the lobular acini lined by cells containing secretory vacuoles and filled with pink secretions in their lumens.

BREAST LUMPS AND THEIR ANATOMICAL CORRELATION

Frequency of Breast Lumps

A breast mass in a woman is likely to occur in decreasing frequency due to fibrocystic disease (40%), miscellaneous benign lesions (13%), carcinomas (10%) or fibroadenomas (7%) and no disease (30%). The presence of myoepithelial cells in breast typically indicates a benign disease and useful in diagnosis. The p63 is a reliable marker for myoepithelial cells. Other myoepithelial cell markers include S-100, GFAP (glial fibrillary acidic protein) and α-smooth muscle actin. Structure of the adult female breast shows major components and location of various lesions (Fig. 25.5).

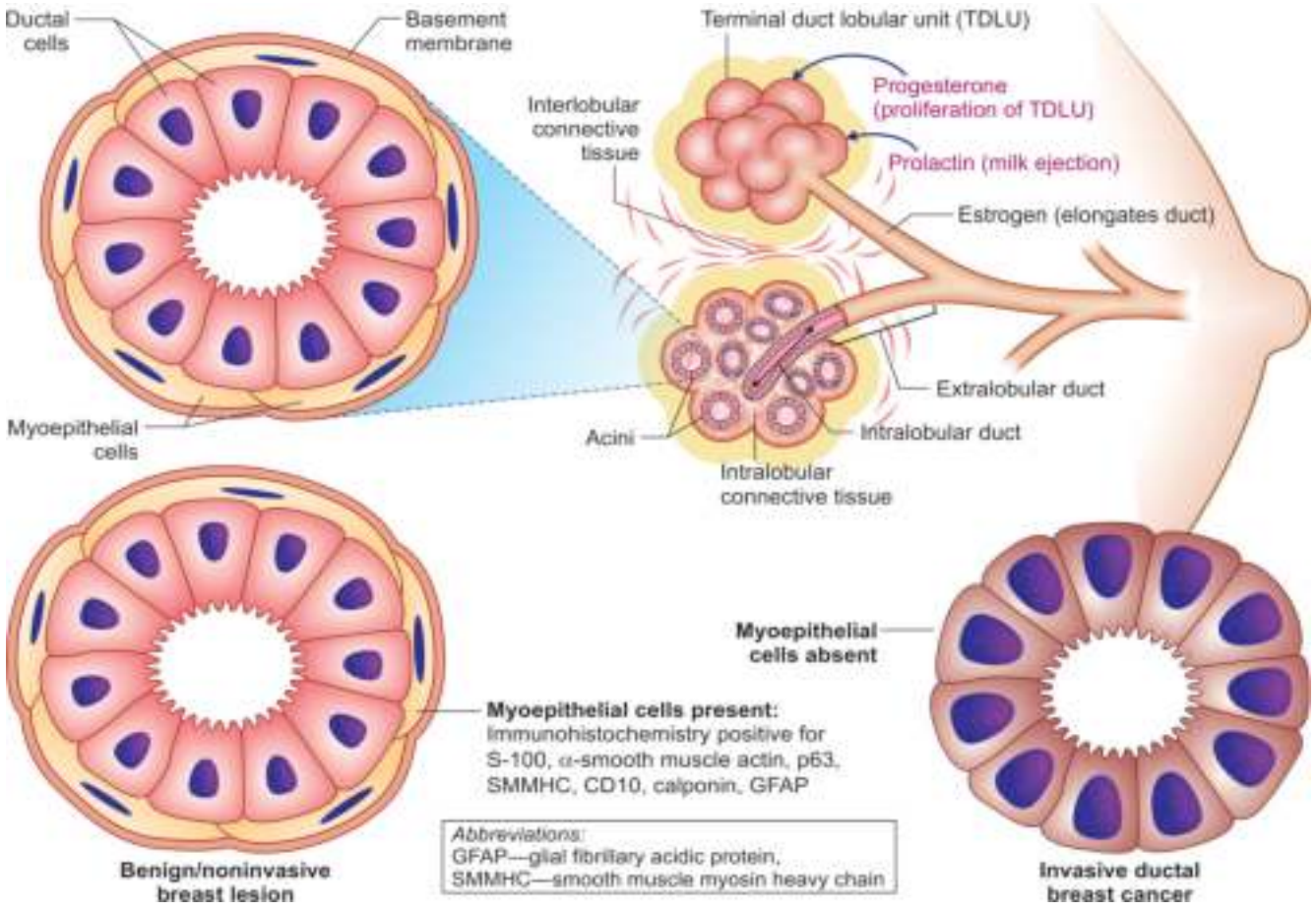


Fig. 25.4: A breast lobule shows the different components. The acinus is lined by epithelial cells surrounded by myoepithelial cells and the basement membrane.

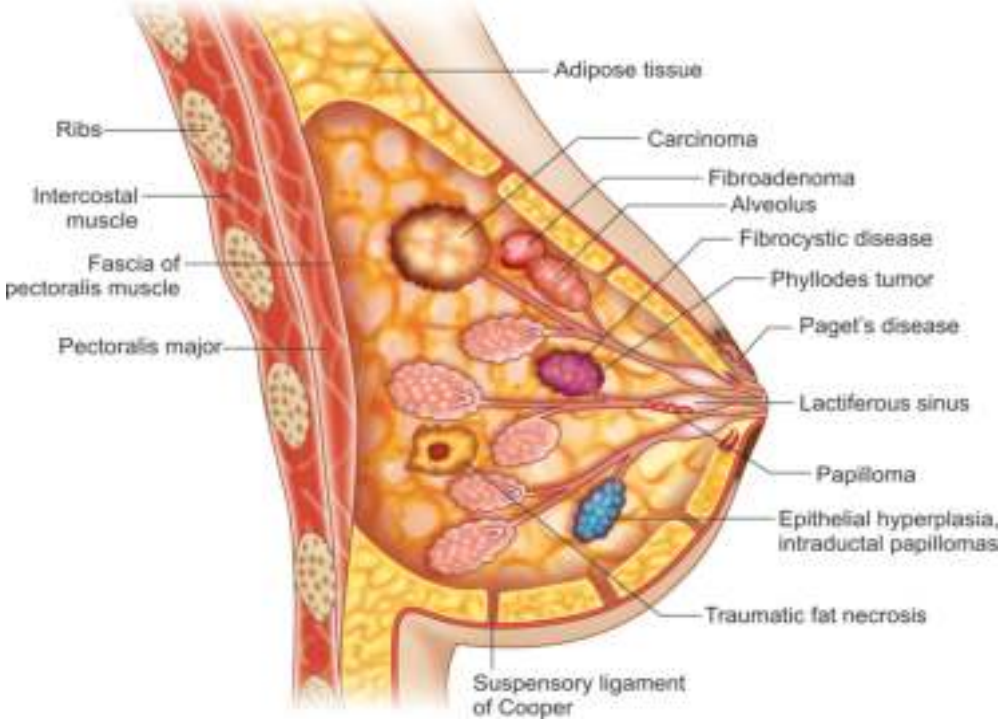


Fig. 25.5: Structure of the adult female breast shows major components and location of various lesions.

Nipple

The nipple may be site for Paget's disease of breast (ductal carcinoma involving nipple skin), nipple adenoma and breast abscess.

Lactiferous Ducts and Sinuses

The lactiferous ducts are the most common site for intraductal papilloma, galactocele (blocked lactiferous duct in a lactating woman), breast abscess, or plasma cell mastitis.

Large Ducts of Female Breasts

Large ducts are the most common site for **fibrocystic disease** and most ductal carcinoma of breast.

Terminal Duct Lobular Unit

The terminal lobules are involved in sclerosing adenosis (variant of fibrocystic change), lobular carcinoma and tubular carcinoma of breast.

Interlobular or Intralobular Stroma of the Breast

Breast stroma can be divided into interlobular and intralobular stroma. Interlobular stroma surrounds large ducts and terminal duct lobular units (TDLUs), and is more collagenous and dense than intralobular connective tissue.

- Interlobular stroma becomes more fatty after puberty; and its increase in volume of this compartment is responsible for majority of breast tissue. **Fibroadenoma** and **phyllodes tumor** are derived from intralobular stroma.
- The breast tumor stroma mainly consists of the basement membrane, fibroblasts, extracellular matrix, immune cells and blood vessels. Although, most host cells in the stroma possesses certain tumor-suppressing abilities. The stroma will change during breast carcinoma and eventually promote growth, invasion and metastasis.

CLINICAL PRESENTATION OF BREAST LUMPS

Women with breast disease most often complains of pain, a palpable lump without a discrete lump and nipple discharge. It is most important to evaluate these women because of the possibility of breast carcinoma.

- Patient with breast carcinoma may present with breast lump, change in the symmetry of the breast, and nipple itching, burning, erosion or retraction, pathological bone fractures and increased serum calcium levels.
- A spontaneous nipple discharge of any kind in a non-breastfeeding and non-lactating women warrants investigation for breast disease (Table 25.2).

Painful Breasts

Tightness, tenderness or sharp pain in one or both breasts can occur not due to underlying disease. Examples include a tight-fitting bra, pregnancy, menstruation, breastfeeding or exercise.

- **Premenopausal women:** These women present with cyclic pain in bilateral breasts, increasing in severity from mid-cycle onwards, and pain improving at menstruation. Women often report fullness,

Table 25.2 Pathological basis of breast signs and symptoms in women

Clinical Presentation	Pathological Correlation	
Breast lump frequency	▪ Fibrocystic disease (40%) ▪ No disease (30%) ▪ Miscellaneous benign lesions (13%)	▪ Breast carcinomas (10%) ▪ Fibroadenomas (7%)
Onset of lump	▪ Fat necrosis-associated with trauma ▪ Fibrocystic disease-associated with menstrual cycle	▪ Duration and rate of growth of lump (fibroadenoma shows slow growth no apparent change in size in 6+ months and rapid growth in breast carcinoma)
Pain and tenderness in breasts	▪ Mastitis (acute or chronic) ▪ Mammary duct ectasia ▪ Breast abscess	▪ Galactocele ▪ Fibrocystic disease (cyclic pain)
Nipple discharge	▪ Milky nipple discharge (galactorrhea) occurs due to increased prolactin level during pregnancy, pituitary adenoma, oral contraceptive therapy, tricyclic antidepressant, methyldopa	▪ Serous/bloody (intraductal papilloma/cancer) ▪ Nipple with rash, eczema-like or blood stained discharge in Paget's disease of nipple (ductal carcinoma involving overlying skin)
Manual examination of breasts	▪ Diffuse: Fibrocystic disease ▪ Discrete: Neoplasm or cyst ▪ Mobile lump: Fibroadenoma	▪ Bulky tumor: Phyllodes tumor and giant fibroadenoma ▪ Immobile lump: Invasive breast carcinoma

Contd...

Table 25.2 Pathological basis of breast signs and symptoms in women (*Contd...*)

Clinical Presentation	Pathological Correlation	
Nipple retraction	▪ Breast carcinomas ▪ Fat necrosis of breast	▪ Inflammatory breast lesions undergoing fibrosis
Skin features	▪ Peau d' orange: Lymphatic blockage by carcinoma cells and puckering ▪ Tethering: Due to invasion of Cooper's ligament in breast cancer	▪ Erythema: Acute mastitis and Paget's disease of nipple
Microcalcifications on mammography	▪ Dystrophic calcification associated with fibrocystic changes such as cysts and adenosis	▪ Carcinoma *in situ* or invasive carcinoma
Metastases	▪ Lymph nodes involvement in axillary and supraclavicular regions ▪ Visceral metastases	▪ Bone pain and pathological fractures occur due to metastases

heaviness, areas of tenderness and increased breast size during 3–7 days before each menstruation.

- **Postmenopausal women:** These women present with continuous localized breast pain not related to menstrual cyclic changes. Approximately 90% of painful breast diseases are benign and 10% of breast carcinomas present with pain.

Breast Lumps

The most common palpable breast lumps are fibrocystic disease, fibroadenoma and invasive breast carcinoma. Premenopausal women most often develop benign palpable masses in 90% and breast carcinoma in 10%. Risk for breast carcinoma increases with advancing age. In clinical practice, Indian women with breast carcinomas report late with evidence of metastases.

Nipple Discharge

Nipple discharge is a part of breast function during pregnancy or breastfeeding. It may also be associated with abnormal response of hormones during menstrual cycle and fibrocystic disease of breast.

- **Milky nipple discharge:** It occurs due to increased prolactin level in pregnant women, oral contraceptive therapy, pituitary adenoma, tricyclic antidepressant and methyldopa.
- **Serous/bloody discharge:** It occurs due to intraductal papilloma or breast carcinoma.
- **Eczema-like lesion with blood stained discharge:** A rash, often eczema-like lesion on the nipple or surrounding area and blood stained discharge from nipple is observed in Paget's disease of breast (ductal carcinoma involving overlying skin).

Nipple Retraction

Indrawing (retraction) of the nipple due to desmoplasia in an underlying advanced scirrhous breast carcinoma is a late feature. Nipple retraction also occurs due to fibrosis in chronic inflammation of the breast.

Overlying Skin Edema of Female Breast

The "**peau d'orange**" (skin edema) appearance of the breast skin occurs due to obstruction of the dermal lymphatics by tumor cells in a case of breast carcinoma.

CLINICAL EXAMINATION OF BREAST LUMPS

Clinician should note these characteristics in women with breast diseases:

- **Location:** Quadrant involved due to breast lump
- **Size:** Dimensions of breast lump
- **Shape:** Lump oval, lobulated or indistinct breast lump
- **Consistency:** Soft, firm or hard breast lump
- **Movable:** Freely movable or fixed breast lump
- **Distinction:** Solitary or multiple breast lumps
- **Nipple:** Displaced or retracted or nipple discharge
- **Skin over the lump:** Erythematous, dimpled or retracted
- **Tenderness:** Presence or absence of tenderness on palpation of breasts
- **Lymphadenopathy:** Lymph nodes palpable or not
- **Distant metastases:** Organs involved, i.e. lungs, liver, bones or brain.

INFLAMMATORY DISORDERS OF BREAST

INFLAMMATORY BREAST DISEASES

Inflammatory breast diseases include acute mastitis, periductal mastitis, mammary duct ectasia, fat necrosis and granulomatous mastitis. Lactational mastitis is common and requires antibiotic therapy. Aspiration is the preferred initial management of lactational breast abscess. Periductal mastitis may be associated with tobacco smoking and requires surgery. Tuberculous mastitis responds to antitubercular treatment.

Pathology Pearls: Inflammatory Disorders of Breast

- Inflammatory diseases of the breast are rare.
- Acute infection occurs only in the lactating breast.
- Periductal mastitis is also known as Zuska's disease (squamous metaplasia of lactiferous ducts).
- Fat necrosis occurs due to trauma to breast especially in obese women.
- Duct ectasia shows many thick-walled dilated ducts filled with yellow-brown cheesy secretions.
- Granulomatous mastitis occurs in systemic granulomatous disease, e.g. tuberculosis, sarcoidosis, Wegener's granulomatosis, fungal infection, breast implants or unknown etiology.
- There is involvement of lobular epithelium of breast.
- Lipogranulomas are caused by rupture of a paraffin-filled polythene sac implanted silicone prosthesis previously as a device for breast augmentation.

ACUTE MASTITIS

Milk stasis is the main predisposing factor for acute mastitis. If disorder is not treated appropriately, breast abscesses can recur, which may be complicated by a fistulous tract. Acute mastitis is caused by *Staphylococcus aureus* that can enter the breast tissue through cracks and fissures in the nipple. Acute mastitis disorder is usually secondary to obstruction of the duct system by inspissated secretions.

Age Group

In acute mastitis, infection of overlying breast skin commonly affects women aged between 18 and 45 years, which may be primary or secondary due to infected sebaceous cyst in overlying skin. Females with pituitary prolactinomas occasionally are associated with galactorrhea.

Clinical Features

In acute mastitis, the breast becomes tense, hot, and very painful. Axillary lymph nodes may become enlarged and tender.

Therapeutic Correlation

Acute mastitis may be treated by mechanical suction, frequent emptying of the breasts, and administration of antibiotics.

PERIDUCTAL MASTITIS

Periductal mastitis is also known as Zuska's disease or squamous metaplasia of lactiferous ducts and characterized by different degrees of periductal inflammation, and periductal fibrosis.

- Periductal mastitis occurs especially in tobacco smokers. Tobacco smoke alters the epithelium of lactiferous sinuses. Keratin is trapped into ducts.
- Nipple inversion occurs due to fibrosis. Recurrences in women with recurrent periductal mastitis are common.

Surgical Pathology: Periductal Mastitis

Light Microscopy

- Histologic examination of biopsy reveals chronic and granulomatous inflammation.
- Foamy histiocytes are typically present within the luminal secretions and within wall of the involved ducts.
- Periductal fibrosis occurs in the late stage.

MAMMARY DUCT ECTASIA

Duct ectasia of major subareolar ducts is characterized by inflammation and dilatation of the major ducts. The ducts are filled with debris, resulting in dilatation, rupture and inflammation. Possible etiological factors of duct ectasia are infections and cigarette smoking.

Clinical Features

Some women present with greenish brown cheesy nipple discharge, slit-like nipple retraction, or palpable lump simulating breast carcinoma that may be hard or doughy. There is no increased risk for breast carcinoma.

Complications

Complications of mammary duct ectasia include breast abscess, fistulous tract and nipple retraction. Associated fibrosis and calcification in mammary duct ectasia simulate breast carcinoma.

Surgical Pathology: Mammary Duct Ectasia

Gross Morphology

- Cut section of mammary duct ectasia lesion shows many thick-walled dilated ducts filled with yellow brown cheesy secretions.

- In women above 50 years of age, frequent incidental pathologic finding is fibrocystic disease in 30–40% of cases in surgically excised breast tissue and in autopsy specimens.

Light Microscopy

Histologic examination shows dilated ducts with fibrosis of wall, inflammatory cell infiltrate with plasma cells, and inspissation of lipid-rich material within duct lumen.

Management

Antibiotics and surgical removal of dilated duct cures these patients with periductal mastitis.

FAT NECROSIS OF BREAST

Fat necrosis of breast most often occurs in women >55 years. It is most common nonsuppurative chronic inflammatory lesion which follows foreign body giant cell reaction and fibrosis due to lipid released from traumatized adipocytes.

Pathogenesis

Trauma to the breast is most common cause of fat necrosis, followed by prior surgical intervention and radiation therapy. Fat necrosis of breast occurs when lipase enzyme breaks down intracellular triglycerides into free fatty acids, which combine with sodium, magnesium or calcium ions to form **soaps** (saponification). The tissue becomes opaque and chalky white due to fat necrosis.

Clinical Features

Patient develops unilateral localized breast mass, which may be painful in acute stage. Clinical examination of affected breast reveals a firm, superficial irregular mass, erythema of the overlying skin, dimpling and nipple retraction, which mimics breast carcinoma.

Radiologic Findings

Fat necrosis of the breast is a challenging diagnosis due to various appearances on mammography, ultrasonography, CT, PET-CT and MRI. Although mammography is more specific in diagnosing the disease. Mammography reveals calcified lesion in the breast.

Surgical Pathology: Fat Necrosis of Breast

Gross Morphology

- Fat necrotic tissue shows chalky white areas of fat saponification. Variegated color and areas of hemorrhage are demonstrated on the cut surface of this lump.
- It is gritty to cut because of the presence of spotty calcification.

Light Microscopy

- In the response to fat necrosis, there is an initial acute inflammatory reaction consisting of necrosis of adipocytes and hemorrhage. It is followed by chronic inflammatory response in which numerous plasma cells are seen.
- Macrophages phagocytose lipid released from adipocytes, forming multinucleated foreign body giant cells, as well as foam cells, also termed '**lipophages**'.
- There is presence of **dystrophic calcification** demonstrated by imaging techniques.
- Fibroblastic proliferation leads to fibrosis resulting in extension into the surrounding tissue. As a result, an irregular, fixed, hard mass may ensue and clinically fat necrosis of breast carcinoma. Thus, the fat necrosis of breast often requires biopsy to establish its benign character.

GRANULOMATOUS MASTITIS

Granulomatous mastitis is a group of immunologic mediated disorder of breast lobules, which leads to alteration in the breast lobular epithelium during lactation in multiparous women.

Etiology

Granulomatous mastitis occurs in systemic granulomatous disease, e.g. tuberculosis, sarcoidosis, Wegener's granulomatosis, fungal infection, breast implants and unknown etiology. There is involvement of breast lobular epithelium.

Surgical Pathology: Granulomatous Mastitis

Light Microscopy

In granulomatous mastitis, breast lobules show granulomas, histiocytes, lymphocytes, plasma cells and giant cells by sparing interlobular stromal region.

- **Breast tuberculosis:** Breast tuberculosis usually occurs due to tubercular lesion extension from rib in females. Patient presents with breast abscess and fever. On cut section, breast abscess contains caseous material. Light microscopy reveals epithelioid cell granulomas, caseous necrosis and Langhans type of giant cells.
- **Sarcoidosis:** Breast sarcoidosis is an idiopathic disorder in which abnormal immune system leads to formation of noncaseating epitheliod cell granulomas and collection of macrophages. These trigger an inflammatory response that causes extensive tissue damage and scarring in the breasts. **Kveim test** is performed by intracutaneous injection of saline suspension of human sarcoidal spleen or lymph nodes, which induces appearance of erythematous nodules.
- **Wegener's granulomatosis:** It is a systemic necrotizing granulomatous vasculitis of unknown etiology or due to inhalation of some infectious agents. Antineutrophil cytoplasmic antibodies (c-ANCA type) play a pivotal role in its pathogenesis. Immune complexes formed are deposited in small vessels resulting in necrotizing vasculitis.

SILICONE BREAST IMPLANTS

Silicone breast implants are used for cosmetic augmentation. Silicone is a polymer of silica, O_2 and

hydrogen. Due to leakage of silicone implants, chronic inflammation takes place. Lipogranulomas are caused by rupture of a paraffin-filled polythene sac implanted prosthesis previously as a device for breast augmentation. This is a very old-fashioned type of breast implantation used for cosmetic augmentation.

SCLEROSING LYMPHOCYTIC LOBULITIS

Sclerosing lymphocytic lobulitis is also known as lymphocytic mastopathy, which most often occurs in women with type I diabetes mellitus or autoimmune thyroid diseases. It is considered to be an autoimmune disorder. Patient presents with hard palpable lumps. It is difficult to obtain breast tissue with needle biopsy due to presence of dense collagenous stroma. Sclerosing lymphocytic lobulitis should be differentiated from breast carcinoma.

DIABETIC MASTOPATHY

Diabetic mastopathy is uncommon complication of diabetes mellitus characterized by painless breast lump mistaken for breast carcinoma.

Surgical Pathology: Diabetic Mastopathy
Light Microscopy
Histologic examination shows keloid-like fibrosis, lymphocytic ductitis, lymphocytic lobulitis and lymphocytic vasculitis.

FIBROCYSTIC DISEASE OF BREAST

FIBROCYSTIC DISEASE OF BREAST: OVERVIEW

Fibrocystic disease is the most common benign disorder of the female breasts. It is caused by abnormal response of breast to ovarian hormones. Patient develops painful multifocal lumps in both breasts. The frequency of fibrocystic disease of breast decreases progressively after menopause.

Although it is a benign condition, the gross and mammographic appearance may mimic breast carcinoma, and is often difficult to distinguish from breast carcinoma on frozen section (Fig. 25.6).

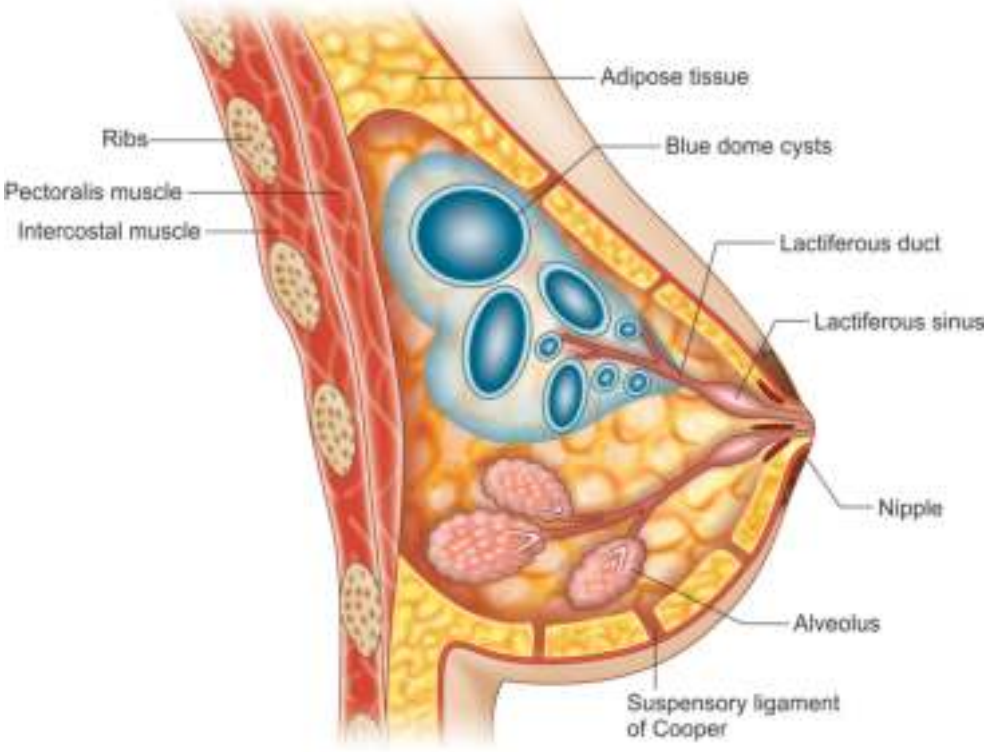


Fig. 25.6: Fibrocystic disease of breast. Structure of the adult female breast shows fibrocystic disease in 45-year-old woman showing one large cyst and multiple smaller ones, with areas of fibrosis.

Pathology Pearls: Fibrocystic Disease of Breast

- Fibrocystic disease of breast is caused by abnormal response of breast to ovarian hormones.
- Fibrocystic changes occur in glands and stroma, which include fibrosis, duct ectasia, apocrine metaplasia, duct hyperplasia and sclerosing adenosis.
- There is increased risk for development of breast carcinoma related to the presence of atypical hyperplasia of the glands.
- Sclerosing adenosis can be clinically and radiologically confused with breast carcinoma.

Fibrocystic disease of breast affects women in 20–50 years of age. About 10% of women have clinically evident disease. It is uncommon before adolescence or after menopause. Approximately 60–90% of breasts show fibrocystic disease at autopsy.

- **Etiopathogenesis:** It is postulated that fibrocystic disease of breast occurs due to hormonal imbalance, i.e. increased uncontrolled response of estrogens on terminal duct lobular unit (TDLU) or to decreased progesterone activity. This hormonal imbalance occurs in functional ovarian granulosa cell tumors and anovulatory cycles. Environmental toxins inhibiting cyclic guanosine monophosphate enzymes by methylxanthines (e.g. caffeine, tea, chocolate), tyramine (e.g. cheese, wine, nuts) and tobacco smoking may also cause fibrocystic disease of breast.
- **Clinical features:** Patient presents with bilateral breast palpable lumps (irregular nodularity) with mid-cyclic tenderness varying during the menstrual cycle. Pain is present in the upper outer quadrant of bilateral breasts. Occasionally, there is history of greenish brown to black nipple discharge containing fat, proteins, ductal cells and erythrocytes.

Surgical Pathology: Fibrocystic Disease of Breast

Gross Morphology

- Cut surface of lesion is firm grey white fibrous tissue.
- Some cysts may be quite large, which undergo hemorrhage into the cyst fluid called blue domed cysts.
- These cysts vary in size during the menstrual cycle.
- These are most often enlarged and tender one week before menstruation.

Light Microscopy

- Fibrocystic changes occur in glands and stroma, which include fibrosis, duct ectasia, apocrine metaplasia, duct hyperplasia and sclerosing adenosis.
- Histologic examination shows cystic dilatation of the terminal ducts and increased surrounding collagen fibers.
- Fibrocystic disease of breast changes may be nonproliferative or proliferative.

FIBROCYSTIC DISEASE OF BREAST: HISTOPATHOLOGIC CHANGES

NONPROLIFERATIVE CHANGES IN BREASTS

Nonproliferative changes in fibrocystic disease of breast include dense fibrous stroma encompassing a number of variable size cystic dilatation of the terminal duct lobular unit (duct ectasia) and mild hyperplasia. Alteration in epithelial lining of ducts is termed 'apocrine metaplasia'.

- Apocrine cells are large and more eosinophilic than that usually line the ducts and resemble apocrine sweat gland epithelium.
- On clinical examination, the breasts are lumpy. In fibrocystic disease of breast, there is no risk for breast carcinoma (Figs 25.7 to 25.11).

PROLIFERATIVE CHANGES IN BREASTS

Proliferative changes in fibrocystic disease of breast are associated with epithelial hyperplasia of ducts and lobules, with or without features of atypia, and sclerosing adenosis. Atypical hyperplasia of ducts and lobules is associated with a five-fold increase in the risk of breast ductal carcinoma. When proliferative changes in fibrocystic disease of breast are associated with a family history of breast carcinoma; the risk of breast carcinoma is ten-fold.

- **Inflammation and fibrosis:** Rupture of the cysts with extravasation of fluid in the stroma results in inflammation and fibrosis. Dense fibrous interlobular stroma expands into the lobules, and replaces the loose intralobular connective tissue. Strands of fibrous tissue constrict the ducts, so that the normal secretions cannot pass out. Terminal ducts become dilated and filled with secretions.
- **Duct ectasia:** Paste-like material in subareolar ducts produces sticky purulent discharge that may be white, gray, brown, green or bloody, which is caused by stagnation of cellular debris and secretions in the ducts. Cysts filled with bluish fluid are known as 'blue dome cysts' when examined through the cyst wall. Histologic examination shows cysts lined by uniform benign cuboidal to columnar epithelial cells of variable height with microcalcifications in their lumen. These blue dome cysts do not have malignant potential. Clinical examination of the breasts reveals cystic feel on palpation.
- **Apocrine metaplasia:** The cells lining large cysts undergo metaplastic change consisting of tall, pink, columnar benign epithelial cells with small nuclei and brightly eosinophilic cytoplasm. Chromosomal abnormalities in apocrine epithelium suggest possible precursor of apocrine carcinoma.

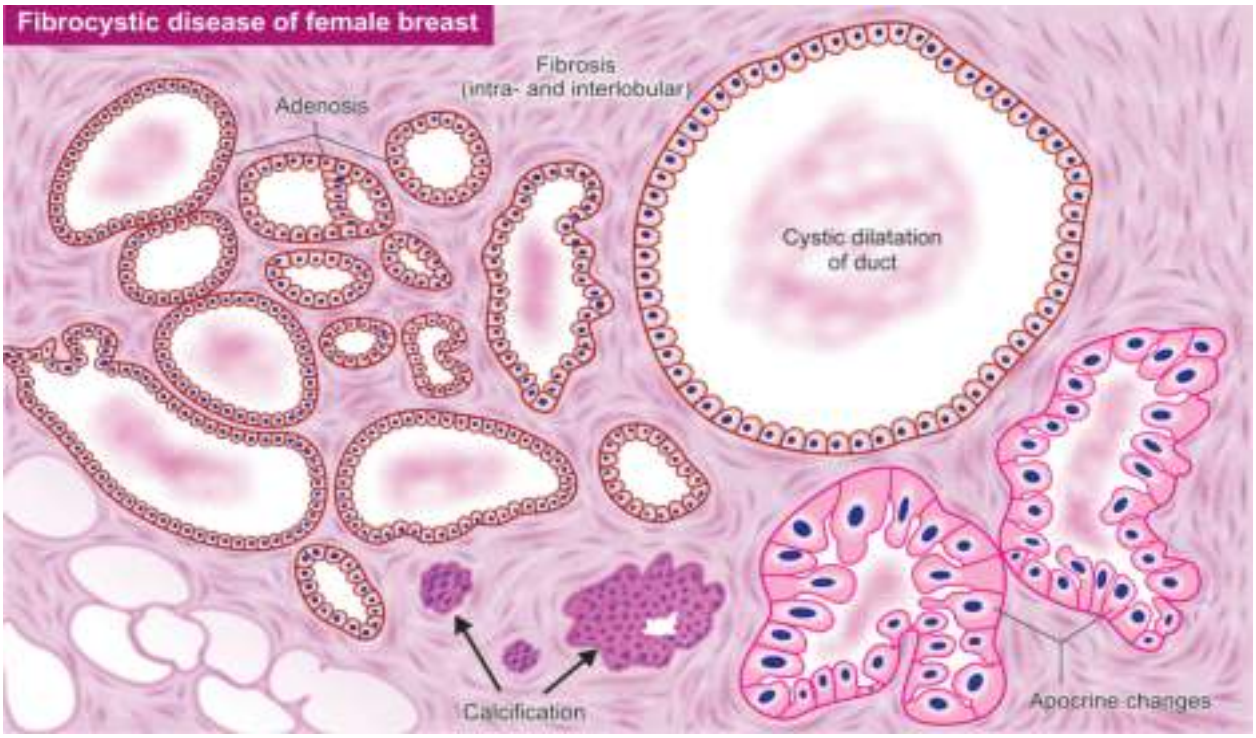


Fig. 25.7: Structure of the adult female breast shows fibrocystic disease (e.g. cystic dilatation of ducts, apocrine change, fibrosis and sclerosing adenosis). In sclerosing adenosis, there are glandular structures with intervening cords of cells in a fibrous stroma. Areas of calcification (arrows) are also present; these would render the lesion visible on mammography.

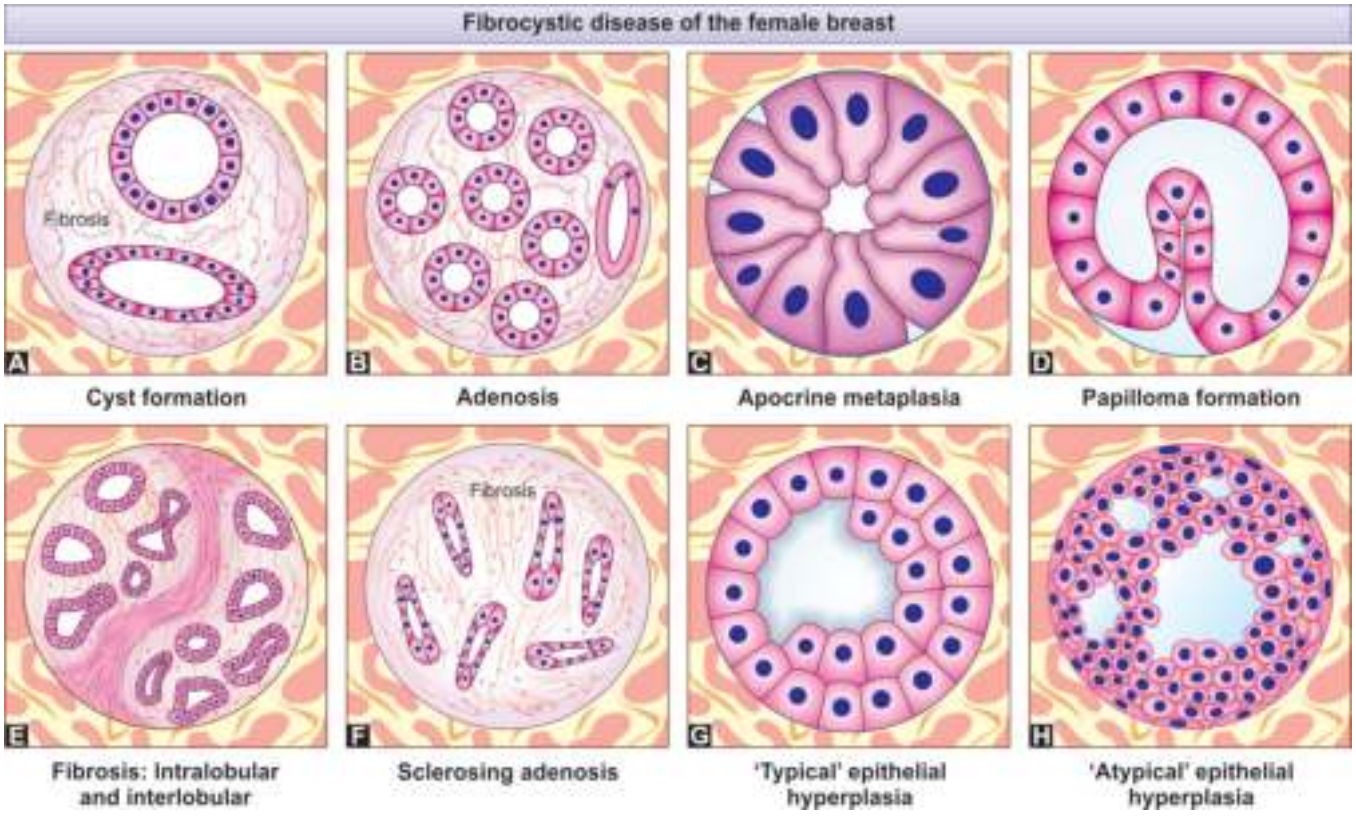


Fig. 25.8: Fibrocystic disease of breast. It shows (A) cyst formation, (B) adenosis, (C) apocrine metaplasia, (D) papilloma formation, (E) fibrosis (intralobular and interlobular), (F) sclerosing adenosis, (G) typical epithelial hyperplasia, and (H) atypical epithelial hyperplasia. In epithelial hyperplasia, the duct lumen is filled by hyperplastic epithelium. In atypical hyperplasia, there is increased risk of development of breast cancer.

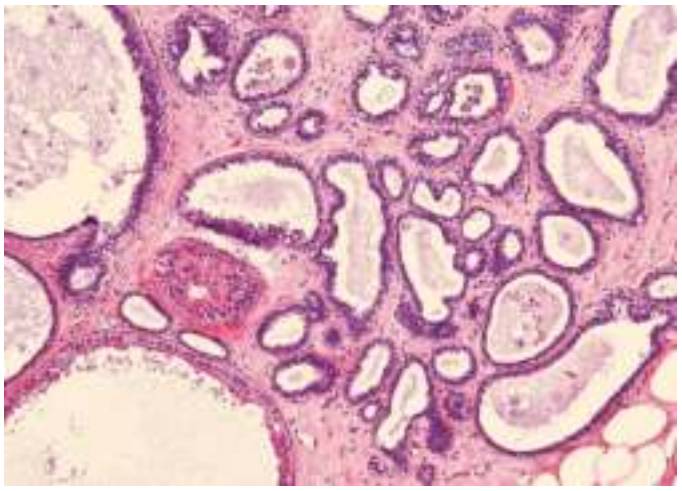


Fig. 25.9A: Spectrum of morphological changes in fibrocystic disease of breast shows duct dilatation, adenosis, fibrosis (intralobular and interlobular), and apocrine change (400X).

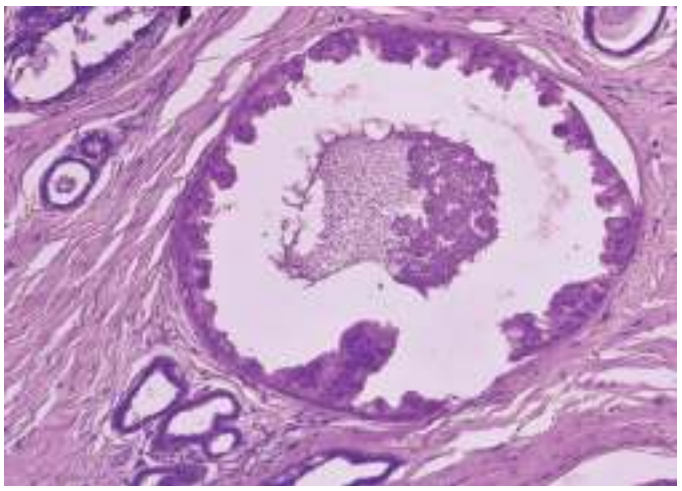


Fig. 25.9B: Apocrine metaplasia in fibrocystic disease of breast. It is characterized by enlarged ductal and lobular epithelial cells with apical sprouting, abundant granular eosinophilic cytoplasm, round nuclei of variable size with vesicular chromatin and prominent nuclei (400X).

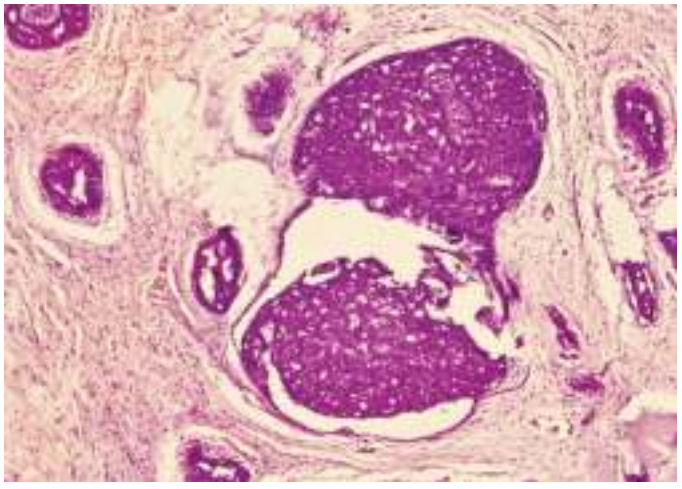


Fig. 25.10: Fibrocystic disease of breast. It shows typical ductal hyperplasia.

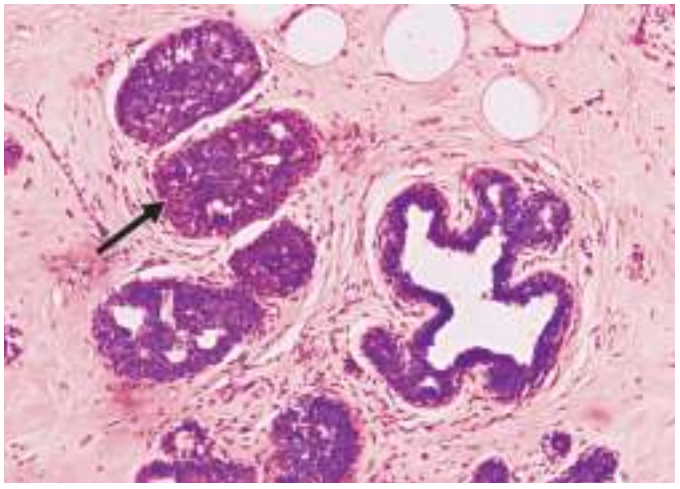


Fig. 25.11: Fibrocystic disease of breast. It shows atypical ductal hyperplasia (arrow) (100X).

- **Sclerosing adenosis:** Sclerosing adenosis associated with fibrosis of the intralobular stroma results in compression of the epithelial structures to give a pseudoinfiltrative growth pattern. The number of acini per terminal duct lobular unit (TDLU) in sclerosing adenosis is more than double the normal found in normal breast lobules. Tubules are lined by two layers of epithelial cells giving lobular configuration. There is more often presence of numerous microcalcifications. Sclerosing adenosis lesion in breast has become significant in modern clinical practice, as sclerosing adenosis in breast can be confused with breast carcinoma on mammographic screening.
- **Ductal epithelial hyperplasia:** As the ducts are estrogen sensitive, florid ductal epithelial hyperplasia occurs within areas of fibrocystic changes. The epithelial lining of ductal cells are multilayered, filling and expanding the ducts or acini. There is a slightly increased risk (1.5–2 times) for breast carcinoma.
- **Atypical ductal hyperplasia:** It occurs in ducts and lobules lined by multilayered pleomorphic atypical cells with hyperchromatic nuclei resembling ductal carcinoma *in situ* (DCIS) or lobular carcinoma *in situ* (LCIS). These atypical cells do not fill the entire lumen of ducts or lobules. The atypical ductal hyperplasia is indicative of an increased risk for subsequent breast carcinoma (Table 25.3).

FIBROCYSTIC DISEASE OF BREAST: DIAGNOSIS

Since the introduction of mammographic and ultrasound imaging of the breast, this condition can be diagnosed without performing surgical excision.

Table 25.3 Comparison of histologic features of ductal hyperplasia and ductal carcinoma *in situ* (DCIS)

Histologic Features	Ductal Hyperplasia (Usual Type)	Atypical Hyperplasia/DCIS (Low Nuclear Grade)
Size	Variable size, rarely extensive when associated with papilloma or radical scar	May be extensive, rarely <3 mm
Cellular composition	Epithelial cells along with spindle cells, lymphocytes, macrophages. Myoepithelial cell hyperplasia around periphery	Single cell population. Absence of spindle cells. Myoepithelial cells around periphery
Architecture	Variable	Well-developed micropapillary, cribriform or solid patterns
Lumina	Lumina irregular often ill-defined slit-like spaces common	Lumina well-delineated, regular, punched out in cribriform pattern
Cell orientation	Streaming pattern with long axis of nuclei arranged parallel to direction of cellular bridges, which often have a 'tapering appearance	Micropapillary structures with indiscernible fibrovascular cores or smooth, well-delineated geometric spaces. Cell bridges 'rigid' in cribriform type with nuclei oriented towards the luminal type
Nuclear spacing	Uneven nuclear spacing	Even nuclear spacing
Epithelial cell character	Small ovoid with variation in shape	Small uniform monotonous appearance
Nucleoli	Indistinct	Single small
Mitoses	Infrequent	Infrequent, abnormal form
Necrosis	Rare	If present, confined to small particulate debris in cribriform and/or luminal spaces

Ultrasonography is done to distinguish cystic fluid filled lesions in fibrocystic disease of breast from solid lumps. Fine needle aspiration cytology of bloody aspirate is done to rule out malignant change. Histologic examination distinguishes benign from malignant changes in fibrocystic disease of breast.

REVISED 2019 WHO CLASSIFICATION OF BREAST TUMORS

The classification of breast tumors continues to evolve with integration of new knowledge from research rapidly translated into the clinical practice. The 2019 WHO of breast tumors series' 5th edition is an update of the 4th edition breast tumors volume published in 2012. Major changes have been updated within the new 2019 WHO classification of breast tumors. Revised 2019 WHO classification of breast tumors is given in Table 25.4.

STROMAL TUMORS OF BREAST

FIBROADENOMA OF BREAST

Fibroadenoma is the most common benign tumor of the breast derived from intralobular stroma (Fig. 25.12), which affects females <25 years of age. In some teenagers, it may reach enormous size (10–15 cm), so-called juvenile giant fibroadenoma. Complex fibroadenoma is associated with increased risk for subsequent breast carcinoma.

Pathogenesis

Proliferation of intralobular stroma of breast leads to compression of the ducts. Intralobular stroma also synthesizes growth factors, which may stimulate proliferation of ducts. The basis of ductal proliferation is not clear; perhaps the neoplastic stromal cells secrete growth factors that induce proliferation of epithelial cells. Fibroadenoma develops in 50% of women, who receive cyclosporine after renal transplantation due to synthesis of growth factor by intralobular stroma of breast.

Molecular Genetic Alterations

Molecular technique reveals that the stromal cells are monoclonal and representing the neoplastic element of fibroadenoma of breast.

Clinical Features

Patient presents with solitary, well-circumscribed, firm rubbery and painless lump most often in the upper

Table 25.4 Revised 2019 WHO classification of breast tumors

Epithelial Tumors	
Benign tumors	▪ Tubular adenoma ▪ Lactating adenoma ▪ Apocrine adenoma ▪ Ductal adenoma ▪ Pleomorphic adenoma
Malignant tumors	▪ Ductal carcinoma *in situ* or invasive (NOS) ▪ Lobular carcinoma (*in situ* or invasive) ▪ Inflammatory carcinoma ▪ Medullary carcinoma ▪ Colloid (mucinous) carcinoma ▪ Tubular carcinoma ▪ Cribriform carcinoma (*in situ* or invasive) ▪ Papillary carcinoma ▪ Micropapillary carcinoma ▪ Scretory carcinoma ▪ Metaplastic carcinoma shows chondroid or bone formation or squamous differentiation ▪ Apocrine carcinoma ▪ Lipid-rich carcinoma ▪ Adenoid cystic carcinoma ▪ Sebaceous carcinoma ▪ Paget's disease of breast
Proliferative lesion	Ductal hyperplasia (typical or atypical)
Stromal Tumors	
Benign and malignant tumors	▪ Biphasic stromal benign tumors • Fibroadenoma • Phyllodes tumor • Periductal stromal tumor ▪ Pure stromal benign tumor • Pseudoangiomatous stromal hyperplasia • Nodular fasciitis • Fibromatosis ▪ Malignant stromal tumor with predominant myofibroblastic differentiation ▪ Malignant stromal tumor with predominant fibroblastic differentiation ▪ Lipoma ▪ Liposarcoma ▪ Leiomyoma ▪ Leiomyosarcoma ▪ Neural origin tumors • Granular cell tumor • Neurofibroma • Schwannoma ▪ Vascular lesions/tumors • Angioma • Angiomatosis • Angiosarcomas (primary, post-irradiation, post-radical mastectomy)
Lymphoid Neoplasm	
NHL	Diffuse large B cell lymphoma (DLBCL)
Metastatic Tumors	
Malignant tumors	Malignant melanoma and gastrointestinal carcinoma

Contd...

In clinical practice, breast tumors include fibroadenoma, phyllodes tumor, invasive ductal carcinoma (70–75%), invasive lobular carcinoma (5–14%), medullary carcinoma, mucinous (colloid) carcinoma, Paget's disease of nipple, papillary carcinoma, and tubular carcinoma.

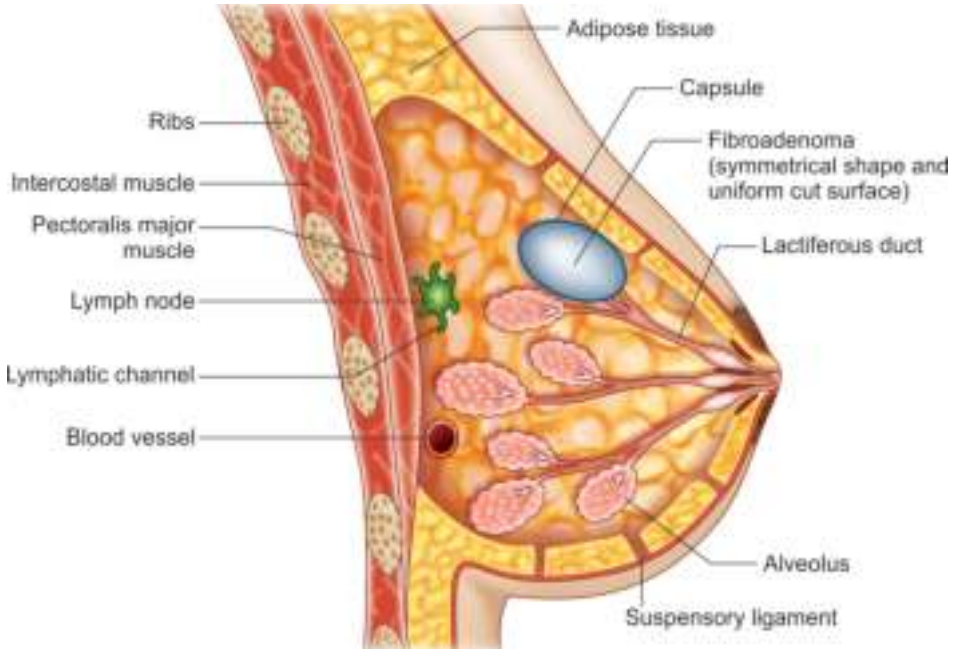


Fig. 25.12: Structure of the adult female breast shows fibroadenoma (well circumscribed tumor) in young female.

outer quadrant. However, fibroadenoma may be present in any quadrant of breast. Multiple or bilateral fibroadenomas may be observed in 10–15% of patients. There is no dimpling or retraction of overlying skin.

During menstrual cycle, pregnancy and lactation, fibroadenoma may undergo transient enlargement associated with pain in response to increase in estrogen/progesterone levels. Fibroadenoma of breast may regress and calcify after menopause.

Surgical Pathology: Fibroadenoma of Breast

Gross Morphology

- Fibroadenoma is delineated from adjacent breast tissue.
- Cut surface is gray white, smooth, firm with rubbery consistency.
- Tumor most often varies in size 1–3 cm in diameter, but may grow larger. Rarely tumors growing >10 cm in diameter are called giant fibroadenoma (Fig. 25.13A and B).

Light Microscopy

- Fibroadenoma of breast shows proliferation and expansion of the stroma is of usually low cellularity.
- The ductal element sometimes forms an intracanalicular pattern with a leaf-like pattern (Figs 25.14 to 25.16). Delicate loose fibrous stroma encloses the epithelial component consisting of gland-like or duct-like spaces lined by cuboidal or columnar cells. The stromal cells of loose fibrous stroma are neoplastic, and the epithelial cells lining ducts to be reactive.
- Tumor shows biphasic patterns: intracanalicular and pericanalicular patterns.
  - **Intracanalicular pattern:** Excessive proliferation of intralobular stroma compressing and distorting glands (ducts) and forming slit-like spaces is known intracanalicular pattern with a leaf-like pattern.
  - **Pericanalicular pattern:** The glands (ducts) retain round shape. Because ducts are not compressed by fibrosis resulting in pericanalicular pattern.

Treatment

Treatment of fibroadenoma of breast depends on severity of disease, which may include monitoring for changes in the size and biopsy evaluation. Fibroadenoma is treated by surgical excision.

GIANT JUVENILE FIBROADENOMA OF BREAST

Giant juvenile fibroadenoma is considered >5 cm, 500 g and replacing 80% of the breast in young girls between the ages of 10 and 18 years. It is characterized by massive and rapid enlargement of a rubbery mobile and a non-tender breast lump. The etiology is not well understood and believed to be an increased sensitivity to normal estrogen level.

- Differentiating giant juvenile fibroadenoma from phyllodes tumor may be difficult with histopathology.
- Surgical excision is highly recommended to establish the diagnosis. Patient is managed by surgical excision with conservation of normal breast tissue, nipple and the areola.
- Hematoxylin and eosin stained section from giant juvenile fibroadenoma tumor shows biphasic proliferation of fibroblastic elements with no cytologic atypia. Clinical photograph of giant juvenile fibroadenoma is shown in Fig. 25.17.

PHYLLODES TUMOR OF BREAST

Phyllodes tumor is a large fleshy bulky tumor measuring 10–15 cm distorting the breast, and overlying skin ulceration and related to fibroadenomas. Phyllodes tumor of breast is more cellular than fibroadenoma.

The distinction of phyllodes tumor from fibroadenoma is made not on the size, but on the histologic and cytologic characteristics of the stromal component.

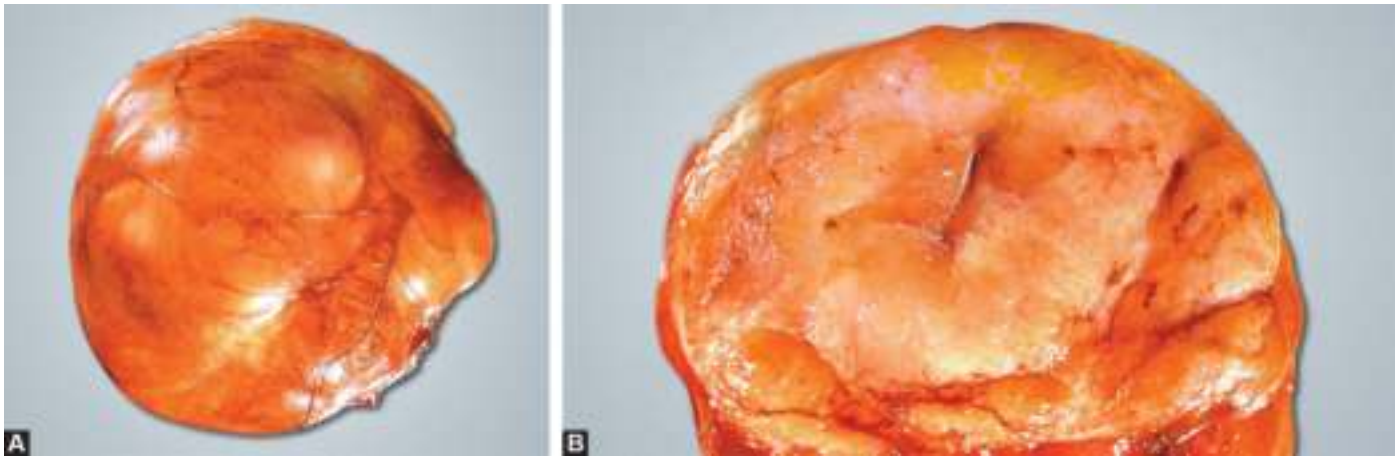


Fig. 25.13A and B: (A) Fibroadenoma—this well circumscribed mobile lump was removed surgically, (B) cut surface of fibroadenoma shows lobulated appearance of tumor bulging outwards, gray white, smooth, firm with rubbery consistency. Most often varies in size from 1 to 3 cm in diameter.

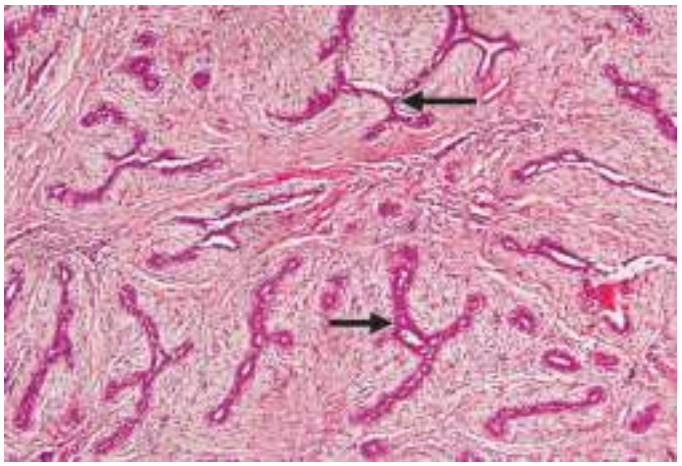


Fig. 25.14: Fibroadenoma breast with intracanalicular pattern. Light microscopy shows intracanalicular pattern. Glands are compressed cords to form slit-like spaces in fibrotic stroma (arrows) (100X).

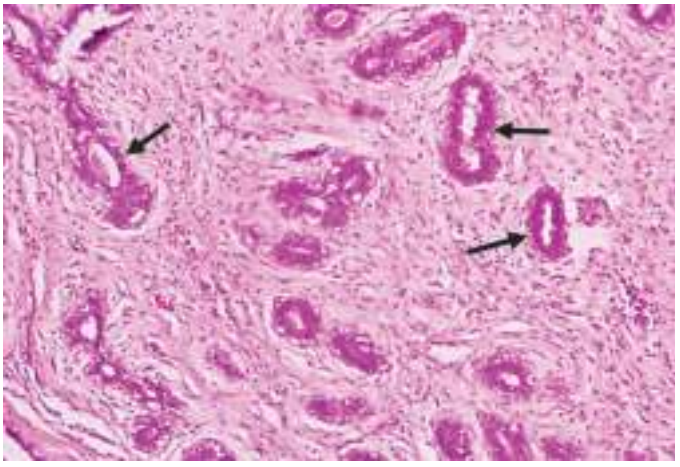


Fig. 25.15: Fibroadenoma breast with pericanalicular pattern—the glands (ducts) retain round shape. Because ducts are not compressed by fibrosis result in 'pericanalicular pattern' (arrows) (100X).

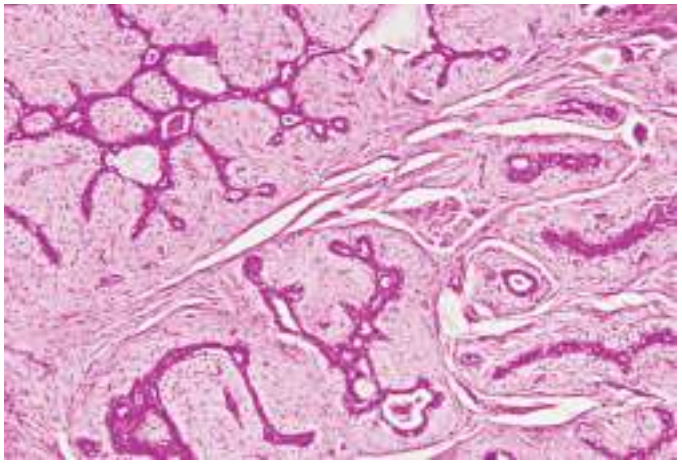


Fig. 25.16: Fibroadenoma. It shows intracanalicular and pericanalicular patterns. Glands compressed by stroma result in slit-like spaces known as intracanalicular pattern. Ducts not compressed by stroma have round shape known as pericanalicular pattern (100X).

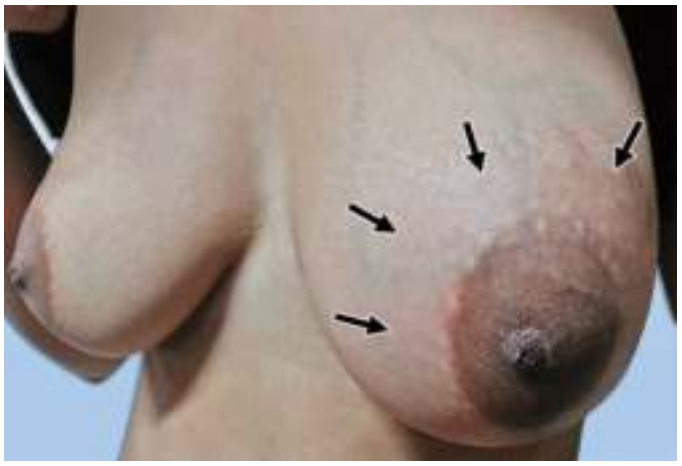


Fig. 25.17: Clinical photograph of giant juvenile fibroadenoma. A 16-year-old girl presented with rapid large lump in the left breast. Clinical examination of breasts revealed a mobile large lump in left breast measuring >5 cm in size stretching the overlying skin and dilatation of superficial veins. Lumpectomy was done. Histologic examination revealed features of fibroadenoma (arrows). (*Courtesy:* Dr. Permeet Kaur Bagga, Senior Professor and Head, Department of Pathology, Govt. Medical College, Amritsar.)

Origin of Tumor

Phyllodes tumor of the breast arises from intralobular stromal cells of the breast, not the ducts or glands. It is most often low-grade neoplasm accompanied by a benign growth of ductal structures. Phyllodes tumor of breast may be benign, intermediate and malignant. Most phyllodes tumors are benign and only a small number are malignant.

Age Group

Phyllodes tumor of breast most often occurs in older age group. It may affect 30–70 years aged women.

Clinical Features

Patient presents with bulky lump distorting the breast with overlying skin ulceration.

Surgical Pathology: Phyllodes Tumor of Breast

Gross Morphology

- Like fibroadenoma, benign phyllodes tumor is sharply circumscribed, lobulated large size tumor measuring 10–15 cm. Cut surface is firm, glistening, and grayish white with cystic spaces containing leaf-like extensions.
- Benign phyllodes tumor has pushing margin.
- Malignant phyllodes tumor has infiltrating margin. Tumor is poorly circumscribed with invasion into the surrounding breast tissue (Figs 25.18 and 25.19).

Light Microscopy

Following histologic features may help to differentiate benign phyllodes tumor from malignant phyllodes tumor, which include cellularity, margin of tumor, mitoses and heterologous elements.

- **Benign phyllodes tumor of breast:** Tumor comprises highly cellular myxoid stroma projecting into the ducts lined by benign epithelial cells, creating the leaf-like pattern (Greek word phyllodes meaning leaf-like clefts and slits) and presence of <5 mitoses per 10 high power field (HPF) in the stroma. The stroma is more cellular than a fibroadenoma.
- **Borderline phyllodes tumor of breast:** Histologic examination of tumor shows 5–10 mitoses per 10 HPF.
- **Malignant phyllodes tumor of breast:** A malignant phyllodes tumor has a sarcomatous stroma that predominates over the ductal elements and invades the breast. Tumor has abundant mitotic activity. Stromal component is abundant in comparison to benign duct elements.
  - Light microscopy shows malignant change in the stromal component of tumor, numerous mitoses >10 per 10 HPF in the stroma, hemorrhage and necrosis (Fig. 25.20).
  - Diagnostic features of biphasic neoplasms of breast are given in Table 25.5. Differentiating pathologic features of cellular fibroadenoma and malignant phyllodes tumor are given in Table 25.6.

Biologic Behavior

It is difficult to predict biologic behavior of phyllodes tumor of breast, which may recur locally after incomplete excision. High grade phyllodes tumor may metastasize to lymph nodes or lung in 33% of cases.

Management

Treatment of phyllodes tumor is wide excision of tumor with wide margin of normal surrounding tissue. Inadequate excision of tumor results in recurrences.

JUVENILE PAPILLOMATOSIS OF BREAST

Juvenile papillomatosis is also known as ('Swiss cheese disease') affecting young girls. Patient presents with solitary, unilateral breast lump. Juvenile papillomatosis is often mistaken for a phyllodes tumor clinically. A family history of breast carcinoma has been observed in 30–50% of juvenile papillomatosis. In addition, increased risk for development of breast carcinoma during follow-up of patient diagnosed with juvenile papillomatosis.

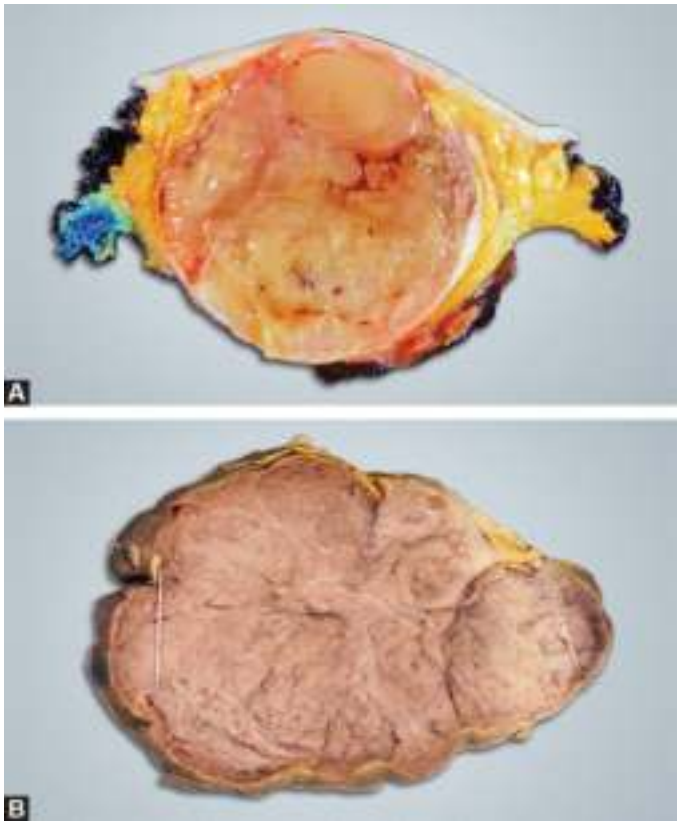


Fig. 25.18A and B: Phyllodes benign tumor. Tumor is large bulky with pushing margin. Cut surface is firm, glistening, and grayish white with cystic spaces containing leaf-like extensions. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru.)

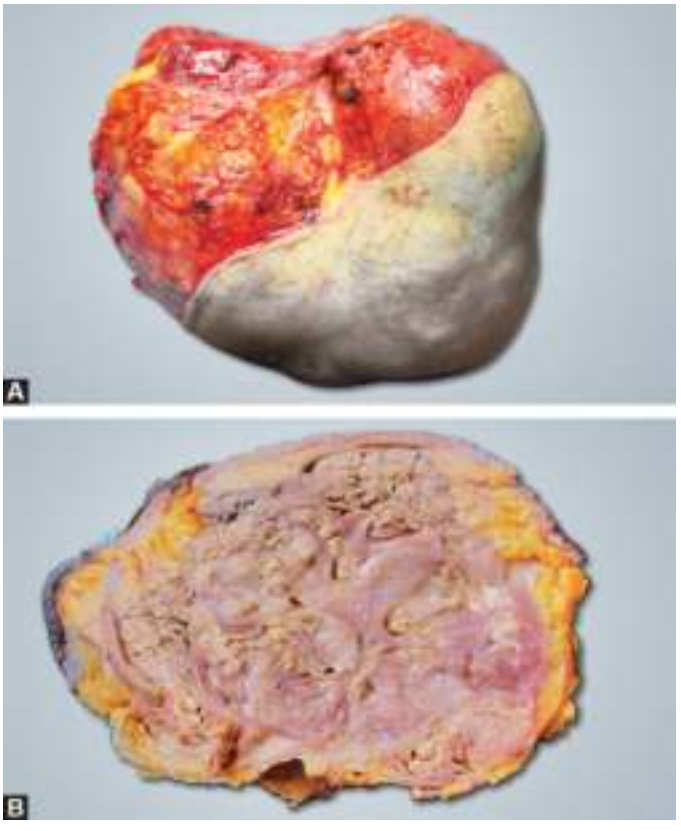


Fig. 25.19A and B: Phyllodes malignant tumor. Tumor is large bulky distorting breast with areas of hemorrhage and necrosis. On right side, surgical excised tumor shows firm, glistening, and grayish white with cystic spaces containing leaf-like extensions. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru.)



Fig. 25.20: Phyllodes tumor—it comprises highly cellular myxoid stroma projecting into the ducts creating the leaf-like pattern (Greek word *phyllodes* meaning leaf-like clefts and slits) and presence of <5 mitoses per 10 HPF in the stroma. The stroma is more cellular than a fibroadenoma (arrows) (400X).

Surgical Pathology: Juvenile Papillomatosis of Breast

Gross Morphology

- Juvenile papillomatosis lesion is circumscribed, but not encapsulated containing large multiple cysts. It strongly resembles fibrocystic disease of the breast.
- Differential diagnosis also pubertal macromastia, and juvenile fibroadenoma of breast.

Light Microscopy

Juvenile papillomatosis shows dilated breast ducts lined by a proliferation of highly atypical-looking epithelium that would be regarded as precancerous in an older woman.

PSEUDOANGIOMATOUS STROMAL HYPERPLASIA

Pseudoangiomatous stromal hyperplasia is a rare benign stromal lesion, which arises from myofibroblast cells lining the slit-like spaces. Lesion is positive for vimentin, actin, CD34 and estrogen receptors (ER).

Table 25.5 Diagnostic features of biphasic neoplasms of breast

Features	Fibroadenoma	Low-grade Phyllodes Tumor	High-grade Phyllodes Tumor
Gross morphology			
Size of the tumor	Variable size	Variable size	Variable size
Margins	Pushing	Pushing	Infiltrative
Cut surface	Gray-white	Gray-white with cystic spaces	Gray-white with cystic spaces
Histologic features			
Cystic spaces	Tubular (pericanalicular) or slit (intracanalicular)-like	Leaf-like cystic spaces	Leaf-like cystic spaces
Stromal cellularity	Variable stromal cellularity	Moderate to high stromal cellularity	Moderate to high stromal cellularity
Stromal overgrowth	Absent	Absent	Present in some cases
Stromal nuclear atypia	Minimal	Mild to moderate	Moderate to severe
Mitotic figures	Variable	<5/10 HPF	>10/10 HPF
Benign heterogenous elements	Rare	Frequent	Frequent

Table 25.6 Differentiating pathologic features of cellular fibroadenoma and malignant phyllodes tumor

Features	Cellular Fibroadenoma	Malignant Phyllodes Tumor
Gross morphology	Well circumscribed without invasion into surrounding breast tissue	Poorly circumscribed with invasion into a surrounding breast tissue
Margins on light microscopy	Well circumscribed margins	More often well circumscribed than infiltrative; adipose tissue infiltration
Nuclear pleomorphism	Absent	Marked
Stromal proliferation/overgrowth	Present	Present
Epithelial lined clefts—epithelium at the age of tissue fragmentation	Usually absent or less prominent	Present
Stromal cellularity	Heterogenous stromal cellularity	Heterogenous; periductal/subepithelial condensation
Mitotic activity	<3/10 HPF	>10/10 HPF

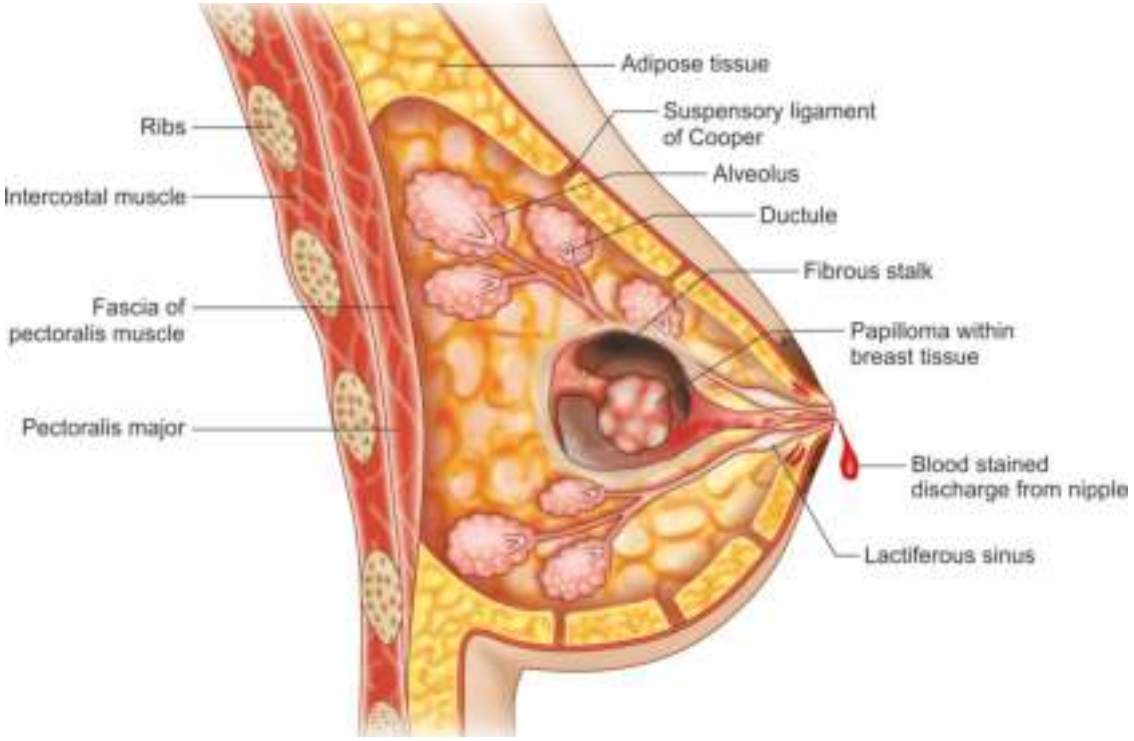


Fig. 25.21: Breast intraductal papilloma has a stalk. Tumor is present within breast substance.

BENIGN EPITHELIAL TUMORS OF BREAST

INTRADUCTAL PAPILLOMA OF BREAST

Intraductal papilloma is a benign proliferative solitary lesion within the subareolar lactiferous duct of female breast, which consists of benign ductal epithelial cell proliferation with branching fibrovascular core and underlying myoepithelial cells. Central intraductal papilloma is well-circumscribed polypoid nodule in a cystically dilated lactiferous duct. Peripheral intraductal papillomas are not grossly visible. Schematic representation of intraductal papilloma of female breast is shown in Fig. 25.21.

Age Group

Intraductal papilloma of breast occurs in the middle-aged (30–50 years) and elderly women.

Pathology Pearls: Intraductal Papilloma of Breast

- Intraductal papilloma of the breast occurs within breast ducts in middle aged and elderly women.
- Patient with central intraductal papilloma within lactiferous duct presents with hemorrhagic or serous nipple discharge, however peripheral tumor lacks nipple discharge.
- There is increased risk for development of cancer in patients with multiple intraductal papillomas.
- Tumor is detected on a mammogram or ultrasound and diagnosed by needle biopsy.
- Tumor is composed of fibrovascular core lined by benign ductal cells and myoepithelial cells.

Clinical Features

Clinical examination may reveal solitary large tumor within large lactiferous duct in the central region or multiple small tumors in the peripheral region of the breast. Patients with large central intraductal papilloma of breast may present with hemorrhagic or serous nipple discharge. Peripheral intraductal papillomas are most often multiple but smaller in size, which do not present with nipple discharge, but can undergo malignant change.

Diagnostic Modalities

Diagnostic modalities of intraductal papilloma of female breast include mammography, biopsy (core needle biopsy, vacuum-assisted biopsy, excisional biopsy), histologic examination and immunohistochemistry.

Surgical Pathology: Intraductal Papilloma of Breast

Gross Morphology

The tumor is tan-pink, friable, usually <1.0 cm in size.

Light Microscopy

- Intraductal papilloma is attached to the wall of the duct by a fibrovascular stalk. It shows multiple branching papillae composed of thin fibrovascular core lined by cuboidal to columnar ductal cells toward lumen and outer myoepithelial cells. Ductal cells may show squamous metaplasia or apocrine change.
- Intraductal papilloma with <3 mm focus of atypical hyperplasia is termed ductal carcinoma *in situ*.

- Myoepithelial cells are demonstrated in intraductal papillomas.
- Intraductal papilloma must be distinguished from papillomatosis, occurring in the peripheral ducts as a component of proliferative fibrocystic change (Fig. 25.22). It should be differentiated from noninvasive papillary carcinoma of breast (Table 25.7).
- Comparative pathologic features among benign intraductal papilloma, atypical papilloma, papilloma with DCIS and papillary DCIS are given in Table 25.8.

Immunohistochemistry Technique

- Myoepithelial cells are smooth muscle-like cells present in the glandular epithelium as a thin layer above the basement membrane and beneath the luminal cells of breast.
- Myoepithelial cell layer is intact in benign (e.g. breast papilloma) and *in situ* breast lesions; but absent in invasive breast carcinoma. Microglandular adenosis lacks myoepithelial cells.
- Panel of markers is used on paraffin-embedded tissue section of breast lesions to demonstrate myoepithelial cells in breast.

Markers	Expression
Smooth muscle actin (α-actin)	Positive
Calponin	Positive
Smooth muscle myosin heavy chain (SMMHC)	Positive
p63	Positive
CK14 (HMWCK)	Positive
S-100 (calcium binding protein)	Positive
Glial fibrillary acidic protein (GFAP)	

TUBULAR ADENOMA OF BREAST

Tubular adenoma of the breast is a benign epithelial tumor predominantly composed of bilayered tubules with sparse intervening stroma. Tubules are lined by an inner layer of epithelial cells and an outer layer of myoepithelial cells. Tumor most often occurs in young women (20–40 years) in the upper outer quadrant of the breast. Patient presents with firm, mobile, well defined and painless breast lump. There is no increased risk for developing subsequent breast carcinoma. Tumor is curable by complete surgical excision.

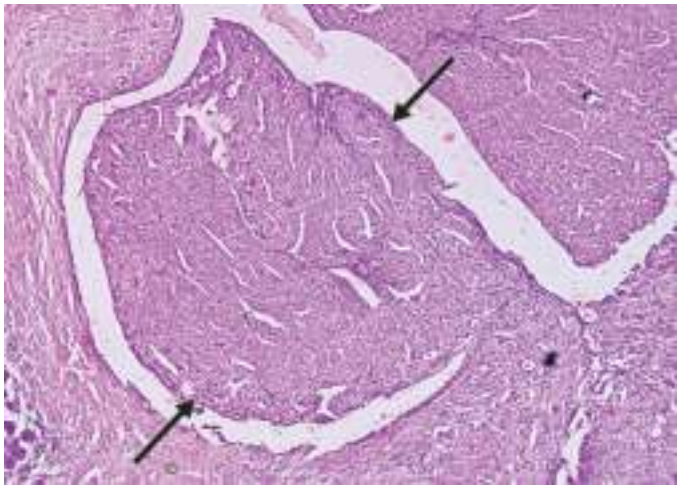


Fig. 25.22: Intraductal papilloma. It shows multiple branching papillae composed of thin fibrovascular core lined by cuboidal to columnar ductal cells toward lumen and outer myoepithelial cells (arrows) (200X).

Surgical Pathology: Tubular Adenoma of Breast

Gross Morphology

Tubular adenoma of breast is well circumscribed mass, sharply demarcated but without capsule.

Light Microscopy

- Tumor is composed of densely packed uniform round tubules lined by luminal cuboidal epithelial and outer myoepithelial cells without cytological atypia.
- There is scant intervening stroma without compression of ducts (Fig. 25.23).

LACTATING ADENOMA OF BREAST

Lactating adenoma of breast may represent the same lesion under different physiologic conditions. Lactating adenoma has been identified in postpartum biopsies that first presented during pregnancy.

- Lactating adenoma predominantly occurs in the breast, however, it may develop in ectopic breast tissue along milk line extending from axilla to vulva.
- Patient presents with painless, soft, palpable, solid mobile and discrete mass. Tumor may be bilateral

Table 25.7 Comparison of intraductal papilloma and noninvasive papillary carcinoma of breast

Parameters	Intraductal Papilloma	Noninvasive Papillary Carcinoma
Cell morphology	Benign cells with normochromatic nuclei	Atypical cells with hyperchromatic nuclei
Myoepithelial cells	Present	Absent
Apocrine change	Present	Absent
Adjacent stroma	Thick prominent stroma	Delicate stroma
Sclerosing adenosis	Unusual finding	Usual finding

Table 25.8 Comparative pathologic features among benign intraductal papilloma, atypical papilloma, papilloma with DCIS and papillary ductal carcinoma *in situ* (DCIS)

Parameters	Benign Intraductal Papilloma	Atypical Papilloma	Papilloma with DCIS	Papillary DCIS
Architectural pattern	Papillary pattern	Architectural pattern of ADH or DCIS (mm in size) or atypical population constitutes 10 to <33% of the lesion	Architectural pattern of ADH or DCIS (>mm in size) or atypical cells involving one-third but <90% of the lesion	Papillary, cribriform, solid or spindle
Fibrovascular core	Present	Present	Present	Present, may be obscured
Cellular components	Epithelial and myoepithelial components present	Epithelial and myoepithelial components may be lost	Epithelial component present, loss of myoepithelial component	Only epithelial component present
Atypia	Present	Present, can be focal	Usually present; variable dregree	Usually present
Necrosis	Absent	Usually absent	May be present	May be present
Cytomorphologic features	Heterogenous	Monotonous	Monotonous	Monotonous
Myoepithelial cell layer	Present	Absent	Usually absent	Usually absent
Immunohistochemistry	CD5, CD6 positive	CD5, CD6 negative	CD5, CD6 negative	CD5, CD6 negative

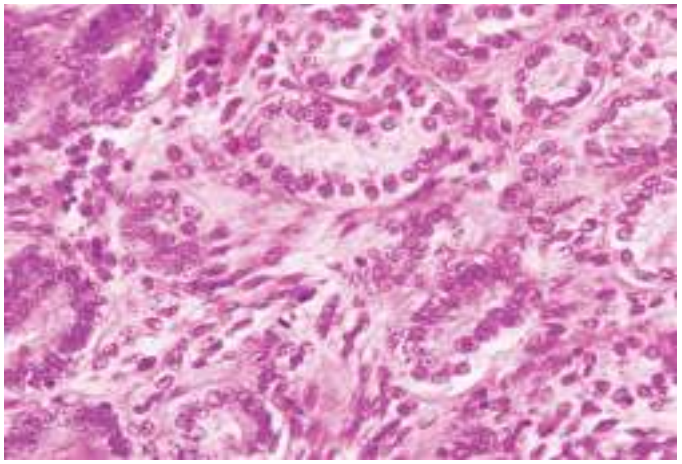


Fig. 25.23: Tubular adenoma. It shows densely packed uniform round tubules lined by luminal cuboidal epithelial and outer myoepithelial cells without cytological atypia. There is scant intervening stroma without compression of ducts (400X).

and multifocal. Infarction of tumor may result in pain, tenderness and rapid enlargement.

- Lactating adenoma of breast is diagnosed by mammography, MRI, fine needle aspiration cytology and histopathologic examination.

Surgical Pathology: Lactating Adenoma of Breast

Gross Morphology

- Lactating adenoma of breast lacks true capsule. It is well circumscribed, lobulated, solitary or multiple firm/rubbery mass.
- Cut surface shows gray-tan appearance. It may have areas of necrosis or infarction.

Light Microscopy

- On histologic examination, tumor is composed of well circumscribed proliferation of hyperplastic closely packed lobules with both epithelial and myoepithelial cell layers separated by thin, delicate connective tissue.
- Glands are lined by actively secreting cuboidal or hobnail-shaped cells with small round nuclei and clear to granular vacuolated cytoplasm. Cells contain nuclei with prominent nucleoli but lack cytologic atypia.
- Tumor resembles pregnancy-like changes, which may resemble fibroadenoma or tubular adenoma with lactational change (Fig. 25.24).

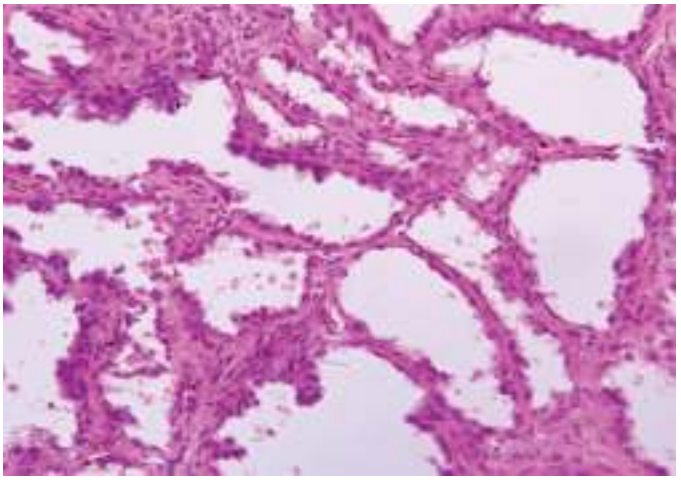


Fig. 25.24: Lactating adenoma of the female breast occurring in pregnancy and lactation. Tumor most often requires biopsy to exclude malignancy. Tumor is composed of mammary ducts lined by epithelial and myoepithelial cells and eosinophilic secretions within the lumen (200X).

COMPLEX FIBROADENOMA OF BREAST

Complex fibroadenoma of breast shows features of fibroadenoma with cysts measuring >3 mm in size, sclerosing adenosis, papillary apocrine change and calcification. Patient presents with round breast lump with distinct borders, mobile and firm or rubbery.

ADENOMA OF THE NIPPLE

Adenoma of the nipple is benign epithelial proliferation arising near the collecting ducts of the nipple.

- Patient presents with serous or bloody nipple discharge or nipple mass or with skin changes such as ulceration and crusting similar to Paget disease of the nipple.
- Initial diagnosis of adenoma of the nipple is established on histologic examination of skin punch biopsy of the nipple.

Surgical Pathology: Adenoma of the Nipple

Gross Morphology

- Adenoma of the nipple is ill-defined.
- Nipple skin shows crusting, erythema, erosion.

Light Microscopy

- In adenoma of breast, adenosis or ducts with usual hyperplasia or papillomatosis are present just below the nipple epidermis associated with collagenous stroma.
- Tumor may demonstrate cysts, focal necrosis associated with ductal hyperplasia or florid ductal hyperplasia, but no cellular atypia.
- Myoepithelial cell layer is intact (Fig. 25.25).

Immunohistochemistry Technique

Myoepithelial cells around duct-like structures are demonstrated by S-100, α-smooth muscle actin (α-SMA), p63 and glial fibrillary acidic protein (GFAP) immunohistochemical markers.

Markers	Expression
S-100	Positive
α-Smooth muscle actin	Positive
p63	Positive
Glial fibrillary acidic protein (GFAP)	Positive

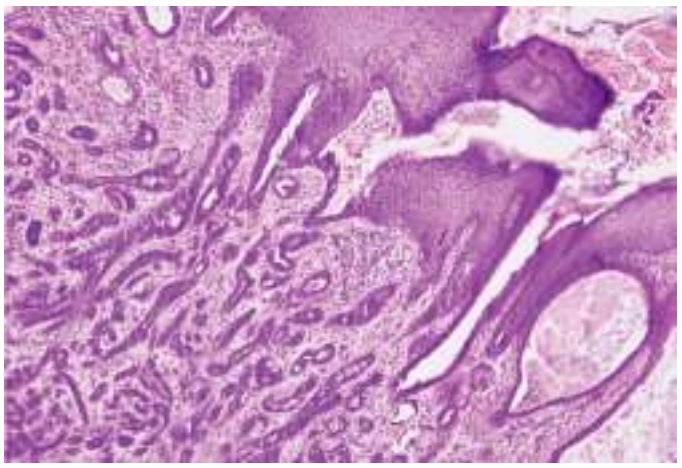


Fig. 25.25: Adenoma of the nipple. It is composed of duct-like structures associated with collagenous stroma (400X).

MALIGNANT EPITHELIAL TUMORS OF BREAST

BREAST CARCINOMA: OVERVIEW

Breast carcinoma is most important disease especially in postmenopausal women and second most frequent cause of death in women. As most breast diseases present as palpable lump, hence it is important to distinguish breast carcinoma from benign breast disease.

- True nature of a breast lump is ascertained by histopathologic examination of many areas of the excised lump. Good idea of nature of breast lump before surgery may be obtained from clinical features (size, texture, relation to adjoining tissues), mammographic examination of the affected breast, and cytologic examination of smears obtained by fine needle aspiration cytology.
- Early detection is the most important factor in breast carcinoma survival. Most important risk factors for breast carcinoma are estrogen stimulation of breasts by ovarian hormones and advancing age. Breast carcinoma occurs due to accumulation of DNA mutations.

Clinical Features

Breast lump is the presenting symptom in 85–90% of patients with breast carcinoma. Approximately 60% of breast masses are discovered by patient on self-examination. Clinical examination of breast is a necessary complement for screening mammography.

Distribution

Most glandular tissue of breast is located in the upper outer quadrant and beneath the nipple. Thus, breast carcinoma is most commonly located in upper outer quadrant. It occurs most commonly in the left breast than right.

Location

Breast carcinoma in decreasing frequency is located in upper outer quadrant (50%), central region beneath nipple (20%), lower outer quadrant (10%), upper inner quadrant (10%) and lower inner quadrant (10%), respectively.

Origin of Tumor

Breast carcinoma arises in the lining cells (epithelium) of the ducts (80–85%) or lobules (10–15%) in the glandular tissue of the breast, i.e. terminal duct lobular unit (TDLU). Initially, breast carcinoma is confined to the

duct or lobule (*in situ*), which has minimal potential to spread. Invasive breast carcinoma has metastatic potential to distant organs. Breast carcinomas are classified according to their site of origin into ductal and lobular. Ductal carcinoma is the most common subtype followed by lobular carcinoma second most common (most often bilateral) with distant metastases. Histologic types and frequency of breast carcinoma are shown in Table 25.9.

- ***In situ* (noninvasive) ductal/lobular carcinoma:** Ductal carcinoma *in situ* and lobular carcinoma *in situ* feature neoplastic cells are confined within the basement membrane of ducts and lobules, respectively. Myoepithelial cells can be demonstrated in ductal carcinoma *in situ* unlike invasive ductal carcinoma of breast.
  - Noninvasive breast carcinomas include ductal carcinoma *in situ*, lobular carcinoma *in situ*, mixed intraductal carcinoma and lobular carcinoma *in situ* and papillary carcinoma and comedocarcinoma (ductal carcinoma *in situ* with central necrosis).
  - Ductal carcinoma *in situ* is detected by screening mammography.
- **Invasive ductal/lobular carcinoma:** When ductal/lobular carcinoma breaches basement membrane and invades the normal breast parenchyma, it is termed invasive breast carcinoma. Lobular carcinomas are usually multifocal in origin.
  - Serum tumor markers for breast carcinoma include CA15.3, CA27.29, BRCA, mammaglobin, PS_2, CEA and gross cystic disease fluid protein 15 (GCDFP-15).
  - CA125 is a cell surface glycoprotein originally identified in mucinous epithelial ovarian tumors, carcinomas of endometrium, gastrointestinal tract, thyroid, and breast (Figs 25.26 and 25.27).

Table 25.9 Histologic types and frequency of breast carcinoma

Histologic Types of Breast Carcinoma	Frequency
Carcinoma *in situ*	
Ductal carcinoma *in situ*	3.6%
Lobular carcinoma *in situ* (LCIS)	1.6%
Intraductal and lobular carcinoma *in situ*	0.2%
Comedocarcinoma with central necrosis	0.3%
Invasive carcinoma	
Ductal carcinoma (not otherwise specified)	70–75%
Lobular carcinoma	5–15%
Ductal and lobular carcinoma	1.6%
Tubular carcinoma	5%
Mucinous (colloid) carcinoma	2–3%
Medullary carcinoma	1–2%
Micropapillary carcinoma	1–2%
Metaplastic carcinoma	1%
Papillary carcinoma	0.8%

Paget's disease constitutes 1.0% of breast cancer. It may be ductal carcinoma in situ or invasive ductal carcinoma involving nipple epidermis.

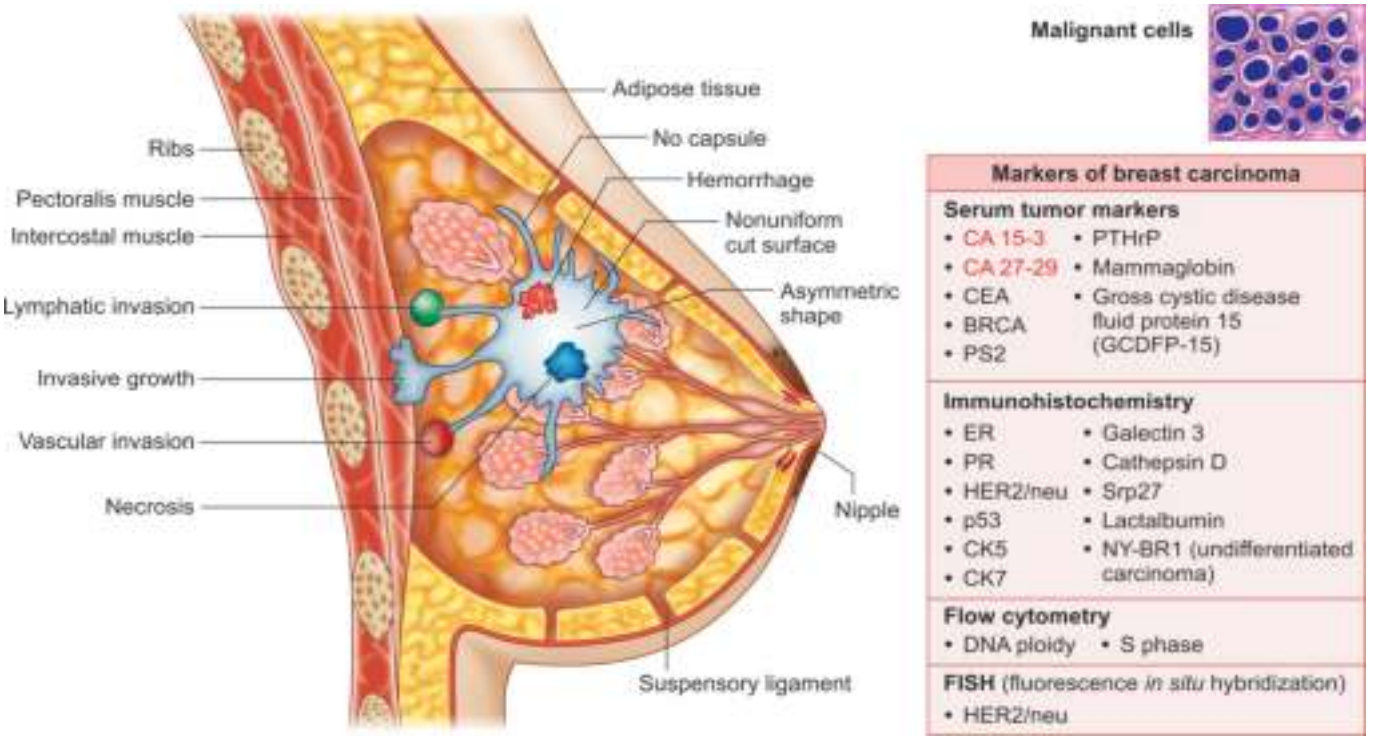


Fig. 25.26: Breast carcinoma shows invasive duct carcinoma with infiltrating margins and areas of hemorrhage and necrosis.

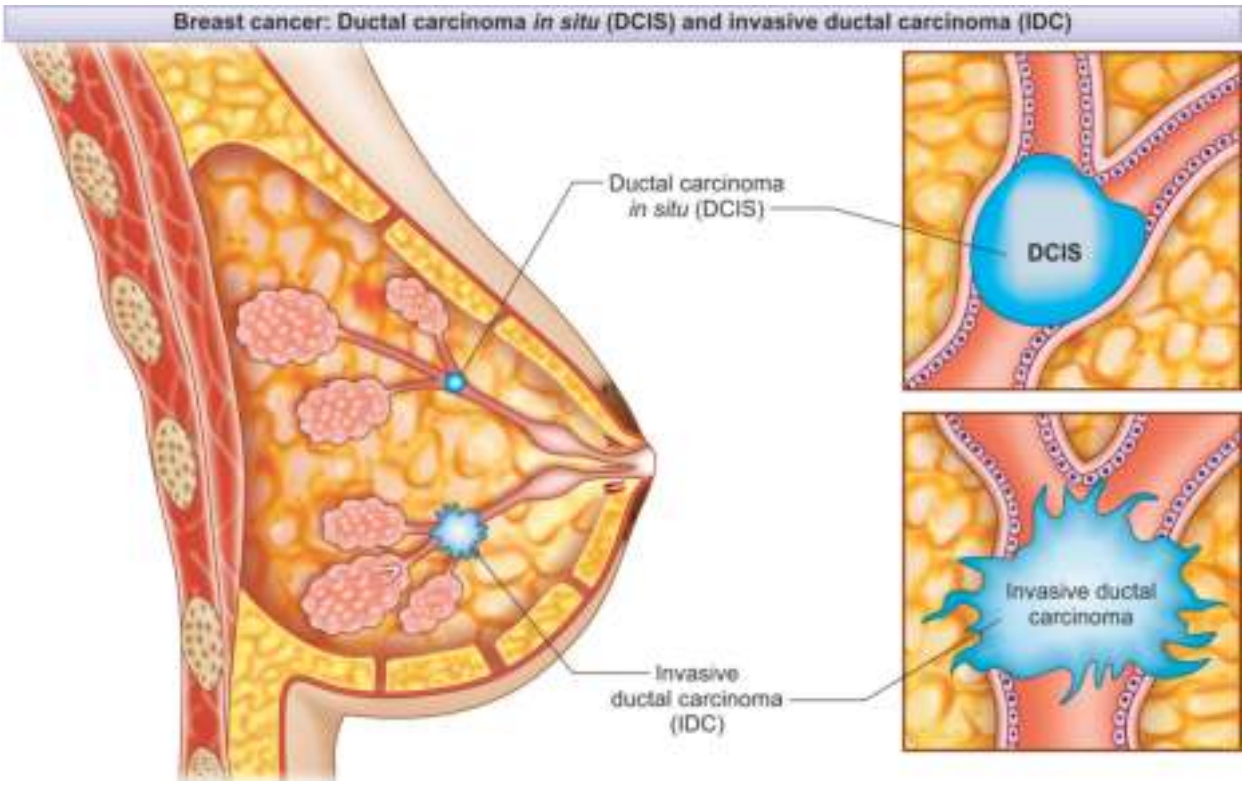


Fig. 25.27: Breast cancer—it shows ductal carcinoma *in situ* in lower inset and invasive ductal carcinoma with infiltrating margins in upper inset.

Pathology Pearls: Myoepithelial Cells

- The acinus is lined by epithelial cells surrounded by myoepithelial cells and the basement membrane.
- On immunohistochemistry, myoepithelial cells show positivity for S-100, α-smooth muscle actin (α-SMA), p63, glial fibrillary acidic protein (GFAP) and keratin 14.
- Myoepithelial cells are most often demonstrated in benign breast tumors and carcinoma *in situ*.

BREAST CARCINOMA: RISK FACTORS

About 50% breast carcinomas develop in women have no identifiable breast carcinoma risk factors other than gender (female) and age (over 40 years). Certain factors increase the risk for breast carcinoma including positive family history, longer reproductive span, nulliparity, obesity after menopause, excessive exposure to estrogens, atypical ductal hyperplasia of breast, radiation exposure, dietary factors, and radical scar in breast.

Incidence of sporadic, familial and hereditary breast carcinomas is given in Table 25.10. Risk for development of invasive carcinoma of breast related to biopsy for histologic findings is given in Table 25.11.

Positive Family History and Breast Carcinoma

Incidence of breast carcinoma is greatly increased in first-degree female relatives of patients (mother, sister,

Table 25.10 Incidence of sporadic, familial and hereditary breast carcinomas

Breast Carcinoma	Incidence	Genes Involved
Sporadic breast carcinoma	65–75%	–
Familial breast carcinoma	20–30%	–
Hereditary breast carcinoma	5–10%	–
▪ BRCA1		45%
▪ BRCA2		35%
▪ TP53 (Li-Fraumeni) syndrome		1%
▪ PTEN (Cowden disease)		<1%
▪ MSH_2/MLH-1 (Muir-Torre syndrome)		<1%
▪ ATM (ataxia-telangiectasia)		<1%
▪ STK-11/4KB1 gene in Peutz-Jeghers syndrome		<1%
▪ Other genes		20%
• CHEK2 gene		
• RB gene		
• FGF3 gene		
• INT2 gene		
• ERBB2 (HER2/neu)		
• NOTCH 1 gene		
• RAS gene		
• c-Myc gene		
• BCL-1 gene		
• Cyclin D1 gene		
• CCND-1 gene		
• E-cadherins		
• Galectin 2		

Table 25.11 Risk of development of invasive carcinoma for breast related to biopsy for histologic findings

Breast Lesion	Clinical Features	Histologic Features	Risk of Breast Carcinoma
Nonproliferative fibrocystic disease	▪ Lumpy bumpy breast ▪ Cyclic breast pain	▪ Cysts formation ▪ Apocrine metaplasia ▪ Fibrosis	No risk
Duct ectasia	Nodule with calcification	Inflammatory breast lesion	No risk
Fat necrosis	Traumatic firm breast lump	▪ Foreign body giant cell reactions ▪ Calcification	No risk
Fibroadenoma	Mobile lump	Stromal-epithelial benign breast neoplasm	No risk
Proliferative fibrocystic disease	▪ Lumpy bumpy breast ▪ Cyclic breast pain ▪ Calcification	▪ Atypical ductal epithelial hyperplasia ▪ Sclerosing adenosis	2-fold (slight risk)
Papilloma	Blood stained nipple discharge in subareolar mass	Epithelial cells lining fibrovascular core	2-fold (slight risk)
Atypical ductal hyperplasia	Mass lesion with or without calcifications	Atypical ductal cells, not enough for ductal carcinoma *in situ*	4-fold (moderate risk)
Ductal carcinoma *in situ*	Breast mass with or without calcifications	▪ Cribriform pattern ▪ Papillary pattern ▪ Solid pattern with atypical nuclei	8-fold (high risk)

or daughter with history of breast carcinoma) at a younger age.

Family members may also transmit the abnormal susceptible gene (autosomal dominant with limited penetrance) without developing breast carcinoma themselves. Approximately 5% of cases are associated with a penetrant dominant genetic predisposition. Prophylactic bilateral mastectomy is performed in familial positive history of breast carcinoma.

BRCA1 Tumor Suppressor Gene Mutation

All women in the family with strong family history of breast carcinoma may have mutation of BRCA1 tumor suppressor gene located on chromosome 17q2, which regulates DNA repair by binding to RAD51, a molecule that mediates DNA double-strand repair breaks.

BRCA1 gene mutation is responsible for 52% familial and rarely sporadic breast carcinomas (medullary carcinoma and metaplastic carcinoma). This is also associated with development of carcinoma of the ovary and prostate.

BRCA2 Tumor Suppressor Gene Mutation

BRCA2 gene is a tumor suppressor gene located on chromosome 13q12–13, which regulates DNA repair by binding to RAD51, a molecule that mediates DNA double-strand repair breaks. BRCA2 gene mutation is responsible for 32% familial breast carcinoma. BRCA2 gene mutation is rarely demonstrated in sporadic breast carcinoma.

TP53 Tumor Suppressor Gene Mutation

TP53 gene is tumor suppressor gene mapped on chromosome **17p13.1**. Approximately 5% of families with breast carcinoma have mutation of TP53 tumor suppressor gene.

Genetic Disorders and Breast Carcinoma

Women with genetic disorders are at increased risk for development of breast carcinoma. These are described as under.

Li-Fraumeni Syndrome

Germline mutations of TP53 tumor suppressor gene cause Li-Fraumeni syndrome. Patients may develop multiple human cancers such as breast carcinoma, leukemia, sarcoma, brain tumors, adrenal tumors, laryngeal and lung carcinoma.

Cowden Disease

PTEN gene is located on chromosome 10q. Phosphatase and tensin homologue are proteins of PTEN gene. Normally, PTEN gene products inhibit AKT/PI3K signaling pathway. PTEN gene mutation leads to Cowden disease, characterized by fibroadenomas, fibrocystic lesions, ductal epithelial hyperplasia, and nipple malformations. There is increased risk for breast carcinoma in Cowden disease.

Inherited Ataxia-telangiectasia

Under physiologic state, ATM gene is a caretaker tumor suppressor gene located on chromosome 11p22.

It downregulates tyrosine kinase activity. Patient with ATM gene mutation has reduced capacity to repair DNA breaks resulting in accumulation of numerous genetic mutations overtime. Patient develops inherited ataxia-telangiectasia characterized by cerebral ataxia, immunodeficiency and dilations of small blood vessels. There is increased risk for breast carcinoma, gastric carcinoma, leukemia and lymphomas in ataxia-telangiectasia.

Age Group

Advancing age is the second most important factor for breast carcinoma. Incidence of breast carcinoma increases with advancing age in women >30 years of age until menopause.

Longer Reproductive Span

Early menarche and late menopause increase duration of reproductive life and associated with increased hormonal activity. Prolonged exposure to endogenous estrogen increases risk for breast carcinoma.

Nulliparous Women

Nulliparous women are at greater risk for breast carcinoma. Women, who had their first child over 30 years of age, are at greater risk. Pregnant women are at lower risk for breast carcinoma.

Age of Women at First Pregnancy

Women, who had their first child over age 30, are at greater risk for breast carcinoma. An early age at birth of a second child further reduces the risk for breast carcinoma.

Obesity after Menopause

Postmenopausal obesity and increased dietary fat increases the risk for breast carcinoma due to aromatization of androstenedione to estrogens in adipose tissue.

Excessive Exposure to Estrogens

Postmenopausal estrogen therapy and oral contraceptive use increase the risk for breast carcinoma. Use of oral contraceptives for >4 years by younger women before their first trimester of pregnancy almost increases risk of breast carcinoma in postmenopausal women.

- Risk for breast carcinoma increases in women, who receive hormonal replacement therapy for a period of 10–15 years. Hormonal replacement therapy increases breast density, which make detection of breast carcinomas more difficult.
- Hormones via blood circulation interact either with nuclear estrogen receptors of breast terminal duct lobular units (TDLUs). The hormone–receptor interactions cause activation of DNA response elements leading to proliferation and differentiation of luminal cells leading to development of breast carcinoma.

Atypical Ductal Hyperplasia of Breast

Presence of atypical changes in ductal epithelium in fibrocystic disease of breast does increase the risk for breast carcinoma.

History of Breast Carcinoma in Opposite Breast

History of breast carcinoma one side of breast is associated with increased incidence of breast carcinoma involving opposite breast.

History of Endometrial Carcinoma

Women with history of endometrial or ovarian carcinoma are at increased risk for breast carcinoma.

Exposure to Diethylstilbestrol

Diethylstilbestrol (DES) was administered to some pregnant women in the United States to prevent miscarriage. Women, who took DES or whose mothers took DES while pregnant with them have a higher risk for getting breast carcinoma.

Radiation Exposure

During Second World War of radiation exposure, risk for breast carcinoma among teenage girls has been observed due to low level ionizing radiation later in life. Women who had prior radiation therapy to the chest or breast region for Hodgkin disease before the age of 30 years have a higher risk for breast carcinoma later in life.

Dietary Factors

Diet high in animal fat increases five times risk for breast carcinoma in the United States than in Japan.

Breastfeeding

Reducing period of breastfeeding increases risk for breast carcinoma.

Longer period of breastfeeding reduces the risk for breast carcinoma. Lactation suppresses ovulation and may trigger terminal differentiation of luminal cells.

Radical Scar in Breast

Radical scar is a benign lesion mimicking breast carcinoma

- There is increased risk for development of breat carcinoma.
- It is believed that most tubular carcinomas of breast arise from radical scar of >1 cm in size.
- Finding of a radical scar on a core biopsy is an indication for resection of the lesion.

MOLECULAR GENETIC ALTERATIONS

Approximately 12% of breast carcinomas occur due to inherited gene mutations. Most of the genes play interrelated roles in maintaining genome integrity (Tables 25.12 and 25.13).

- Loss of genes have been identified in relation to breast carcinoma. Mutations and abnormal amplification of both oncogenes and tumor suppressor genes play key roles in the pathogenesis of tumor initiation and progression. Hereditary syndromes associated with breast carcinoma are given in Table 25.14.
- Sporadic breast carcinomas are associated with estrogen hormone exposure, age of menarche, age of menopause, reproductive history, hormone replacement therapy and breastfeeding. Estrogen has been identified as promoter of breast caracinomas. Estrogen stimulates breast growth during puberty, menstrual cycles and pregnancy, thereby increasing breast cells potential to malignant change.

BRCA1 Tumor Suppressor Gene

BRCA1 is a tumor suppressor gene located on chromosome 17q21.

- **Physiologic state:** BRCA1 regulates DNA repair by binding to RAD51, a molecule that mediates DNA double-strand repair breaks.
- **Pathologic state:** BRCA1 gene mutation is responsible for 52% of familial and 1–2% of all breast caracinomas. Risk is increased in age group by 70 years. Mutation of BRCA1 gene occurs by methylation.

Table 25.12 Oncogenes associated with breast carcinomas

Category	Proto-oncogene	Mode of Activation	Associated Carcinomas
Growth factors			
Fibroblast growth factors	▪ FGF3 ▪ INT2	▪ Amplification ▪ Increased baseline activity	▪ **Breast carcinoma**, osteosarcoma, gastric carcinoma, urinary bladder carcinoma and melanoma ▪ **Breast carcinoma** and melanoma
Growth factor receptors			
EGF receptor	ERBB2 (HER2/neu ERBB2)	Amplification	**Breast carcinoma** (marker of aggressiveness)
Signal transduction proteins			
NOTCH signal transduction	NOTCH 1	Point mutation, translocation	**Breast carcinoma,** leukemia and lymphoma
Cyclin and cyclin-dependent kinase (cell cycle regulators)			
Cyclin-dependent kinase	CCND1 (cyclin D1)	Amplification	**Breast carcinoma,** multiple myeloma and esophageal cancer

Table 25.13 Tumor suppressor gene associated with breast carcinomas

Gene	Proteins	Function	Familial Tumors	Sporadic Carcinomas
Mitotic signaling pathway inhibitors				
PTEN	Phosphatase and tensin homologue	It inhibits AKT/PI3K signaling pathway	*Cowden syndrome* (carcinomas of breast, endometrium and thyroid gland)	Lymphoid neoplasms, carcinomas
Cell cycle progression inhibitors				
CDKN2A	P16/INK4a and P14ARF	P16 inhibits CDK. P14 indirectly activates p53 tumor suppressor gene	Familial melanoma	Breast carcinoma, pancreas carcinoma, and esophagus carcinoma
RB gene	Retinoblastoma protein (locus 13p14) mutation by deletion and nonsense	It codes for pRB protein, master brake on cell cycle. It inhibits G1 to S transition during cell cycle. It inhibits nuclear transcription factor	Retinoblastoma, osteosarcoma	Breast carcinoma, osteosarcoma, colon carcinoma, prostate carcinoma, urinary bladder carcinoma and lung carcinoma
Inhibitors of invasiveness and metastases				
CDH1	E-Cadherin	It participates in cell adhesion. It inhibits cell motility	Gastric carcinoma	Breast lobular carcinoma and gastric carcinoma

There is increased risk of breast and endometrial carcinomas in women on oral contraceptive therapy.

Table 25.14 Hereditary syndromes associated with breast carcinoma

Syndrome	Gene	Inheritance	Carcinomas	Other Features
Breast/ovarian carcinoma syndrome	BRCA1	Autosomal dominant	▪ Breast ▪ Ovary	Absent
Cancer syndrome	BRCA2	Autosomal dominant	▪ Breast ▪ Ovary ▪ Prostate ▪ Pancreas	Fanconi anemia in homozygotes
Li-Fraumeni syndrome	TP53	Autosomal dominant	▪ Breast ▪ Brain ▪ Leukemia ▪ Soft tissue sarcoma	Absent
Cowden syndrome	PTEN	Autosomal dominant	▪ Breast ▪ Ovary ▪ Colon ▪ Thyroid follicular carcinoma	▪ Thyroid adenoma ▪ GI polyps ▪ Leiomyomas
Peutz-Jeghers syndrome	STK-11	Autosomal dominant	▪ GIT ▪ Breast	▪ Hamartomas bowel ▪ Pigmentation buccal mucosa
Ataxia-telangiectasia	ATM	Autosomal dominant	Breast	▪ Leukemia ▪ Lymphoma ▪ Cerebellar ataxia ▪ Telangiectasia ▪ Immune deficiency
Muir-Torre syndrome	MSH-2/ MLH-1	Autosomal dominant	▪ Breast ▪ Colorectal region	Absent
Site specific	CHEK-2	Autosomal dominant	Breast	Low penetration

- BRCA1 gene mutation is rare in sporadic medullary carcinoma and metaplastic carcinoma of breast.
- BRCA1 gene mutation may occur in carcinomas of ovary, male and female breast, prostate, pancreas and fallopian tube; but less than due to BRCA2 gene mutation.

BRCA2 Tumor Suppressor Gene

BRCA2 is a tumor suppressor gene located on chromosome 13q12–13.

- **Physiologic state:** BRCA2 regulates DNA repair by binding to RAD51, a molecule that mediates DNA double-strand repair breaks.
- **Pathologic state:** BRCA2 gene mutation is rare in sporadic cases of breast carcinomas.

BRCA2 gene mutation also occurs in carcinomas of ovary, male and female breasts, prostate, pancreas, stomach, gallbladder, bile duct, pharynx and melanoma.

TP53 Tumor Suppressor Gene

TP53 is tumor suppressor gene located on chromosome 17p13.1.

- **Physiologic state:** TP53 gene product (p53 protein) halts cell cycle in G1 phase by inhibiting nuclear transcription factor, until the DNA is repaired, which activates BAX gene-induced cell suicide (apoptosis). The p53 protein interacts with at least 17 cellular and viral proteins.
- **Pathologic state:** TP53 is responsible for 3% of familial and 20% in sporadic breast carcinomas. There is increased risk for breast carcinoma in 90% of cases by the age of 70 years.

TP53 gene mutations are also demonstrated in cancers of pancreas (50–70%), lung, colon, and breast. TP53 gene mutation also occurs in **Li-Fraumeni syndrome** (breast carcinoma, sarcoma, leukemia, brain tumors and adrenal gland cancer).

CHEK2 Tumor Suppressor Gene

CHEK2 is a tumor suppressor gene located on chromosome 22q12.1.

- **Physiologic state:** CHEK2 protein induces cell cycle arrest and repairs damaged DNA. It activates BRCA1 and p53 tumor suppressors by phosphorylation.
- **Pathologic state:** Gene mutation of CHEK2 is responsible for 5% of familial as well as sporadic breast carcinomas. Due to CHEK2 gene mutation, there is increased risk for breast carcinoma in 10–20% of cases by 70 years of age especially after radiation exposure. It is worth mentioning that CHEK2 gene mutation is

also demonstrated in carcinomas of prostate, thyroid and kidney.

RB Tumor Suppressor Gene

RB is a gatekeeper tumor suppressor gene located on long arm of chromosome 13q14. Normal people have two alleles of the RB gene.

- **Physiologic state:** RB gene codes for pRB protein, master brake of cell cycle. Dephosphorylated pRB protein inhibits cell division. When the pRB gene is phosphorylated, cell division takes place.
- **Pathologic state:** RB gene mutation is demonstrated in breast carcinoma, familial/acquired retinoblastoma, and familial/acquired osteosarcoma. RB gene mutation is also demonstrated in carcinomas of colon, prostate, breast and urinary bladder.

Fibroblast Growth Factor 3 (FGF3)

- **Physiologic state:** FGF3 belongs to growth factor category. Cells have the ability to synthesize the same growth factors to which they are responsive, in generating an autocrine loop.
- **Pathologic state:** Amplification of FGF3 is associated with breast carcinoma, osteosarcoma, gastric carcinoma, urinary bladder carcinoma and melanoma.

INT2 Oncogene

- **Physiologic state:** INT2 belongs to growth factor category. Cells have the ability to synthesize the same growth factors to which they are responsive, generating an autocrine loop.
- **Pathologic state:** Increased baseline activity of INT2 has been demonstrated in breast carcinoma and melanoma.

ERBB2 (HER2/neu) Gene

- **Physiologic state:** Human epidermal growth factor ERBB2 (HER2/neu) belongs to growth factor receptor family, which encodes for an epithelial growth factor receptor on the cell membrane that delivers continuous mitogenic signals to the cells.
- **Pathologic state:** Amplification of ERBB2 (HER2/neu) delivers continuous mitogenic signals to the neoplastic cells of breast carcinoma even in the absence of growth factor in the environment. It is most often observed in ductal carcinoma and rarely in lobular carcinoma of breast.
- **Immunohistochemistry technique:** ERBB2 (HER2/neu) is demonstrated by immunohistochemistry technique on the cell membrane or using fluorescent *in situ* hybridization (FISH). It is marker of breast carcinoma associated with aggressive behavior.

NOTCH1 Gene

- **Physiologic state:** NOTCH 1 gene belongs to NOTCH signal transduction protein category.
- **Pathologic state:** Point mutation or translocation of NOTCH 1 gene is linked to breast carcinoma, leukemia and lymphomas.

RAS (from Rat Sarcoma Virus) Gene

- **Physiologic state:** RAS oncogene activates several intracellular signal transduction pathways and synthesis of transcription factors 'Fos' and 'Jun'.
- **Pathologic state:** RAS gene mutation is demonstrated in breast carcinoma, pancreatic carcinoma, lung carcinoma and colorectal carcinoma.

c-Myc Oncogene (Nuclear Transcription Factor)

- **Physiologic state:** c-Myc is nuclear regulatory protein that controls the expression of several genes.
- **Pathologic state:** Mutation of c-Myc is demonstrated in breast carcinoma, colorectal carcinoma and gastric carcinoma.

BCL-1 Cell Cycle Regulatory Gene

- **Physiologic state:** BCL-1 gene codes for cyclin D1, acts as stimulatory protein of the cell cycle.
- **Pathologic state:** Mutation of BCL-1 gene causes loss of regulation of cell cycle resulting in breast carcinoma.

Cyclins and Cyclin-dependent Kinase

- **Physiologic state:** CCND1 and cyclin D1 are cell cycle regulators.
- **Pathologic state:** CCND1 and cyclin D1 are activated by translocation and amplification. Deregulation of cyclins due to mutation is associated with breast carcinoma.

E-Cadherins

- **Physiologic state:** E-cadherins belong to CDH1 category, which are inhibitors of invasiveness and metastases of breast lobular carcinoma.
- **Pathologic state:** E-cadherins are irreversibly lost in invasive lobular breast carcinoma, but expressed in invasive ductal breast carcinoma. Germline and somatic mutations of E-cadherin also play an important role in the pathogenesis of gastric carcinoma (diffuse type).

PTEN Tumor Suppressor Gene

- **Physiologic state:** PTEN gene is located on chromosome 10q, which inhibits mitogenic signaling pathway.
- **Pathologic state:** PTEN gene mutation is associated with **Cowden disease** characterized by fibroadenoma,

Table 25.15 Molecular subtypes of breast carcinoma on the basis of DNA microarray

Molecular Subgroup and Frequency	Luminal A Breast Carcinoma (50%)	Luminal B Breast Carcinoma (20%)	HER2/neu Enrich Breast Carcinoma (15%)	Triple Negative Basal Type Breast Carcinoma (up to 15% in African Women)
ER	+++	+	–	–
PR	+	+	–	–
HER2/neu	–	–/+	++	–
Proliferation index	Low	Moderate	High	Very high
Distant metastases	Bones, viscera and brain	Bones, viscera and brain	Bones, viscera and brain	Bones, viscera and brain
Gene mutations	GATA-3, LIV-1, CCND-1, FOX-1	–	Amplification of gene located on 17q12	Basal epithelial genes, TP53, BRCA1
Others	CK8/18 (cytokeratin)	CK8/18 (cytokeratin)	Androgen receptors	CK5/6, CK14, vimentin, EGFR, c-KIT
Ki-67	+ (≤10%)	+ (≥30%)	+ High ≥20%	+ (≥20%)
CD20	+	+	+	+
Prognosis	Excellent (recurrence low)	Intermediate (recurrence high)	Poor (recurrence high)	Poor (recurrence high)
Treatment	Hormonal therapy	Hormonal + chemotherapy	Trastuzumab (Herceptin) and anthracyclines	Platinum- and anthracycline-based chemotherapy, PARP inhibitors

PARP: Poly (adenosine diphosphate-ribose) polymerase, ER (estrogen), PR (progesterone).
Histologic variants of luminal A: Low-grade invasive ductal carcinoma (NOS), classic lobular, tubular and cribriform carcinomas.
Histologic variants of luminal B: Invasive ductal carcinoma and micropapillary carcinoma.
Histologic variants of HER2/neu enrich: Invasive ductal carcinoma, NOS (not otherwise specified).
Histologic variants of basal type: Invasive ductal carcinoma, NOS (not otherwise specified), medullary and metaplastic carcinomas.
Patients with breast carcinoma with Ki-67 >20% show poorest prognosis.

fibrocystic disease of breast, ductal epithelial hyperplasia, nipple malformations, breast carcinoma and endometrial carcinoma.

Galectin 3 Encoded by 4GALS3 Gene

- **Physiologic state:** Galectin 3 is a member of lectin family and encoded by LGALS3 gene located on chromosome 14, locus q21–q22. Galectin 3 is expressed in the nucleus, cytoplasm and extracellular space.
- **Pathologic state:** Galectin 3 overexpression promotes neoplastic transformation and the maintenance of transformed phenotypes as well as enhances adhesion of malignant tumor cells to the extracellular matrix in breast carcinoma.

MAJOR MOLECULAR PATHWAYS: EVOLUTION OF BREAST CARCINOMA

Aims of molecular profiling of breast carcinoma to study the patient's risk for stratification, specific treatment modality, reduction therapy-induced toxicity and ensurig high chance of survival.

- In a simplified approach, some authors used ER, PR, ERBB2 (HER2/neu) plus Ki-67 to classify breast carcinoma into four molecular clusters.
- Recently, breast carcinoma is classified based on expression of estrogen (ER), progesterone (PR) and human epidermal growth factor (HER2/neu) markers with or without the addition of molecular gene signatures. Subtypes of breast carcinoma have different biology and prognosis with accumulating evidence of risk factors.
- Proposed molecular subtypes of breast carcinoma on the basis of DNA microarray include as mentioned in Table 25.15.

Molecular Subtypes of Breast Carcinoma

Luminal 'A' Breast Carcinoma

- ER/PR positive
- Ki-67 proliferative activity ≤10%
- Low-grade tumor
- Prognosis good

Luminal 'B' Breast Carcinoma

- ER/PR positive
- ERBB2/HER2/neu expression in some cases
- Ki-67 proliferative activity ≥20%
- Relatively high-grade tumor
- Prognosis is not good.

Triple Negative Basal-like Breast Carcinoma
▪ ER/PR negative ▪ ERBB2/HER2/neu negative ▪ Also called triple negative breast carcinoma (TNBC) ▪ Most BRCA1 breast carcinomas are basal-like TNBC ▪ High-grade tumor ▪ Prognosis worse.
ERBB2/HER2/neu Enriched Breast Carcinoma
▪ ER/PR positive/negative ▪ ERBB2/HER2/neu amplification seen ▪ Most common type of breast carcinoma with germline TP53 gene mutations occurring in Li-Fraumeni syndrome ▪ Ki-67 proliferative activity ≤14% ▪ High-grade tumor ▪ Prognosis poor
Normal Breast-like Breast Carcinoma
▪ ER/PR often negative ▪ ERBB2/HER2/neu often negative ▪ Claudin low breast carcinoma ▪ Low expression of cell to cell junction proteins, i.e. E-cadherin ▪ Tumor showing infiltration by lymphocytes ▪ Prognosis poor

ESTROGEN POSITIVE MOLECULAR PATHWAY (LUMINAL A AND LUMINAL B)

Estrogen (ER) positive breast carcinomas are termed luminal A and luminal B breast carcinomas, which resemble normal breast luminal cells in terms of their mRNA expression regulated by estrogen. Breast carcinoma occurs due to inheritance of germline BRCA2 gene mutation associated with gain of 1q chromosome, loss of 16q. ER positive pathway is the most common molecular pathway, which expresses estrogen receptors (Figs 25.28 and 25.29).

Luminal 'A' Breast Carcinoma

Luminal A breast carcinoma is low-grade tumor with low proliferative activity Ki-67 ≤10% seen in <50% of cases.

- Recurrence rate of breast carcinoma is low.
- Immunohistochemistry shows ER/PR positivity. ERBB2 (HER2/neu) expression is negative.
- Prognosis is excellent, because these patients respond to endocrine therapy, i.e. estrogen blocking drug tamoxifen and aromatase inhibitors.

Luminal 'B' Breast Carcinoma

Luminal B breast carcinoma is relatively high-grade tumor with high proliferative activity Ki-67 ≥30 than luminal A tumor.

- Immunohistochemistry technique reveals low expression of ER/PR in breast carcinoma.

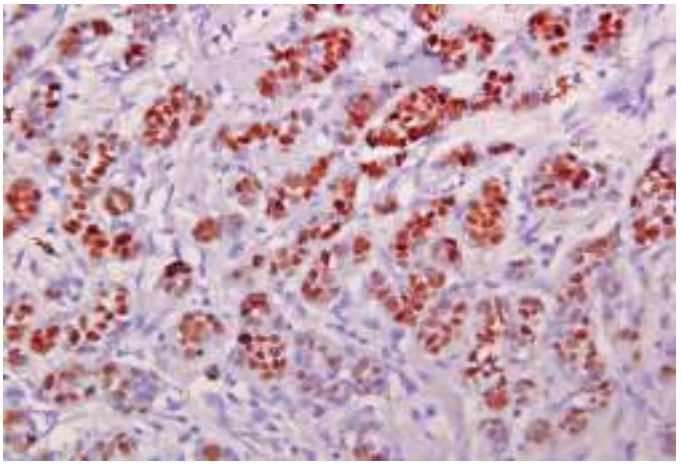


Fig. 25.28: Breast carcinoma—infiltrative duct carcinoma shows estrogen nuclear receptor positivity. Hormone therapy with tamoxifen is used to block the effects of estrogen that may otherwise help breast carcinoma cells to survive and grow. Most women with breast carcinomas expressing estrogen or progesterone benefit from treatment with tamoxifen (400X).

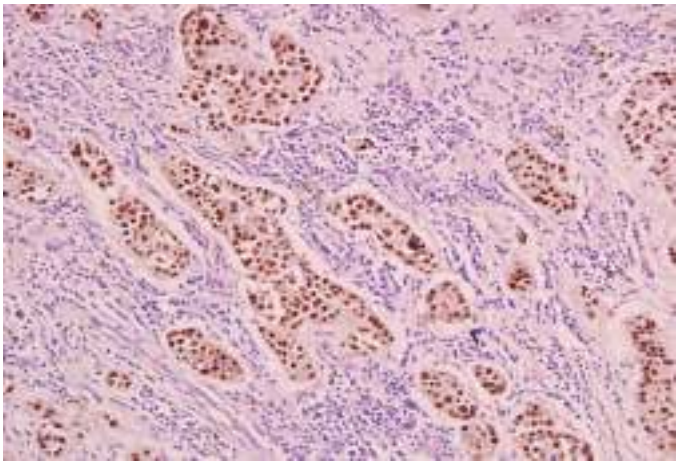


Fig. 25.29: Breast carcinoma—infiltrative duct carcinoma shows progesterone receptor nuclear positivity (100X).

- ERBB2 (HER2/neu) expression in tumor cells may be positive or negative in breast carcinoma.
- Prognosis may not be as good as in luminal A breast carcinoma.

Pathology Pearls: Sequence of Events: Luminal 'B' of Breast Carcinoma
▪ **Development of flat lesion:** Patient develops flat lesion in breast as a result of gain of 1q chromosome and loss of 16q. ▪ **Progression to breast carcinoma:** Due to mutation of PIK3CA gene, flat lesion progresses to atypical hyperplasia of breast, ductal carcinoma *in situ* and invasive breast carcinoma. PIK3CA gene product encodes phosphoinositide-3 kinase (PI3K), major component of signal pathway, down regulating growth factor receptor.

Immunohistochemistry Technique		
▪ Immunohistochemistry technique of luminal type A breast carcinoma shows positivity for ER/PR and cytokeratin (8;18). ▪ Immunohistochemistry technique of luminal type B breast carcinoma shows low expression of ER/PR. Tumor cells are immunoreactive to cytokeratin (8;18).		
Markers	**Luminal type A**	**Luminal type B**
▪ Estrogen receptor (ER)	+++	+
▪ Progesterone receptor (PR)	++	+
▪ HER2/neu	–	–/+
▪ Cytokeratins (8;18)	+	+

HER2/NEU ENRICHED MOLECULAR PATHWAY

HER2/neu is located on chromosome 17q. Amplification of HER2/neu is responsible for 20% cases of breast carcinomas. It is the most common type of breast carcinoma in patient with germline mutations in TP53 gene (Li-Fraumeni syndrome). Patient develops atypical ductal hyperplasia progressing to ductal carcinoma *in situ* resulting in invasive breast carcinoma (Fig. 25.30).

Clinical Course

HER2/neu enriched invasive ductal carcinoma is high-grade tumor with higher proliferative rate and poor prognosis. It most often shows involvement of axillary lymph nodes.

Pathology Pearls: HER2/neu Enriched Molecular Pathway	
Immunohistochemistry Technique	
Immunohistochemistry technique of HER2/neu enriched molecular pathway of breast carcinoma shows HER2/neu positivity. Tumor cells may be ER positive/negative.	
Markers	**Expression**
▪ HER2/neu ▪ Estrogen receptor (ER)	▪ Positive ▪ Positive or negative

Treatment

These patients with HER2/neu enriched invasive ductal carcinoma respond to Herceptin and anthracycline based chemotherapeutic agents.

ER, PR AND HER2/NEU TRIPLE NEGATIVE BASAL-LIKE MOLECULAR PATHWAY

Triple negative molecular basal-like pathway is least understood pathway due to inheritance of BRCA1 gene mutation responsible for 15% of breast carcinomas. These tumors have a basal-like pattern of mRNA expression present in normal myoepithelial cells.

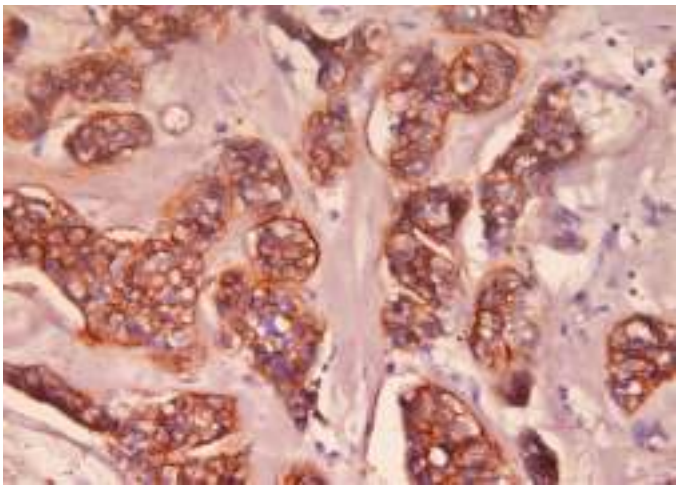


Fig. 25.30: Breast carcinoma. Infiltrative ductal carcinoma of breast shows HER2/neu membrane positivity. These patients are treated with Herceptin (anti-HER2/neu monoclonal antibody) (400X).

- Basal cell type of breast carcinoma has high proliferative rate with positive lymph nodes and high recurrence rate. TP53 gene mutation is very common.
- These breast carcinomas show high expression of basal epithelial genes and TP53 gene mutations, and dysfunctional BRCA1 gene.
- Ki-67 is expressed in <14% cases of triple negative breast carcinomas.
- Triple negative breast carcinomas (TNBCs) are associated with a younger age at clinical presentation, higher-grade tumor and poor prognosis.

Progression of Ductal Carcinoma *in situ* (DCIS) to Invasive Ductal Breast Carcinoma

TP53 gene mutation and inactivation of BRCA1 directly cause ductal carcinoma *in situ* progressing to invasive breast carcinoma. These breast carcinomas are triple negative, i.e. ER/PR and HER2/neu negative.

Treatment

The patients with triple negative breast carcinoma do not respond to endocrine therapy (tamoxifen), aromatase inhibitors or Herceptin. Prognosis is poor. Trials are going onto treat these patients with platinum-based chemotherapy or poly (ADP-ribose) polymerases (PARPs) inhibitors.

NORMAL BREAST-LIKE MOLECULAR PATHWAY

Normal breast-like breast carcinoma lacks the expression of ER, PR and HER2/neu and can also be classified as triple negative but it is not considered to be basal-like breast carcinoma as it is negative for CK5 and EGFR. There are few studies on this subtype and its clinical significance remains under determined.

BREAST CARCINOMA: HISTOLOGIC VARIANTS

More than 90% of breast carcinomas arise in ducts or lobules as carcinoma *in situ* (Figs 25.31 and 25.32). When malignant epithelial cells invade the basement membrane and extends in surrounding tissue, it is known as invasive ductal carcinoma. Ductal carcinoma *in situ* (DCIS) is associated with the development of small calcified deposits in the breast demonstrated by mammography. Differentiating features of invasive ductal carcinoma versus invasive lobular carcinoma of breast are given in Table 25.16.

DUCTAL CARCINOMA: BASIC CONCEPT

Ductal Carcinoma *in situ* of Breast

Ductal carcinoma *in situ* (DCIS) of breast is a neoplastic proliferation of mammary ductal epithelial cells confined to the ductal–lobular system without evidence of invasion through basement membrane into the surrounding stroma. Microcalcification on mammography raises concern and need for obtaining biopsy. Diagnosis is confirmed on histologic examination of tissue obtained via needle core biopsy, lumpectomy or mastectomy.

Ductal carcinoma *in situ* has two histologic variants: comedocarcinoma and noncomedocarcinoma (Table 25.17). Tumors may be low, intermediate and high-grades. Low-grade DCIS shows monomorphic cells looking like normal breast cells with small size nuclei. High-grade DCIS is composed of pleomorphic neoplastic cells with vesicular hyperchromatic nuclei and prominent nucleoli. Intermediate-grade DCIS has intermediate features.

- **Comedocarcinoma:** Comedocarcinoma is high-grade ductal carcinoma *in situ* of breast and composed

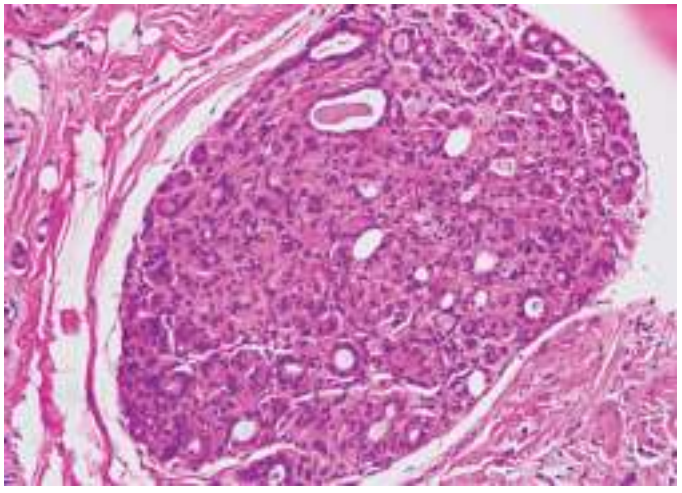


Fig. 25.31: Comedocarcinoma breast. It is high-grade ductal carcinoma *in situ* with highly pleomorphic tumor cells present within and distending the duct space, associated with central comedonecrosis (cheesy material) (400X).

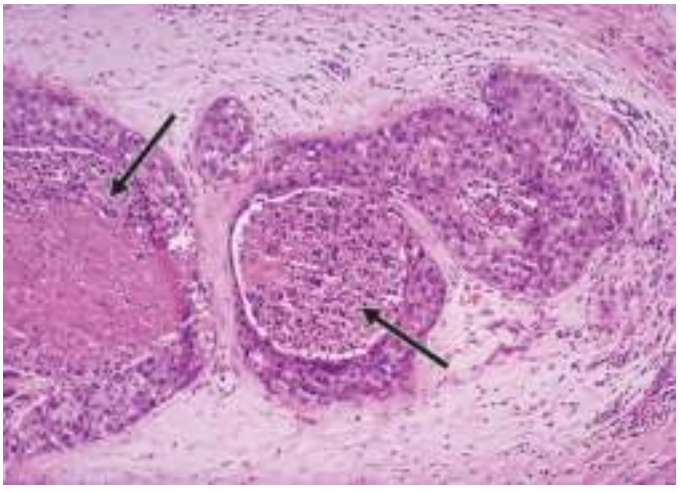


Fig. 25.32: Breast comedocarcinoma. Breast comedocarcinoma is high-grade ductal carcinoma *in situ* (DCIS) that demonstrates comedonecrosis (arrows), which is the central necrosis of cancer cells within involved ducts (400X).

Table 25.16 Differentiating features of invasive ductal carcinoma versus invasive lobular carcinoma of breast

Features	Invasive Ductal Carcinoma	Invasive Lobular Carcinoma
Relative frequency	▪ 80–85% ▪ Usually unifocal involving unilateral breast	▪ <10% ▪ Multifocal and involving bilateral breasts
Pattern of infiltration	Neoplastic cells arranged in nests/sheets	Neoplastic cells arranged in single 'Indian file pattern'/tagetoid pattern
Intervening stroma	Often scant	Extensive
Tubule formation	Present	Absent
Cell size	Moderate to large	Usually small
Signet ring cells	Rare	Frequent
Associated lesions	Ductal carcinoma *in situ*	Lobular carcinoma *in situ*

Presence of in situ component is not essential for diagnosis. Some tumors may show both elements of invasive ductal carcinoma as well as invasive lobular carcinoma.
Mutations in BRCA1 gene located on 17q21 and BRCA2 gene located on 13q12-13 are responsible for familial early onset breast carcinoma.
Patient with HER2/neu oncogene amplification is associated with poor prognosis.

Table 25.17 Comparison of comedocarcinoma and noncomedocarcinoma

Characteristics	Comedocarcinoma	Noncomedocarcinoma
Nuclear grade	High	Low
Estrogen receptor	Negative	Positive
ERBB2/HER2/neu overexpression	Present	Absent
Distribution	Continuous	Multifocal
Necrosis	Persent	Absent
Local recurrence	High	Low
Prognosis	Worse	Favorable

of highly pleomorphic tumor cells present within and distending the duct space, associated with central comedonecrosis (cheesy material), which may show lymph node metastases. The ducts are lined by pleomorphic neoplastic cells with hyperchromatic irregular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm. Tumor cells are arranged in solid pattern with central necrosis in the ducts. The necrotic debris may undergo dystrophic calcification. The tumor cells incite chronic inflammatory cell infiltration and fibroblastic response in the surrounding stroma.

- **Noncomedocarcinoma:** Noncomedocarcinoma of breast is ductal carcinoma *in situ* (DCIS) without central necrosis. Malignant cells have regular outlines and small nuclei in contrast to comedocarcinoma. Tumor cells may be arranged in solid pattern or cribriform (multiple lumens within duct) or micropapillary pattern with delicate fibrovascular cores lined by tumor cells.

Invasive Ductal Carcinoma of Breast

Invasive ductal carcinoma is most common breast carcinoma constituting 65–80% of all breast carcinomas. Tumor breaches basement membrane and invades the normal breast at random. Its precursor lesion is ductal carcinoma *in situ*. Its architecture ranges from tubule formation to solid sheets (Fig. 25.33).

LOBULAR CARCINOMA: BASIC CONCEPT

Lobular carcinoma is most common breast carcinoma in the terminal duct lobular unit (TDLU). Tumor is often bilateral and multifocal in 20–50% of cases. On light microscopy, lobular carcinoma of breast may be classified as *in situ* or invasive types.

Lobular Carcinoma *in situ* (LCIS) of Breast

Lobular carcinoma *in situ* of breast is considerably less common (1%) than ductal carcinoma *in situ* (DCIS). It is characterized by distension of the terminal lobules by small- to intermediate-sized cells with a few mitoses. Approximately untreated 20–30% of cases, patients with lobular carcinoma *in situ* develop invasive lobular carcinoma (Fig. 25.34).

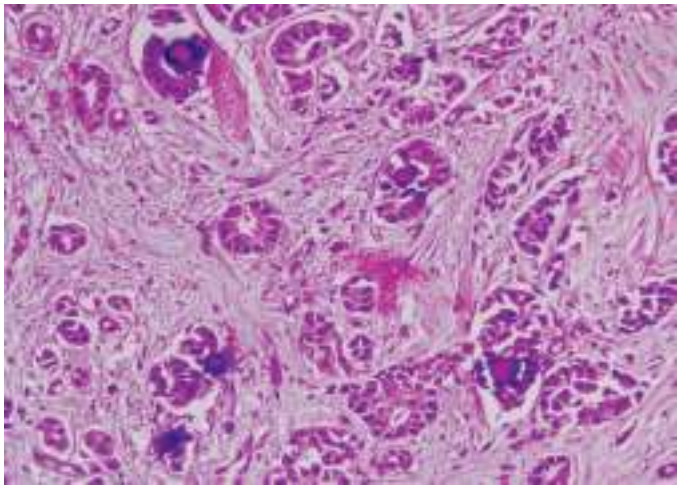


Fig. 25.33: Histgology of breast invasive ductal edenocarcinoma. Tumor is composed of atypical pleomorphic cells with hyperchromatic nuclei and arranged in glandular structures, clusters, and sheets invading surrounding connective tissue stroma of breast and microcalcifications (400X).

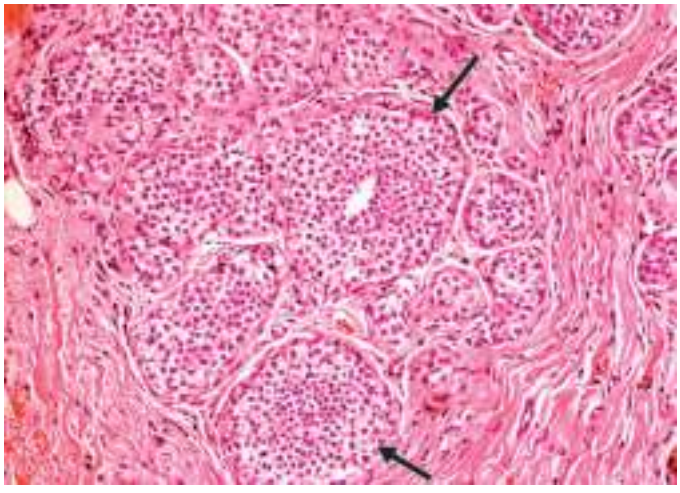


Fig. 25.34: Lobular carcinoma in situ of the breast. Tumor is composed of loosely cohesive uniform population of cells and within the acini (arrows) with indistinct cell borders and pale cytoplasm, small uniform nuclei, evenly distributed chromatin, and inconspicuous nucleoli (400X).

Invasive Lobular Carcinoma of Breast

Invasive lobular carcinoma is less common breast carcinoma constituting 5–10% of cases. Its precursor lesion is lobular carcinoma *in situ*. Although microcalcifications are uncommon, yet mammography detects the lesion. It is luminal A type of breast carcinoma, which shows low proliferative rate. Recurrence rate is low. Immunohistochemistry of invasive lobular carcinoma shows positivity for ER/PR and cytokeratin (8;18). These patients respond to tamoxifen, aromatase inhibitors and Herceptin associated with good prognosis.

Molecular Genetic Alterations

Amplification of ERBB2 (HER2/neu) is rarely detected in noninvasive or invasive lobular carcinoma of breast by immunohistochemistry on the cell membrane or using fluorescent *in situ* hybridization (FISH) technique.

Surgical Pathology: Lobular Carcinoma of Breast

Gross Morphology

Lobular carcinoma *in situ* of breast is rubbery poorly circumscribed, firm to hard tumor or not readily palpable.

Light Microscopy

- Classic variant of invasive lobular carcinoma of breast comprises small- to moderately-sized atypical cells that lack cohesion.
- Neoplastic cells are individually dispersed singly in linear fashion 'single Indian file pattern' through fibrous tissue around preexisting benign ducts.
- Presence of mucin in malignant cells pushes the nucleus against the membrane creating signet ring appearance (Fig. 25.35).

Histologic Variants

Lobular invasive carcinoma of breast shows five histologic types: classic, alveolar (aggregates of more than 20 cells), tubuloalveolar (cells in cords and microtubules), pleomorphic, solid (cells arranged in diffuse sheets) and mixed (70% cells in Indian file pattern) variants.

Immunohistochemistry Technique

Tumor cells show ER, PR and cytokeratin (8;18) positivity in all cases of invasive lobular carcinomas of breast. In addition, a substantial number of these tumors is reactive for CEA.

Markers	Expression
Estrogen receptor (ER)	Positive
Progesterone receptor (PR)	Positive
Cytokeratin (8;18)	Positive
Carcinoembryonic antigen (CEA)	Positive

Clinical Correlation

Patient with invasive lobular carcinoma of breast presents with bilateral multiple breast lumps in 20–50% of cases. Tumor may spread to axillary lymph nodes and distant organs such as lungs, liver, and bone, cerebrospinal fluid, ovaries (Krukenberg tumor) and uterus.

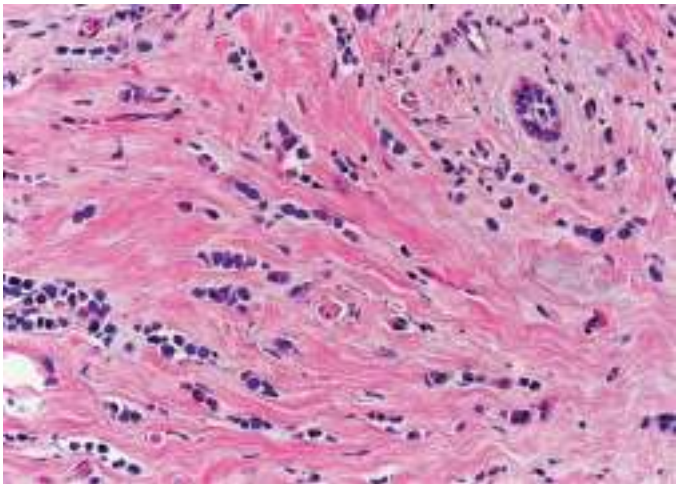


Fig. 25.35: Classic invasive lobular carcinoma of the breast. It shows poorly cohesive uniform round cells infiltrating into surrounding stroma and arranged in rows or in Indian file pattern (400X).

Management

Management of invasive lobular carcinoma of breast depends on stage with treatment similar to that with invasive ductal carcinoma. These patients respond well to tamoxifen, aromatase inhibitors and Herceptin.

INVASIVE DUCTAL CARCINOMA (NOS) OF BREAST

Invasive ductal carcinoma (not otherwise specified) is commonest type of breast carcinoma. Most invasive ductal carcinomas may evoke a dense fibroblastic response (proliferation of fibroblasts producing collagen fibers) in the host tissue that adds to the lesion's bulk.

Tumor replaces normal breast adipose tissue. Prognosis is stage dependent. Tumor has firm to hard in consistency (schirrhous), and chalky white discoloration due to desmoplasia. Nipple retraction due to desmoplasia in an underlying advanced disease is a late feature of invasive ductal carcinoma of breast.

Clinical Features

Patient with invasive ductal carcinoma (NOS) of breast presents with poorly defined solitary, nontender breast lump of variable consistency and mobility with nipple retraction. Tumor is solid or hard, dense and fixed to the underlying tissues or skin due to invasion of breast carcinoma.

Nipple Retraction

A recent nipple retraction suggests breast carcinoma, which causes fibrosis of the whole duct system and pulls

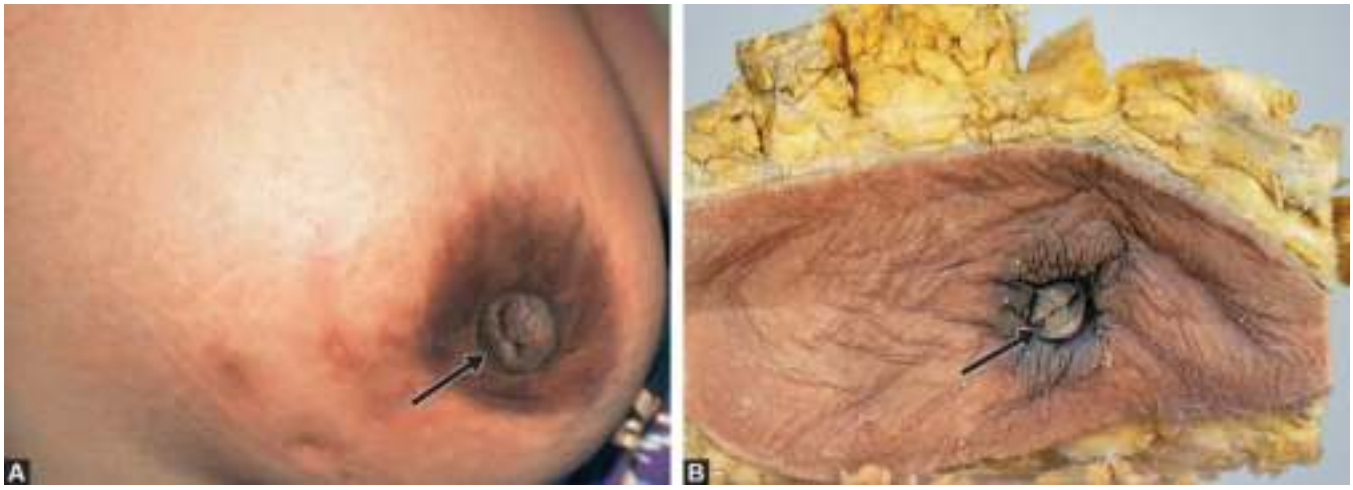


Fig. 25.36: Breast carcinoma. (A) Photograph shows a tumor that has caused nipple retraction (arrow), (B) mastectomy specimen shows nipple retraction (arrow). (*Courtesy*: Department of Pathology, Dr. DY Patil Medical College, Pune, Maharashtra.)

in the nipple, known as retraction of the nipple. Fibrosis also contracts the suspensory ligament, that pulls the nipple inward is known as dimpling (also called a skin tether) (Fig. 25.36A and B).

Overlying Skin

The '**peau d'orange**' (skin edema) appearance of the breast skin occurs due to obstruction of the dermal lymphatics by breast carcinoma cells (Fig. 25.37).

Surgical Pathology: Invasive Ductal Carcinoma (NOS) of Breast

Gross Morphology

- Tumor is firm to hard, irregular, and fibrous extension into the adjacent breast stroma that create stellate (crab-like) outline measuring 1–4 cm (rarely 4–5 cm).
- Cut section reveals gray chalky white tissue yellow crab-like satellites extending into the yellow fat tissue.
- Skin surface may show retraction of nipple (Fig. 25.38A and B).

Light Microscopy

- Tumor is composed of poorly differentiated pleomorphic neoplastic cells with hyperchromatic nuclei and abundant mitoses.
- Tumor cells are arranged in irregular nests and cords or glandular structures within a dense fibrous stroma.
- Tumor cells invading lymphatic channels, blood vessels and perineural spaces may be seen.
- Microcalcifications may be demonstrated.

Immunohistochemistry Technique

Panel of markers is used on paraffin-embedded tissue sections for invasive ductal carcinoma, which include ER/PR, gross cystic disease fluid protein 15 (GCDFP-15), mammaglobin, HER2/neu, CK7, Ki-67, p53, E-cadherin and N-BRI mentioned below. E-cadherin is expressed by invasive ductal carcinoma. E-cadherin expression is absent in lobular carcinoma of breast.

Markers	Expression
▪ ER/PR	▪ Positive (60–75%)
▪ GCDFP-15	▪ Positive (50%)
▪ Mammaglobin	▪ Positive (50%)
▪ HER2/neu	▪ Positive (10–30%)
▪ CK7	▪ Positive
▪ Ki-67	▪ Positive
▪ p53	▪ Positive
▪ E-cadherin	▪ Positive
▪ NYBRI	▪ Positive (specific in undifferentiated carcinoma)

E-cadherin expression is positive in invasive ductal carcinoma but absent in lobular carcinoma.

Molecular Pathways of Breast Carcinoma

The current molecular classification divides breast carcinoma into five groups as luminal A, luminal B, HER2/neu, basal-like and normal breast-like. The three



Fig. 25.37: Breast carcinoma—peau d'orange appearance and nipple retraction. Peau d' orange appearance occurs due to obstruction of dermal lymphatics by tumor cells.

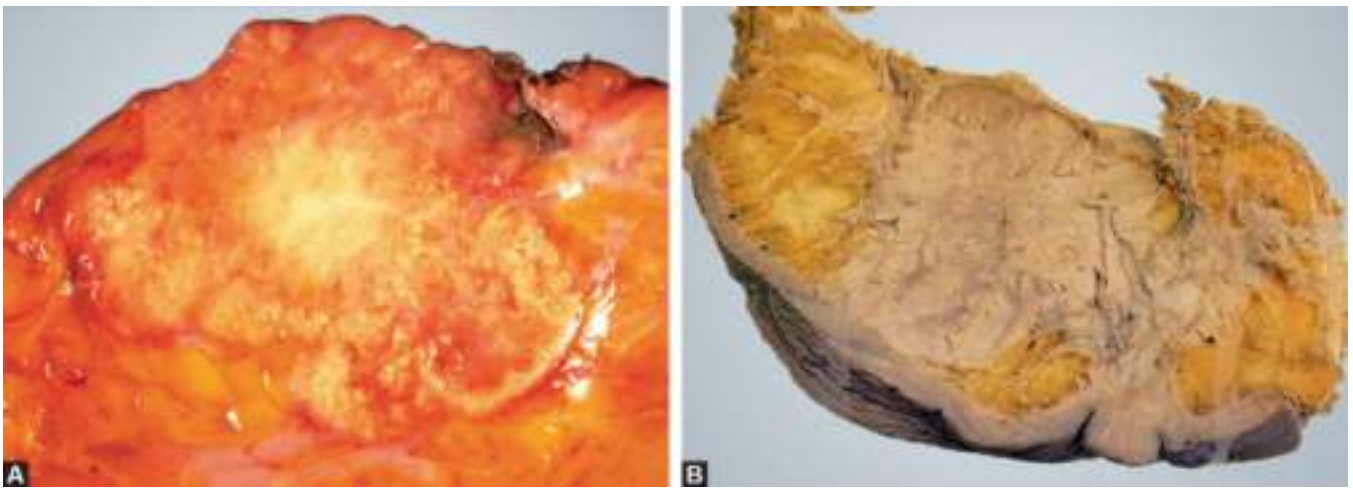


Fig. 25.38A and B: Invasive ductal carcinoma of breast. Cut surface shows gray white tumor with infiltrating margins.

main subtypes of breast carcinoma are: (a) hormone receptor positive breast carcinoma, (b) HER2/neu positive breast carcinoma and (c) triple negative breast carcinoma.

- **Luminal type A breast carcinoma:** It is low-grade breast carcinoma seen in <50% of cases. It shows low proliferative rate (Ki-67 ≤10%). Recurrence rate is low. Prognosis is good.
- **Luminal type B breast carcinoma:** It is relatively high-grade breast carcinoma seen in <15% of cases. It has higher proliferative rate (Ki-67 ≥30%) than luminal A type. Recurrence is high. Prognosis may not be as good as in luminal type A breast carcinoma.
- **HER2/neu enrich breast carcinoma:** It is high-grade breast carcinoma with high proliferative rate Ki-67 seen in <20% of cases. It most often shows involvement of axillary lymph nodes. These patients respond to Herceptin- and anthracycline-based chemotherapeutic agents.
- **Basal-like breast carcinoma:** It is high-grade variant of invasive ductal carcinoma with high proliferative rate with positive lymph nodes. Recurrence rate is high. TP53 gene mutation is very common. It shows dysfunction of BRCA1 gene. Ki-67 is expressed in <14% of cases. On immunohistochemistry, it is triple negative, i.e. ER, PR and HER2/neu negative. These patients do not respond to endocrine therapy (tamoxifen), aromatase inhibitors and Herceptin. Prognosis is poor. Trials are going onto treat these patients with platinum-based chemotherapy or poly ADP-ribose polymerase (PARP) inhibitors.
- **Normal breast-like breast carcinoma:** As it lacks expression of ER, PR and HER2/neu and can also be classified as triple negative but it is not considered to be basal-like breast carcinoma as it is negative for CK5 and EGFR. There are few studies on this subtype and its clinical significance remains undetermined.

Prognostic Implications

Tumor grade, DNA content and proliferative index, and presence of ERBB2 (HER2/neu) amplification also have prognostic implications.

Metastases

Tumor spreads to axillary lymph nodes via lymphatic route and then to lungs, liver and bones via hematogenous route.

Management

Management of invasive ductal carcinoma (NOS) of breast depends on tumor size, grade, metastases status to lymph nodes or distant organs, estrogen and progesterone receptor status. The patients are managed by surgery, irradiation, chemotherapy or tamoxifen or Herceptin.

PAGET'S DISEASE OF BREAST

Paget's disease of breast shows intraepidermal carcinoma cells in overlying epidermis of nipple and areola derived from an intraductal breast carcinoma. Patient presents with eczematous lesion with crusted, eroded surface over nipple and areola. Malignant cells in epidermis are known as '**Paget's cells**'. The tumor is most often poorly circumscribed. Paget's disease of breast is more frequently found in older women (Fig. 25.39A and B). Paget disease of breast is treated by radical mastectomy.

Etiopathogenesis

The synthesis of heregulin-α by keratinocytes, acts via the HER2/neu receptor, may play a role in its pathogenesis of Paget's disease of breast.

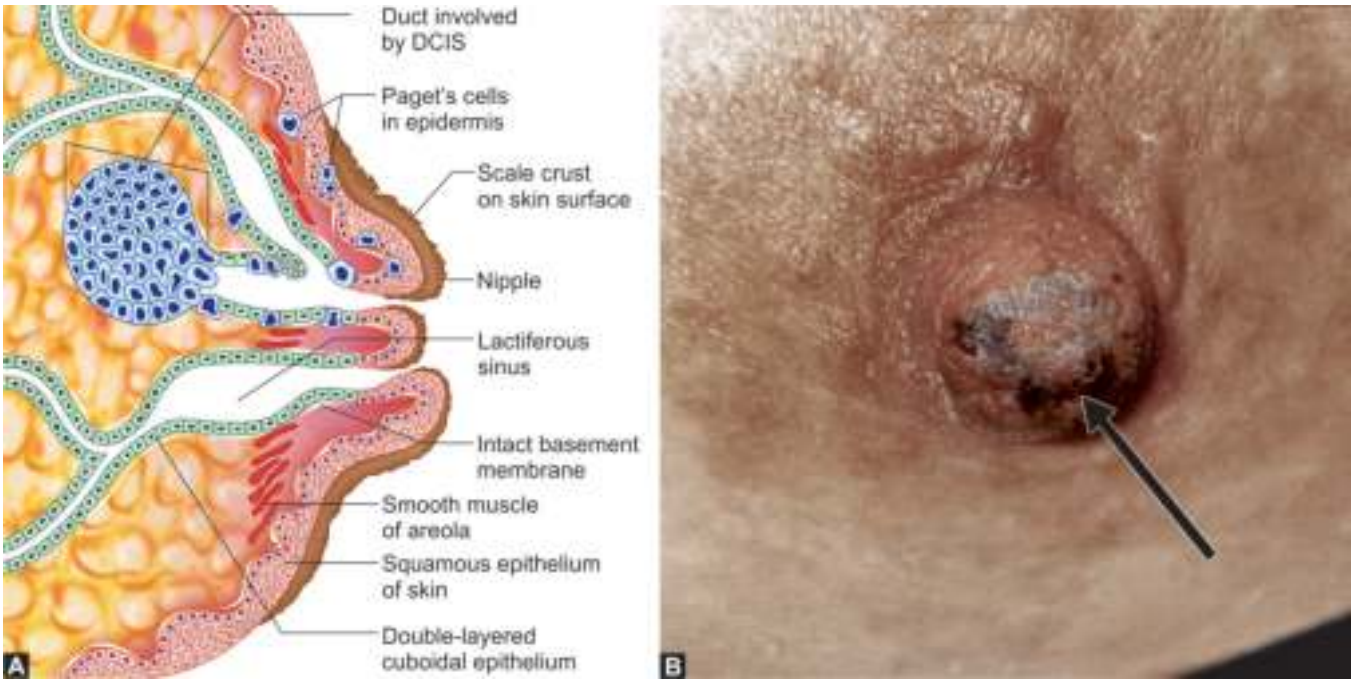


Fig. 25.39: Paget's disease of breast. (A) Structure of the adult female breast shows Paget's disease of the breast. Malignant cells infiltrating overlying epidermis are known as Paget's cells, (B) eczematous lesion with crusted, eroded surface over nipple and areola (arrow).

Molecular Genetic Alterations

Amplification of ERBB2 (HER2/neu) gene is observed in Paget's disease of breast, which encodes an epithelial growth factor receptor (EGFR) on the cell membrane that delivers continuous mitogenic signals to the cell resulting in breast carcinoma, i.e. Paget's disease of breast, even in the absence of growth factor in the environment. HER2/neu is demonstrated by monoclonal antibody on the cell membrane by using fluorescent *in situ* hybridization (FISH) technique.

Clinical Features

Paget's disease of the breast affects the nipple from the start, whereas eczema affects the areolar region first and only rarely affects the nipple skin, which eventually leads to erosion and ulceration of the nipple in elderly women. On clinical examination, a painless mass is palpable in the underlying breast in 50% of cases, and often associated with delay in its diagnosis.

Surgical Pathology: Paget's Disease of Breast

Gross Morphology

Nipple skin in Paget's disease of breast demonstrates scaling crust erythema and exudate resembling eczema. Nipple may show ulceration.

Light Microscopy

- Histologic examination of Paget's disease of breast reveals either *in situ* or invasive ductal carcinoma, which involves overlying epidermis of nipple and areola. The tumor cells in the overlying epidermis are called Paget's cells.
- Paget's cells within the surface epithelium of the nipple and areola are large neoplastic cells with abundant clear cytoplasm and large nuclei with prominent nucleoli, arranged singly or in clusters; represent intraepithelial extension of an underlying ductal carcinoma *in situ* or invasive ductal carcinoma (Fig. 25.40).

Histochemistry

Periodic acid–Schiff (PAS) stain demonstrates mucin within the Paget's cells of Paget's disease of the breast. This is evidence for their origin from an underlying ductal carcinoma.

Immunohistochemistry Technique

By immunohistochemical staining, Paget cells will also be cytokeratin (low molecular weight), HMB-45, CAM 5.2, and epithelial membrane antigen (EMA) positive.

Markers	Expression
▪ Cytokeratin (low MW)	▪ Positive
▪ HMB-45	▪ Positive
▪ CAM 5.2	▪ Positive
▪ Epithelial membrane antigen (EMA)	▪ Positive

Mammography

If Paget's disease of breast is suspected on clinical examination, mammography should be performed to determine if there is an underlying tumor.

Diagnosis

Diagnosis of Paget's disease of breast is established by cytology (e.g. smears from imprint and scrapping) or wedge biopsy of nipple.

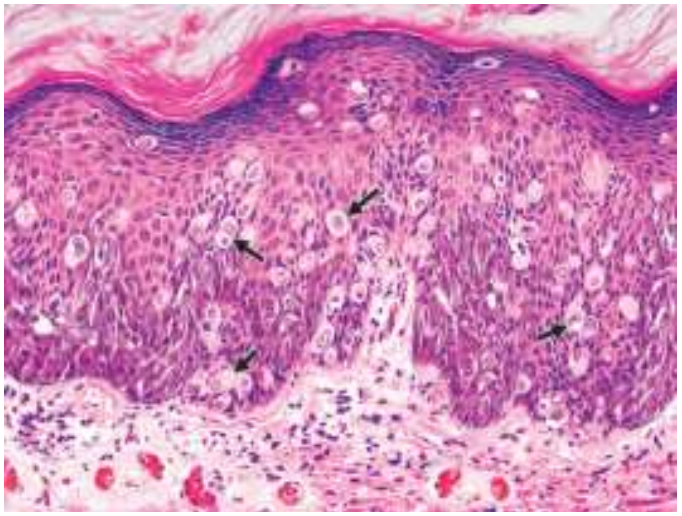


Fig. 25.40: Paget's disease of breast. The tumor cells in the overlying epidermis are called Paget's cells. These are large cells with abundant clear cytoplasm and large nuclei with prominent nucleoli, arranged singly or in clusters; represent intraepithelial extension of an underlying ductal carcinoma *in situ* or invasive ductal carcinoma (arrows) (400X).

Pathology Pearls: Toker Cells Versus Paget Cells in Differential Diagnosis

- Toker cells are epithelial cells with clear cytoplasm most often free of cytologic atypia localized within the nipple epidermis.
- Toker cells normally occur in small number within the nipple epidermis.
- Rarely, Toker cells can be so numerous and atypical as to require a careful distinction from malignant cells of Paget's disease of breast.
- Phenotype markers can be helpful in differential diagnosis.
- On immunohistochemical analysis, Toker cells show positivity for CK7 and ERBB2, while negativity for CD138 and p63.

INFLAMMATORY CARCINOMA OF BREAST

Inflammatory carcinoma of breast shows diffuse erythema and edema (peau d'orange) of the breast skin.

- Rapidly growing breast carcinoma metastasizing in the dermal lymphatic channels results in thickened, erythematous (red), rough, swollen, hot skin as a consequence of inflammatory process.
- Edema (peau d'orange) usually begins in the skin around and beneath the areola, the most dependent area of breast (Fig. 25.41).
- Prognosis is poor in inflammatory breast carcinoma. Patient is treated by chemotherapy followed by surgery and irradiation.

MEDULLARY CARCINOMA OF BREAST

Medullary carcinoma is uncommon breast carcinoma affecting young females. Patient presents with fleshy, bulky tumor measuring 5–10 cm in diameter. It is basal-like subtype of breast carcinoma. It has high proliferative rate with positive lymph nodes. Recurrence rate is high in these patients.



Fig. 25.41: Mastectomy specimen in a case of breast carcinoma. It shows inflammatory carcinoma of breast (arrow).

Molecular Genetic Alterations

BRCA1 gene mutations are observed in some cases. TP53 gene mutation is very common. Ki-67 is expressed in <14% of cases. Amplification of ERBB2 (HER2/neu) is not seen.

Surgical Pathology: Medullary Carcinoma of Breast

Gross Morphology

- The tumor is well circumscribed, with a soft, fleshy and uniform consistency with rounded pushing margins.
- There is no evidence of a fibrous reaction. It most often measures between 1 and 4 cm in diameter.

Light Microscopy

Medullary carcinoma of breast has following diagnostic features based on histology and gross morphology (Fig. 25.42):

- Tumor comprises pleomorphic atypical cells arranged in interconnecting sheets, forming a syncytial network. Cells contain hyperchromatic nuclei with scant to moderate cytoplasm arranged in sheets forming '**syncytial network**'. It shows large bizarre nuclei with high mitotic count.
- The intervening stroma is scant. There is presence of lymphocytes and plasma cells (immune response) around tumor cell groups (and not between individual tumor cells). Foci of necrosis and hemorrhage may be found.

Immunohistochemistry Technique

Medullary carcinoma of breast is triple negative for ER, PR and HER2/neu by immunohistochemistry technique.

Markers	Expression
▪ Estrogen receptor (ER) ▪ Progesterone receptor (PR) ▪ HER2/neu	▪ Negative ▪ Negative ▪ Negative

Treatment

Patients do not respond to tamoxifen, aromatase inhibitors and Herceptin. Trials are going onto treat these patients with platinum-based chemotherapy or PARP inhibitors.

Prognosis

Medullary carcinoma of breast has much better prognosis than usual invasive ductal carcinoma even with lymph node metastases. Approximately 70% of patients have overall 10-year survival.

COLLOID (MUCINOUS) CARCINOMA OF BREAST

Colloid (mucinous) carcinoma of breast is a special type of invasive ductal carcinoma (not otherwise specified). In fact, focal mucin production is quite common in invasive ductal carcinoma (NOS). The mucinous component must comprise >90% of the tumor for diagnosis of colloid carcinoma is made. Colloid carcinoma accounts for 1–4% of breast carcinomas, which features abundant extracellular mucin production and is associated with a favorable prognosis.

Molecular Genetic Alterations

BRCA1 gene mutation is demonstrated in some cases. Amplification of ERBB2 (HER2/neu) is seen in some cases.

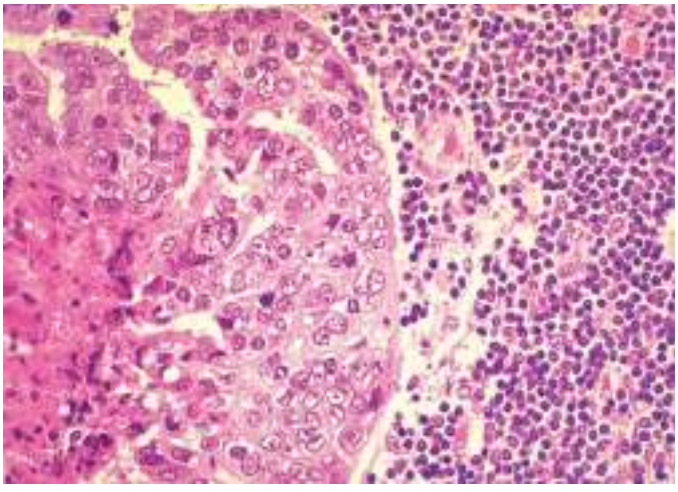


Fig. 25.42: Medullary carcinoma of breast. Carcinoma with medullary features shows a rounded margin and an intense lymphocytic infiltrate at the periphery. The tumor cells form a sheet-like pattern with high degree of nuclear pleomorphism and brisk mitotic activity (400X).

Clinical Features

Colloid carcinoma of breast is uncommon slow-growing neoplasm primarily seen in elderly women as a small well circumscribed mass.

Surgical Pathology: Colloid Carcinoma of Breast
Gross Morphology
▪ Tumor is well circumscribed tumor with soft consistency measuring 1–5 cm in diameter with an average of 2.8 cm. ▪ Cut surface of tumor is completely replaced by glistening gelatinous blue to gray-colored mucoid material (Fig. 25.43).
Light Microscopy
▪ Tumor is composed of small islands or clusters of generally uniform, round epithelial cells (10–20 cells) arranged in trabeculae, nests, sheets or acini with glandular lumen, floating within extensive lakes of extracellular mucin pool synthesized by tumor cells. ▪ Mucin dissects into the surrounding stroma. Tumor contains >90% of mucinous component (Fig. 25.44).
Immunohistochemistry Technique
Most tumors express hormone ER (estrogen receptors) in 80% of cases.

Prognosis

Colloid carcinoma of breast is low-grade malignant tumor with low incidence of metastases. It has better prognosis than conventional invasive ductal carcinoma of breast.

TUBULAR CARCINOMA OF BREAST

Tubular carcinoma of breast accounts for 1–4% of invasive breast carcinomas. A higher frequency (up to 19%) has been reported in small and screen-detected breast carcinomas in whites compared with blacks. It affects younger age at onset (40s). It is luminal type A



Fig. 25.43: Colloid carcinoma of the breast. This sagittal slice of the breast shows that it is completely replaced by mucoid tumor.

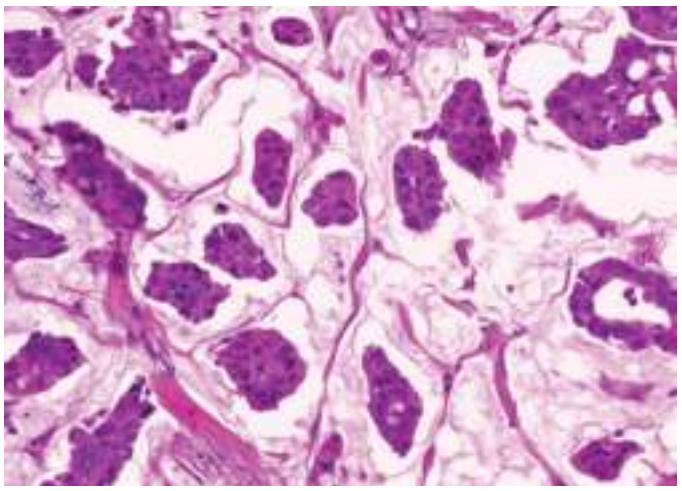


Fig. 25.44: Mucinous/colloid carcinoma of breast. Mucinous carcinoma shows large sheets of low-grade malignant cells present within pools of accumulated extracellular mucinous material (400X).

low-grade invasive ductal cell carcinoma. It shows low proliferative rate. Recurrence rate is low after surgical excision of tumor.

Molecular Genetic Alterations

Amplification of ERBB2 (HER2/neu) is unusual in tubular carcinoma of breast.

Clinical Features

Patient with tubular carcinoma of breast presents with breast lump measuring 2 mm to 1.5 cm in diameter. Most are 1 cm or less, but rarely 2 cm or above is encountered.

Surgical Pathology: Tubular Carcinoma of Breast

Gross Morphology

- The tumor is usually small (<2 cm), firm to hard in consistency, and often detected on mammography.
- There is increased incidence of tubular carcinoma in opposite breast in 10–40% of cases.

Light Microscopy

- Diagnosis of tubular carcinoma of breast is made when 75% of neoplasm shows well-formed small duct structures embedded in cellular stroma.
- Tumor shows bland-looking tumor cells arranged in tubules that possess widely patent lumens lined by low-grade single cell layer.
- Myoepithelial cells are absent, and the malignant tubules are arranged in an irregular stellate pattern.
- The intervening stroma is densely fibrotic. Mitoses are rare.
- Tubular carcinoma may be difficult to defferentiate from sclerosing adenosis or radial scar but generally spread outside the confines of enlarged lobules (Fig. 25.45).
- Distinguishing features of tubular carcinoma, sclerosing adenosis and microglandular adenosis of breast are given in Table 25.18.

Immunohistochemistry Technique

Virtually all tubular carcinomas express ER and PR hormone receptors and **cytokeratin (8;18).**

Markers	Expression
▪ Estrogen (ER)	▪ Positive
▪ Progesterone (PR)	▪ Positive
▪ Cytokeratin (8;18)	▪ Positive

Treatment

Patient presents tubular carcinoma of breast responds to tamoxifen, aromatase inhibitors and Herceptin.

Prognosis

Prognosis of tubular carcinoma of breast is better despite multifocal nature and bilateral involvement. Lymph node metastases are rare.

PAPILLARY CARCINOMA OF BREAST

Papillary carcinoma of breast is a rare subtype of invasive ductal carcinoma (IDC).

- Papillary carcinoma of breast comprises 0.5–0.7% of all invasive breast carcinomas. Tumor has infiltrative papillary growth in women in their sixth to eighth decades of life.
- Approximately 50% of women present with breast lump beneath nipple and bloody nipple discharge.
- Tumors should be graded according to the Nottingham grading system.
- Tumors should be staged according to their pathologic size and lymph node status.
- Poor prognostic factors include old age, advanced pathologic stage and patients without radiation.

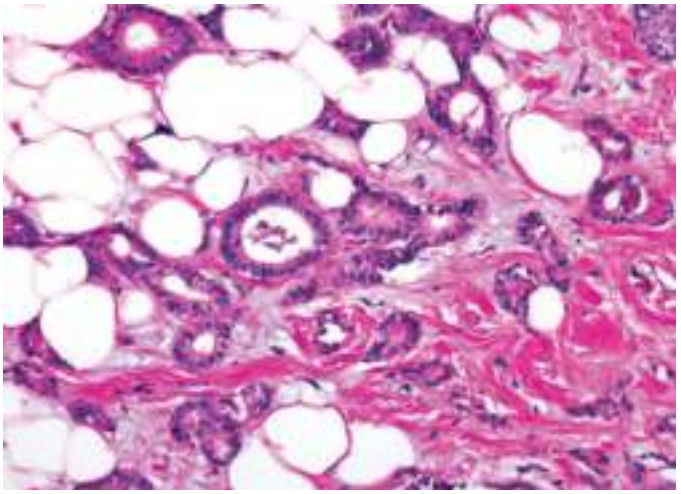


Fig. 25.45: Tubular carcinoma of breast. Tumor is composed of bland-looking tumor cells arranged in tubules that possess widely patent lumens. Myoepithelial cells are absent, and the malignant tubules are arranged in an irregular stellate pattern. The intervening stroma is densely fibrotic (400X).

Table 25.18 Distinguishing features of tubular carcinoma, sclerosing adenosis and microglandular adenosis of breast

Features	Tubular Carcinoma	Sclerosing Adenosis	Microglandular Adenosis
Shape of the lesion	Stellate, irregular	Lobular, round	Irregular
Shape of the glands	Angulated	Round to oval	Round
Luminal contents	Sometimes	Rarely	Frequent 'colloid'
Cell layers	One layer of epithelial cell	Two layers composed of epithelial and myoepithelial components	One layer of epithelial cells
Cell luminal surface	Snouting	Occasional snouting	Smooth
Cytoplasm	Eosinophilic	Inconspicuous	Clear
Stroma	Desmoplastic	Around lobules	Dense collagenous
Basement membrane	Usually none	Present	Prominent

Surgical Pathology: Papillary Carcinoma of Breast

Gross Morphology

- The tumor is usually 2–3 cm in size, and well-circumscribed.
- Cystic tumor contains brown mixture of blood clot and neoplastic tissue.

Light Microscopy

- The tumor is composed of delicate fibrovascular tissue lined by atypical epithelial cells with hyperchromatic nuclei and mitoses forming papillary structures with the absence of an outer myoepithelial cell layer.
- It must be differentiated from intraductal papilloma that is characterized by thick fibrovascular tissue and presence of myoepithelial cells.
- Periphery of the cystic tumor is fibrotic and distinguishing invasion may be difficult unless neoplasm reaches the adipose tissue (Fig. 25.46).

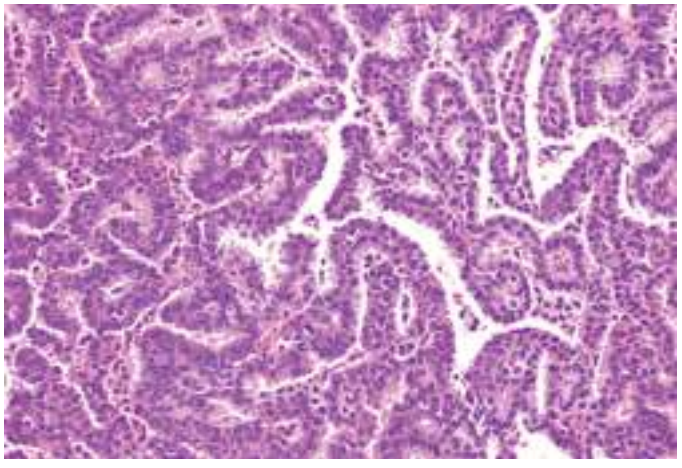


Fig. 25.46: Invasive papillary carcinoma breast. It is comprised of delicate fibrovascular tissue lined by atypical epithelial cells with hyperchromatic nuclei and mitoses forming papillary structures with the absence of an outer myoepithelial cell layer (400X).

INVASIVE MICROPAPILLARY CARCINOMA OF BREAST

Invasive micropapillary carcinoma of breast is luminal type B invasive breast carcinoma, which accounts for seen in <20% of cases. It has higher proliferative rate than luminal type A. Recurrence of tumor after surgery is high. Hormone receptor negativity may explain the more aggressive behavior of invasive micropapillary carcinoma. It should be differentiated from duct papilloma of breast (Table 25.19).

Surgical Pathology: Invasive Micropapillary Carcinoma of Breast

Gross Morphology

- Tumor has no specific gross features.
- Tumor with 75% of micropapillary growth tends to be large (mean 6 cm).
- Tumor has lobulated outline.

Light Microscopy

Invasive micropapillary carcinoma shows rounded groups of tumor cells with a peripheral clear rim and lacks true fibrovascular core (Fig. 25.47).

Immunohistochemistry Technique

Tumor cells may show low expression of ER and PR. Immunohistochemistry shows cytokeratin positivity (8;18).

Markers	Expression
▪ Estrogen receptor (ER)	▪ Negative or low expression
▪ Progesterone receptor (PR)	▪ Negative or low expression
▪ Cytokeratins (8;18)	▪ Positive

Prognosis

Invasive micropapillary carcinoma of breast has aggressive behavior. Prognosis may not be as good as in luminal A breast carcinoma.

Table 25.19 Comparison of intraductal papilloma and noninvasive ductal papillary carcinoma of breast

Parameters	Ductal Papilloma	Noninvasive Ductal Papillary Carcinoma
Cell morphology	Benign cells with normal nuclear chromatin	Atypical cells with hyperchromatic nuclei
Myoepithelial cells	Present	Absent
Apocrine changes	Present	Absent
Surrounding stroma	Thick prominent stroma	Delicate stroma
Sclerosing adenosis	Unusual	Usual

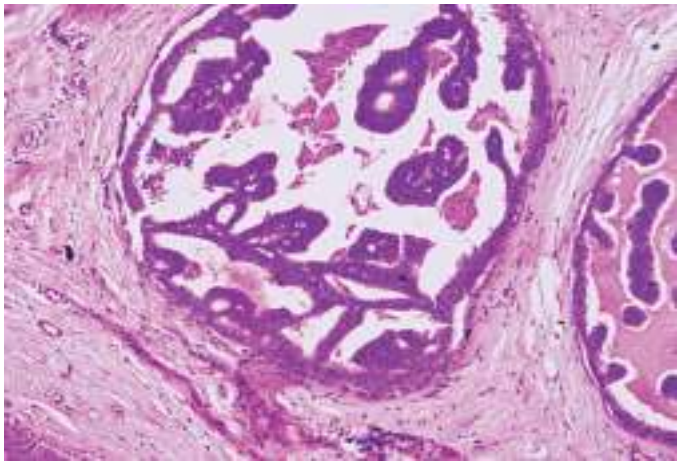


Fig. 25.47: Micropapillary carcinoma of breast. It shows large duct with micropapillary tufts of epithelial cells lacking true fibrovascular core (400X).

Pathology Pearls: Papillary Lesions of Breast

- Papillomas (single or multiple)
- Papilloma with atypical ductal hyperplasia or ductal carcinoma *in situ*
- Solid papillary carcinoma
- Encapsulated papillary carcinoma
- Invasive papillary carcinoma
- Papillomatosis

METAPLASTIC CARCINOMA OF BREAST

Metaplastic carcinoma of the breast is a form of breast carcinoma that shows differentiation towards malignant squamous epithelium, cartilaginous, or bony tissue. This term is used to describe a heterogeneous group of neoplasms. It is also known as carcinosarcoma. It is basal-like subtype of breast carcinoma. It has high proliferative rate with positive lymph nodes. Recurrence rate is high.

Molecular Genetic Alterations

TP53 gene mutation is very common in metaplastic carcinoma of breast. It shows dysfunction of BRCA1 gene. **Ki-67** is expressed in <14% of cases.

Treatment

Patients with metaplastic carcinoma of breast do not respond to tamoxifen, aromatase inhibitors and Herceptin. Trials are going onto treat these patients with platinum-based chemotherapy or poly-ADP ribose polymerase (PARP) inhibitors. PARPs play an important role in various cellular processes such as modulation of chromatin structure, transcription, replication, recombination and DNA repair.

Surgical Pathology: Metaplastic Carcinoma of Breast

Gross Morphology

Metaplastic carcinoma of breast varies in size, i.e. ≤2 cm (31%), 2–5 cm (50%) and ≥5 cm (19%). Cut surface shows variable consistency.

Light Microscopy

- Metaplastic carcinoma of breast has monophasic or biphasic sarcomatoid carcinoma variants.
- Tumor is admixture of malignant epithelial component (adenocarcinoma grade 2 or 3) with malignant mesenchymal elements including cartilage, bone, and myxoid stroma, and/or squamous or spindle cell elements (Figs 25.48 and 25.49).

Immunohistochemistry Technique

Metaplastic carcinoma of breast is '**triple negative**' for ER, PR and HER2/neu. Neoplastic cells show positivity for cytokeratins and vimentin. Spindle cell component is positive for vimentin. Neoplastic cells show positivity for CK903 (HMWCK), p53, CAM 5.2 (most reliable marker), CK7 (most reliable marker) and AE1/AE3 (variable expression).

Markers	Expression
CK903 (HMWCK)	Positive
p53	Positive
CAM 5.2 (most reliable marker)	Positive
CK7 (most reliable marker)	Positive
AE1/AE3 (variable expression)	Positive
ER (estrogen receptor)	Negative
PR (progesterone receptor)	Negative
HER2/neu	Negative

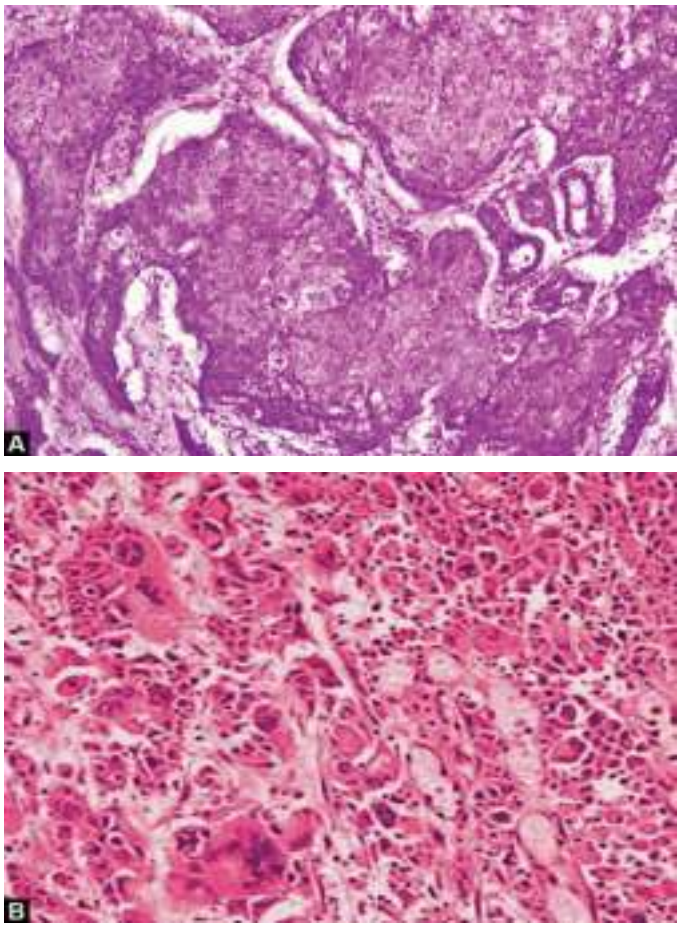


Fig. 25.48: Metaplastic carcinoma of breast with squamous differentiation. (A) Tumor shows mixed squamous cell carcinoma and ductal carcinoma components, (B) the intervening areas show malignant spindle cell proliferation. It is high-grade breast carcinoma with poor prognosis (400X).

APOCRINE CARCINOMA OF BREAST

Apocrine carcinoma of breast consists of tumor cells with apocrine morphology in >90% of neoplastic cells.

Surgical Pathology: Apocrine Carcinoma of Breast
Gross Morphology
▪ Apocrine carcinoma of breast is indistinguishable from invasive ductal carcinoma. ▪ Tumor is often multicentric in breast.
Light Microscopy
Tumor is composed of large cells with well-defined cellular outlines, vesicular nuclei, prominent nucleoli, abundant granular cytoplasm and foci of necrosis (Fig. 25.50).
Electron Microscopy
Tumor contains prominent mitochondria with abnormal cisternae, large membrane bound vesicles and dense cores.

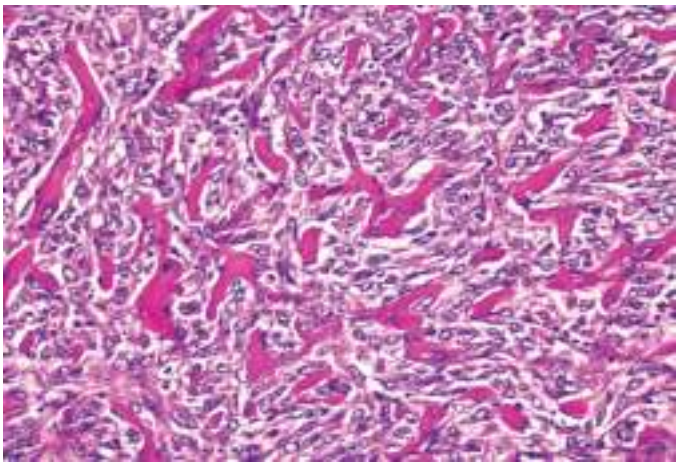


Fig. 25.49A: Metaplastic carcinoma of breast. Tumor is composed of heterogenous malignant mesenchymal components such as osseous element and spindle cells (400X).

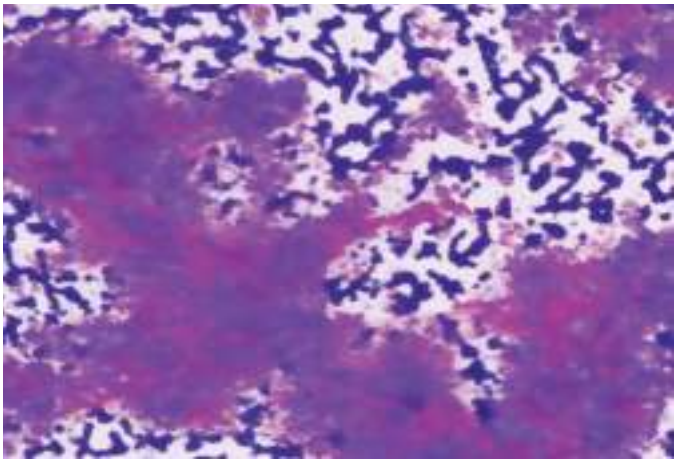


Fig. 25.49B: Breast metaplastic carcinoma with osseous metaplasia. Metaplasia in mammary ductal epithelium and carcinoma is a well-known phenomenon. There seems to be morphological overlap between various components. Smears prepared from fine needle aspiration cytology of breast lump revealed malignant cells and matrix producing metaplastic component. Mastectomy was performed and metaplastic carcinoma of breast with osseous metaplasia (400X).

Prognosis

Apocrine carcinoma of breast has similar prognosis that of infiltrating duct carcinoma (NOS) of similar grade.

SECRETORY CARCINOMA OF BREAST

Originally, secretory carcinoma was named juvenile breast carcinoma. Terminology was changed to secretory carcinoma after the reports of the tumor occurring in the adults of both sexes.

- Tumor size, rates of axillary lymph nodes involvement, stage at presentation, pattern of surgical therapy are similar to invasive ductal carcinoma of breast.
- Tumor is often located in the central region near the areola.

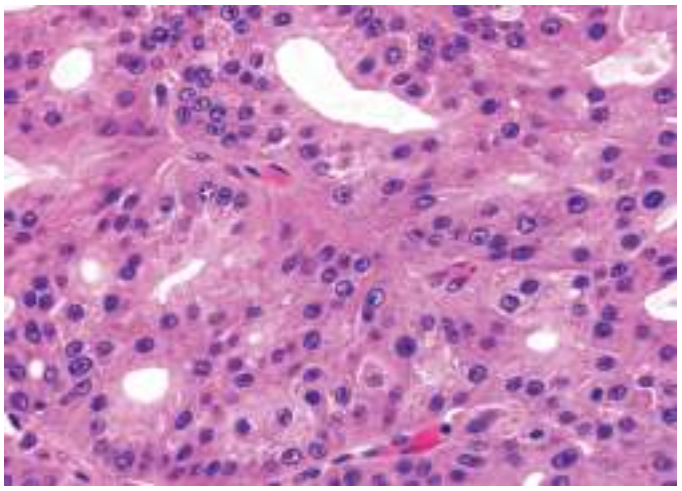


Fig. 25.50: Apocrine breast carcinoma is composed of large cells with well-defined cellular outlines, vesicular nuclei, prominent nucleoli, abundant granular cytoplasm and foci of necrosis (400X).

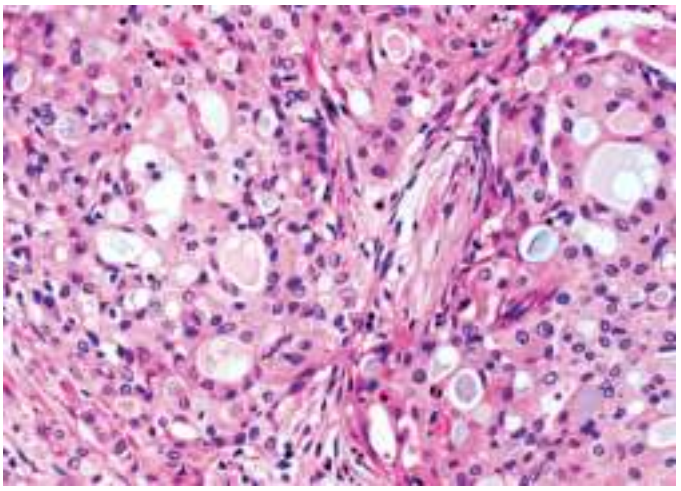


Fig. 25.51: Secretory carcinoma of breast. It mimics lactating breast by forming multiple dilated spaces filled with amorphous eosinophilic secretions. The cystic spaces are lined by cuboidal epithelium with clear or pale cytoplasm (400X).

Clinical Course

Patient with secretory carcinoma of breast has indolent clinical course and excellent prognosis. Five-year survival rate is seen in 87% of cases.

Surgical Pathology: Secretory Carcinoma of Breast

Gross Morphology

- Tumor is small with median size 1.9–2.36 cm, and well circumscribed.
- Tumor is located in the central region near the areola having pushing border but may be infiltrative at periphery.

Light Microscopy

On histologic examination, secretory carcinoma mimics lactating breast.

- Architecture of tumor is most often microcystic, solid or tubular or mixture or all three patterns.
- Tumor cells show low-grade cytologic atypia bland uniform nuclei and low mitotic rate.
- Histologic hallmark of secretory carcinoma of breast is the presence of tumor cells with vacuolated foamy cytoplasm and abundant intracellular and extracellular secretions, which are positive with periodic acid–Schiff (PAS) and diastase resistant (Fig. 25.51).

Histochemistry

Presence of intracellular and extracellular secretions in secretory carcinoma are demonstrated by periodic acid–Schiff (PAS) diastase resistant.

Immunohistochemistry Technique

Tumor cells do not express ER and HER2/neu.

Markers	Expression
▪ Estrogen receptors (ER)	▪ Negative
▪ HER2/neu	▪ Negative
▪ S-100	▪ Positive

Treatment

Surgical treatment of secretory carcinoma of breast and use of antihormonal target therapy are similar to invasive ductal carcinoma.

CRIBRIFORM CARCINOMA OF BREAST

Cribriform carcinoma of breast is luminal A low-grade invasive ductal carcinoma, which shows low proliferative rate. Recurrence rate is low after surgery. These patients respond to endocrine therapy (tamoxifen, aromatase inhibitors and Herceptin). Prognosis is excellent even with nodal metastasis.

Surgical Pathology: Cribriform Carcinoma of Breast

Light Microscopy

- Cribriform carcinoma of breast is a low-grade tumor with cribriform appearance (well-defined spaces formed by arches of cells) similar to ductal carcinoma *in situ* (DCIS) but with large cells, more vesicular nuclei.
- Tumor cells are small, which often have apical snouts, mild/moderate nuclear pleomorphism, which invade stroma.
- Tumor may have predominantly solid appearance.
- It is worth mentioning that pure cribriform pattern is defined as 90% of tumor cribriform appearance. It is also considered 'classic' even if minor component of tubular carcinoma of breast is also present.

Immunohistochemistry Technique

Immunohistochemistry technique shows tumor cells positivity for ER, PR and cytokeratin (8;18).

Markers	Expression
▪ Estrogen receptor (ER)	▪ Positive
▪ Progesterone receptor (PR)	▪ Positive
▪ Cytokeratin (8;18)	▪ Positive

CUTANEOUS ANGIOSARCOMA OF BREAST

Stewart Treves syndrome is also known as cutaneous angiosarcoma, which occurs in long-standing lymphedema.

- Radical mastectomy is performed for treatment of angiosarcoma of breast. Women develop gross lymphedema of the arm, which is a usual consequence of radical mastectomy.
- Occasionally, angiosarcomas arise in the skin and subcutaneous tissue of the grossly edematous arms some years after the surgery.

Surgical Pathology: Cutaneous Angiosarcoma of Breast

Gross Morphology

Angiosarcoma of the breast is seen as a reddish, cavitated hemorrhagic tumor within the subcutaneous mammary tissue.

Light Microscopy

Histologic grades of angiosarcoma of female breast described as under:

- **Low-grade angiosarcoma:** Tumor is composed of predominant freely anastomosing channels. Papillary growth and endothelial tufting is minimal.
- **Intermediate grade angiosarcoma:** Tumor is composed of freely anastomosing vascular channels. Papillary growth and endothelial tufting are present. It may show focal solid areas composed of polygonal and spindle cells.
- **High-grade angiosarcoma:** Tumor is composed of polygonal and spindle cells. Blood lakes and necrosis are common. Vascular channels may be difficult to identify.

Diagnostic features of major histologic types of invasive breast carcinoma are given in Table 25.20.

BREAST CARCINOMA: MODE OF SPREAD, PROGNOSTIC AND PREDICTIVE FACTORS

BREAST CARCINOMA: ROUTES OF METASTASIS

Most breast carcinomas arise from lining epithelium of ducts, which becomes invasive and metastasizes early to axillary lymph nodes, lungs, liver, bones, brain, adrenal glands and ovaries.

Women presenting with an isolated enlarged axillary node must be taken seriously because 33–50% will have occult breast carcinoma. Mode of spread of breast carcinoma by various routes is described as under.

Table 25.20 Diagnostic features of major histologic types of invasive breast carcinoma

Histologic Type	Incidence (%)	Clinical/Gross Findings	Light Microscopy	Prognosis	Typical Biomarkers
Invasive ductal carcinoma, NOS (not otherwise specified)	70–75	Speculated mass, variable	Variable, devoid of special type of histologic features	Variable	Variable
Invasive lobular carcinoma	5–15	Architectural distortion, density (no distinct mass), multifocal/bilateral	Uniform cells, eccentric nuclei, intracytoplasmic lumina, Indian filing, targetoid pattern	Unfavorable	▪ ER (+), PR (+) ▪ HER2/neu (–)
Tubular carcinoma	5	Speculated mass, small size	Angulated tubules with tapered ends, open lumina, dense stroma	Favorable	▪ ER (+), PR (+) ▪ HER2/neu (–)
Mucinous or colloid carcinoma	2–3	Well-circumscribed lobulated mass, gelatinous cut surface	Tumor cells floating in pools of extracellular mucin	Favorable	▪ ER (+), PR (+) ▪ HER2/neu (–)
Medullary carcinoma	1–2	Young women, well-circumscribed mass, no calcifications	Syncytial growth, pushing borders, infiltrated by lymphocytes and plasma cells, high nuclear grade	Favorable (if pure)	Triple negative: ER (–), PR (–) HER2/neu (–); (triple negative)
Micropapillary carcinoma	1–2	Similar to IDC, not otherwise specified (NOS)	Tumor clusters within empty spaces, reverse polarity, no fibrovascular cores	Higher rate of LN metastasis	Variable
Metaplastic carcinoma	<1	Large tumor	Tumor transformation to squamous or mesenchymal (bone, cartilage) elements	Lower LN metastasis; prognosis is very poor	Triple negative: ER (–), PR (–), HER2/neu (–) ER and PR can be positive in glandular elements, coexpression of cytokeratins (5, 6) and vimentin

Local Tumor Invasion Route of Metastasis

Invasive breast carcinoma can infiltrate and cause fixation to adjacent structures. If the tumor is deep, fixation may occur to the pectoralis muscles. If tumor is superficial, the skin may be involved.

- Skin involvement can result in nipple retraction and inversion when the tumor arises near the central zone.
- Invasion and obstruction of small cutaneous lymphatic channels results in edema of the skin giving it the appearance of the surface of an orange peel (**peau d'orange**).
- Once skin fixation occurs, cutaneous ulceration may follow. Breast carcinoma may spread to opposite breast.

Lymphatic Route of Metastasis

Normally lymphatic drainage of breast occurs via axillary and internal mammary nodes. Approximately 75% of breast lymphatic drainage takes place in axillary lymph nodes, while rest 25% via internal mammary lymph nodes adjacent to vertebrae and skin lymphatic channels. That is the reason that occult breast carcinoma presents with isolated axillary lymphadenopathy (Fig. 25.52).

Axillary Lymphatic Drainage

- Axillary group consists of approximately 21 lymph nodes, which lie below the axillary vein. Axillary lymph nodes include anterior, apical, internal (Rotter's nodes), central and lateral lymph nodes.
- Axillary group lymph nodes are divided into three groups in relation to pectoralis minor muscle proceeding from level I (13 nodes) lateral to muscle, level II (5 nodes) behind muscle and level III (3 nodes) medial to pectoralis minor muscle. Level I and level II lymph nodes are removed in the standard axillary dissection of breast carcinoma.



Fig. 25.52: Breast carcinoma—axillary lymph nodes show metastases (arrows).

Sentinel Lymph Node Biopsy

Sentinel lymph nodes are the first group of nodes that drain lymph from the breast to the axilla.

- Sentinel lymph node is usually in level I. It is unusual for breast carcinoma to skip to higher levels of lymph nodes without involving the lower lymph nodes.
- Sentinel lymph node biopsy has been designed for axillary staging in breast carcinoma patients, who have clinically negative lymph nodes. Sentinel lymph node biopsy offers accuracy equivalent to that of axillary lymph node dissection.
- According to the American College of Breast Surgeons (ACBS), lymph node biopsy is suitable for clinically lymph node negative T1–T2 invasive ductal carcinoma of breast.
- Sentinel lymph node demonstration involves injecting radioisotope (technetium-99m sulphur colloid) alone or radioisotope plus a patent blue dye (lymphazurin blue or methylene blue) into the tissues of the breast.
- With sentinel lymph node dissection, 1–3 lymph nodes are removed and tested for nodal metastases with hematoxylin and eosin (H&E) stain and immunohistochemistry.

Lymph Node Skip Metastases

Lymph node skip metastases are seen in <5% of patients breast carcinoma associated with axillary lymph node involvement. It is due to lymphatic drainage from level II to level III under surface of pectoralis minor muscle.

Lymph Node Micrometastases

Micrometastases in lymph node were originally defined as occult metastases <0.2 cm in diameter in patient with breast carcinoma. Nowadays, micrometastasis term also includes presence of disseminated tumor cells in peripheral blood, bone marrow or lymph nodes.

Hematogenous Route of Metastasis

Metastasis occurs as a result of vascular permeation in the region of the breast carcinoma, in areas of lymph node metastases. Neoplastic cells break lymph nodal barriers, invade blood vessels in stroma and enter into systemic circulation.

- Neoplastic cells metastasize through the blood vessels to various distant organs preferentially to the **lung**,

liver and **bones**. Breast carcinoma metastasizing to bones may show either **osteolytic** or **osteoblastic lesions**.

- Patients with breast carcinoma frequently develop metastases to other multiple sites, e.g. adrenal glands, brain, pleura, gastrointestinal tract, peritoneum, skin, spleen, pericardium, ovary, kidney, uterus and heart in decreasing frequency.

Transcoelomic Route of Metastasis

Breast lobular carcinoma spreading to ovaries by transcoelomic route (retrograde lymphatic channels) in elderly women is known as 'Krukenberg tumor'. It is rapidly developing bilateral ovarian metastases from carcinoma of breast, stomach and intestine (Fig. 25.53).

Surgical Pathology: Krukenberg Tumor of Ovary

Gross Morphology

- Tumor is large, solid, lobulated, with smooth surface and freely mobile.
- Cut surface is gray white, gelatinous, areas of hemorrhage and cystic spaces.

Light Microscopy

Tumor shows sheets of malignant epithelial cells in a dense fibromyxomatous stroma.

Immunohistochemistry Technique

Positive immunostains depend on primary tumor. Gastrointestinal carcinomas are positive for CK20 and CDX2. Primary breast carcinoma is positive for GcDFP-15.

BREAST CARCINOMA: PROGNOSTIC AND PREDICTIVE FACTORS

There are a number of pathologic parameters capable of predicting prognosis and guiding new strategies or treatment of cases with breast carcinoma. Prognosis depends on molecular features or histologic type, extent of spread of breast carcinoma (stage) at the time of diagnosis.

Strong independent prognosis factors of breast carcinoma include tumor grade, lymph node status, lymphovascular invasion and tumor size.

Invasiveness of Breast Carcinoma

Breast carcinoma *in situ* (ductal or lobular) has better prognosis after surgery than invasive (ductal or lobular) carcinoma. Exception is comedocarcinoma, a ductal carcinoma *in situ* (DCIS) with central necrosis and lymph node metastases.

Histologic Variant of Breast Carcinoma

Breast carcinomas, i.e. tubular, papillary, mucinous, microinvasive, medullary and adenoid cystic are associated with better prognosis. Patients with scirrhous breast carcinoma, Paget's disease of breast, secretory carcinoma and apocrine carcinoma have poor prognosis.

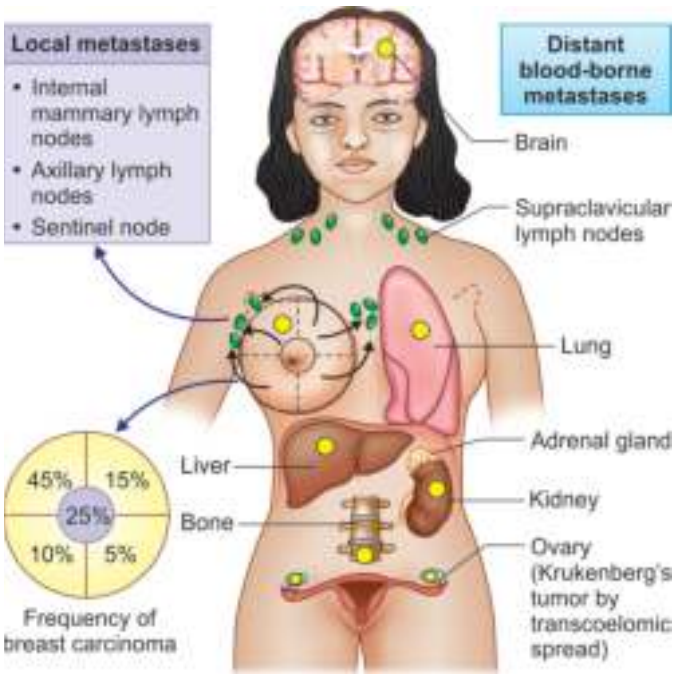


Fig. 25.53: Breast carcinoma—mode of spread of breast cancer. It also shows frequency of breast carcinoma in various quadrants of breast. Breast carcinoma spreads by local invasion, lymphatic, hematogenous and transcoelomic routes.

Distant Metastases of Breast Carcinoma

Breast carcinoma metastases via hematogenous route to lungs, pleura, liver, bones, brain, adrenal glands and ovaries (**Krukenberg tumor**) with fatal outcome.

Status of Axillary Lymph Nodes in Breast Carcinoma

The single best prognostic factor is the presence or absence of axillary nodal metastases in breast carcinoma. There is a direct correlation between survival and the number of axillary lymph nodes involved. Breast carcinoma involving beyond the axillary or internal mammary lymph nodes has a poor prognosis. Breast carcinoma limited to supraclavicular nodes has a good prognosis.

Pathology Pearls: Lymph Nodes Isolation in Mastectomy Specimens

- Lymph nodes should be isolated from mastectomy specimen.
- All the lymph nodes should be examined under microscope to observe number of lymph nodes showing metastatic deposits, size of the metastatic deposit, extracapsular extension and histologic subtype of metastases resembling primary breast carcinoma.

Tumor Size of Breast Carcinoma

Tumor size correlates directly with survival. Patient with smaller tumor <2 cm has better survival rate than with large tumor. Large size breast carcinoma invades locally into adjacent skeletal muscles of breast.

Grading and Staging Systems of Breast Carcinoma

Grading system refers to classification of tumors according to their light microscopic characteristics. Staging system is a clinical exercise that classifies tumors according to their size, invasiveness and spread. Tumors are staged according to the TNM system (T: Tumor size, N: Lymph node involvement, M: Metastases to distant organs). Schemes for TNM classification vary according to tumor type and organ involved (Fig. 25.54).

A grading system (Nottingham modification of the Bloom Richardson Grading System) utilizes histologic characteristics of the breast carcinoma.

- **Basis of Nottingham modification grading system:** It is based on assessment of entire tumor differentiation (e.g. glandular formation), nuclear pleomorphism and frequency of mitotic figures studied in formalin fixed H&E stained sections.
- **Scoring of Nottingham grading system:** For example, a tumor with >75% glandular differentiation would score 1, 10–75% glandular differentiation as score 2, and <10% glandular differentiation would score grade 3. These values are combined and converted into three groups: grade I (scores 3–5), grade II (scores 6–7), and grade III (scores 8 and 9). This histologic grade is often known as Nottingham modification of the Bloom and Richardson grading system (Tables 25.21 to 25.23).

Pathology Pearls: Nottingham Grading System in Breast Carcinoma

High percentage of tubule formation (>75%) would indicate a better prognosis in patient with invasive ductal carcinoma, not otherwise specified (NOS) of breast.

Lymphovascular Invasion of Breast Carcinoma

Invasion of lymphatic channels and blood vessels is demonstrated in 25% of breast carcinoma on light microscopy. These patients have poor prognosis. Breast carcinoma metastasis and its prognostic markers are given in Table 25.24.

Age of the Patient with Breast Carcinoma

Younger women <35 age have a poorer prognosis than older patients with breast carcinoma of equivalent stage.

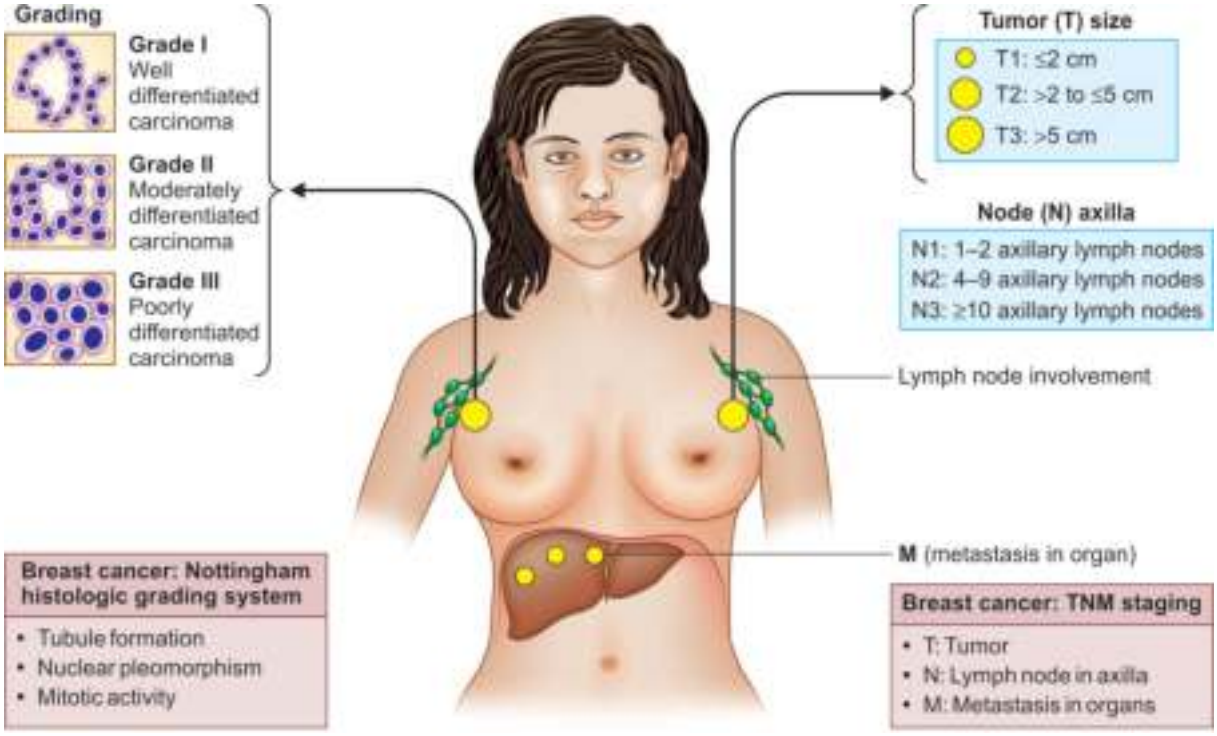


Fig. 25.54: Breast carcinoma shows grading and staging. TNM stands for tumor, lymph node metastases and distant metastases. Grading and staging of breast carcinoma [grading classifies tumors according to their light microscopic characteristics. Staging is a clinical exercise that classifies tumors according to their size, invasiveness and spread. Tumors are staged according to the TNM system (T: Tumor size, N: Lymph node involvement, M: Metastases to distant organs). Schemes for TNM classification vary according to tumor type and organ involved].

Table 25.21 Nottingham modification of the Bloom and Richardson grading system in breast carcinoma

Histology	Characteristics	Scores	Comments
% of tubules formation by tumor cells	▪ >75% ▪ 10–75% ▪ <10%	1 2 3	Based on assessment of entire tumor
Nuclear pleomorphism	▪ Small uniform cells ▪ Moderate increase in size of cells ▪ Marked variation in size of cells	1 2 3	Based on the worst area of tumor
Mitotic count per 10 HPF	▪ ≤6 ▪ 7–13 ▪ ≥14	1 2 3	Mitotic count at the leading edge of tumor growth

The grading is calculated by adding the above scores.

Table 25.22 Correlation of grade and survival rate in breast carcinoma

Grade	Score	Differentiation of Tumor	5-Year Survival (%)	7-Year Survival (%)
1	3 to 5	Well differentiated tumor	95	90
2	6 or 7	Moderately differentiated tumor	75	65
3	8 or 9	Poorly differentiated tumor	50	45

Table 25.23 Staging of breast carcinoma

Stage	Primary Tumor (T)	Positive Axillary Lymph Nodes (N)	Distant Metastases	5-Year Survival
0	▪ DCIS (ductal carcinoma *in situ*) ▪ LCIS (lobular carcinoma *in situ*)	None	None	93%
I	Invasive carcinoma, ≤2 cm	None	None	88%
II	▪ Invasive carcinoma, >2 cm ▪ Invasive carcinoma, <5 cm	▪ 1–3 nodes ▪ 1–3 nodes	None	74–81%
III	▪ Invasive carcinoma, >5 cm ▪ Invasive carcinoma, any size ▪ Invasive carcinoma, attached to skin or chest wall or inflammatory carcinoma	▪ ≥4 nodes ▪ ≥4 nodes ▪ 0–>10 nodes	None None None	41–67%
IV	Any invasive carcinoma ▪ *IVa:* Pectoralis major muscle involvement ▪ *IVb:* Skin involvement: Skin shows ulceration, satellite nodule and peau d' orange ▪ *IVc:* Combination of IVa + IVb ▪ *IVd:* Inflammatory breast carcinoma	Any lymph node status	Present	15%

Markers of Cell Proliferation in Breast Carcinoma

Breast carcinoma with high rate of proliferation of atypical tumor cells in S-phase of cell cycle is associated with local recurrence and associated with fatal outcome in comparison to slow proliferative rate of tumor. S-phase of cell cycle fraction above 5% indicates poor prognosis, which is estimated by the use of monoclonal antibodies such as Ki-67, KiS1, and MIB-1; and identification of proliferating atypical tumor cells by the use of tracers such as bromodeoxyuridine. Measurement of proliferation of tumor cells alone does not give complete information, but prognosis also depends on the metastatic potential of a breast carcinoma.

Table 25.24 Breast carcinoma metastasis and its prognostic markers

Prognostic Markers	Details of Marker	Use in Clinical Practice	Metastatic Determinants
Tumor size	Independent prognostic marker	Established tumor size	▪ Tumor ≤2 cm in diameter has low risk of metastasis ▪ Tumor of 2–5 cm has a high risk of metastasis ▪ Tumor >5 cm has a very high risk of metastasis
Histologic grade	Related to tumor size	Established histologic grade	▪ Grade 1 tumor has a low risk of metastasis ▪ Grade 2 tumor has an intermediate risk of metastasis ▪ Grade 3 has a high risk of metastasis
Axillary lymph node status	Related to tumor size	Established axillary lymph node metastasis	▪ Absence of lymph node metastasis has low risk of metastasis ▪ Presence of lymph node metastasis has high risk of metastasis ▪ Presence of >4 lymph node metastasis has very high risk of metastasis
Angioinvasion	In patient with lymph node negative tumor	Established in patient with lymph node negative tumor	Presence of tumor emboli in >3 blood vessels is associated with metastasis
UPA (urokinase type plasminogen activator) protein and PAI-1 (plasminogen activator inhibitor 1) protein level	Independent prognostic markers	Newly established markers	High protein levels are associated with high risk of metastatic disease
Steroid receptors ER/PR expression	Short-term predictor of metastasis risk (5-year) related to histologic grade of tumor	Established for adjuvant therapy decision	Low steroid receptor (ER/PR) level is associated with metastasis
ERBB2/HER2/neu gene amplification and protein expression	In patient with lymph node positive tumor	Established for adjuvant therapy decision	ERBB2/HER2/neu amplification/overexpression is associated with metastasis
Gene profiling	Currently being analyzed	Analyzed in patient with lymph node negative tumor	▪ A good signature of 70 genes is associated with low risk of metastasis ▪ A poor signature of 70 genes is associated with high risk of metastasis

DNA Content Analysis of Breast Carcinoma

Amount of DNA content in breast carcinoma of atypical tumor cells measured by flow cytometry provides indication of malignant potential. Homogenous population of cells with euploid DNA content is seen in normal or benign cells. Poorly differentiated breast carcinoma shows increased DNA content. Neoplastic atypical cells with aneuploid indicate poor outcome. Diploid tumor has better prognosis than aneuploid tumor.

Internalized Factors Analysis of Breast Carcinoma

Chromosomal aneuploidy and proliferative index parameters determined by flow cytometry or fluorescence *in situ* hybridization (FISH) technique provide data on the aggressiveness of breast carcinoma. Flow cytometry to performed on the atypical cells obtained by fine needle aspiration, excisional biopsy, or resection of breast carcinoma to determine the characteristics of the DNA content. The presence of an increased proliferative rate (high S-phase of cell cycle) and aneuploidy in the flow cytometric pattern of breast carcinoma, suggest a worse prognosis.

Hormone Receptor Status of Breast Carcinoma

The hormone receptor status (estrogen and progesterone) of the breast carcinoma of atypical cells can be useful information for treatment and prognosis. Breast carcinoma with positive estrogen receptor (ER) treated by estrogen receptor blocker (tamoxifen) has better prognosis. In general ER positive breast carcinoma has progesterone positive status. Significance of progesterone status is less well understood.

Amplification of Proto-oncogenes

There is increased expression of oncogenes in breast carcinoma; which include HER2/neu, LGALS3, INT2, and c-Myc.

HER2/neu Proto-oncogene

HER2/neu proto-oncogene encodes for an epithelial growth factor receptor on the cell membrane that stimulates cellular proliferation. Amplification of HER2/neu oncogene is associated with poorly differentiated breast carcinoma (high nuclear grade), metastasizing to lymph nodes, with high rate of recurrence, a small chance of survival and aneuploidy (abnormal number of chromosome).

- **Associated carcinomas:** Amplification of HER2/neu is identified in 10–35% of primary breast carcinoma (high-grade tumors). It has also been demonstrated in carcinomas of the lung, ovary, and stomach.
- **Immunohistochemistry technique:** It is demonstrated as c-erbB2 protein by immunohistochemistry technique on the cell membrane or by analysis of the HER2/neu using fluorescent *in situ* hybridization (FISH). These patients exhibiting HER2/neu gene amplification are treated with monoclonal antibody (Herceptin) that selectively binds to the extracellular domain of the protein.

Galectin 3 Encoded by LGALS3 Proto-oncogene

- **Physiologic state:** Galectin 3 is a member of lectin family and encoded by LGALS3 gene located on chromosome 14, locus q21–q22. Galectin 3 is expressed in the nucleus, cytoplasm and extracellular space.
- **Pathologic state:** Galectin 3 overexpression promotes neoplastic transformation and the maintenance of transformed phenotypes as well as enhances adhesion of tumor cells to the extracellular matrix in breast carcinoma.

Tumor Suppressor Gene Mutations

- **Physiologic state:** RB, TP53 and NM23 are tumor suppressor genes.
- **Pathologic state:** Mutations of RB, TP53 and NM23 genes lead to overexpression of oncogenes resulting in breast carcinoma. These gene mutations are significantly related to poor differentiation, disease recurrence, diminished survival and absence of steroid hormone receptors.
- **Associated carcinomas:** RB tumor suppressor gene mutation is also seen in retinoblastoma, breast carcinoma, osteosarcoma, glioblastoma, urinary bladder carcinoma and small cell lung carcinoma. Inherited TP53 gene mutation is known as Li-Fraumeni syndrome that can increase risk for breast carcinoma, osteosarcoma, leukemia and soft tissue sarcoma. NM3 gene mutation is linked to breast carcinoma metastasis.

Epidermal Growth Factor Receptor (EGFR)

- **Physiologic state:** EGFR is a polypeptide expressed within membrane of breast carcinoma cells. EGFR participates in cell growth.
- **Pathologic state:** Breast carcinoma with overexpression of EGFR is associated with poor survival. There is no role of estrogen receptor blocker (tamoxifen) in these patients.

Alterations in Cell Structure

There is increased expression of **vimentin**, and decreased expression of '**fodrin**' in some cases of breast carcinoma.

Loss of Cell Adhesion Molecules

There is loss of integrin in poorly differentiated ductal carcinoma of breast; and E-cadherin in lobular variant of breast carcinoma in some cases.

Protease (Cathepsin D) Overexpression

- **Physiologic state:** Protease (cathepsin D) is an estrogen-induced acidic lysosomal protease 16 demonstrated in the cytoplasm of nonductal carcinomas of breast. Cathepsin D is also found in the stroma between the cells, which has growth promoting activity and extracellular proteolytic activity.
- **Pathologic state:** Overexpression of cathepsin D is associated with lymph node metastases and poor prognosis in patients with breast carcinoma.

Stress Response Protein 27 (Heat Shock Protein 27)

- **Physiologic state:** Stress response protein 27 (Srp27) is estrogen-related heat shock protein or stress response protein.
- **Pathologic state:** Overexpression of Srp27 is associated with breast carcinoma nodal and widespread hematogenous metastases to distant organs.

Secondary Messenger Systems

- **Physiologic state:** Cyclic AMP binding proteins are the regulatory subunits of a major second messenger system—protein kinase A.
- **Pathologic state:** Cyclic AMP binding proteins are overexpressed in 10–15% of cases. Breast carcinoma expressing high concentration of cyclic AMP is associated with poor outcome.

Prognostic and predictive factors are given in Table 25.25.

Table 25.25 Prognostic and predictive factors of breast carcinoma

Features	Good Prognosis	Poor Prognosis
Age group	30–50	<30 and >50
Diagnosis	Early diagnosis	Late diagnosis
Treatment	Early treatment	Late treatment
Metastases	Absent	Present
Histologic types of breast carcinoma	▪ Ductal carcinoma *in situ* except comedocarcinoma ▪ Low-grade invasive ductal carcinoma (NOS): Luminal type A ▪ Lobular carcinoma *in situ*/invasive: Luminal type A ▪ Tubular carcinoma: Luminal type A ▪ Cribriform carcinoma: Luminal type A ▪ Colloid carcinoma ▪ Papillary carcinoma	▪ Invasive ductal carcinoma of breast ▪ High-grade invasive ductal carcinoma (NOS): It is HER2/neu enrich breast carcinoma. HER2/neu is sensitive to Herceptin. It may be of basal type of breast carcinoma ▪ Paget's disease of breast ▪ Micropapillary carcinoma: Luminal type B ▪ Metaplastic carcinoma: Basal type and luminal type B ▪ Medullary carcinoma: Basal type ▪ Secretory carcinoma
Tumor morphology	▪ <2 cm size ▪ Pushing margin ▪ Absence of necrosis	▪ >2 cm size ▪ Infiltrative margin ▪ Presence of necrosis
Hormonal status	▪ Estrogen positive ▪ Progesterone positive	▪ Estrogen negative ▪ Progesterone negative
C-erbB2	Negative	Positive
p53	Negative	Positive
Srp27	Negative	Positive
Cathepsin D	Negative	Positive
EGFR	Negative	Positive

BREAST LUMPS: LABORATORY DIAGNOSIS

Clinical history includes duration of lump, mode of onset, duration of symptoms, rate of growth, pain in breast, nipple discharge and bone pain.

CLINICAL HISTORY AND EXAMINATION

Nipple, areola, overlying skin, swelling and draining lymph nodes should be examined.

- **Nipple retraction:** Nipple retraction occurs in association with both breast carcinoma and inflammatory lesions in the breast.
- **Skin dimpling or a change in contour:** Skin dimpling is present in a high percentage of patients with breast carcinoma.
- **Nipple and areola eczema:** Paget's disease of breast affects the nipple from the start, whereas eczema affects the areolar region first and only rarely affects the nipple skin, which eventually leads to erosion and ulceration of the nipple in elderly women. Eczema also occurs due to inflammation in the areolar and nipple regions.
- **Peau d'orange skin:** Inflammatory carcinoma of breast is a descriptive term referring to a clinical appearance resembling diffuse acute mastitis, which implies dermal lymphatic channels invasion by underlying usually infiltrating ductal carcinoma.
- **Axillary lymph nodes:** Axillary lymph nodes are palpable in 40% of patients with breast carcinoma.

IMAGING DIAGNOSTIC TECHNIQUES

Radiological investigations done in breast pathology include mammography, nuclear imaging (scintigraphy), Doppler flow studies, thermography, magnetic resonance imaging and positron emission tomography (PET) scan.

Digital Mammography

Screening mammography is done in asymptomatic women, when breast carcinoma is not suspected.

Diagnostic mammography is performed in symptomatic women, when a breast lump or nipple discharge is observed during self-examination by women.

- Digital mammography is screening procedure to detect nonpalpable 2–3 mm breast masses. Most mammographic calcification in benign breast lesions is indeterminate. A few benign lesions such as oil cysts, calcified fibroadenomas and vascular calcification have diagnostic mammographic features. Directional vacuum-assisted core biopsies can remove small benign calcified lesions. BIRADS (breast imaging and radiological analysis data system) is used for reporting digital mammograms (Table 25.26, Figs 25.55 and 25.56).
- Microcalcifications most often occur in ductal carcinoma *in situ* and sclerosing adenosis of breast. The risk of breast carcinoma increases with increase in the number of calcium specks in a cluster.
- Presence of ≥5 tightly clustered microcalcifications within 1 cm area of breast lump is considered a threshold for suspicion of breast cancer. Fibroadenoma shows popcorn calcification.

Table 25.26 BIRADS used for reporting mammograms

Seven Categories	BIRADS is Used for Reporting Mammograms: It has Seven Categories	Management	Likelihood of Breast Carcinoma
0	Incomplete assessment: It needs to additional imaging or prior examinations	Recall for additional imaging and/or await prior examinations	Not applicable
1	Negative assessment	Routine screening	Essentially 0%
2	Benign lesion	Routine screening	Essentially 0%
3	Probably benign lesion	Short interval follow up for 6 months and then continued	>0% and <2%
4	Suspicious lesion	Tissue diagnosis	4a. Low suspicion for malignancy (>2% to <10%) 4b. Moderate suspicion of malignancy (>10% to <50%) 4c. High suspicion of malignancy (>50% to <95%)
5	Highly suspicious of malignancy	Tissue diagnosis	>95%
6	Biopsy proved breast carcinoma	Surgical excision when clinical appropriate	Not applicable

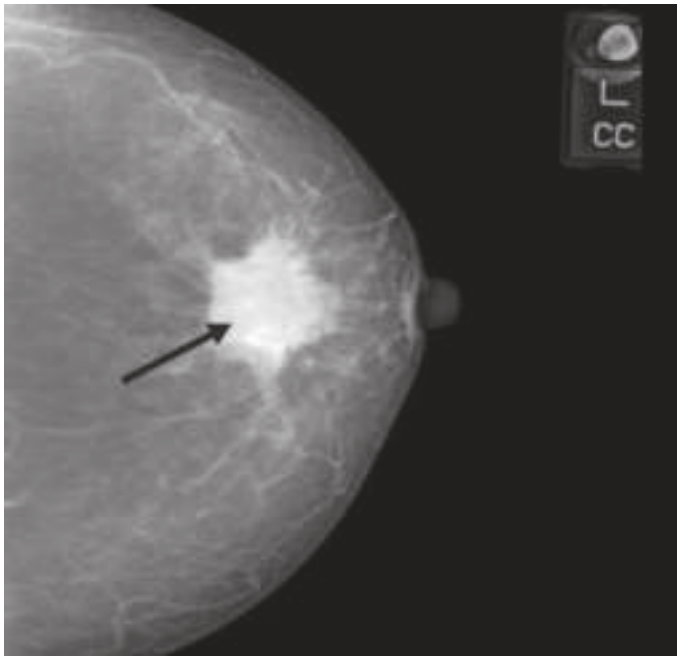


Fig. 25.55: Digital mammogram. It shows breast carcinoma: BIRADS 5 (highly suspicious) (arrow).



Fig. 25.56: Digital mammogram. It shows breast infiltrating carcinoma (biopsy proved breast carcinoma: BIRADS 6) (arrow).

- Mammographic densities are formed due to replacement of adipose tissue with radio-dense tissue. Fibrocystic disease of breast shows rounded densities. Breast carcinomas form irregular masses.
- Approximately 10% of breast carcinomas are not detected by digital mammography.

Xenomammography

Xenomammography is identical with digital mammography except the image produced on xenographic plate rather than a conventional transparency.

- The image produced is positive rather than negative.
- Edge enhancement and wide recording latitude permit details of the soft tissues of the breast, chest wall, and thinner peripheral portions of the breast to be recorded with one exposure.

Magnification Mammography

Magnification mammography enhances the sharpness of details and increases diagnostic accuracy for breast carcinoma.

- Optimum magnification is 1.5 times.
- Margins of breast lumps, degree and specificity of microcalcifications are clearly defined.
- Magnification mammography technique may significantly reduce the number of patients referred for biopsy.

Mammary Ultrasonography

Ultrasonography is the best investigation to differentiate cysts (transparent lesion) from solid lesions such as benign lesions (well demarcated) and breast carcinomas (indistinct outlines). Ultrasonography is also useful for guiding the aspiration of cysts to provide cytologic specimens on fine needle aspiration.

Bone Scan (Bone Scintigraphy)

Bone scan is also known as **bone scintigraphy**, which evaluates breast carcinoma metastases in bone by injecting radioactive substance by intravenous route. After 2–4 hours of absorption of radioactive substance, body scans (films) are taken. This radioactive substance is collected in areas of new bone formation formed by metastases, which appear as dark patches on the film. Any part of the bone may be affected by breast carcinoma.

Computed Tomography Scan

Computed tomography scan can detect breast carcinomas and permit visualization of both axillary and internal mammary lymph nodes. High cost and irradiation exposure are drawbacks of CT scan.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is better for assessing lesions in dense breasts than other methods. MRI is used for evaluation of breast carcinoma, staging of disease (metastases in lungs, bones, liver, brain and ovaries) and detection of silicon prosthesis.

Color Doppler Ultrasonography

Color Doppler ultrasonography serves as a useful imaging modality to evaluate internal structure, blood flow and breast lesions with a hope to differentiate benign nodules from malignant solid masses in breasts. Angiogenesis defined as the emergence of new blood vessels to further growth of the tumor, has stimulated interest in the potential uses of color Doppler ultrasonography in patients with breast carcinoma. Imaging techniques used to detect breast lesions are given in Table 25.27.

Table 25.27 Imaging techniques used to detect breast lesions

Imaging Techniques	Interpretation
Digital mammography: It detects 2–3 mm lesion. BIRADS is used for reporting mammograms. Seven categories are described	▪ Assessment incomplete: 0 ▪ Negative assessment: 1 ▪ Benign lesion assessment: 2 ▪ Probably benign lesion: 3 ▪ Suspicious lesion assessment: 4 ▪ Highly suspicious of malignancy: 5 ▪ Biopsy proved breast carcinoma: 6 **Note:** Popcorn calcification is seen in fibroadenoma. Breast tumor shows ≥5 dense calcified deposits in 1 × 1 cm area is highly suspicious of cancer
Ultrasonography	Ultrasonography detects cysts (transparent lesion), benign lesions (well demarcated) and breast carcinomas (indistinct outlines)
Bone scan (scintigraphy)	Bone scan is used for staging of breast carcinoma
CT scan	Detection of breast carcinoma and involvement of draining axillary and internal mammary lymph nodes
MRI	Evaluation of breast carcinoma and staging disease (metastases in lungs, bones, liver, brain and ovaries)
Color Doppler ultrasonography	Visualization of blood supply to the lump and to distinguish benign nodules from malignant sold masses in breasts.

Positron Emission Tomography Scanning

Positron emission tomography (PET) scanning is the most sensitive and specific imaging modality by using a radioactive substance for studying potential spread of breast cancer.

- At present, its main utility is to detect recurrences of breast carcinoma in scarred breasts.
- PET scanning is also useful in multifocal disease in detecting axillary involvement and equivocal cases of systemic metastases.
- PET scanning also assists in identification of nonaxillary lymph node metastases, i.e. internal mammary or supraclavicular lymph nodes for staging locally advanced and inflammatory breast carcinoma before starting neoadjuvant therapy.

TRIPLE ASSESSMENT OF BREAST LUMPS

Triple assessment in breast cancer is the combination of clinical examination, imaging (e.g. mammography for women aged 35 or over and ultrasonography for women under 35), and fine needle aspiration cytology.

SERUM TUMOR MARKERS

The potential uses of serum tumor markes in breast cancer include aiding early diagnosis determining prognosis, prospectively predicting response or resistance specific therapies surveillance after primary surgery and monitoring therapy in patients with advanced disease. Various serum tumor markers analyzed for breast carcinoma include CA 15-3, CA 27-29. BRCA1, BRCA2, PS2, CEA (carcinoembryonic antigen), PTHrp, GCDFP-15 (gross cystic disease fluid protein 15) and mammaglobin. CA 15-3 is a cell surface glycoprotein demonstrated in breast carcinoma. Serum tumor markers of breast carcinoma are given in Table 25.28.

Table 25.28 Serum tumor markers of breast carcinoma

Markers
CA 15-3
CA 27-29
BRCA1, BRCA2
PS2
Carcinoembryonic antigen (CEA)
PTHrP
Gross cystic disease fluid protein 15 (GCDFP-15)
Mammaglobin

Flow cytometry: DNA ploidy, and S-phase markers.
FISH (fluorescence in situ hybridization): HER2/neu marker.

FINE NEEDLE ASPIRATION CYTOLOGY

Fine needle aspiration cytology (FNAC) is widely used in the diagnosis of breast lumps, which has been reliable, rapid cost effective, complication free and an accurate diagnostic modality for the preoperative evaluation or management of spectrum of benign lesions and malignant tumors. Evaluation of breast lumps involves the rational use of a detailed clinical history, clinical examination imaging modalities and tissue diagnosis.

- Fine needle aspiration cytology is performed using a 23-gauge needle and 10 or 20 ml disposable syringe. Cellular material from brast lump is aspirated into syringe and expelled onto glass slides to prepare smears. All the smears are wet fixed in 95% methanol, and air-dried and stained with two stains, i.e. hematoxylin and eosin (H&E) and Papanicolaou stain. Slides are examined under light microscope by cytopathologist.
- Fibroadenoma can be usually distinguished by invasive ductal carcinoma of breast. Fibroadenoma consists of three components; invasive carcinoma has loosely cohesive and singly dispersed cells with highly atypical morphology. Fine needle aspiration cytology carries high specificity when diagnosing breast malignancy, with a false positive rate of 0–2%. Intraductal papilloma is cytologically distinguishable florid ductal hyperplasia; however, nipple discharge or a mass in the subareolar region may favor the diagnosis of intraductal papilloma. It is difficult to distinguish fibroadenoma from phyllodes tumor of breast on fine needle aspiration cytology, reflecting the fact that these two breast lesions exhibit many overlapping histologic features in common.
- Fine needle aspiration cytology findings in fibroadenoma, fibrocystic disease of breast, ductal carcinoma of breast and medullary carcinoma of breast are shown in Figs 25.57 to 25.60.

BREAST LUMP BIOPSY

Breast lump biopsy is recommended if digital mammogram shows suspicious area in breast. In addition, ultrasound and magnetic resonance imaging (MRI) reveals suspicious area in breast. Biopsy is done if patient has nipple or areolar change including crusting, scaling, dimpling skin or bloody nipple discharge.

- **Tru-cut core needle biopsy:** A small core is removed from the breast mass by means of a cutting needle technique. It is to interpret results on small biopsy measuring 2 cm in length and 1.0 mm wide.
- **Open biopsy:** Open biopsy should be performed only in patients with breast lump, who have been appropriately investigated by imaging, fine needle

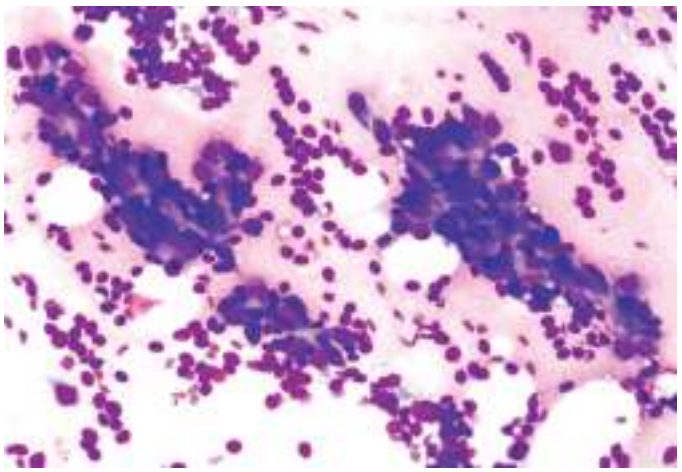


Fig. 25.57: Fibroadenoma of breast—fine needle aspirate smear shows benign ductal cells arranged in clusters. Stromal cells are also seen (400X).

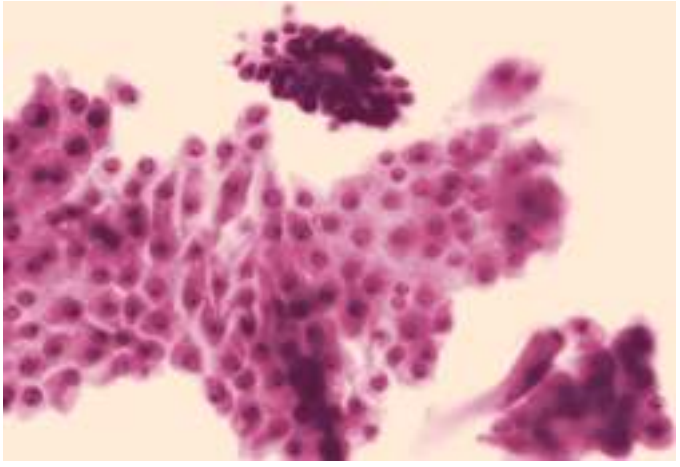


Fig. 25.58: Fibrocystic disease of breast—fine needle aspirate smear shows benign ductal cells arranged in clusters. Apocrine change is seen in the ductal cells (400X).

aspiration cytology, and tru-cut core needle biopsy. The specimen is fixed in 10% buffered formalin to obtain paraffin-embedded sections. Hematoxylin and eosin stained sections are studied by light microscopy. Freshly prepared 2% buffered glutaraldehyde is recommended for fixation of tissues for electron microscopy.

- **Skin biopsy:** Skin biopsy from areola or nipple is important in diagnosing Paget's disease of breast (ductal carcinoma involving epidermis) and inflammatory carcinoma of breast in which dermal lymphatic channels are involved.
- **Sentinel lymph node biopsy:** Sampling of initial lymph node that drains breast carcinoma is done. If sentinel lymph node is negative for metastases, the other lymph nodes in that group are usually negative. If sentinel lymph node is positive for metastases, there is one-third chance that other lymph nodes in the group have metastases.

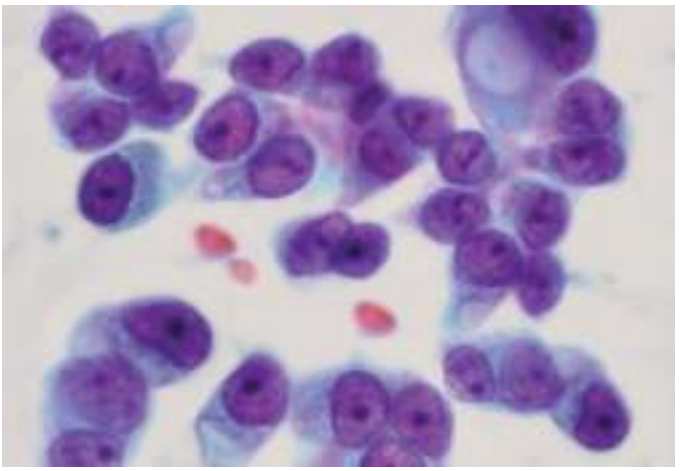


Fig. 25.59: Breast cancer—fine needle aspirate smear shows pleomorphic atypical cells with hyperchromatic nuclei and prominent nucleoli arranged in clusters and dissociated forms (400X).

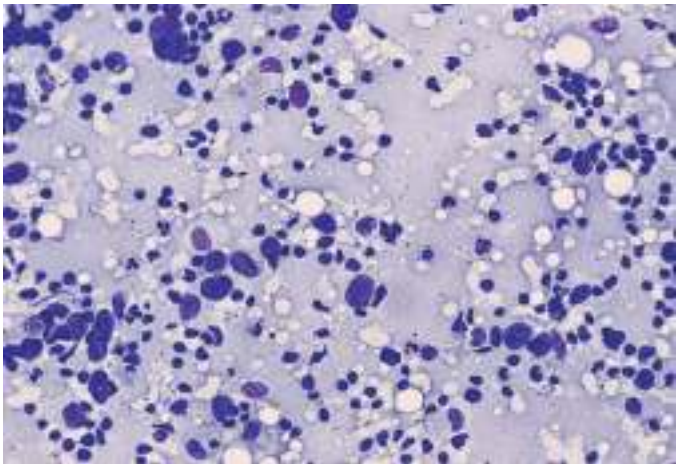


Fig. 25.60: Medullary carcinoma of breast—fine needle aspirate smear shows pleomorphic atypical cells with hyperchromatic nuclei and prominent nucleoli arranged in clusters and dissociated forms. In the background, there is presence of lymphocytes (400X).

FROZEN/CRYOSTAT SECTION TECHNIQUE

Frozen/cryostat section technique is a rapid diagnostic technique before proceeding to definite surgery to confirm breast carcinoma, assessment of margins around tumor, and lymph node involvement. Tissues are quickly frozen, sectioned, mounted on slides, stained with hematoxylin and eosin (H&E) and interpreted within a few minutes, which enable surgeon to take appropriate decisions about the extent of surgery.

Diagnostic difficulties occur in the presence of papillary lesions, sclerosing adenosis, florid adenosis, atypical ductular hyperplasia, fat necrosis, and lobular carcinoma *in situ* (LCIS) cause diagnostic problems on frozen section evaluation. These lesions are best diagnosed with paraffin-embedded sections.

HISTOLOGIC EXAMINATION

Tru-cut core needle biopsy, incisional biopsy, lumpectomy and mastectomy specimens including lymph nodes are fixed in 10% buffered formalin. Sections obtained from paraffin-embedded blocks are stained with hematoxylin and eosin (H&E) studied by light microscopy. The following histologic features of breast tumors are important in deciding course of treatment described as under:

- Size of tumor
- Gross examination of tumor
- Histologic type (*in situ* or invasive)
- Margin of tumor
- Lymph node status (number, size and extracapsular invasion)
- Nottingham modification of Bloom-Richardson (MBR) grade
- Nipple involvement
- Pathologic stage (tumor and lymph node status)
- DNA content
- S-phase fraction
- Immunohistochemistry (ER, PR, ERBB2/HER2/neu).

IMMUNOHISTOCHEMISTRY TECHNIQUE

Immunohistochemistry is a newer technique to detect antigens (markers) by applying monoclonal antibodies in a sample of tissue. It plays an important role in differentiating poorly differentiated carcinoma from lymphoma; and benign lesions from malignant tumors. It is performed for assessment of estrogen receptor (ER), progesterone receptor (PR), HER2/neu for the purpose of therapy of breast carcinoma. Applications of immunohistochemistry in breast carcinoma are given in Table 25.29. Immunoprofile in histologic variants of breast carcinoma is given in Table 25.30.

Estrogen and Progesterone Receptors Analysis

Estrogen and progesterone receptors (ER/PR) are present in normal and neoplastic epithelial cells of breast. It is worth mentioning that carcinomas of ovary and endometrium are also positive for ER/PR.

- ER immunostaining has become a standard protocol in management of breast cancer. In addition, ER/PR immunostaining is performed to predict response to hormonal therapy and identify metastases in lymph nodes and other organs from breast carcinoma.

Table 25.29 Applications of immunohistochemistry in breast carcinoma

Immunomarker	Immunomarker Locations	Features	Applications
E-cadherin	Membranous staining	Adhesion molecule expressed in ducts and lobules of breast	Loss of E-cadherin staining is hallmark of breast *in situ* lobular or invasive lobular carcinoma. E-cadherin staining is negative in breast *in situ* ductal or invasive ductal carcinoma
ER/PR	Nuclear staining	ER/PR expression in normal ductal cells and neoplastic cells of breast	ER/PR expression in breast carcinoma associated with favorable prognosis treated with tamoxifen. ER/PR immunomarkers are used to diagnose metastatic breast cancer
Gross cystic disease fluid protein 15 (GCDFP-15)	Cytoplasmic staining	GCDFP-15 immunomarker for breast and sweat glands showing apocrine differentiation	GCDFP-15 and ER used to detect metastatic breast carcinoma
Mammaglobin	Cytoplasmic staining	Mammaglobin immunomarker for breast and sweat glands showing apocrine differentiation	Mammaglobin more sensitive and less specific than GCDFP-15
HER2/neu	Cytoplasmic staining	HER2/neu is growth factor receptor expressed in neoplastic cells but absent in normal cells	Overexpression of HER2/neu in breast carcinoma associated with poor prognosis. Patients are treated by Herceptin
Myoepithelial cell markers	Variable staining	Smooth muscle actin (α-actin), calponin, smooth muscle myosin heavy chain (SMMHC) and p63 usual immunomarkers for myoepithelial cells. Other immunomarkers are CK14 (HMWCK), S-100 protein, glial fibrillary acidic protein (GFAP) and CD10	Myoepithelial cell layer is intact in benign and *in situ* breast lesions; but absent in invasive breast carcinoma

Microglandular adenosis lacks myoepithelial cells.

Table 25.30 Immunoprofile in histologic variants of breast carcinoma

Immunomarkers	Expression
Breast *in situ* ductal carcinoma/invasive ductal carcinoma (not otherwise specified)	
ER/PR	Positive (65–75%)
GCDFP	Positive (50%)
Mammaglobin	Positive (50%)
HER2/neu	Positive (10–30%)
CK7	Positive
Ki-67	Positive
p53	Positive
E-cadherin	Positive
NY-BR1	Positive (specific marker for undifferentiated breast cancer)
CK20	Negative
Breast *in situ* lobular carcinoma/invasive lobular carcinoma	
GCDFP	Positive (100%)
E-cadherin	Negative
Metaplastic carcinoma of breast	
CK903 (HMWCK)	Positive
p53	Positive
AE1/AE3	Variable
CAM 5.2	Positive (most reliable marker)
CK7	Positive (most reliable marker)
ER/PR	Negative
HER2/neu	Negative
Medullary carcinoma of breast	
ER/PR	Negative
HER2/neu	Negative
Basal-like carcinoma of breast	
ER/PR	Negative
HER2/neu	Negative
Micropapillary carcinoma of breast	
ER/PR	Negative
HER2/neu	Negative

GCDFP (gross cystic disease fluid protein), triple negative, i.e. ER, PR, HER2/neu (medullary carcinoma, basal-like carcinoma, micropapillary carcinoma).

- ER expression correlates better differentiation and favorable predictive factor for treating breast carcinoma with estrogen blocker 'tamoxifen'.
- Risks associated with tamoxifen are endometrial carcinoma and thromboembolism. Tamoxifen is contraindicated in active smokers and previous history of thromboembolism.
- Tamoxifen is administered for five years in cases of ductal carcinoma *in situ* (DCIS) and lobular carcinoma *in situ* (LCIS) for prevention of development of invasive carcinoma of breast.
- While analyzing ER/PR expression in breast carcinoma, percentage of neoplastic cells immunostaining intensity (strong, medium or weak) and internal control tissue should be taken into consideration.
- ER/PR positive breast carcinomas are treated with tamoxifen. Prognosis is excellent in these cases. ER/PR expression and interpretation in breast carcinoma is given in Table 25.31.

HER2/neu Growth Factor Receptor Analysis

HER2/neu is a growth factor receptor, which is absent in normal breast. Due to amplification of HER2/neu, its overexpression is demonstrated in 10–30% of breast carcinomas. HER2/neu positive breast carcinomas are poorly differentiated and associated with poor prognosis.

- HER2/neu positive patients are treated by anti-HER2/neu '**Herceptin**' chemotherapeutic agent.
- On the other hand, well differentiated breast carcinomas are ER/PR positive/HER2/neu negative.
- Triple negative breast carcinomas are medullary carcinoma, basal-like carcinoma and micropapillary carcinoma of breast.
- Analysis of HER2/neu is done by immunohistochemistry, fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH) and silver *in situ* hybridization technique. Factors adversely affecting analysis of HER2/neu by FISH technique

Table 25.31 ER/PR expression and interpretation in breast carcinoma

ER/PR	Interpretation
≥1% of neoplastic cells showing staining of any intensity	Positive
<1% of cells showing staining of internal control tissue	Negative

While analyzing ER/PR expression, percentage of neoplastic cells immunostaining intensity (strong, medium or weak) and internal control tissue should be taken into consideration.

are prolonged fixation of tissue in formalin for more than one week and insufficient protease treatment of the tissue section.

- Scoring method of HER2/neu expression in breast carcinoma by immunohistochemistry technique is based on the cell membrane staining pattern (Table 25.32). Algorithm of HER2/neu status in diagnosis of breast carcinoma is shown in Fig. 25.61.

E-Cadherin Cell Adhesion Molecule Analysis

E-cadherin is cell adhesion molecule responsible for cell-to-cell adhesion of epithelial cells. E-cadherin requires calcium ions to function. It is expressed in normal breast cells lining the ducts and lobules.

- Main application of E-cadherin immunostaining is to differentiate breast *in situ* ductal carcinoma/invasive ductal carcinoma, atypical ductal hyperplasia from *in situ* lobular carcinoma and invasive lobular carcinoma of breast.
- E-cadherin is expressed in breast *in situ* ductal carcinoma/invasive ductal carcinoma, atypical ductal hyperplasia of breast.
- Loss of E-cadherin immunostaining is a hallmark of breast *in situ* lobular carcinoma and invasive lobular carcinoma of breast. It is worth mentioning that neoplastic cells are discohesive in this histologic variant of breast carcinoma. Application of E-cadherin in normal breast and breast carcinoma is given in Table 25.33.

Gross Cystic Disease Fluid Protein 15 Analysis

Gross cystic disease fluid protein 15 (GCDFP-15) is a cytoplasmic immunomarker for breast and sweat glands showing apocrine cell differentiation. GCDFP-15

Table 25.32 Scoring method of HER2/neu expression in breast carcinoma by immunohistochemistry based on the cell membrane staining pattern

Criteria of Cell Membrane Staining Pattern	Score	Interpretation
<30% of neoplastic cells showing membranous immunostaining	0	Negative
>30% of neoplastic cells showing partial membranous immunostaining	1	Negative
>30% of neoplastic cells showing moderate to complete membranous immunostaining	2	Weakly positive
>30% of neoplastic cells showing moderate to strong complete membranous immunostaining	3	Strongly positive

Breast carcinomas showing weakly positive HER2/neu expression or equivocal cases should be further analyzed by FISH or CISH.

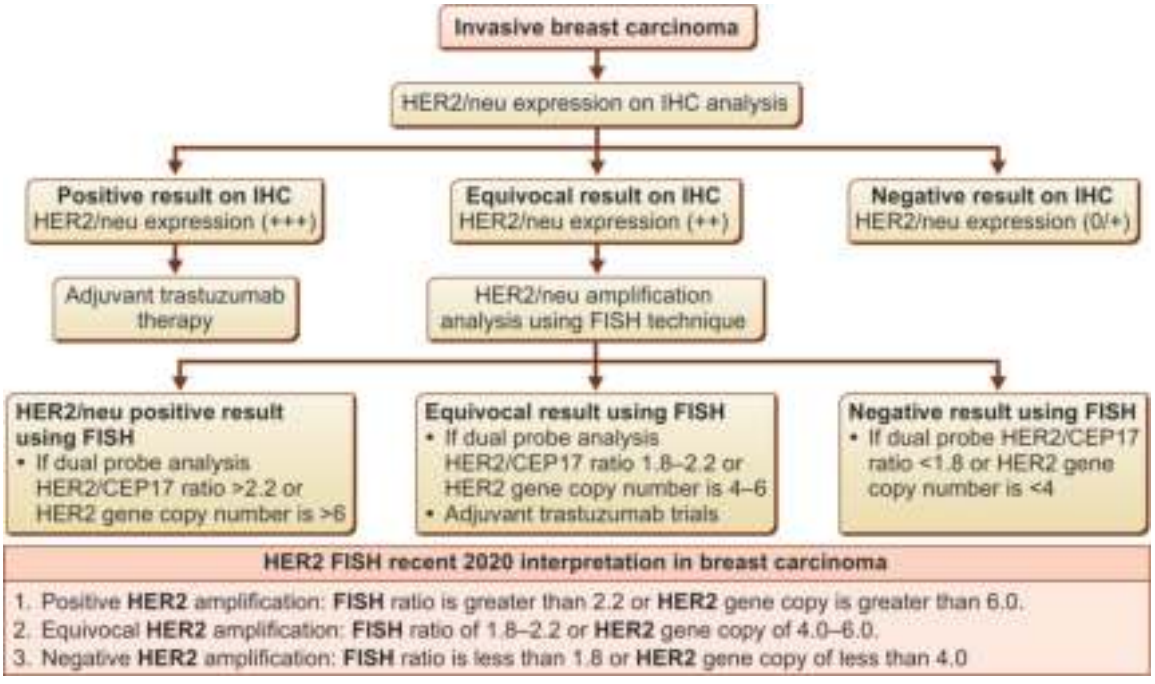


Fig. 25.61: Algorithm of oncotype DX in diagnosis of breast carcinoma.

Table 25.33 Application of E-cadherin in normal breast and breast carcinoma

Breast Epithelial Cells	E-cadherin Expression
Epithelial cells lining ducts and ductules	Positive
Breast *in situ* ductal carcinoma/invasive ductal carcinoma, atypical ductal hyperplasia	Positive
Breast *in situ* lobular carcinoma and invasive lobular carcinoma	Negative

immunomarker is analyzed to demonstrate breast carcinoma metastases. ICDFP-15 immunostaining pattern is patchy in the cytoplasm of neoplastic cells in ≤50% of breast carcinoma cases.

- GCDFP-15 is also expressed in lung carcinoma, salivary duct carcinoma and some skin adnexal tumors.
- Therefore, GCDFP-15 is always analyzed in conjunction with ER and PR. Breast lobular carcinoma showing apocrine differentiation shows positivity for GCDFP-15.

Mammaglobin Analysis

Mammaglobin gene encodes a 10 kilodalton glycoprotein, which is expressed in adult female mammary glands. Like gross cystic disease fluid protein 15 (GCDFP-15), mammaglobin recognizes apocrine differentiation of breast and sweat glands. Mammaglobin is a cytoplasmic immunostaining marker. Mammaglobin expression is increased in breast carcinoma, and carcinomas of ovary, endometrium and cervix. It is less specific but more sensitive immunomarker than GCDFP-15.

Myoepithelial Cell Immunomarkers

Myoepithelial cells are smooth muscle-like cells present in the glandular epithelium as a thin layer above the basement membrane and beneath the luminal cells of breast. Myoepithelial cell layer is intact in benign and *in situ* breast lesions; but absent in invasive ductal carcinoma. Microglandular adenosis also lacks myoepithelial cells.

Pathology Pearls: Myoepithelial Cell Markers

Panel of markers is used on paraffin-embedded tissue section of breast lesions to demonstrate myoepithelial cells in breast. Smooth muscle actin (α-actin), calponin, smooth muscle myosin heavy chain (SMMHC) and p63 are usual immunomarkers for myoepithelial cells. Other immunomarkers are CK14 (HMWCK), S-100 protein, glial fibrillary acidic protein (GFAP) and CD10.

Myoepithelial Cell Immunomarkers	Expression
▪ Smooth muscle actin (α-actin)	▪ Positive
▪ Calponin	▪ Positive
▪ Smooth muscle myosin heavy chain (SMMHC)	▪ Positive
▪ p53	▪ Positive
▪ CK14 (HMWCK)	▪ Positive
▪ S-100 protein	▪ Positive
▪ Glial fibrillary acidic protein (GFAP)	▪ Positive
▪ CD10	▪ Positive

Other Immunomarkers

Other immunomarkers include p53, galectin 3, NY-BR1, cathepsin D, stress response protein 27 (Srp27), milk fat globulin membrane antigen and lactalbumin.

RECENT ADVANCES: MOLECULAR GENETIC TECHNIQUES IN BREAST CANCER

Oncotype DX Assay

Oncotype DX is a RT-PCR based assay of 16 cancer-related genes and 5 normal comparator genes.

- Oncotype DX has been designed to use in estrogen positive breast carcinoma. The test is run on formalin-fixed paraffin-embedded tissue.
- The assay computes a recurrence score (RS) from 0 to 100. Oncotype DX results are reported as a recurrence score
  - Recurrence rate <18 is considered low risk for breast carcinoma recurrence.
  - Recurrence rate 18–30 is considered intermediate risk for breast carcinoma recurrence.
  - Recurrence rate >30 is considered high risk of breast carcinoma recurrence. It is associated with poor prognosis referring to the likelihood of recurrence without treatment. Higher recurrence rate for breast carcinoma is also associated with a higher probability of response to chemotherapy, which is termed a positive predictive factor.

Algorithm of oncotype DX in diagnosis of breast carcinoma is shown in Fig. 25.62.

MammaPrint Gene Expression Profile Test

MammaPrint gene expression profile test analyzes 70 genes from an early-stage breast carcinoma tissue sample to figure out if the breast carcinoma has a low or high risk of recurrence within 10 years after diagnosis.

- MammaPrint is the first FDA approved gene microarray based prognostic score performed on fresh frozen tissue of breast carcinoma.

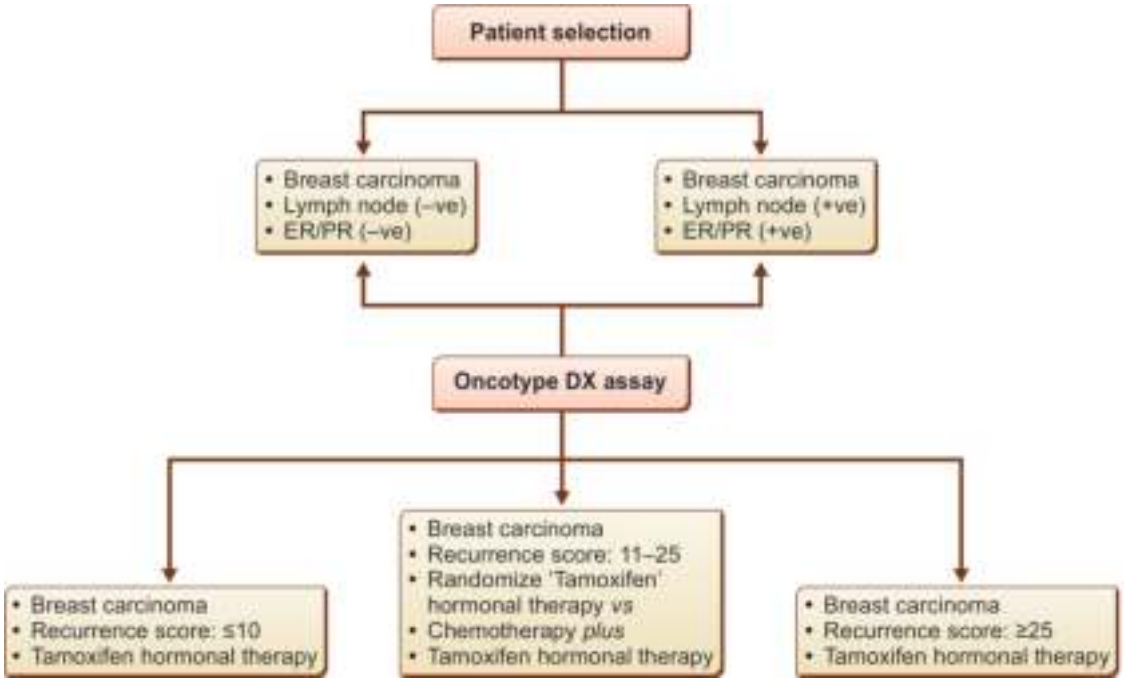


Fig. 25.62: Algorithm of oncotype DX in diagnosis of breast carcinoma.

- Top 70 genes that regulate cell cycle, invasion and metastasis and angiogenesis are evaluated in cases of breast carcinoma. A tumor has been categorized as low or high risk for breast carcinoma.
- MammaPrint can determine whether or not patient with breast carcinoma will be benefited from chemotherapy.
- MammaPrint can be used to analyze breast carcinomas that are in stage I or stage II of invasive ductal carcinoma of breast measuring <5 cm and involvement of three or more lymph nodes.

Algorithm of MammaPrint in diagnosis of breast carcinoma is shown in Fig. 25.63.

Prosigna Kit Test (PAM50) Risk of Recurrence Score

Prosigna kit utilizes formalin-fixed paraffin-embedded tissue from breast carcinoma to quantify mRNA expression of 50 genes used in the PAM50 molecular classification algorithm and 50 housekeeping genes.

Prosigna kit is indicated for *in vitro* diagnostic use in postmenopausal women with hormone receptor positive, lymph node negative (stage I or II), or lymph node positive (stage II) of breast carcinoma to be treated with adjuvant endocrine therapy for assessment of risk of distant recurrence of disease.

Breast Carcinoma Index Test

The breast carcinoma index (BCI) test analyzes the activity of seven genes, that predict the risk for lymph node negative, hormone receptor positive breast carcinoma in patients, who visit hospital after 5–10 years after diagnosis of breast carcinoma.

- BCI test can help the patient or attending oncologist to decide whether extending hormonal therapy 5 more years (for a total of 10 years of hormonal

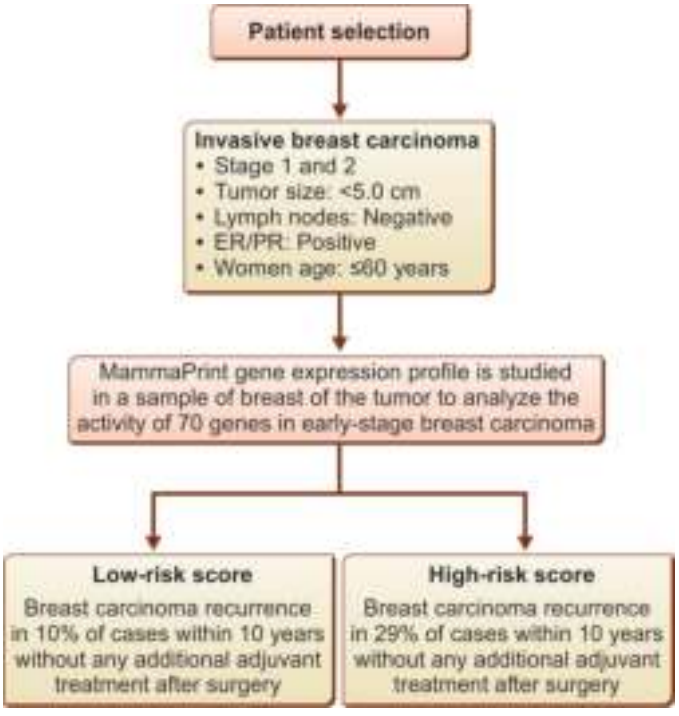


Fig. 25.63: Algorithm of MammaPrint in diagnosis of breast carcinoma.

therapy) would be beneficial to the breast carcinoma patient.
- BCI test is performed on formalin-fixed paraffin-embedded (FFPE) tissue from breast carcinoma that was removed after surgery.

EndoPredict Breast Cancer Prognostic Test

The EndoPredict test is a genomic test for patients newly diagnosed with early stage, estrogen-receptor positive and ERBB2/HER2/neu negative breast carcinoma.
- EndoPredict test is an RT-PCR-based assay using formalin-fixed paraffin-embedded tissue, which measures the expression of 8 breast carcinoma-related genes and 3 housekeeping genes.
- The EndoPredict test provides a score that is either low risk or high risk for breast carcinoma recurring as distant metastases. Test helps the patient and doctor to decide whether chemotherapy or other treatment required to reduce risk for breast carcinoma after surgery.

Next-Generation Sequencing in Breast Cancer

Molecular biomarkers indicating disease diagnosis, prognosis and treatment decisions in patients with breast carcinoma are being covered by next-generation sequencing (NSG) technologies. Over the past many years, various molecular profiling technologies such as immunohistochemistry (IHC), gene expression microarrays and germline DNA sequencing have had considerable impact on the clinical care of patients with breast carcinoma. Although the molecular subtyping of breast carcinoma has been extensively studied and incorporated into clinical care guidelines. Next-generation sequencing has improved our understanding of breast cancer with the genomic landscapes of early- and advanced-stage disease being well documented.
- Next-generation sequencing (NGS) is applied in a variety of ways such as whole genome sequencing, target sequencing, gene expression profiling, chromatin immunoprecipitation sequencing and small RNA sequencing to accelerate biomedical and biological research.
- Next-generation sequencing is applied to analyze genomic alterations such as insertion, deletions and copy number changes in breast carcinomas.
- Next-generation sequencing with breast carcinoma successfully reveals actionable genetic alterations in BRCA1, BRCA2, PIK3CA, HER2/neu, CCND1, ESR1, CDK6, FGFR1, EGFR, K-RAS, PDGFRA and AKT. Gene mutations are more frequently detected by plasma-based tests, whereas gene amplifications are analyzed by tissue-based tests. Plasma-based testing has utility in monitoring for resistance mutation status in patients with breast carcinoma.
- It is expected to provide insights on personalized treatment of breast carcinoma patients. With its increasing use, novel therapeutic target therapy is expected to be identified.

Microarray Studies in Breast Cancer

Microarray studies on prognostic gene expression profiles in breast carcinoma are done by using multiple microarray platforms that use varying parameters.
- These parameters include distinct sets of genes either cDNA of variable lengths or small oligonucleotide sequences.
- Different methods to determine gene include fluorescently labeled cDNA from breast carcinoma neoplastic cells to the microarray or hybridization of both a test and reference set of differently labeled cDNA to single microarray and measure the ratio.

cDNA Microarray in Breast Cancer

cDNA microarray study is performed to analyze 456 intrinsic genes in breast carcinoma.
- Luminal 'A' breast carcinoma has better prognosis than luminal 'B' breast carcinoma.
- Worst prognosis is for basal-like and ERBB2 (HER2/neu) positive breast carcinomas.

Oligonucleotide Microarray in Breast Cancer

Oligonucleotide microarray study is done to analyze 70 genes, 76 genes and 442 genes by different methods.
- Good signature genes are related to low metastasis risk for breast carcinoma.
- Poor signature genes are associated with a high metastasis risk for breast carcinoma.

BREAST CARCINOMA: MANAGEMENT

Treatment of breast carcinoma depends on the stage of the disease. Several options for treatment are most often used in combination for best results. These are described as under.

Surgical procedures include lumpectomy, mastectomy (partial, total, or radical).

Clinical Pearls: Levels of Intervention in Breast Carcinoma

Levels of intervention in breast carcinoma important in management are mentioned below.

- Identification of risk factors and risk reduction therapy
- Screening of cases
- Staging and management
- Hormonal therapy
- Chemotherapy
- Radiotherapy
- Follow-up

BREAST LUMPECTOMY

Lumpectomy is a segmental mastectomy with microscopically free margins, plus sentinel lymph node and low level I and II axillary node resection (breast conservative therapy) that is followed by radiation therapy of breast carcinoma.

MODIFIED RADICAL MASTECTOMY

Modified radical mastectomy in patient with breast carcinoma is removal of all ipsilateral breast tissue, the pectoralis minor muscle, the nipple–areola complex, and levels I, II and III axillary lymph nodes below axillary vein.

Lymph nodes are examined for signs of further metastases. A winged scapula may occur due to damage of the long thoracic nerve. There is also a danger for developing lymphedema in patients with breast carcinoma, who have undergone modified radical mastectomy.

Clinical Pearls: Adjuvant Therapy in Breast Carcinoma

- After surgical excision, adjuvant therapy for breast carcinoma is based on tumor size, modified Bloom-Richardson (MBR) grade, lymphovascular invasion and ERBB2/HER2/neu.
- Adjuvant therapy includes cytotoxic chemotherapeutic and/or endocrine therapy or radiotherapy of the primary tumor.
- Adjuvant therapy is used to ensure that all microscopic disseminated carcinoma cells are destroyed.

RADIATION THERAPY

Radiation therapy can be directed at the breast carcinoma tumor in the breast, the chest wall, or other tissues known or suspected to have remaining breast carcinoma neoplastic cells. Radiation therapy is aimed to slow or shrink breast carcinoma tumor to make surgery easier.

CHEMOTHERAPY

Chemotherapy is used to reduce the size of breast carcinoma growth for making surgery easier. It is used to help eliminate breast carcinoma neoplastic cells that may still remain in the breast or that may have already spread to other parts of the body.

HORMONAL THERAPY

Hormone therapy with **tamoxifen** is used to block the effects of estrogen that may otherwise help breast carcinoma neoplastic cells to survive and grow.

Most women with breast carcinoma neoplastic cells expressing estrogen or progesterone benefit from treatment with tamoxifen.

AROMATASE INHIBITORS THERAPY

A new class of medicines called *aromatase inhibitors*, such as **aromasin** has been shown to be as good as or possibly even better than tamoxifen in women with stage IV breast carcinoma.

BIOLOGICAL THERAPY

Monoclonal antibodies may be administered to attack potential breast carcinoma neoplastic cells and reduce the risk for recurrence. **Herceptin** monoclonal antibody binds HER2/neu on breast carcinoma neoplastic cell surface and induces receptor internalization. Investigation of a breast lump is shown in Fig. 25.64.

MISCELLANEOUS DISORDERS OF THE FEMALE BREASTS

CONGENITAL HYPOPLASIA OF BREAST

Absence of the underlying pectoralis muscles is often associated with congenital hypoplasia of female breast.

GIANT JUVENILE HYPERTROPHY OF BREAST

The redness of breast in patient with juvenile hypertrophy of breast occurs due to cellulitis from ulceration caused by wearing a bra that is too tight. This benign lesion is removed followed by reconstruction of breast.

ACCESSORY NIPPLE

Accessory nipple occurs in both sexes and may be found anywhere on the 'milk line' from the axilla to the groin.

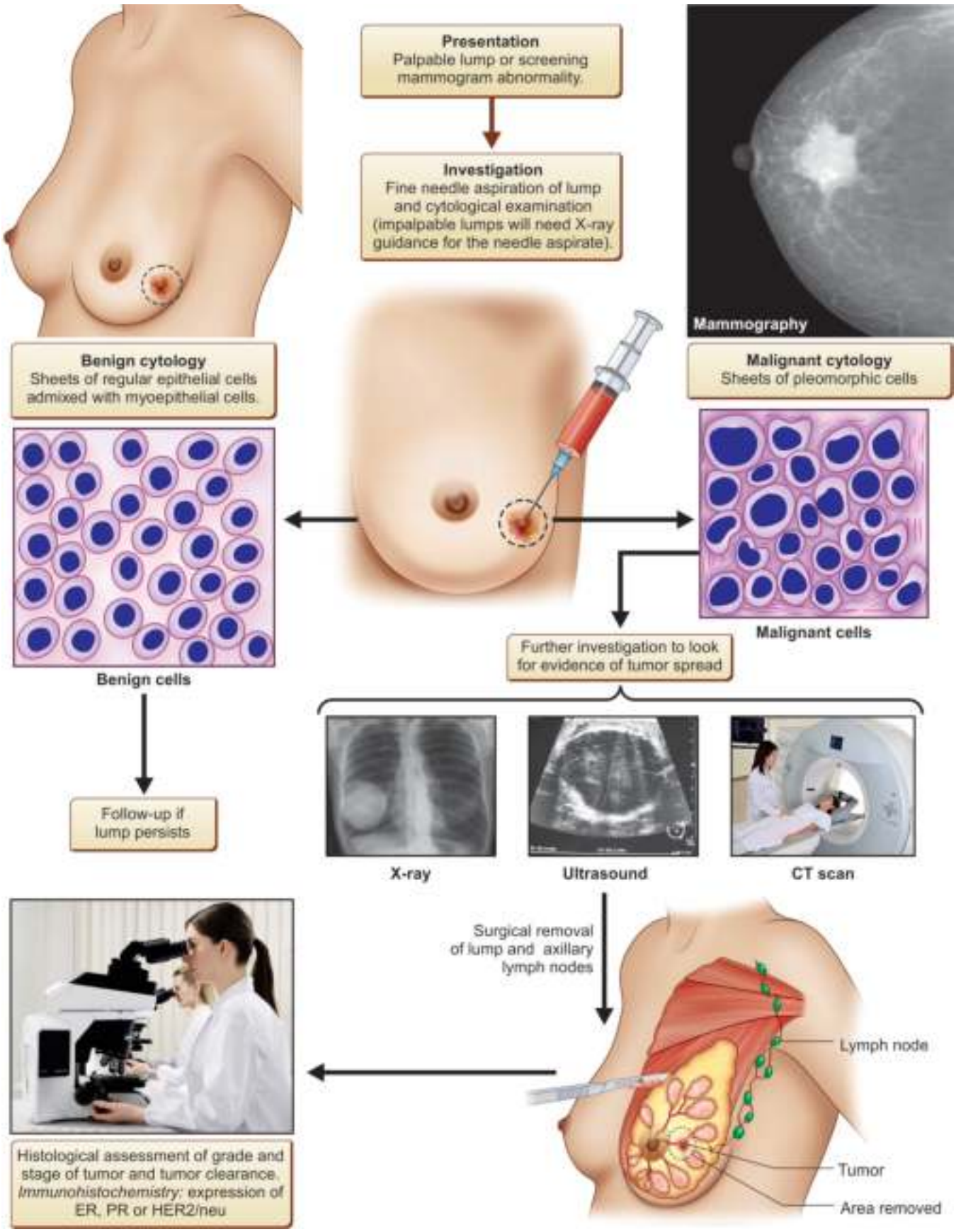


Fig. 25.64: Investigation of a breast lump.

MALE BREASTS

NON-NEOPLASTIC DISORDERS OF MALE BREASTS

GYNECOMASTIA

Gynecomastia is enlargement of the male breast analogous to giant juvenile hypertrophy of the female breast, which occurs most commonly at about puberty, presumably resulting from mild hormonal imbalance. Gynecomastia may occur also during adult life. The patient presents with unilateral or bilateral breast enlargement initially in the subareolar region.

Etiopathogenesis

Unilateral gynecomastia is most often idiopathic. Only very occasional case of gynecomastia is caused by an endocrine tumor. Gynecomastia is most often bilateral due to secondary causes, which occurs due to excess of circulating estrogens or a relative increase in the estrogen and androgen ratio. Estrogen excess relative to androgens results in proliferation of both ducts and stroma of male breasts.

- Gynecomastia is associated with: (a) hormone-secreting adrenal or testicular neoplasms (estrogenic), (b) paraneoplastic production of gonadotropins by carcinomas, (c) Klinefelter's syndrome, and (d) liver cirrhosis resulting in increased conversion of androstenedione into estrogens.
- Liver normally inactivates estrogen in men. Male patients with cirrhosis no longer inactivate estrogen. Hyperestrinism in men manifests gynecomastia, loss of chest and pubic hair, impotence, testicular atrophy and palmar erythema (liver palms); spider nevi (capillary telangiectasia) of the face, upper arms, and chest.

Surgical Pathology: Gynecomastia

Gross Morphology

- Gynecomastia is soft rubbery to firm gray-white subareolar mass.
- Gynecomastia can be ill-defined subareolar induration

Light Microscopy

- Histologic examination of gynecomastia specimen shows active epithelium within a proliferation of mammary ducts with piling of nuclei in the ducts, periductal fibrosis with numerous fibroblast nuclei.
- No lobules are found in the male breast.

Immunohistochemistry Technique

- Luminal epithelium of ducts expresses CK5/CK6 and KC14.
- Intermediate cuboidal to columnar cells express ER, PR and AR (androgen receptor).
- Outer myoepithelial cells express CK5/CK6 and CK14.

NEOPLASTIC DISORDERS OF MALE BREASTS

INVASIVE DUCTAL CARCINOMA

Invasive ductal carcinoma occurs in elderly men of 60–70 years of age when compared to women. Most invasive ductal carcinoma of male breast is ER positive. Clinical presentation, imaging findings and ultimately image-guided biopsies of the lesion provide tissue for accurate histologic diagnosis.

Risk Factors

BRCA1 is the strongest familial risk factor for invasive ductal carcinoma of male breast in 17% of cases. Prognosis is very poor to that of female breast carcinoma. Klinefelter's syndrome and BRCA1 mutation increase risk for breast carcinoma in men. Other risk factors linked to invasive ductal carcinoma of male breast include hereditary BRCA2 mutation, PTEN mutation (Cowden syndrome) and CHEK2 mutation carriers; radiation, antiandrogen therapy, testicular atrophy, occupational exposure to high temperatures, pituitary gland dysfunction, and therapeutic drugs such as digitalis, tricyclic antidepressants. Annual screening of invasive ductal carcinoma of male breast is recommended in men with BRCA mutations.

Clinical Features

Patient with invasive ductal carcinoma of male breast presents with nipple retraction, painless, firm subareolar mass or a mass in the outer quadrant of the breast, and serous nipple discharge. Lymph node metastases are more common in men than women due to paucity of breast tissue in men. Axillary lymph nodal staging is done by sentinel lymph node biopsy. The tumor invades the chest wall muscles.

Surgical Pathology: Invasive Ductal Carcinoma of Male Breast

Gross Morphology

Male breast invasive ductal carcinoma is grossly identical to female breast carcinoma. Cystic papillary carcinoma can be striking.

Light Microscopy

- About 85% of breast carcinomas of male breast are of invasive ductal type. Tumor is poorly differentiated invasive ductal carcinoma followed by papillary carcinoma and other subtypes similar to female breast carcinoma.
- Tumor is composed of pleomorphic atypical cells with hyperchromatic nuclei and prominent nucleoli. Cells are arranged in sheets and clusters with presence of mitotic figures.

Immunohistochemistry Technique

- Older patients over 65 years are most likely to have ER positive, PR positive and AR positive tumors. ER positive invasive ductal carcinoma has better prognosis than ER negative.

Markers	Expression
ER	Positive (85–90%)
PR	Positive (85%)
AR	Positive (90–95%)
BCL-2	Positive (84% mostly luminal A and luminal B)
HER2/neu	Positive (younger patients)

Prognostic Factors

Poor prognostic factors for invasive ductal carcinoma of male breast include lymphovascular invasion, tumor size over 2 cm, poor histologic differentiation, HER2/neu overexpression, TP53 inactivation/biallelic loss, and amplification of CCND1 (11q13), contralateral breast involvement, androgen receptor (AR) positive luminal type A male breast carcinoma (ER positive, HER2/neu negative, high tumor grade) has better survival rate.

Management

Modified radical mastectomy followed by external radiation therapy is the standard treatment for breast invasive ductal carcinoma of male breast. Hormonal therapy, as an adjuvant treatment, is the first-line approach in a majority of patients, and chemotherapy is reserved for patients with poor prognostic factors.

CHAPTER

26

Endocrine System: Pancreas, Thyroid, Parathyroid, Adrenal and Pituitary Glands

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

DISORDERS OF ENDOCRINE PANCREAS

- Endocrine pancreas: embryology, anatomy and physiology
  - Embryology of pancreas
  - Anatomy of pancreas
  - Physiology of pancreas
- Insulin hormone
  - Insulin synthesis
  - Insulin release
  - Insulin actions
  - Insulin signaling pathways
- Diabetes mellitus
  - Diabetes mellitus: classification
  - Acute diabetic complications
  - Diabetes mellitus and complications: cellular metabolic signaling pathways
  - Long-term complications of diabetes mellitus
  - Laboratory diagnosis of diabetes mellitus

DISORDERS OF THYROID GLAND

- Anatomy of thyroid gland
- Physiology of thyroid gland
  - Regulation of thyroid hormones
  - Synthesis of thyroid hormones
- Thyroiditis
  - Hashimoto's thyroiditis
  - Subacute painless lymphocytic thyroiditis
  - Subacute granulomatous thyroiditis
  - Riedel's thyroiditis
  - Acute bacterial thyroiditis
- Hypothyroidism
  - Congenital hypothyroidism
  - Cretinism (thyroid dwarf)
  - Myxedema (hypothyroidism) in adults
- Hyperthyroidism
  - Toxic nodular goiter
  - Graves' disease
  - Hyperthyroidism: other causes
- Goiter
  - Simple nontoxic goiter
  - Multinodular goiter
- Neoplastic disorders of thyroid gland
  - General considerations
  - Follicular thyroid adenoma
  - Hyalinizing trabecular tumor
  - Papillary thyroid carcinoma
  - Follicular thyroid carcinoma
  - Oncocytic thyroid neoplasms
  - Medullary thyroid carcinoma
  - Poorly differentiated thyroid carcinoma
  - Anaplastic thyroid carcinoma
  - Secondary thyroid tumors
- Thyroid gland anomalies
  - Thyroglossal duct cyst
  - Ectopic thyroid tissue
  - Lingual thyroid
- Thyroid disorders: diagnostic approach
  - Thyroid function tests
  - Imaging techniques
  - Fine needle aspiration cytology
  - Histopathologic examination
  - Molecular genetic testing
  - Serum tumor markers
  - 24 hours urinary catecholamines assay

DISORDERS OF PARATHYROID GLANDS

- Anatomy of parathyroid glands
- Physiology of parathyroid glands
  - Calcium metabolism: regulation
  - Vitamin D metabolism: regulation
- Hyperparathyroidism
  - Primary hyperparathyroidism
  - Secondary hyperparathyroidism
  - Tertiary hyperparathyroidism
  - α-Hydroxylase deficiency (vitamin D-resistant rickets)
- Hypoparathyroidism
  - Tetany (tetanic seizure)
  - Pseudohypoparathyroidism
- Neoplastic disorders of parathyroid glands
  - Parathyroid adenoma
  - Parathyroid carcinoma

DISORDERS OF ADRENAL GLANDS

- Anatomy and physiology
  - Adrenal cortex
  - Adrenal medulla
- Adrenal cortex hyperfunctioning
  - Cushing's syndrome
  - Hyperaldosteronism
- Adrenocortical insufficiency
  - Addison's disease
  - Waterhouse-Friderichsen syndrome
  - Adrenogenital syndrome
- Neoplastic disorders of adrenal medulla
  - Pheochromocytoma
  - Neuroblastoma
- Neoplastic disorders of adrenal cortex
  - Adrenocortical adenoma
  - Adrenocortical carcinoma

DISORDERS OF PITUITARY GLAND

- Anatomy and physiology
- Anterior pituitary gland: hypofunctioning
  - Primary hypopituitarism
  - Other causes of hypopituitarism
- Posterior pituitary gland: hypofunctioning
  - ADH deficiency
    - Central diabetes insipidus
    - Nephrogenic diabetes insipidus
- Syndrome of inappropriate ADH secretion (SIADH)
- Neoplastic disorders of pituitary gland
  - Pituitary adenomas
  - Lactotroph adenoma (prolactinoma)
  - Somatotroph adenoma
  - Corticotroph adenoma
  - Mixed somatotroph–lactotroph adenoma
  - Craniopharyngioma

DISORDERS OF ENDOCRINE PANCREAS

ENDOCRINE PANCREAS: EMBRYOLOGY, ANATOMY AND PHYSIOLOGY

EMBRYOLOGY OF PANCREAS

The pancreas is formed during embryonic development from two pancreatic buds that originate from the duodenal part of the foregut of endodermal origin.

- The pancreas development results from rotation of the ventral bud and the fusion of the two buds.
  - Ventral pancreatic bud gives rise to main pancreatic duct, uncinate process, and head of the pancreas.
  - Dorsal pancreatic bud ultimately gives rise to accessory pancreatic duct, the body, isthmus and tail of the pancreas.
- Annular pancreas is a congenital condition in which the head of the pancreas surrounds the second portion of the duodenum, which may be associated with duodenal atresia. Infants present with feeding disorders and growth retardation.

ANATOMY OF PANCREAS

Pancreas is a compound gland with exocrine and endocrine functions. Pancreas consists of the head, neck, body and tail. The widest part is the head, which is attached to the first part of small intestine, known as the duodenum through which pancreatic duct of Wirsung.

- Pancreas has a rich blood supply, with blood vessels originating as branches of both celiac artery and superior mesenteric artery.
- Pancreas receives parasympathetic innervation from the vagus nerve and sympathetic innervation from the greater and lesser splanchnic nerves (T5–T12).

PHYSIOLOGY OF PANCREAS

Pancreas has two main functions: (a) **exocrine function** helps in digestion by releasing peptidases, lipases and amylase into the duodenum; and (b) **endocrine function** regulates blood sugar by releasing hormones such as insulin (β cells 60%), glucagon (α cells 25%) and somatostatin (δ cells 10%) of islets of Langerhans. Islets of Langerhans constitute 1–2% of pancreatic tissue (Fig. 26.1). Flowchart demonstrates actions of insulin and glucagon on glucose, lipid and protein metabolism (Fig. 26.2).

Pancreatic Islets of Langerhans

The pancreatic islets of Langerhans contain endocrine hormone producing cells. These cells constitute 1–2% of the pancreas volume. Islets of Langerhans cells secrete glucagon (α cells), insulin (β cells) and somatostatin (δ cells), which regulate glucose, lipid and protein metabolism.

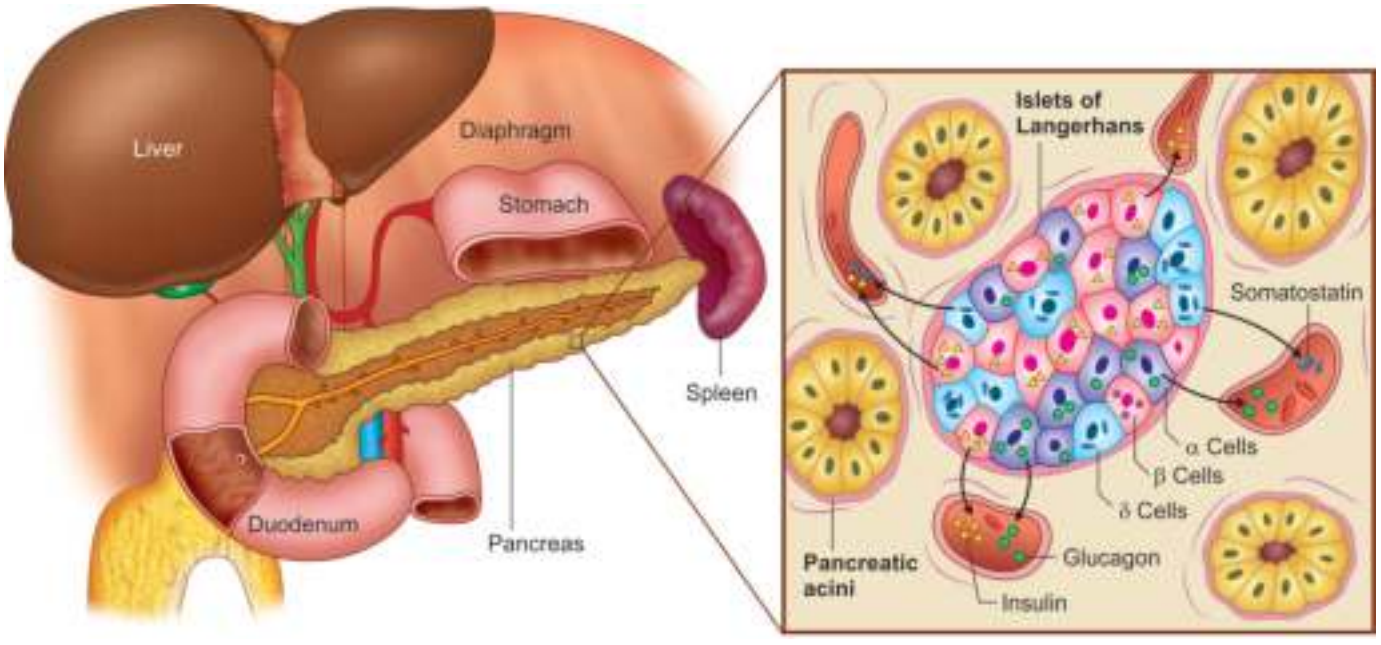


Fig. 26.1: Pancreas demonstrates arrangement of α, β and δ cells of islets of Langerhans. These cells secrete glucagon (α cells), insulin (β cells) and somatostatin (δ cells).

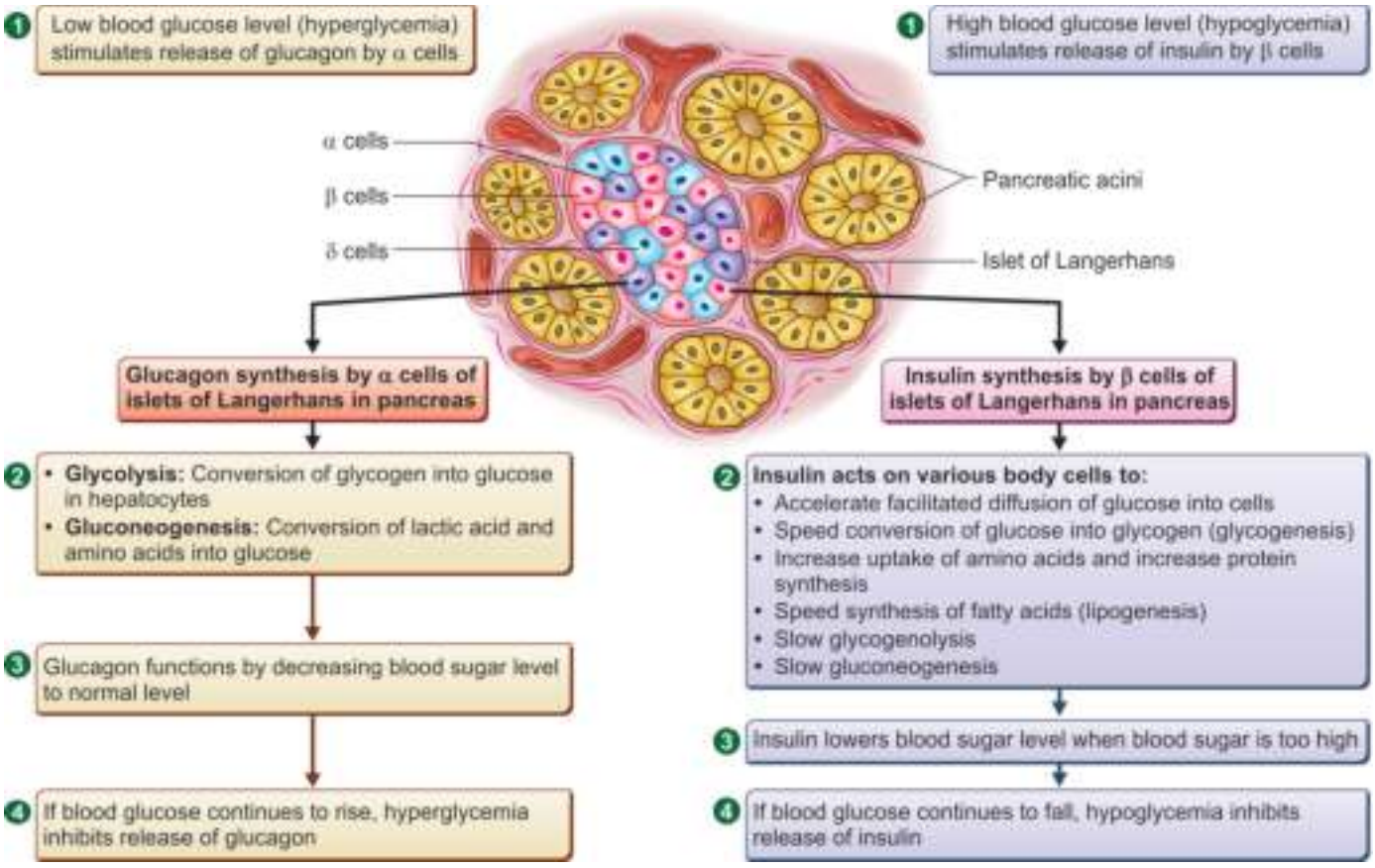


Fig. 26.2: Flowchart demonstrates actions of insulin and glucagon on glucose, lipid and protein metabolism.

α Cells of Islets of Langerhans

Under hypoglycemic state, α cells secrete glucagon that increases blood glucose levels. Hyperglycemia inhibits release of glucagon. Glucagon acts on liver and adipose and causes hyperglycemia by breaking down of glycogen into glucose (glycogenolysis) and making glucose derived from lipids or proteins in the liver (gluconeogenesis). Functional effects of glucagon on blood glucose in tissues are given in Table 26.1.

β Cells of Islets of Langerhans

β Cells of islets of Langerhans are situated around capillaries within pancreatic lobules, which synthesize insulin. Hyperglycemia stimulates synthesis of insulin that acts on liver, adipose tissue and skeletal muscles. Insulin synthesis and its actions are given in Table 26.2.

- **Glucose metabolism:** Insulin facilitates diffusion of glucose into the cells and coverts glucose into glycogen (glycogenesis) by glucose synthase enzyme in liver and thus decreases blood sugar levels. Insulin inactivates liver phosphorylase enzyme that causes liver glycogen to split into glucose and this step prevents breakdown of the glycogen that has been stored in the hepatocytes. Insulin increases the activity of the glucokinase enzyme that causes initial phosphorylation of glucose after it diffuses into the

Table 26.1 Functional effects of glucagon on blood glucose in tissues

Metabolic Effects	Target Organ	Target Enzyme
↑Glycogen breakdown (Glycogen → Glucose)	Liver	↑Glycogen phosphorylase
↓Glycogen synthesis	Liver	↓Glycogen synthase
↓Glycolysis	Liver	↓Phosphofructose 1
↑Gluconeogenesis	Liver (amino acids, glycerol and oxaloacetate → glucose synthesis)	▪ ↑Fructose-1,6-bisphosphatase ▪ ↓Pyruvate kinase
↑Fatty acid mobilization	Adipose tissue (less glucose used as fuel by liver and striated muscle)	↑Triacylglycerol lipase

Table 26.2 Insulin synthesis and its actions

Parameters	Comments
Site of synthesis	β Cells in pancreas
Chemical nature	51-amino acids peptide (A chain and B chain)
Biosynthesis	Peptide hormone
Transport in the blood circulation	Insulin dissolved in the plasma
Half-life of insulin	5–9 minutes
Insulin degradation	Maximum insulin is degraded by insulinase enzyme in liver and least in vascular endothelium
Insulin release inducing conditions	▪ ↑Blood glucose level (>100 mg) ▪ ↑Blood amino acids ▪ ↑Gastrointestinal tract hormones ▪ ↑Parasympathetic activity ▪ ↓Sympathetic activity
Target cells/tissues	▪ Liver ▪ Skeletal muscle ▪ Adipose tissue ▪ Brain ▪ Kidney
Target insulin receptor on cells/tissues	Cell membrane receptor with tyrosine kinase activity; insulin signaling pathways by binding insulin to its insulin-receptor substrate (IRS)
Insulin action on cells/tissues	▪ Carbohydrate metabolism • ↑Glucose uptake by cells/tissues via GLUT4 • ↑Glycogen synthesis (glucokinase, glycogen synthase, phosphorylase) • ↓Glycogenolysis • ↓Gluconeogenesis ▪ Lipid metabolism • ↑Lipogenesis (↑triglyceride, fatty acids and cholesterol synthesis) • ↓Lipolysis, ↓ketogenesis by HMG-CoA synthetase • ↑Utilization of acetyl-CoA in Krebs's cycle ▪ Protein metabolism • ↑Transcription of proteins • ↑Amino acids uptake

hepatocytes and the phosphorylated glucose cannot diffuse back through the cell membrane.

- **Lipid metabolism:** Insulin promotes the uptake of fatty acids and synthesis of fatty acids (lipogenesis), whilst inhibiting metabolic breakdown of lipids (lipolysis). Recent studies indicate that lipid synthesis requires an increase in the transcription factor steroid regulatory element-binding protein-1c (SREBP-1c) due to unknown mechanism. Insulin inhibits lipid metabolism through downregulating cellular concentrations of cAMP by a cAMP specific phosphodiesterase in adipocytes.
- **Protein metabolism:** Insulin increases uptake of amino acids and promotes protein synthesis by liver. Insulin inhibits breakdown of proteins.

δ Cells of Islets of Langerhans

δ Cells secrete somatostatin hormone, that inhibits the release of insulin from β cells of islets of Langerhans. Somatostatin also inhibits the release of growth hormone from anterior pituitary gland. Somatostatin decreases secretion, motility and absorption in the digestive tract.

INSULIN HORMONE

Insulin is synthesized by β cells in the pancreas via transcription and translation of DNA into mRNA and amino acid chains (polypeptide chains). Thereafter, the protein undergoes structural changes to achieve its final form. Insulin synthesis is regulated by several mechanisms: glucose stimulates nuclear translocation of PDX-1 → PDX1-1+RIPE3b1/MAFA binding → transcription regulation (insulin gene) → mRNA formation → mRNA stability → translation regulation of mRNA to polypeptide chains → regulation of post-translational modifications and formation of quaternary structure.

INSULIN SYNTHESIS

Insulin is synthesized by β cells of islets of Langerhans in pancreas. Insulin messenger RNA is translated as a single chain precursor in inactive form called preproinsulin. Thereafter the removal of its signal peptide during insertion into the endoplasmic reticulum

generates proinsulin. Synthesis of human insulin is shown in Fig. 26.3.

- **Proinsulin:** Proinsulin has 82 amino acids consists of three domains: (a) a carboxy-terminal A chain (21 amino acids), (b) an amino-terminal B chain (30 amino acids), and (c) a connecting peptide in the middle known as the C peptide. Chain A and chain B are linked by two disulfide bridges (Cys7A–

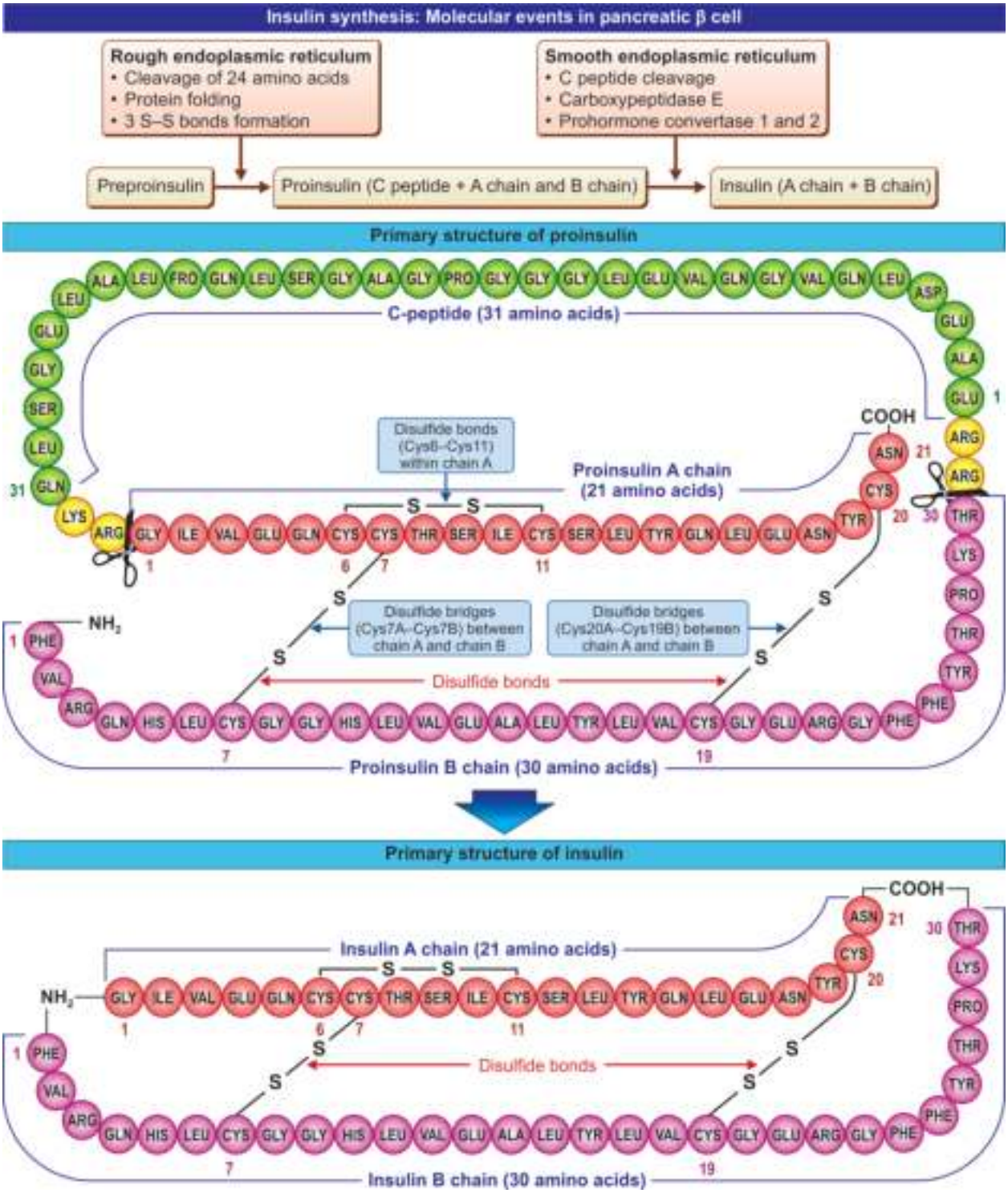


Fig. 26.3: Synthesis of human insulin. Proinsulin is an 82-polypeptide hormone consisting of C peptide (31 amino acids), chain A (21 amino acids) and chain B (30 amino acids) linked by two disulfide bonds (Cys7A–Cys7B/Cys20A–Cys19B). One more intrachain disulfide bond is located between Cys20A and Cys19B between chain A and chain B. Proinsulin is exposed to several specific endopeptidases which excise the C peptide, thereby generating mature form of insulin distributed only in extracellular compartment. Human insulin consists of chain A (21 amino acids) and chain B (30 amino acids) linked by disulfide bonds.

Cys7B/Cys20A–Cys19B). One or more intrachain disulfide bonds is located between Cys6 and Cys11 within the A chain.

- **Proinsulin conversion to mature insulin:** Within the endoplasmic reticulum, proinsulin is exposed to several specific endopeptidases, which excise the C peptide, thereby generating mature form of insulin distributed only in extracellular compartment of islets of Langerhans. Insulin and free C peptide are packaged in the Golgi apparatus into secretory granules, which accumulate in the cytoplasm of the β cells in the pancreas.
- **Mature insulin:** Mature insulin is a protein composed of two chains: (a) A chain with 21 amino acids and (b) B chain with 30 amino acids, which are linked together by sulfur atoms.

INSULIN RELEASE

When the β cell in pancreas is appropriately stimulated in response elevated blood glucose levels, insulin is secreted and released from the cell by the process of exocytosis. The insulin then diffuses into blood vessels of the pancreas. The 'C peptide' is also secreted into the blood circulation, but has no known biological activity. Insulin release from the pancreatic β cell in response to hyperglycemia is shown in Fig. 26.4.

- **Insulin release:** Glucose is transported into the pancreatic β cells via glucose transporter 'GLUT2' in an insulin-independent mechanism of concentration gradient. Then glucose is phosphorylated by glucokinase to glucose-6-phosphate, that takes part in the metabolic process to generate ATP. Elevated ATP level triggers accumulation of K^+ ions along with membrane depolarization. Membrane depolarization then activates Ca^{++} channels resulting in increased intracellular concentration of Ca^{++} ions inside the β cell and consequent release of insulin along with C peptide from the vesicles by the process of exocytosis and diffuses into capillary blood around islets of Langerhans. C peptide molecule is also secreted into the blood, but has no known biological activity.
- **Insulin degradation:** Maximum insulin entering portal vein from pancreas is degraded in the liver

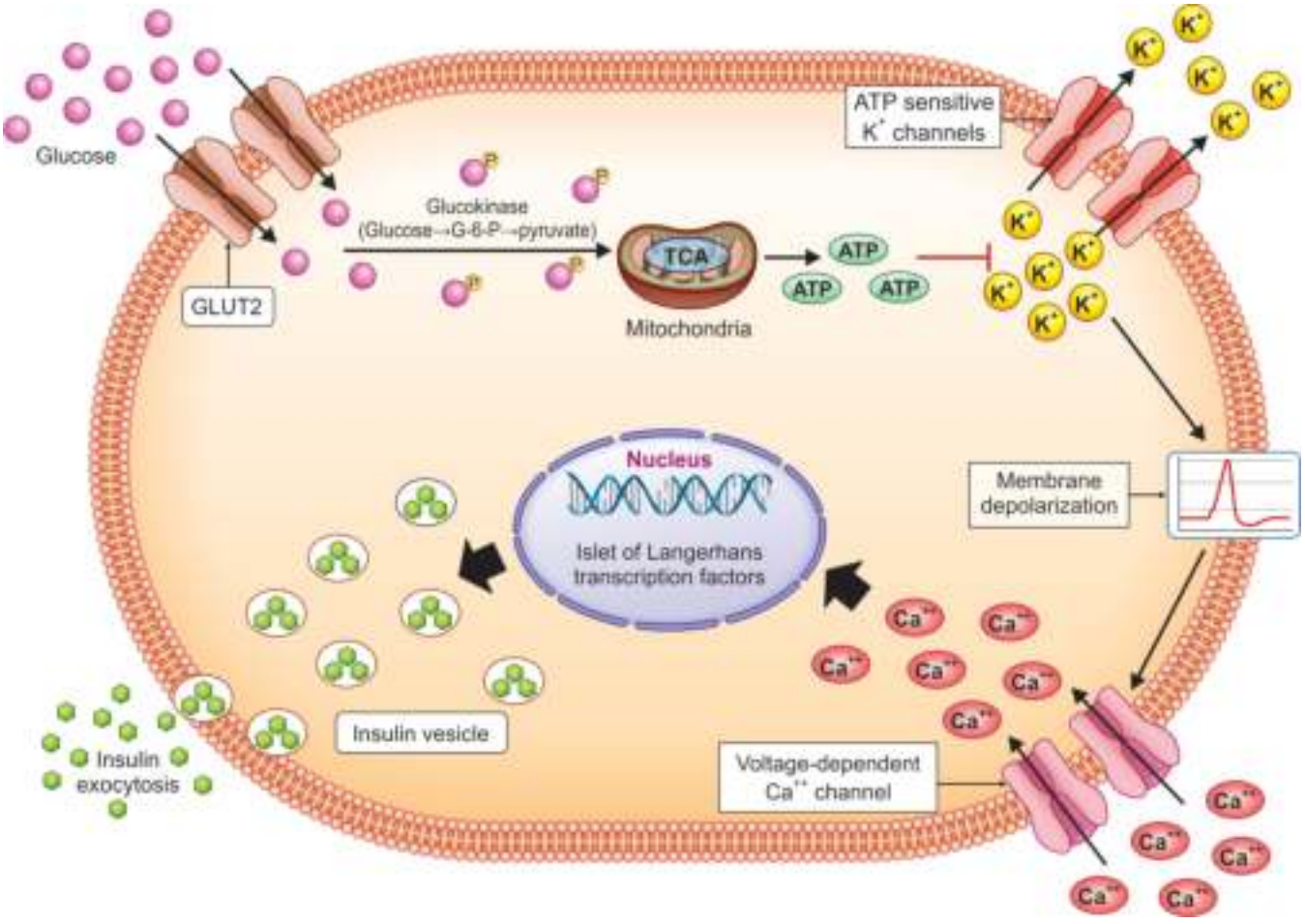


Fig. 26.4: Insulin release from the pancreatic β cell in response to hyperglycemia. Pancreatic β cell releases insulin in response to hyperglycemia in the extracellular fluid bathing the β cells. Insulin release is stimulated by growth hormone, cortisol, prolactin and the gonadal steroids.

and least in the vascular endothelium. During biotransformation, the sulfide bonds are reduced. A and B chains of insulin are separated and further broken down to the constituent amino acids. Half-life of the mature insulin is 5–9 minutes.

- **C peptide analysis:** 'C peptide' analysis in blood is used to analyze endogenous insulin synthesis and to differentiate type 1 diabetes mellitus from type 2 diabetes mellitus. Type I diabetes mellitus is characterized by the body's inability to produce insulin due to the autoimmune destruction of the β cells of islets of Langerhans in the pancreas. Type 2 diabetes mellitus is characterized by lack of functional response to insulin in tissues and/or insulin deficiency. Blood concentration of C peptide in type 1 and type 2 diabetes mellitus is given in Table 26.3.

INSULIN ACTIONS

Insulin is a peptide hormone that predominantly functions to reduce blood glucose concentration. It is synthesized by pancreatic β cells of islets of Langerhans in response to elevated nutrients in the blood. Insulin triggers the uptake of glucose, amino acids and lipids into liver, striated muscle and adipose tissue, and promotes the storage of nutrients in the form of glycogen, proteins and lipids respectively. Insulin is essential for cell proliferation, metabolism and survival.

- **Insulin receptor substrate (IRS):** The insulin receptor substrate (IRS) is a tyrosine kinase, that functions as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins, which in turn phosphorylation of target intracellular proteins alters its activity, thereby generating a biologic response in maintaining basic cellular functions such as growth, survival and normal homeostasis of glucose, fat and protein metabolism. At least four mammalian insulin receptor substrate molecules are well studied (IRS1, IRS2, IRS3, IRS4).
- **Binding of insulin-to-insulin receptor substrate (IRS):** When insulin binds to its receptors on target cells (e.g. skeletal muscle cells and adipocytes, a signaling cascade is initiated by phosphorylation of target intracellular proteins, which culminates in the translocation of the glucose transporter type 4 (GLUT4) from intracellular vesicles to the cell membrane. Once GLUT4 is incorporated into the plasma membrane, it functions to promote the uptake of extracellular glucose and glycogen synthesis, which is then stored as glycogen in the skeletal muscles and adipocytes, thereby regulating blood glucose. Most common GLUT family of glucose transporters is given in Table 26.4.
- **Insulin actions:** Insulin regulates blood sugar concentration through gluconeogenesis (*de novo* glucose production) and glycogenolysis (glycogen breakdown) in the liver. Besides regulating blood sugar concentration, insulin also plays essential role in facilitating synthesis of proteins and lipids; and preventing conversion of protein and lipids to glucose (gluconeogenesis). Metabolic functions of insulin in tissues/organs are shown in Fig. 26.5 and Table 26.5.
- **Insulin deficiency or insulin resistance:** Diabetes mellitus is a metabolic disorder in which the body does not produce enough insulin or body becomes resistant to its effects and causing abnormally high blood glucose levels. Type 1 diabetes mellitus is characterized by the inability to synthesize insulin, whereas in type 2 diabetes mellitus, the body becomes resistant to the effects of insulin, presumably because of defects in the insulin signaling pathway.

Pathology Pearls: Family of Insulin Receptor Substrates

- **Insulin receptor substrate 1 (IRS1):** IRS1 is predominantly found in the cytoplasm. Tyrosine phosphorylated IRS1 is an intracellular signaling adaptor protein that binds to signaling effectors, activates signaling cascades, integrates and coordinates numerous biologically key extracellular signals within the cell. IRS1 regulates several biological processes including cell proliferation and survival.
- **Insulin receptor substrate 2 (IRS2):** IRS2 belongs to the insulin receptor substrate protein family that is an alternative route for signaling mediated by the insulin receptor. IRS2 mediates mitogenic and antiapoptotic signaling from important cellular receptors. IRS2 regulates important biological processes involved in cell proliferation, metabolism and survival.
- **Insulin receptor substrates 3 and 4 (IRS3 and IRS4):** IRS-3 and IRS-4 play central role in insulin signaling by impairing IRS1 and IRS2.

Table 26.3 Blood concentration of C peptide in type 1 and type 2 diabetes mellitus

Parameters	Type 1 Diabetes Mellitus	Type 2 Diabetes Mellitus
β Cell mass	β Cell mass reduced/ depleted	β Cell mass normal
Endogenous insulin synthesis	Absent or reduced	Normal synthesis of insulin, but peripheral tissue resistance to insulin
Blood C-peptide concentration	Absent/low concentration	Increased concentration

Table 26.4 Most common GLUT family of glucose transporters in human tissues

Glucose Transporter (GLUT) Name	Insulin Sensitivity	GLUT in Tissues	Function(s)
GLUT1	Insulin-independent	RBC membrane, brain barrier, recently identified in human β cells	Basal glucose uptake isoform for cellular metabolism and glucose transport
GLUT2	Insulin-independent	▪ Liver ▪ Pancreatic β cells ▪ Renal proximal tubule ▪ Intestinal cells	▪ GLUT2 removes excess glucose from the blood ▪ GLUT2 regulates insulin in pancreatic β cells ▪ GLUT2 regulates transmembrane glucose transport
GLUT3	Insulin-independent	All tissues	Basal glucose uptake
GLUT4	Insulin-dependent	Skeletal muscles, adipose tissue	Insulin stimulates translocation of GLUT4 to plasma membrane
GLUT5	Insulin-dependent	Small intestine, brain	Primarily fructose transporter

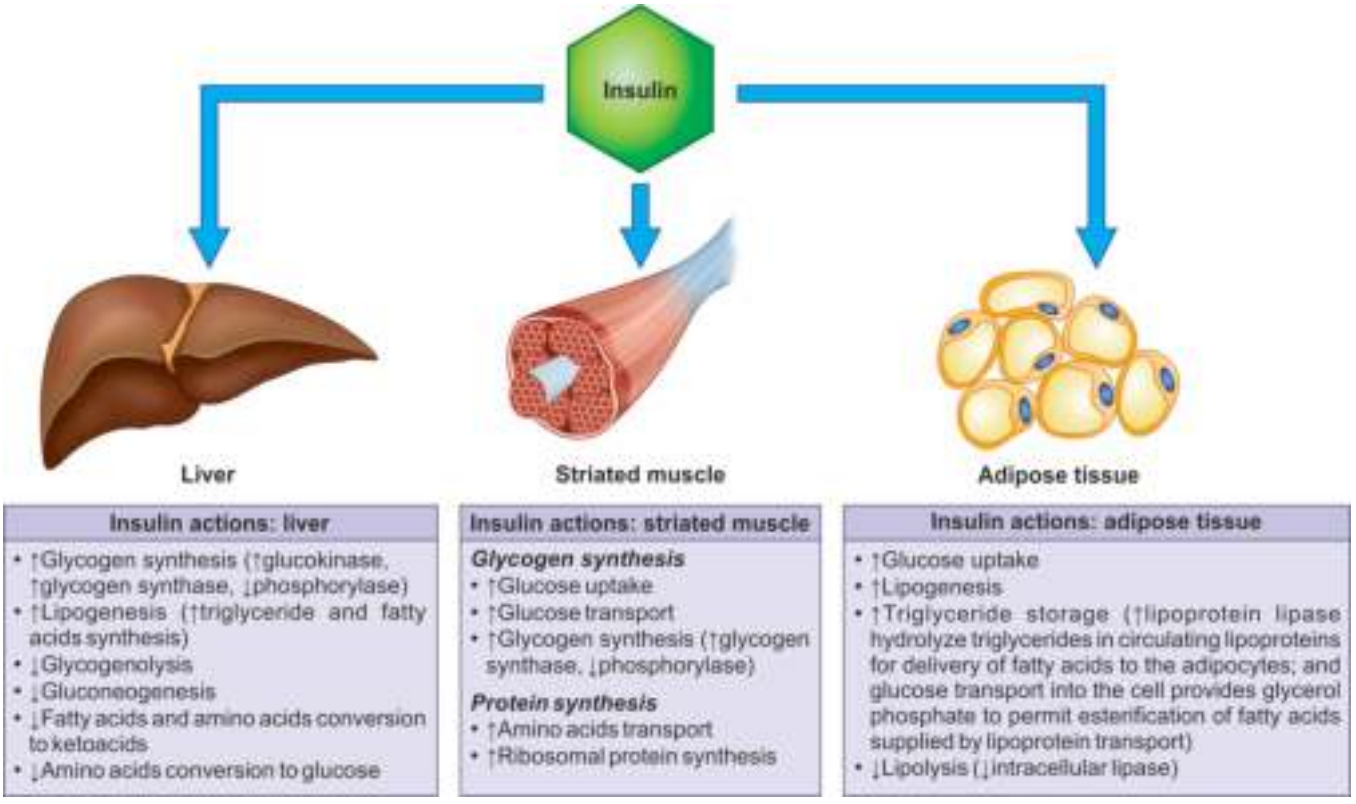


Fig. 26.5: Metabolic functions of insulin in various tissues/organs (liver, striated muscle and adipose tissue).

Table 26.5 Metabolic functions of insulin in tissues/organs

Tissues/Organs	Insulin Actions
Liver	▪ ↑Glycogen synthesis (↑glucokinase, ↑glycogen synthase, ↓phosphorylase) ▪ ↑Lipogenesis (↑triglyceride and fatty acids synthesis) ▪ ↓Glycogenolysis (glycogen → glucose) ▪ ↓Gluconeogenesis ▪ ↓Fatty acids and amino acids conversion to ketoacids ▪ ↓Amino acids conversion to glucose

Contd...

Table 26.5 Metabolic functions of insulin in tissues/organs (*Contd...*)

Tissues/Organs	Insulin Actions
Striated muscle	▪ Glycogen synthesis • ↑Glucose uptake • ↑Glucose transport • ↑Glycogen synthesis (↑glycogen synthase, ↓phosphorylase) ▪ Protein synthesis • ↑Amino acids transport • ↑Ribosomal protein synthesis
Adipose tissue	▪ ↑Glucose uptake ▪ ↑Lipogenesis ▪ ↑Triglyceride storage (↑lipoprotein lipase hydrolyze triglycerides in circulating lipoproteins for delivery of fatty acids to the adipocytes; and glucose transport into the cell provides glycerol phosphate to permit esterification of fatty acids supplied by lipoprotein transport) ▪ ↓Lipolysis (↓intracellular lipase)
Other insulin actions	▪ ↑Gene expression ▪ ↑DNA synthesis ▪ ↑Na^+ and K^+ pump ▪ ↓Apoptosis ▪ ↓Autophagy

Glycolysis is a process of breaking down a glucose molecule in pyruvate, ATP and NADH. Glycogenolysis is the process of breakdown glycogen into glucose.

Pathology Pearls: GLUT Family of Glucose Transporters in Human Tissues

- SLC2 gene codes for a family of GLUT transport proteins thereby cooperatively function to provide a supply of glucose in the direction needed for cell metabolic processes. There are 14 known glucose transporters present in different concentrations in tissues.
- Glucose transporters are stored within cell in membrane GLUT vesicle. When insulin interacts with its receptor, membrane GLUT vesicle moves to surface and fuses with the plasma membrane. GLUT transports glucose inside the cell.
- When insulin level drops, glucose transporter is removed from the plasma membrane by endocytosis forming small GLUT vesicle.
- The small GLUT vesicle fuses with larger endosome. Then patches of endosome enriched with GLUT buds off to become small GLUT vesicle, ready to return to the surface when insulin level rises again.
- Pathophysiologic abnormalities in GLUT transport proteins are associated with type 2 diabetes mellitus, other tissue disorders and cancer.

INSULIN SIGNALING PATHWAYS

Insulin is the major hormone regulating critical energy functions such as glucose, lipid and protein metabolism. The insulin receptor is composed of two extracellular α-subunits and two transmembrane β-subunits linked together by disulfide bonds.

- **Insulin binding to its receptor:** Binding of insulin to the α-subunit of insulin receptor induces a conformational change resulting in the autophosphorylation of a number of tyrosine kinase residues present in the β-subunit. These molecular changes form the binding sites for insulin receptor substrate family adaptor proteins and initiate multiple intracellular signaling pathways leading to diverse metabolic events in almost every tissue.
- **Major insulin signaling pathways:** There are two major routes of intracellular signaling pathways resulting from the insulin-insulin receptor substrate interaction: (a) PI3K (phosphoinositol 3-kinase)/AKT signaling pathway (also known as protein kinase or PKB); and (b) RAS/MAPK signaling pathway (also known as extracellular signal regulated kinase or ERK). Insulin intracellular signaling pathways resulting from the insulin-insulin receptor substrate interaction are shown in Fig. 26.6.
- **Actions of insulin signaling pathways:** PI3K/AKT signaling pathway is linked exclusively through insulin receptor substrate (IRS) and produce insulin's effects in the cells such as synthesis of glycogen, lipids and proteins. RAS/MAPK signaling pathway is involved in regulation of gene expression and in cooperation with PI3K/AKT pathway, also regulates cell proliferation, growth differentiation and survival.

PI3K/AKT Signaling Pathway

Glucose uptake is regulated by several mechanisms, where insulin hormone plays most important role. Insulin regulates the transport of glucose into the cells through translocation of glucose transporter, 'GLUT4' from an intracellular pool in the plasma membrane mainly in metabolically active tissues like liver, skeletal muscle and adipose tissue. GLUT4 translocation occurs through multiple steps of PI3K/AKT signaling pathway.

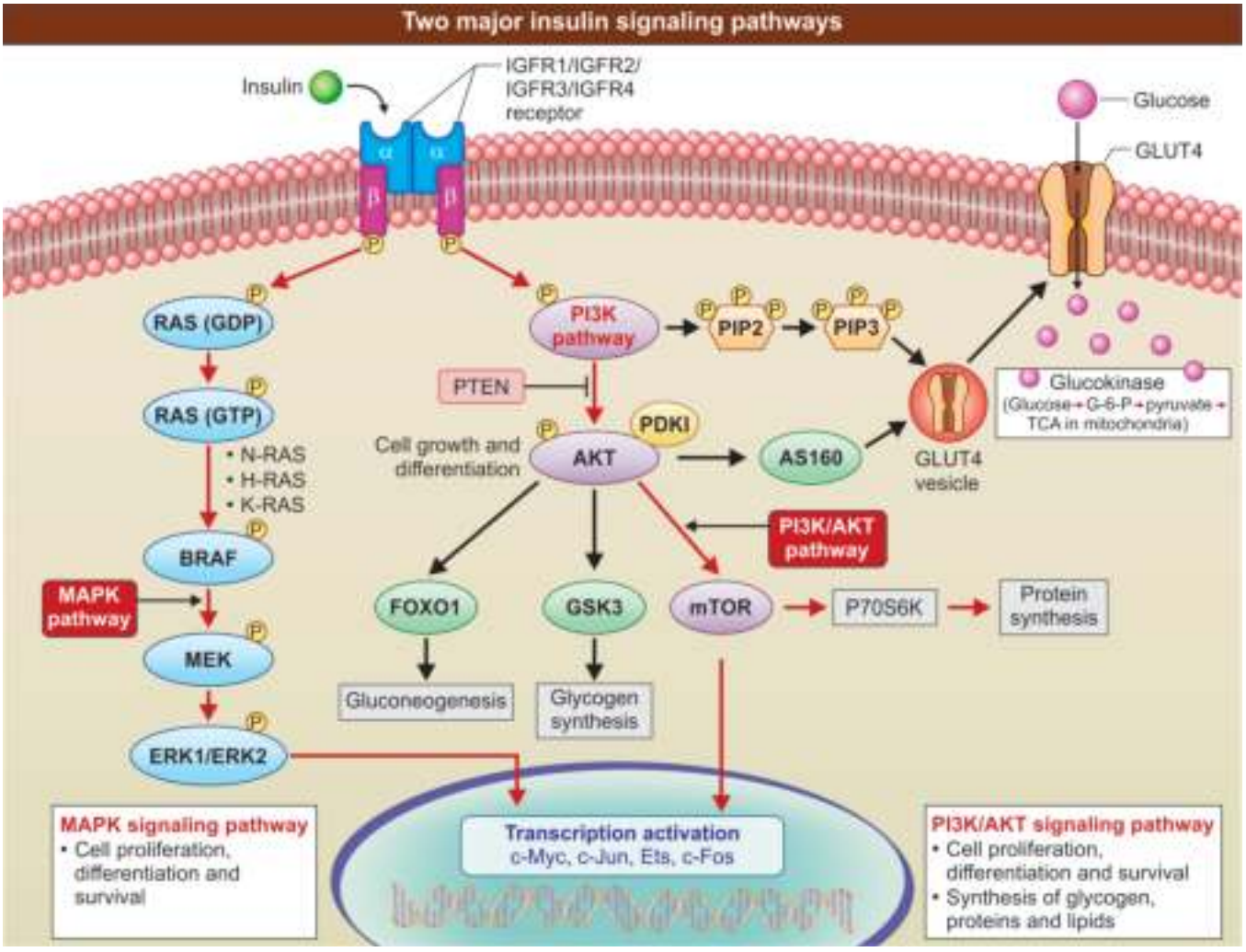


Fig. 26.6: Insulin intracellular signaling pathways resulting from the insulin-insulin receptor substrate (IRS) interaction: (a) PI3K (phosphoinositol 3-kinase)/AKT signaling pathway (also known as protein kinase or PKB) and (b) RAS/MAPK signaling pathway (also known as extracellular signal regulated kinase or ERK) involved in cell proliferation, growth differentiation and survival.

- Insulin binds to insulin receptors via SH2 domains to PI3K (phosphoinositol 3-kinase) regulatory subunits, and triggers dimerization and intracellular autophosphorylation.
- P13 kinase catalyzes the production of PIP3 (phosphatidylinositol 3,4,5-triphosphate) by phosphorylating PIP2 (phosphatidylinositol 4,5-bisphosphate).
- PIP3 is bound to the inner lipid layer of the plasma membrane that recruits and AKT and **PDK1** (phosphoinositide-dependent kinase 1) to the plasma membrane, where phosphorylation of PDK1 occurs. PDK1 then activates serine/threonine residues of AKT, subsequently eliciting phosphorylation of AS-160 substrate protein that stimulates translocation of glucose transporter 4 (GLUT4) vesicles from intracellular pool to the cell membrane surface, thereby increases the insulin-dependent transport of glucose into the cell.
- Activated AKT usually inhibits activity of **GSK-3** (glycogen synthase kinase 3) through phosphorylation of serine 21 in GSK-3 α and serine 9 in GSK-3. AKT promotes MAPK (mitogen activated protein kinase) phosphorylation.
- AKT and GSK-3 (glycogen synthase 3) are serine threonine kinases that cooperatively modulate signaling pathways in the regulation of glycogen synthesis in response to insulin receptor stimulation. Most recent studies indicate that GSK-3 phosphorylates a broad range of substrates, including several transcription factors such as c-Myc, c-Jun and c-Myb and the translator factor elF2B.
- Glycogen synthase (UDP-glucose-glycogen glucosyltransferase) is a key enzyme in glycogenesis, conversion of glucose into glycogen in tissues. Glycogen synthase enzyme is activated by glucose-6-phosphate dehydrogenase (**G6PD**) and inhibited by glycogen

synthase kinase 3 (GSK-3). These two mechanisms play an important role in glycogen metabolism.

- In addition to glycogenesis, insulin downregulates the expression of genes encoding hepatic enzymes, which inhibit gluconeogenesis and glycogenolysis.
- PI3K/AKT signaling pathway regulates synthesis of glycogen, lipids and proteins involved in cell proliferation, growth, differentiation and survival.
- Activated AKT stimulates the activity of translation of initiation factors and upregulates ribosome biogenesis. Insulin stimulates amino acid uptake into cells, inhibits protein degradation and promotes protein synthesis. Upon receipt of insulin signal, inactivation of GSK3 by AKT leads to the dephosphorylation of a guanine nucleotide exchange factor elF2B (a regulator of protein translation), thereby promoting protein synthesis and the storage of amino acids. AKT also activates mammalian target of rapamycin (mTOR), which promotes protein synthesis through p70S6K (p70 ribosomal protein S6-kinase) and inhibition of elf-4EBP1 (eukaryotic translation initiation factor 4E-binding protein 1).
- Aberrant activation of the PI3K/AKT signaling pathway is linked to diabetes mellitus and human cancers.

Pathology Pearls: Insulin-mediated GLUT4 (Glucose Transporter 4)

- Insulin stimulates glucose transport across cell membrane by ATP-dependent translocation of glucose transporter 4 (GLUT4) to the plasma membrane.
- The second messenger PIP3 (phosphatidylinositol 3, 4, 5-trisphosphate) is bound to the inner lipid layer of the plasma membrane and phosphorylated PDK1 (phosphoinositide-dependent kinase 1).
- AKT plays key role in the insulin sensitive translocation of GLUT4 from cytosol to the plasma membrane, especially in skeletal muscles and adipose tissue, thereby increases the insulin-dependent transport of glucose into the cell.
- Over the period of time, insulin also promotes expression of genes directing synthesis of GLUT4.
- Genes encoding large number of enzymes are regulated by insulin through RAS/RAF/MEK/ERK/MAPK and PI3K/AKT signaling pathways.

RAS/RAF/MEK/ERK (MAPK) Signaling Pathway

RAS/RAF/MEK/ERK (MAPK—mitogen-activated protein kinase) signaling pathway is another insulin signaling pathway, which regulates cellular proliferation, differentiation and survival.

- MAPK signaling pathway is activated independently of the PI3K/AKT pathway either through binding of a **Grb2** (growth factor receptor-bound protein 2) to tyrosine-phosphorylated Shc adaptor protein, or through Sh2 adaptor protein binding to insulin receptor. It leads to recruitment of guanine nucleotide exchange factor protein SOS, which promotes the active GTP-bound RAS and subsequent activation of the MAPK signaling pathways.
- Activated RAS-GTP stimulates downstream RAF serine/threonine kinase, the latter proceeds to phosphorylate and activate MEK. Then MEK phosphorylates and activates extracellular signal-regulated kinases (ERK-1 and ERK-2).
  - Activated ERK-1 and ERK-2 are involved multiple cellular processes such as cellular proliferation, differentiation and survival.
  - ERK1/ERK2 act by regulating gene expression as well as extranuclear events such as cytoskeleton reorganization, through the phosphorylation of kinases in the cytoplasm and activation of target proteins in both cytosol and nucleus.
- RAS/RAF/MEK/ERK (MAPK) signaling pathway mediates stimulation of glucose uptake during exercise by elevating the expression levels of glucose transporter 'GLUT4' and increasing PGC1-α activity that entails mitochondrial biogenesis and oxidative metabolism. MAPK signaling pathway regulates cell proliferation, growth, differentiation and survival.

NEGATIVE REGULATORS: TERMINATION OF INSULIN-SIGNALING PATHWAYS

After the insulin acts on its receptor, insulin may be released back into the extracellular environment and then degraded by the cell. Insulin degradation involves endocytosis of the insulin-receptor complex followed by the action of insulin degrading enzymes. Insulin degradation mainly takes place in the liver within one hour after its initial release into blood circulation.

- The enzymes that are important in the attenuation of PIP3 (phosphatidylinositol 3,4,5-trisphosphate) signaling are protein tyrosine phosphatase and family of Src homology 2 containing inositol 5-phosphatase (**SHIP 5-phosphatase**) proteins, which include two gene products, SHIP1 and SHIP2 phosphatases, which degrade PIP3 (phosphatidylinositol 3,4, 5-trisphosphate) and PIP2 (phosphatidylinositol 4, 5-bisphosphate).
- Termination of the insulin-signaling event also occurs when the insulin-insulin receptor substrate (IRS) complex is internalized into endosomes and dephosphorylated leading to its degradation by IDE (insulin-degrading enzyme).

DIABETES MELLITUS

Diabetes mellitus is a metabolic disease characterized by elevated levels of blood glucose, brought about by impaired pancreatic β cell, which leads over time to serious damage to cardiovascular system (heart, brain, blood vessels), kidneys, eyes and nerves. Uncontrolled diabetes mellitus is linked to long-term complications such as atherosclerosis, coronary artery disease (myocardial infarction), cerebral stroke, peripheral vascular disease, nephropathy, retinopathy, and neuropathy.

- Diabetes mellitus is classified as primary or secondary diabetes mellitus. Based on pathogenesis, primary diabetes mellitus can be immune-mediated or nonimmune-mediated mechanism. Secondary diabetes mellitus, there is excess activity of hormones antagonistic to insulin.
- Type 1 diabetes mellitus, once known as juvenile diabetes mellitus or insulin-dependent diabetes mellitus, in which the pancreas produces little or no insulin by itself. Type 2 diabetes mellitus is more common in persons older than 40 years, which occurs when the body becomes resistant to insulin. Diagnostic criteria of diabetes mellitus adapted from the American Diabetes Association are given in Table 26.6.

DIABETES MELLITUS: CLASSIFICATION

Symptoms in diabetes mellitus vary depending on elevation of blood glucose level. Symptoms tend to appear in type 1 diabetes mellitus than type 2 diabetes mellitus. Type 1 diabetes mellitus is an autoimmune disorder that attacks β cells in pancreas leading to little or no insulin production. Type 2 diabetes mellitus happens when body becomes resistant to insulin. Potentially reversible diabetes mellitus conditions include prediabetes and gestational diabetes mellitus (GDM). Classification of diabetes mellitus is given in Table 26.7. Differences between type 1 and type 2 diabetes mellitus are given in Table 26.8.

Prediabetes Stage

Diabetes mellitus evolves through prediabetes stage and is defined as impaired fasting blood sugar and/or impaired glucose tolerance test.

- Person has prediabetes stage if fasting blood glucose is between 100 mg/dl (5.6 mmol/L) and 125 mg/dl (6.9 mmol/L) or if blood glucose level, 2 hours after a oral glucose tolerance test is between 140 mg/dl (7.8 mmol/L) and 199 mg/dl (11.0 mmol/L).
- Prediabetes stage carries high risk for type 2 diabetes mellitus and its long-term complications later in life.
- Reducing body weight by 5–10% through diet and exercise can significantly decrease the risk for diabetes mellitus and its complications later in life.

Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (formerly called insulin-dependent or juvenile-onset diabetes mellitus) is a chronic metabolic disorder characterized by the body's inability to produce very little or no insulin due to the autoimmune destruction of the >90% of β cells of islets of Langerhans in the pancreas.

- Four autoantibodies are markers of β cell autoimmune destruction in type 1 diabetes mellitus: islet cell antibodies (ICA), against cytoplasmic proteins in the β cell, insulin autoantibodies (IAA), autoantibodies to glutamic acid decarboxylase (GAD-65)

Table 26.6 Diagnostic criteria of diabetes mellitus adapted from the American Diabetes Association (any one of these diagnostic criteria)

Test	Abnormal Blood Glucose Level in Diabetes Mellitus	Comments
Fasting blood glucose	≥126 mg/dl (7 mmol/L)	▪ Fasting is defined as no caloric intake for at least 8 hours ▪ Euglycemic fasting blood sugar is <100 mg/dl (5.6 mmol/L)
Blood glucose OGTT (two-hour oral glucose tolerance test with maximum dose of 75 g)	≥200 mg/dl (11.1 mmol/L)	▪ Euglycemic blood glucose is <140 mg/dl (7.8 mmol/L) ▪ Prediabetic state blood glucose is 140–200 mg/dl
HbA1c (glycated hemoglobin) additional diagnostic criteria	≥6.5%	▪ Glycated HbA1c is formed by nonenzymatic combination of glucose with globin of hemoglobin ▪ Euglycemic HbA1c level is <5.6% ▪ Prediabetes HbA1c level is 5.7–6.4% ▪ Diabetes mellitus (HbA1c level is ≥6.5%)
Random blood glucose	≥200 mg/dl (11.1 mmol/L)	Patient has classical signs and symptoms of diabetes mellitus due to hyperglycemia

- *One of the preceding first diagnostic criteria must be present on a subsequent day to confirm the diagnosis of diabetes mellitus.*
- *In the absence of unequivocal hyperglycemia, fasting blood glucose and HbA1c (glycated hemoglobin) should be confirmed by repeat testing.*

Table 26.7 Classification of diabetes mellitus

Diabetes mellitus due to lack of insulin and/or insulin resistance in tissues
▪ Type 1 diabetes mellitus (juvenile onset insulin-dependent DM): It occurs due to β cell destruction (immune-mediated or idiopathic), usually resulting in absolute insulin deficiency associated with ketoacidosis prone, islet cell autoantibodies, glutamic acid decarboxylase autoantibodies and decreased C peptide. ▪ Type 2 diabetes mellitus (adult-onset non-insulin-dependent DM): It occurs as a result of combination of insulin resistance in peripheral tissues and β cell dysfunction associated with high glycosylated hemoglobin, increased C peptide and increased risk for diabetic retinopathy and absence of glutamic acid decarboxylase autoantibodies ▪ Gestational diabetes mellitus (GSD): It occurs due to insulin resistance in peripheral tissues during pregnancy.
Diabetes mellitus due to genetic defects in β cell function
▪ Maturity onset diabetes mellitus of the young (MODY): It occurs due to mutations in MODY genes include: hepatocyte nuclear factor 4α: (HNF4A, MODY1 in 30–60%), glucokinase (GCK: MODY2 in 30–60%), hepatocyte nuclear factor 1 alpha (HNF1A, MODY3 in 30–60%), pancreatic and duodenal homeobox (PDX1, MODY4), hepatocyte nuclear factor 1 β (HNF1B, MODY5 in 50%) and neurogenic differentiation factor (NEUROD1, MODY6). Other gene mutations such as INS, PAX4, ABCC8, KCNJ11, KLF11, CEL, BLK and APPL1 may also be demonstrated in these patients. ▪ Neonatal diabetes mellitus (NDM): It occurs due to activating mutations in KCNJ11 and ABCC8, encoding Kir6.2 and SUR1 respectively ▪ Maternal inherited diabetes mellitus and deafness (MIDD): It is due to mitochondrial DNA mutations (m.3243A–G) ▪ Diabetes mellitus due to defects in proinsulin conversion to insulin ▪ Diabetes mellitus due to mutations in insulin gene
Diabetes mellitus due to genetic defects in insulin action
▪ Type A insulin resistance diabetes mellitus ▪ Lipoatrophic diabetes mellitus
Diabetes mellitus due to exocrine pancreatic defects
▪ Hereditary hemochromatosis ▪ Chronic pancreatitis ▪ Cystic fibrosis ▪ Pancreatic amyloidosis ▪ Fibrocalculous pancreatopathy ▪ Pancreatectomy/trauma
Diabetes mellitus due to endocrinopathies
▪ Pituitary gland (acromegaly) ▪ Adrenal gland diseases (Cushing syndrome, pheochromocytoma) ▪ Thyroid gland disorders (hyperthyroidism) ▪ Pancreatic carcinoma of α cells (glucagonoma)
Diabetes mellitus due to infections
▪ Mumps ▪ Cytomegalovirus ▪ HIV infections ▪ Coxsackie B virus ▪ Congenital rubella
Diabetes mellitus due to drugs and chemical agents
▪ Glucocorticoids ▪ Thyroid hormones ▪ Thiazide drugs ▪ Cyclosporine ▪ Protease inhibitors ▪ Phenytoin (dilantin) ▪ γ-Interferon ▪ β-Adrenergic agonists ▪ Pentamidine ▪ Vacor

and autoantibodies to protein tyrosine phosphatase in susceptible genotype.

- Genetic and environmental factors are thought to play a role in the onset of the disease, which usually occurs in childhood and young adulthood.

Pathogenesis of type 1 diabetes mellitus is shown in Fig. 26.7.

Predisposing Factors

Although the exact cause of type 1 diabetes mellitus is unknown. However, predisposing factors may signal an increased risk for disease discussed here.

- **Geography:** Certain countries, such as Sweden and Finland, have higher rates of type 1 diabetes mellitus.
- **Environmental factors:** Exposure to environmental pathogens in early life is important for the development of immunity. The lack of early priming of the immune system results in deficient of immunoregulation. If pathogens are encountered at a later stage, autoimmune diseases can be triggered. Several viruses (e.g. mumps, Coxsackievirus and rubella) and the dietary factors (e.g. early exposure to gluten or cow's milk) trigger autoimmune destruction of β cells in pancreas leading to deficient insulin

Table 26.8 Differences between type 1 and type 2 diabetes mellitus

Parameters	Type 1 Diabetes Mellitus	Type 2 Diabetes Mellitus
Prevalence	5–10%	90–95%
Age of onset	Children and adolescents <20 years	Adults >30 years
Type of onset	Rapid (abrupt), symptomatic polyuria, polydipsia, polyphagia often with severe ketoacidosis (fruity odor to breath)	Gradual often symptomatic
Body habitus	Normal, recent loss of weight (usually thin)	Usually obese (80%)
Family history	Uncommon (<20%)	Common (>60%)
Monozygotic twins	50% concordant	90% concordant
HLA association	Present	Absent
Insulin gene VNTR (variable number of tandem repeats)	Present	Absent
Diabetogenic and obesity related candidate genes	Present	Absent
Islet lesions	Early inflammation, late fibrosis and atrophy	Late fibrosis and atrophy
Islet cell autoantibodies	Present	Absent
β Cells mass	Markedly reduced	Normal to slightly reduced
Circulating insulin status	Lack of insulin	Peripheral resistance to insulin
Insulin in blood	Decreased	Normal or increased
C-peptide level in blood	Decreased	Increased
Hyperglycemia	Common	Uncommon
Pathogenesis	T cell cytokines and autoantibodies are formed against pancreatic β cells in resulting to destruction	▪ Decreased insulin receptors on adipose tissue ▪ Post-receptor defects due to abnormalities of tyrosine kinase and glucose transport unit (GLUT4) ▪ Fibrosis of pancreatic β cells occurs due to amyloid deposition
Pathology	▪ Insulitis (T cells and macrophages infiltration) ▪ β Cells depletion ▪ Islet cells atrophy present	▪ No insulitis ▪ β Cells normal in number ▪ Islets cells atrophy absent
Ketoacidosis (fruity odor to breath)	Common	Ketoacidosis rare
Hyperosmolar nonketotic coma	Absent	Present
Laboratory findings	Urine analysis shows presence of ketone bodies	Reactive hypoglycemia occurs due to much insulin is released for a glucose load (early finding)
Management	Insulin absolutely required	Insulin usually not required initially. Insulin may be needed at later stage

production. These patients require insulin therapy for their survival.

- **Genetic predisposition:** Type 1 diabetes mellitus is usually present in persons without a family history. Only 10–15% of the patients have a first- or second-degree relative with the disease. HLA complex polymorphic alleles are responsible for 40–50% of the genetic predisposition to development of diabetes mellitus. The human leukocyte antigen locus on chromosome 6 contributes to type 1 diabetes mellitus in 50% of familial cases. The point mutation in the HLA genes DR3-DQ2 and DR4-DQ8 are present in 80% of children with type 1 diabetes mellitus. The HLA gene DR15-DQ6 is thought to have a protective role in <1% of children. The variable number tandem repeats (VNTR) polymorphism is located in the insulin promoter (INS-VNTR) on the short arm of chromosome 11 and is close to the gene encoding proinsulin. VNTR polymorphism upstream is strongly associated with type 1 diabetes mellitus. Short class 1 alleles are predisposing to diabetes mellitus and the long alleles are protective. CTLA-4 gene

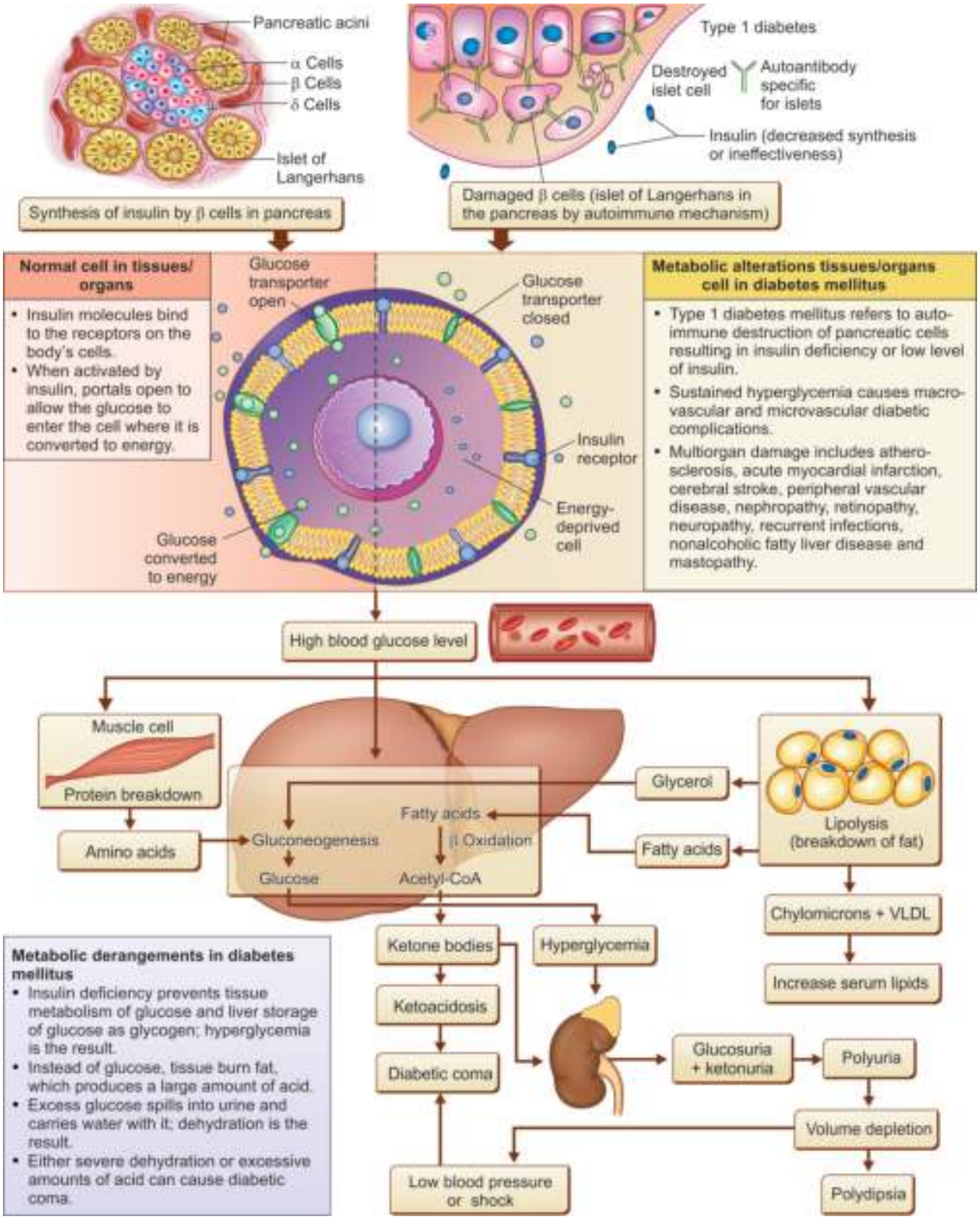


Fig. 26.7: Pathogenesis of type 1 diabetes mellitus. Type 1 diabetes mellitus is thought to be caused by an autoimmune reaction that destroys the insulin making β cells in the pancreas leading to deficient insulin or no insulin production.

(cytotoxic T lymphocyte-antigen-4) polymorphism located on the long arm of chromosome 2 (2q33) may directly influence type 1 diabetes mellitus.

Pathophysiology

Type 1 diabetes mellitus results from the autoimmune destruction of β cells of Langerhans in the endocrine pancreas leading to deficient insulin production. As the β cell mass decline, insulin secretion decreases until the available insulin no longer is adequate to maintain normal blood glucose levels. After 80–90% of the pancreatic β cells are destroyed, before overt type 1 diabetes mellitus becomes evident. On histologic examination, the islets of Langerhans are small with decrease in number or complete absence of β cells. Heavy lymphocytic infiltrate is present within and around islets of Langerhans.

Clinical Features

Unless insulin is replaced in a new case of type 1 diabetes mellitus, patient develops marked carbohydrate intolerance associated with hyperglycemia, resulting to polyuria, polydipsia, dehydration, weight loss, acetone smell and unconsciousness.

- Weight loss occurs due to breakdown of proteins and lipids, despite polyphagia. Osmotic diuresis due to glucose excretion results in dehydration.
- Acetone formed from acetoacetate is exhaled giving acetone smell. Both diabetic ketoacidosis and blood hyperosmolarity impair nervous system function resulting in diabetic coma with fatal outcome.
- Patient may develop flu-like disease, possibly myocarditis due to coxsackievirus infection.

Type 2 Diabetes Mellitus

In type 2 diabetes mellitus (formerly called non-insulin-dependent or adult-onset diabetes mellitus), the pancreatic β cells often continue to produce insulin, however body develops resistance to insulin due to decreased peripheral cell membrane insulin receptors or post-receptor dysfunction.

- As disease progresses, the insulin producing ability of pancreatic β cells decreases. Diabetic ketoacidosis is unusual, but does occur due to unusual stress such as infection and surgical procedure.
- Type 2 diabetes mellitus usually begins in persons older than 30 years and becomes more common in advancing age.

Predisposing Factors

Obesity and sedentary lifestyle accelerate the onset of type 2 diabetes mellitus. Central or visceral obesity (abdominal fat) is strongly linked to insulin resistance. Other predisposing factors for development of type 2 diabetes mellitus include corticosteroid drugs, Cushing syndrome, pregnancy (gestational diabetes mellitus), acromegaly and chronic pancreatitis discussed below.

- **Central or visceral obesity:** Obese persons require very large quantity of insulin to maintain blood sugar levels in type 2 diabetes mellius.
- **Physical inactivity:** Physical inactivity increases risk for type 2 diabetes mellitus. Physical activity helps in weight loss and makes cells more sensitive to insulin.
- **Genetic susceptibility:** Risk increases if a parent or sibling has type 2 diabetes mellitus. Disease incidence is more in monozygotic twins.
- **Race or ethnicity:** Type 2 diabetes mellitus is more prevalent in Blacks, Asian Americans. Indians, Spanish or Latin ancestry compared with White population. Disease can run in families.
- **Advancing age:** Risk for type 2 diabetes mellitus increases as one gets older, because people tend to have less physical activity hence gain weight. However, type 2 diabetes mellitus is also increasing among children, adolescents and young children.
- **Gestational diabetes mellitus:** Pregnant women with gestational diabetes mellitus are at higher risk to develop prediabetes mellitus and type 2 diabetes mellitus. If such pregnant woman gives birth to a baby weighing more than 4 kg, there is increased risk to develop type 2 diabetes mellitus.
- **Polycystic ovary syndrome:** Women suffering from polycystic ovary syndrome present with irregular menstrual cycle, excess hair growth, acne, obesity, greater risk for type 2 diabetes mellitus.
- **Hypertension:** Persons having blood pressure over 140/90 mm Hg is linked to an increased risk for type 2 diabetes mellitus.
- **Abnormal plasma cholesterol and triglyceride levels:** Persons having abnormal plasma cholesterol and triglyceride levels are linked to higher risk for type 2 diabetes mellitus.

Pathophysiology

The β cells of islets of Langerhans in pancreas continue to produce insulin; however, body develops resistance to insulin-mediated by decreased peripheral cell membrane insulin receptors or post-receptor dysfunction. Type 2 diabetes mellitus may be associated with impaired processing of proinsulin to insulin by pancreatic β cells, decreased sensing of glucose by β cells, or impaired function of intracellular carrier proteins. The plasma insulin concentration is normal or increased. On histologic examination, the islets of Langerhans show focal fibrosis and hyalinization due to deposition of amylin, also known as islet amyloid polypeptide (**IAPP**).

- **Peripheral tissue resistance to insulin by NEFAs:** Nonesterified fatty acids (NEFAs) are increased

in liver and striated muscle in obese persons and correlated with peripheral tissue resistance to insulin in type 2 diabetes mellitus. NEFAs intermediate toxic products such as ceramide and diacylglycerol decrease signaling response and thus adversely affect insulin receptors on tissues. Excess of visceral fat accumulation results in altered release of adipokines (i.e. adiponectin, leptin, chemerin, omentin, retinol binding protein 4, resistin, vaspin, and visfatin/PBEF/Nampt), which result in central nervous system-mediated skeletal muscle and hepatic insulin resistance. Leptin stimulates pro-inflammatory cytokines such as IL-6 and TNF-α, that may also reduce insulin sensitivity.

- **β cells dysfunction:** TCF7L2 and other gene alterations cause dysfunctional pancreatic β cells leading to synthesis of more insulin to maintain blood glucose in type 2 diabetes mellitus due to increased resistance to insulin in tissues/organs. Amyloid deposition may be directly toxic to β cells of pancreas.

Treatment

Patient with type 2 diabetes mellitus is managed by diet and oral antidiabetic agents, who does not require insulin therapy. These patients may do reasonably well on diet, exercise and oral hypoglycemic agents for years before they need insulin therapy.

Maturity-onset Diabetes Mellitus of the Young

Maturity-onset diabetes mellitus of the young (MODY) is a monogenic autosomal disorder caused by a single gene mutation that leads to a defect in development of pancreatic islets of Langerhans and impaired insulin secretion in response to glucose stimulation.

- Penetration can vary among family members and the phenotype of MODY is dependent on the gene involved. MODY typically begins before 30 years of persons. Maturity-onset diabetes mellitus of the young is classified on the basis of MODY gene involved. MODY gene mutations affect insulin secretion via impairment of insulin sensing, glucose metabolism in pancreatic β cells, or activation of adenosine triphosphate (ATP)-dependent potassium channels.
- There are about 14 different known MODY mutations which include: hepatocyte nuclear factor 4 α (HNF4A) in MODY1 (30–60%), glucokinase (GCK) in MODY2 (30–60%), hepatocyte nuclear factor 1α (HNF1A) in MODY3 (30–60%), pancreatic and duodenal homeobox (PDX1) in MODY4, hepatocyte nuclear factor 1β (HNF1B) in MODY5 (50%) and neurogenic differentiation factor 1 (NEUROD1) in MODY6. Other mutations in INS, PAX4, ABCC8, KCNJ11, KLF11, CEL, BLK and APPL1 genes may also be demonstrated in these patients. Mutations of several genes adversely affect synthesis of insulin receptors on tissues, insulin binding and intracellular signaling.
- Both HNF1A and HNF4A genes regulate the expression of numerous genes encoding serum proteins such as coagulation factors and apolipoproteins. HNF1A is found in the liver, kidney, intestine and pancreas, which acts by inhibiting the key steps of glucose transport and metabolism as well as mitochondrial metabolism in pancreatic β cells. Glucokinase enzyme enables the β cells and hepatocytes to respond to blood glucose levels. PDX1 is a homeobox-containing transcription factor that affects development of pancreas and insulin gene expression.
- MODY genes associated with various syndromes include: (a) Wolfram syndrome (diabetes mellitus, diabetes insipidus, optic atrophy and deafness), (b) thiamine-responsive megaloblastic anemia, and (c) maternally-inherited diabetes mellitus with sensorineural deafness (MIDD) due to mitochondrial DNA mutations in 3243A–G that results in decreased ATP production leading to diminished insulin release.
- The different MODY gene mutations vary with respect to age of onset, response to treatment and the presence of extra-pancreatic manifestations. Genetic testing for MODY should be performed in cases, where there is a high index of suspicion that the patient does not have type 1 diabetes mellitus or type 2 diabetes mellitus. Genetic defects of maturity-onset diabetes mellitus of the young (MODY) is given in Table 26.9.

Neonatal Diabetes Mellitus (NDM)

Neonatal diabetes mellitus (also known as congenital diabetes mellitus of infancy) occurs due to an underlying monogenic (single gene) defect during first six months of life. Infants do not produce enough insulin leading to an increased glucose accumulation in the tissues.

- **Gene mutations:** NDM occurs due to mutations in KCNJ11 and ABCC8 (affecting the pancreatic β cells K^+ ATP channel). Other gene mutations are also demonstrated in neonatal diabetes that include INS, GATA6, EIF2AK3, GCK, PTF1, FOXP3, ZEP57, GLIS3, PDX1, SLC2A2, SLC19A2, GATA4, NEUROD1, NEUROG3, NKX2-2. RFX6, IER3IP1, MNX1 and HNF1B.
- **Clinical features:** Neonatal diabetes mellitus associated with mutations in KCNJ11 and ABCC8 genes presents with low-birth weight, developmental delay and neonatal diabetes syndrome (DEND syndrome). Monitoring of fetal growth during pregnancy and blood glucose levels in neonates are essential.

Table 26.9 Genetic defects of maturity-onset diabetes mellitus of the young (MODY)

MODY	Genetic Defects	Comments
MODY1	HNF4A (30–60%)	▪ HNF4A (hepatocyte nuclear factor 4 α) regulates expression of genes encoding coagulation factors and apolipoproteins ▪ MODY1 accounts for 30–60% of cases ▪ MODY1 with transient neonatal hypoglycemia, high birth weight and macrosomia
MODY2	GCK (30–60%) (chromosome 7)	▪ GCK (glucokinase) enzyme enables the pancreatic β cells and hepatocytes to respond to blood glucose levels ▪ MODY2 accounts for 30–60% of cases ▪ MODY2 presents with mild and nonprogressive hypoglycemia ▪ Increased risk for macrovascular and macrovascular complications in 10% of cases
MODY3	HNF1A (chromosome 12)	▪ HNF1A (hepatocyte nuclear factor 1 α) is found in the liver, kidney, intestine and pancreas ▪ HNF1A acts by inhibiting the key steps of glucose transport and metabolism as well as mitochondrial metabolism in pancreatic β cells. ▪ MODY3 accounts for 60% cases between 21–26 years of age and responds to oral sulfonylureas and meglitinide drugs
MODY4	PDX1 also known as insulin promoter factor 1 (chromosome 13)	PDX1 (pancreatic and duodenal homeobox 1) is a homeobox-containing transcription factor that affects pancreatic development and insulin gene expression
MODY5	HNF1B (chromosome 17)	▪ HNF1B (hepatocyte nuclear factor 1 β) is found in the liver, kidney, intestine, lungs and ovaries ▪ MODY5 accounts for less than 5% of cases ▪ MODY5 can present with renal cysts. Dysplasia, renal tract malformations or hypoplastic glomerulocystic renal disease. It is sometimes referred to a RCAD (renal cysts and diabetes mellitus) ▪ There can be a progressive loss of renal function independent of diabetic nephropathy ▪ MODY5 patients do not respond to oral hypoglycemic drugs, hence these require insulin therapy
MODY6	NEUROD1 (chromosome 17)	▪ NEUROD1 (neurogenic differentiation factor) is a gene mutation in a basic-loop-helix transcription factor that affects development of pancreas and neurons ▪ MODY6 requires insulin therapy

1. *HNF4A (hepatocyte nuclear factor 4α), HNF1A (hepatocyte nuclear factor 1α), GCK (glucokinase). PDX-1 (pancreatic and duodenal homeobox 1),*
2. *Lipoatrophic diabetes mellitus is accompanied by loss of subcutaneous adipose due to mutation of OPAR-γ.*

Gestational Diabetes Mellitus

Pregnancy associated transient diabetes mellitus is known as gestational diabetes mellitus (GDM) that is usually identified in the 24–28 weeks of gestation. Woman, with GDM, has high-risk for developing type 2 diabetes mellitus later in life.

- During pregnancy, anti-insulin hormones are synthesized by placenta such as estrogen, prolactin, human chorionic somatomammotropin, cortisol, and progesterone.
- Human chorionic somatomammotropin regulates carbohydrate and protein metabolism of the mother to ensure delivery of glucose and protein to fetus for fetal growth. Human chorionic somatomammotropin is also known as chorionic growth hormone-prolactin, and placental lactogen.

Predisposing Factors

Pregnant women can develop gestational diabetes mellitus. Some women are at higher risk than other women.

- **Pregnant women age:** Women older than age 25 are at higher risk for gestational diabetes mellitus.
- **Family and personal history:** Risk for gestational diabetes mellitus increases if women have prediabetes a precursor of type 2 diabetes mellitus or if a close family member such as a parent or sibling has type 2 diabetes mellitus. Women, who had gestational diabetes mellitus during a previous pregnancy or delivered overweight newborn baby are at higher risk for developing gestational diabetes mellitus.
- **Weight:** Overweight women before pregnancy increase risk for gestational diabetes mellitus.
- **Race and ethnicity:** Black pregnant women in Asia or Europe are more likely to develop gestational diabetes mellitus.

Gestational Diabetes Mellitus: Maternal Complications

- **Cesarean delivery:** Due to increased fetal birth weight, there is increased indication of cesarean section in women with gestational diabetes mellitus.

- **Pre-eclampsia and eclampsia of pregnancy and their complications:** Pregnant women can develop pre-eclampsia, eclampsia and its life-threatening complications for both mother and baby.
- **Subsequent gestational diabetes mellitus:** Once the mother has gestational diabetes mellitus, she is more likely develop subsequent gestational diabetes mellitus with next pregnancy.

Gestational Diabetes Mellitus: Complications in Neonates

- **Macrosomia (large baby):** In pregnant women with gestational diabetes mellitus, extra glucose can cross the placenta, which triggers baby's β cells of islets of Langerhans in pancreas to produce more insulin, that can cause baby to grow too large (macrosomia). Such large babies are more likely to require cesarean birth.
- **Low-blood glucose level:** Sometimes babies born in mother with gestational diabetes mellitus develop hypoglycemia shortly after birth because their insulin production is high as a result of pancreatic β cells hyperplasia in response to hyperglycemia and increased demand of insulin during early gestation. Prompt breastfeeding and sometimes an intravenous glucose solution administration can return the baby's blood glucose level within normal range.
- **Higher-risk for type 2 diabetes mellitus:** Babies of mothers who have gestational diabetes mellitus have higher risk for gaining weight and developing type 2 diabetes mellitus. Untreated gestational diabetes mellitus can result in a baby's death either during intrauterine life or shortly after birth.
- **Other newborn complications:** There is increased risk for fetal birth weight, brachial plexus injuries, and neonatal respiratory distress syndrome (hyaline membrane disease).

Diagnostic Approach of Gestational Diabetes Mellitus

Gestational diabetes mellitus is diagnosed with a glucose tolerance test, in which the woman drinks 75 g glucose. Blood glucose level after an hour more than 140 mg/dl is considered positive. The confirmatory test is a 3-hour, 100 g glucose tolerance test in which the blood glucose values are set at highest sensitivity. Urine analysis is done for sugar.

ACUTE DIABETIC COMPLICATIONS

Diabetes ketoacidosis (DKA) and hyperosmolar hyperglycemic state (HHS), also known as hyperosmotic hyperglycemic nonketotic state (HHNK), are the two most serious acute complications of diabetes mellitus. Each represents an extreme in the spectrum of hyperglycemia. Differences between diabetic ketoacidosis and hyperosmolar hyperglycemic state are given in Table 26.10.

Diabetic Ketoacidosis

Diabetic ketoacidosis is mainly a complication of type 1 diabetes mellitus. It produces severe volume depletion due to loss of sodium and water associated with osmotic diuresis and the patient becomes unconscious. Pathophysiology of diabetic ketoacidosis is shown in Fig. 26.8.

Pathophysiology

In the absence of insulin, glucose cannot be used by the body and blood glucose levels rise. The liver responds by increasing hepatic glucose output via gluconeogenesis and glycolysis worsening hyperglycemia.

- Insulin also suppresses lipolysis. In the absence of insulin, fat is consequently broken down to free fatty acids by lipase enzyme. β-Oxidation of fatty acids results into increased production of acetyl-CoA.

Table 26.10 Differences between diabetic ketoacidosis and hyperosmolar hyperglycemic state

Parameters	Diabetic Ketoacidosis	Hyperosmolar Hyperglycemic State (HHS)
Prevalence	Type 1 diabetes mellitus	Type 2 diabetes mellitus
Age	Younger age group	Older age group
Prodromal symptoms appear	Less than 24 hours	Several days
Abdominal pain and Kussmaul respiration symptoms	Present	Absent
Acidosis	Moderate/severe	Absent
Blood glucose	More than 250 mg/dl	High (more than 600 mg/dl)
Serum bicarbonate	Less than 15 mEq/L	More than 15 mEq/L
Blood/urine ketones	++++	+/–
β-Hydroxybutyrate	High	Normal or raised
Arterial blood pH	Low (less than 7.3)	Normal (more than 7.3)
Effective serum osmolarity	Variable	Increased (more than 320)
Anionic gap	More than 12	Variable

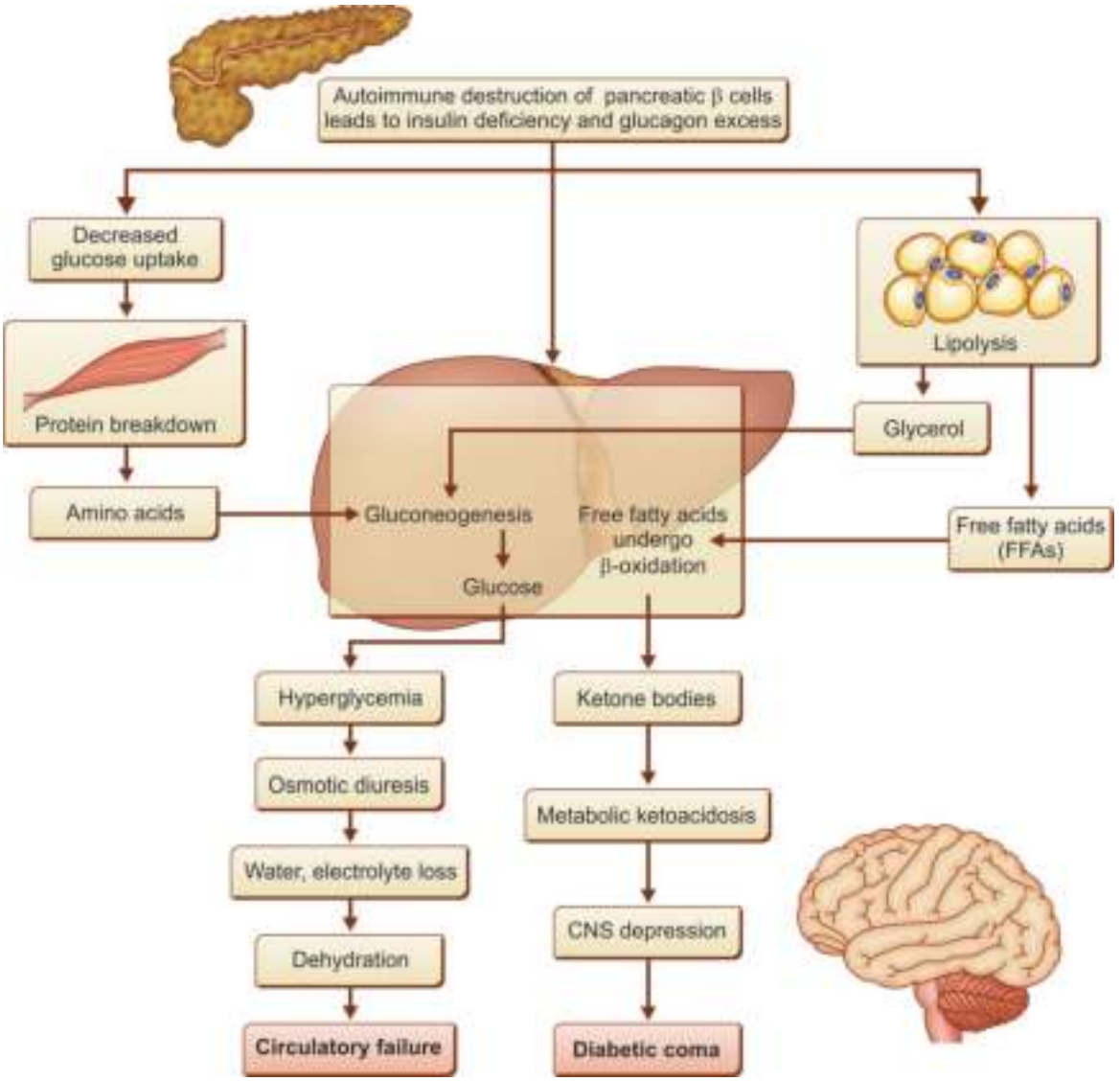


Fig. 26.8: Pathophysiology of diabetic ketoacidosis (DKA). DKA is associated with very low insulin levels and extremely high levels of glucagon, catecholamines, and other counter-regulatory hormones. Increased levels of glucagon and catecholamines lead to mobilization of substrates for gluconeogenesis and ketogenesis by the liver. Gluconeogenesis in excess of that is needed to supply glucose for the brain and other glucose-dependent tissues produces a rise in blood glucose levels. Mobilization of free fatty acids (FFAs) from triglyceride stores in adipose tissue leads to accelerated ketone production and diabetic ketoacidosis.

- Conversion of acetyl-CoA into ketone bodies results into increased production of ketone bodies with acetone-fruity odor (principally β-hydroxybutyric acid, acetoacetic acid along with small quantities of acetone) in liver, giving a fruity odor to the breath. Ketones are acidic and their accumulation results in the life-threatening systemic acidosis. The triad of hyperglycemia, ketosis and acidosis is known as diabetic ketoacidosis.

Clinical Features

Patient with diabetic ketoacidosis develops nausea, vomiting, fatigue, abdominal pain, drowsiness and coma in extreme cases.

- Patient is often profoundly dehydrated at presentation. Breathing tends to become deep and rapid as the body attempts to correct the acidosis. Breath smells fruity odor and nail polish remover.
- Without treatment, patient may progress to coma and death. It is worth mentioning that diabetic ketoacidosis is not limited to type 1 diabetes mellitus, but milder form of ketoacidosis is also seen in prolonged starvation.

Hyperosmolar Hyperglycemic State

Hyperosmolar hyperglycemic state (HHS) is characterized by severe dehydration and severe hyperglycemia without diabetic ketoacidosis. It is one of the two

most serious metabolic derangements that occurs in diabetic patients. Life-threatening complications are associated with high mortality, if not adequately treated. Predisposing factors include acute infections, glucocorticoids, diuretics and nonadherence to diabetes mellitus treatment.

- **Pathophysiology:** Hyperosmolar hyperglycemic state (HHS) occurs when blood glucose of a person with diabetes mellitus becomes >600 mg/dl for long time. The extra glucose is excreted into the urine, which causes the person to urinate frequently. As a result, patient loses a lot of fluid, which can lead to severe dehydration.
- **Clinical features:** Patient presents with signs of severe dehydration, extreme thirst, dry mouth, frequency of micturition, weakness, leg cramps, blurred vision and an altered level of unconsciousness. Onset of HHS is typically over days to weeks. Complications of HHS include coma, seizures and death.
- **Management:** HHS is diagnosed by blood glucose analysis and serum osmolarity. Patient is treated by intravenous fluids, electrolytes and insulin to control blood sugar levels.

DIABETES MELLITUS AND COMPLICATIONS: CELLULAR METABOLIC SIGNALING PATHWAYS

Diabetes mellitus (DM) is a metabolic disorder that is characterized by hyperglycemia and glucose intolerance. It is associated with impaired insulin secretion and peripheral sensitivity, as well as eventual β cell dysfunction.

- **Diabetes mellitus classification:** Diabetes mellitus is classified into type 1 diabetes mellitus (defects in insulin synthesis), type 2 diabetes mellitus (peripheral resistance to insulin), gestational diabetes mellitus during pregnancy and monogenic diabetes mellitus.
- **Hyperglycemic state:** Hyperglycemia promotes cellular glucose uptake and oxidative metabolism, generation of reactive oxygen species (ROS) and subsequent inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme. Additionally, hyperglycemia activates metabolic pathways that eventually promote the release of cytokines such as TGF-β, ICAM-1, VCAM-1, TNF-α, connective tissue growth factor (CTGF) and plasminogen activator inhibitor 1, involved in various diabetic complications.
- **Diabetic complications:** Hyperglycemia activates certain metabolic processes including enzymes and their intermediates that induce diabetic complications such as cardiovascular diseases (coronary artery disease, cerebral stroke, peripheral vascular disease), diabetic nephropathy, diabetic neuropathy, diabetic ocular complications, infectious diseases, skin diseases and nonalcoholic fatty liver disease.

Cellular Metabolic Signaling Pathways

Metabolic pathways are facilitated by enzyme activities of protein kinase C due to diacylglycerol (DAG) synthesis, polyol pathway (sorbitol-aldose reductase pathway), hexosamine biosynthetic pathway (HBP), glucose autooxidation and degradation, as well as being associated with advanced glycated end products (AGEs) synthesis, hexokinase II driven glycolytic overload and enhanced activities of cyclooxygenase (COX) enzyme, lipoxygenase (LOX) enzyme. The inhibition of the enzymes involved in these metabolic pathways and potentially more effective antidiabetic drugs could serve to mitigate and inhibit diabetic complications. Interaction between metabolic pathways leading to diabetic complications is shown in Fig. 26.9.

Protein Kinase C Activation Signaling Pathway

Out of more than eleven isoforms of serine threonine kinases of protein kinase C (PKC) family, PKC activation causes upregulation of extracellular matrix synthesis, cytokines synthesis, contractility, vascular permeability, vascular cell proliferation and cytosolic phospholipase A2; and downregulation of Na^+/K^+ ATPase in diabetic patients. Alterations of metabolic protein kinase C pathway activation cause abnormal retinal hemodynamics resulting disruption in blood retinal barrier (BRB).

- Hyperglycemia is accompanied by PKC activation in heart, retina and renal glomeruli, which eventually escalates complications in diabetic patients.
- Additionally, continuous PKC activation stimulates various growth factors including VEGF (vascular endothelial growth factor), IGF-1 (insulin growth factor 1), PDGF (platelet-derived growth factor), TGF-β (transforming growth factor-β), and HGF (hepatocyte growth factor).

Polyol Pathway Activation (Sorbitol/Aldose Reductase Activation Pathway)

Under physiologic state, glucose is converted to glucose-6-phosphate by hexokinase III. In hyperglycemic milieu the hexokinase II pathway becomes saturated, and rising intracellular glucose level activates aldose reductase (AR) enzyme that eventually converts glucose to sorbitol (alcohol) followed by oxidation of sorbitol to fructose by sorbitol dehydrogenase (SD) enzyme.

- Both aldose reductase and sorbitol dehydrogenase-catalyzed steps involve using nicotinic acid adenine dinucleotide phosphate (NADPH). Once sorbitol is produced, it does not diffuse easily across the cell membrane. Sorbitol permeates the cell membranes

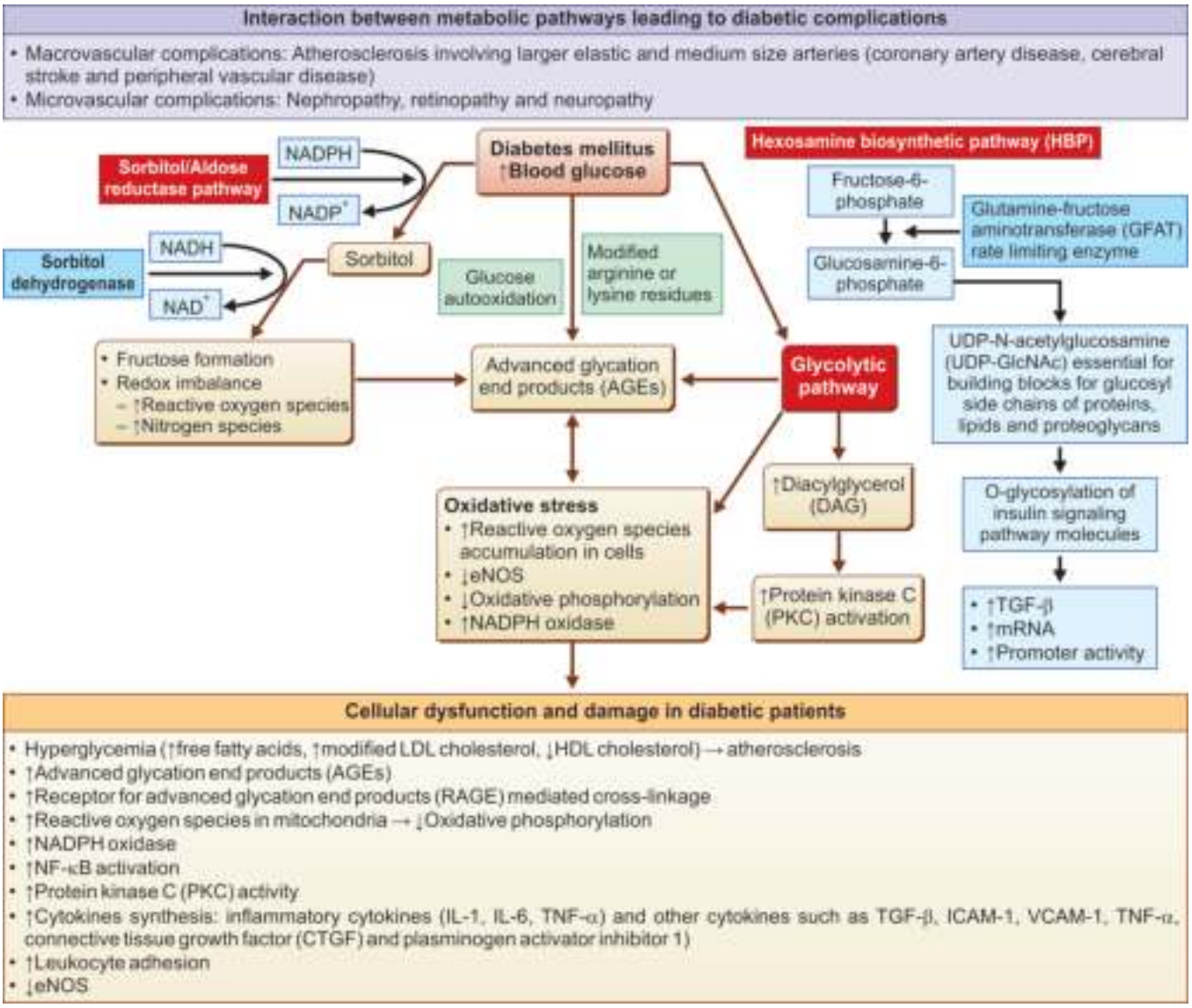


Fig. 26.9: Interaction between metabolic pathways leading to diabetic complications.

leading to disruption of cellular structure and osmotic cell swelling and reduction in ATPase function.

- Sorbitol in hyperglycemic state gives rise to increased intracellular osmolarity, decreases the availability of enzyme cofactor nicotinamide adenine dinucleotide phosphate leading to altered vascular endothelial cell functions that changes the course of leukocyte–endothelial interaction.
- Rising intracellular glucose in hyperglycemic state also directly activates the polyol pathway leading to decreased availability of NADPH (cofactor of GSH), which depletes GSH. It has been reported that NADH produced in the polyol pathway translocates into mitochondria and is oxidized by the respiratory chain, thus generating superoxide oxygen-derived free radicals responsible for diabetic complications.
- Sorbitol also activates pyruvate kinase C (PKC) that plays key role in inducing diabetic complications. Thus, polyol pathway is associated with a vast variety of diabetic complications.

Hexosamine Biosynthetic Signaling Pathway

The hexosamine biosynthetic pathway (HBP) is involved in the development of insulin resistance as well as diabetic vascular complications. It has been demonstrated that transforming growth factor-β (TGF-β) mediated by the hexosamine pathway is involved in the development of diabetic nephropathy.

- Upon cellular uptake of glucose, relatively large glucose concentrations are catabolized and channelized towards glycogenolysis, glycolysis and pentose phosphate metabolism.

- Moreover, a small fraction (2–3%) of glucose molecules is channelized into the hexosamine biosynthetic pathway. Glucose enters insulin-sensitive tissues either by using the insulin-independent glucose transporter (GLUT), and glucose phosphorylating enzyme hexokinase I (HK-I) or with the help of the glucose transporter GLUT4 and hexokinase II (HK-II) is metabolized to hexosamine products in the presence of glutamine (GluN): fructose-6-phosphate amidotransferase (the first and rate-limiting step of hexosamine biosynthetic pathway).
- The end product of hexosamine biosynthetic pathway is uridine diphosphate (UDP)-N-acetylglucosamine (UDP-GlcNAc), which can be covalently linked to serine and threonine residues of specific proteins (O-linked glycosylation) such as insulin signaling molecules and GLUT4. The uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) is the precursor for the biosynthesis of the essential amino sugars required for the generation of glycoproteins, proteoglycans, glycosaminoglycans and glycolipids.
- The O-linked glycosylation seems to decrease insulin action and GLUT4 translocation leading to insulin resistance diabetes mellitus. O-linked glycosylation can modify the functions of many proteins, including transcription factors.
- With hyperglycemia, some of the excess glucose is shunted into the hexosamine biosynthetic pathway. In addition, increased generation of UDP-GlcNAc could alter gene expression and cellular function through the excess glycosylation of proteins contributing to the pathogenesis of microvascular disease.
- Sustained hyperglycemia induces metabolic pathway through elevation of fructose-6-phosphate concentration, which flows into the hexosamine biosynthetic pathway, that eventually promotes the release of cytokines such as TGF-β, ICAM-1, VCAM-1, TNF-α, connective tissue growth factor (CTGF) and plasminogen activator inhibitor 1 involved in inducing diabetic complications.

Glycolytic/Glucose Auto-oxidation Pathway and Formation of Advanced Glycation End Products (AGEs)

Glycolytic/glucose auto-oxidation regulated by phosphofructokinase 1 (PFK-1) and pyruvate dehydrogenase (PDH) is the primary stimulus for insulin secretion, which plays central role in maintenance of glucose homeostasis. The mutation in mitochondrial DNA that disrupts glycolytic/glucose auto-oxidation signaling pathway has been linked to type 2 diabetes mellitus.

- In hyperglycemic state, glycolytic/glucose auto-oxidation of glucose induces functional damage to proteins via modifications of critical arginine residues. Nonenzymatic modification of proteins, termed advanced glycation end products (AGEs) are derived from dicarbonyl species (i.e. 3-deoxyglucosone, methylglyoxal and glyoxal), which perturb protein function, thus contributing to diabetic vascular complications.
- Reactive dicarbonyl species (i.e. 3-deoxyglucosone, methylglyoxal and glyoxal) bind with arginine and lysine residues, the N-terminal of proteins to produce a Schiff base, subsequent rearrangement of Amadori intermediate α-amino groups products and then production of advanced glycation end products (AGEs) at high rate; and thereby provoke pathogenesis of diabetes mellitus. Unlike glucose modifies lysine residues, reactive dicarbonyl species react preferentially with arginine as well as lysine residues with high reaction rates.
- Advanced glycation end products (AGEs) and their receptors (RAGEs) modulate generation of oxidative stress, enhance release of growth factors and cytokines via NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling and stimulate cell adhesion molecules, all of which eventually result in inflammatory response and escalate arteriosclerosis progression. Increased level of advanced glycation end products (AGEs) is linked with unstable angina and myocardial infarction and other diabetic complications.

Clinical Pearls: Drug Inhibitors of Advanced Glycation End Products (AGEs)

Various drugs are administered (e.g. atorvastatin, pravastatin, telmisartan, ramipril, rosiglitazone, exendin 4 and aminoguanidine) to prevent diabetic complications by inhibiting generation of harmful advanced glycation end products.

- **Atorvastatin** is an antioxidant, that acts by blocking the production of AGEs.
- **Pravastatin** acts by reducing destruction of renal tubular cells in diabetic nephropathy, and mitigates cell apoptosis induced by the AGEs.
- **Telmisartan** acts by inhibiting expression of AGEs on renal mesangial cells, endothelial cells and hepatocytes.
- **Telmisartan** and **ramipril** also ameliorate the induction of oxidative stress, inflammation and arteriosclerosis associated with AGEs expression.
- **Rosiglitazone** mitigates the expression of AGEs receptors on tissues.
- **Exendin 4** acts by blocking the interaction between AGEs and their receptors (RAGEs) on tubular cells, thus inhibiting development of diabetic nephropathy.

Hyperglycemia and Cyclooxygenase (COX) Enzyme Activation

The cyclooxygenase enzyme exists in COX-1 and COX-2 isoforms. COX-1 isoform is present in almost all tissues,

whereas COX-2 exists in trace amounts in brain and kidney. Cyclooxygenase 2 (COX-2) enzyme has been implicated in the pathogenesis of several inflammatory diseases.

- Activation of protein kinase C (PKC), oxidative stress, growth factors, inflammatory cytokines and tumor promoters induce synthesis of COX-2. The cyclooxygenase enzyme synthesizes thromboxane A_2 (TXA_2), and prostaglandins (PGI_2, PGD_2, PGE_2).
- Thromboxane A_2 causes vasoconstriction and promotes platelet aggregation. Prostaglandins induce inflammation: (a) prostacyclin (PGI_2) causes vasodilation and inhibits platelet aggregation, and (b) PGD_2 and PGE_2 cause vasodilation and increase vascular permeability.
- Glucose auto-oxidation and aldose reductase (AR) activation pathway leads to increased COX-1, that has been linked to hyperglycemia and onset of diabetes mellitus resulting in diabetic complications. AR activation pathway is accompanied by secondary reduction of nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NAD), activation of protein kinase C, increased expression of receptor for advanced glycation end products (RAGEs), as well as increased generation of reactive oxygen species (ROS).
- Many studies revealed abundant COX-2 in the coronary arterioles and renal podocytes in diabetic patients. The inhibitory effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on COX-2 has been extensively reported.

Lipoxygenase 12 Enzyme Activation Signaling Pathway

Lipoxygenase enzyme has two isoforms: 12-LOX (ALOX12 gene product) and 5-LOX. The 12-lipoxygenase (LOX) enzyme activated by hyperglycemia inserts molecular oxygen into polyunsaturated fatty acids (arachidonic acid and linoleic acid) to generate 12-hydroperoxyl-eicosatetraenoic acid (12-HPETE) and 12-hydroxyeicosatetraenoic acid (12-HETE). The 12-HPETE plays a critical role in the pathogenesis of diabetic retinopathy, a severe microvascular complication in diabetes mellitus is the main cause of vision loss.

- Emerging evidence has demonstrated that 12-LOX and its main product 12-hydroperoxyl-eicosatetraenoic acid (HPETE) activate retinal vascular endothelial cells through activation of nicotinamide adenine dinucleotide phosphate with hydrogen atom (NADPH) and subsequent generation of reactive oxygen species (ROS), mediating multiple pathologic changes in retinal cells and show their potential as future targets for diabetic retinopathy. Further studies are needed on the specific mechanism including 12-LOX pathway related 12-HETE receptors and downstream signaling pathways.
- Eventually, proinflammatory cytokines induce an elevation of reactive oxygen species (ROS), oxidative stress, and endoplasmic reticulum (ER) stress, which act in an autocrine, paracrine and endocrine manner and cause impairment and apoptosis of β cells of islets of Langerhans leading to development of diabetes mellitus.

Hexokinase II (HK-II) Activation Signaling Pathway Driven Glycolytic Metabolic Products Overload

Hexokinase is the rate-limiting first step of glycolysis that catalyzes the first step of glycolysis involving the phosphorylation of glucose to glucose-6-phosphate.

- Under physiologic state, hexokinase II (HK-II) regulates glucose metabolism and also functions as a protective signaling molecule. Glucose transported through glucose transporters (GLUTs) on the plasma membrane is phosphorylated by hexokinase II isoform to produce glucose-6-phosphate (G6P) in insulin-sensitive tissues such as heart, skeletal muscle, and adipose tissue.
  - Glucose-6-phosphate (G6P) serves as a precursor for glycolysis (ATP) as well as the pentose phosphate pathway (NADPH and ribose-5-P), glycogenesis (glycogen) and hexosamine biosynthetic pathway (UDP-GlcNAc). HK-II therefore plays key role in the regulation of anabolic and catabolic processes.
  - HK-II expression is phosphorylated by AKT/mTOR signaling pathway. HK-II binds to voltage-dependent anion channel 1 (VDAC1), the outer mitochondrial membrane protein and provide mitochondrial protection by facilitating coupling between glycolysis and oxidative phosphorylation.
  - The mTOR is a protein kinase that signals via two complexes: TORC1 and TORC2. Hexokinase II also binds and inhibits TORC1 to facilitate autophagy in response to glucose deprivation.
- Under pathologic (hyperglycemic) state, HK-II initiates an abnormal elevation in glycolytic metabolic flux with increase in the level of glycolytic intermediate metabolic products without concurrent transcriptional and functional regulation.
  - This process is known as glycolytic overload and is accompanied by several diabetic complications.
  - Steps in the pathogenic mechanism of diabetes mellitus associated with glycolytic overload include: (a) glucose-6-phosphate (G6P) induces mitochondrial dysfunction, (b) fructose-6-phosphate (F6P) is channelized to the hexamine pathway, and (c) dihydroxyacetone phosphate (DHAP) activates pyruvate kinase C (PKC) through glyceraldehyde 3 phosphate and dihydroxyacetone phosphate (DHAP) intermediate products.

LONG-TERM COMPLICATIONS OF DIABETES MELLITUS

Long-term diabetic complications develop gradually depending on elevation of blood glucose level. Duration and severity of disease are key factors underlying the clinical presentation of diabetes mellitus. Symptoms tend to appear in type 1 diabetes mellitus than type 2 diabetes mellitus. Long-term complications of uncontrolled diabetes mellitus are given in Table 26.11 and Fig. 26.10.

- Long-term diabetic complications result from elevated blood glucose levels due to impairment of metabolic pathways of glucose, lipids and proteins. Hyperglycemia adversely affects blood vessels such as abdominal aorta, coronary arteries, carotid arteries, cerebral arteries, anterior tibial, posterior tibial and popliteal vessels causing them to narrow and therefore restricting blood flow to the tissues/organs.

Table 26.11 Long-term complications of uncontrolled diabetes mellitus

Macrovascular Complications (Large Elastic and Medium-sized Arteries)
▪ Coronary artery disease (atheromatous plaque) ▪ Cerebral vessel disease (atheromatous plaque) ▪ Peripheral vascular disease (atheromatous plaque)
Microvascular Complications (Small Blood Vessels)
▪ Diabetic nephropathy • Diffuse glomerulosclerosis • Nodular glomerulosclerosis • Renal papillary necrosis • Fibrin cap • Capsular drop • Tubulointerstitial disease
▪ Diabetic neuropathy • Polyneuropathies • Amyotrophy (diabetic radiculopathy) • Autonomic nervous system neuropathy • Cranial nerve neuropathy
▪ Diabetic oculopathy • Diabetic retinopathy (nonproliferative and proliferative) • Cataract • Glaucoma • Retinal ganglion cells (RGCs) neuropathy • Ischemic optic neuropathy • Diabetic keratopathy
▪ Diabetic mellitus related infections • Skin infections • Urinary tract infections • Rhinocerebral mucormycosis
▪ Diabetic dermatopathy • Xanthomas • Necrobiosis lipoidica diabeticorum
▪ Nonalcoholic fatty liver disease (NAFLD) in diabetic patients
▪ Diabetic mastopathy

Internal mammary arteries and arteries of the upper limb are always spared of atheromatous plaques.

- Diabetic patients are prone to develop life-threatening complications such as myocardial infarction, cerebral stroke and atherosclerosis, retinopathy, nephropathy and neuropathy. Hyperglycemia also causes disturbances in the body's immune system, so these patients are more susceptible to bacterial and fungal infections.
- Tight control of blood glucose level, hypertension and serum lipids reduces the onset of severity of diabetic complications such as cerebral stroke, myocardial infarction, retinopathy, nephropathy and neuropathy.

Diabetic Macrovascular Complications

Diabetes mellitus is a prime risk factor for cardiovascular diseases. Atherosclerosis is the major threat to the macrovasculature for patients with or without diabetes mellitus. Healthy vascular endothelium is vasodilatory, antithrombogenesis and anti-inflammatory properties. Vascular endothelial dysfunction and dyslipidemia play important role in the pathogenesis of atherosclerosis.

- **Dyslipidemia in diabetes mellitus:** Clinically, dyslipidemia is highly correlated with atherosclerosis. It has been observed that 97% of patients with diabetes mellitus have dyslipidemia (i.e. high triglycerides and low-density lipoproteins and low-HDL cholesterol). Dyslipidemia is the only mechanism by which diabetes mellitus promotes atherosclerosis. Hyperglyceridemia can also lead to increased production of the low-density lipoproteins and decreased HDL transport of cholesterol back to the liver.
- **Low-density lipoproteins:** Low-density lipoproteins are atherogenic, because these can more easily penetrate and form stronger attachments to the large elastic and medium-sized arterial wall, and LDLs are more susceptible to oxidation. Oxidized low-density lipoproteins acquire new properties, which produce several biological responses: (a) chemotaxis of leukocytes to the intima of the arterial wall, (b) necrosis of vascular endothelial and smooth muscle cells, (c) leukocytes ingest lipids and differentiate into foam cells, and (d) proliferation and migration of smooth muscle cells also lead to ingestion of oxidized low-density lipoproteins. All these steps lead to formation of atheromatous plaque. LDL molecules can also become glycated that increases the ability of the LDL to accelerate atherogenesis.

Diabetic Cardiovascular Disease

Hyperglycemia accelerates atherosclerosis in coronary arteries and may cause angina pectoris, acute myocardial infarction and cardiac failure. Clinically, dyslipidemia is highly correlated with atherosclerosis in diabetic

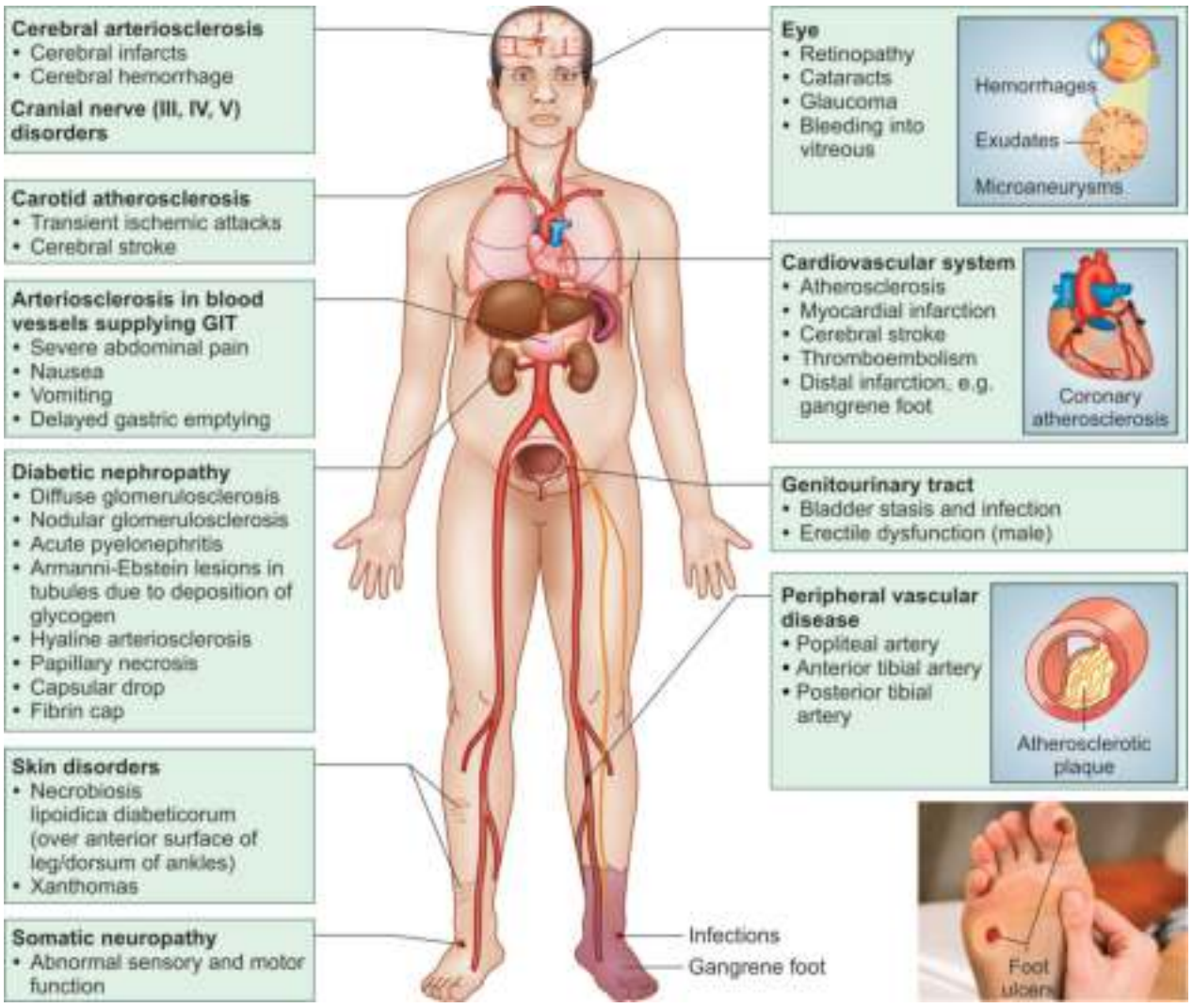


Fig. 26.10: Long-term complications of uncontrolled diabetes mellitus. Macrovascular diabetic complications such as coronary artery disease, cerebral stroke and peripheral vascular disease reflect the combined effects of unregulated blood glucose levels, elevated blood pressure and hyperlipidemia. The most common cause of death with diabetes mellitus is myocardial infarction. Microvascular complications include nephropathy, neuropathy, retinopathy and dermatopathy.

patients. Patient becomes symptomatic if coronary arteries consist of atherosclerotic plaque involving >75% of lumen.

- **Clinical features:** Patient with acute myocardial infarction presents with tightness in the chest, severe pain in the chest, back, jaw and other regions of the upper body that persists for several minutes, shortness of breath, sweating, nausea, vomiting and anxiety.
- **Treatment modalities:** Treatment modalities of acute myocardial infarction include angioplasty, stents and coronary artery bypass graft surgery. Kindly refer acute myocardial infarction discussed in Chapter 16.

Diabetic Cerebral Stroke

Brain tissue needs a constant supply of oxygen and nutrients to keep nerve cells and supporting tissues alive and functioning. Occlusion of the arteries supplying brain results in cerebral stroke. Diabetes mellitus is a well-established risk factor for cerebral stroke.

- **Pathophysiology:** Several possible mechanisms wherein diabetes mellitus leads to cerebral stroke, which include vascular endothelial dysfunction, increased arterial stiffness, at an early age, thickened capillary basement membrane, and systemic inflammation. The C-reactive protein, cytokines (e.g. IL-1, IL-6, TNF-α) and adiponectin are the main serum markers of inflammation. Adiponectin appears to be a modular of lipid metabolism and systemic inflammation. Vascular endothelial function is critical in maintenance of structural and functional integrity of the arterial walls as well as vasomotor control. Nitric oxide (NO) mediates vasodilation, but decreased availability of nitric oxide can cause

vascular endothelial dysfunction and trigger a cascade of atherosclerosis.

- **Cerebral stroke patterns:** There is higher occurrence of atherothrombotic and lacunar cerebral infarctions in diabetic patients.
  - Partial blockage in cerebral arterial may result in temporary reduction of blood supply to a part of the brain (transient ischemic attacks).
  - A complete loss of blood supply to an area of the brain due to clogging or breaking of a cerebral artery results in cerebral stroke.
- **Clinical features:** Cerebral stroke is a medical emergency. Patient presents with sudden numbness or weakness in the face, arm of leg on one side of the body, difficulty in speaking, blurred vision, difficulty in swallowing, dizziness, lack of coordination, paralysis and explained intense headache.

Diabetic Peripheral Vascular Disease

Hyperglycemia accelerates atherosclerosis in popliteal arteries, anterior and posterior tibial arteries resulting to peripheral vascular disease.

- **Pathophysiology:** Endothelial dysfunction, vascular smooth muscle dysfunction, inflammation, hypercoagulability, and decrease in a vasodilator nitric oxide production by dysfunctional endothelium, are the key factors in diabetic arteriopathy in lower legs. All these mechanisms lead to reduction in vascular blood flow, which in turn results in development of skeletal muscle ischemia and peripheral vascular disease. Dysfunctional endothelium induces defective synthesis of bioactive molecules such as potent vasodilator (i.e. nitric oxide), reactive oxygen species (ROS), endothelin, prostaglandins and angiotensin II.
- **Clinical features:** Partial or complete arterial blockage adversely affects the circulation to lower extremities. Patient with partial arterial blockage presents with intermittent claudication (cramps, pain in calf, thigh, hip and buttocks when walking and relieved by taking rest) and ischemic ulcers. Complete arterial blockage causes gangrene foot. Patient condition becomes worse through the development of diabetic neuropathy, leading to propensity for injury. Physical signs in the legs in peripheral vascular disease comprise skeletal muscle atrophy, hair loss, smooth shiny skin, decreased or absent pulses in the feet, non-healing ulcers or wounds in the lower extremities and numbness in the toes. The **six Ps** of acute limb ischemia include: **P**ain, **P**allor, **P**aresthesia, **P**ulselessness, **P**aralysis and **P**erishing cold (poikilothermia).

Diabetic Nephropathy

Morphological changes due to diabetes mellitus are similar in type 1 and type 2 diabetes mellitus and can be observed in all the four structural compartments of the kidney: glomeruli, tubules, interstitial tissue and blood vessels. Renal lesions include diffuse glomerulosclerosis, nodular glomerulosclerosis (also known as Kimmelstiel-Wilson disease), papillary necrosis, hyaline arteriolosclerosis, tubulointerstitial disease, capsular drop and fibrin cap. Renal lesions in diabetic nephropathy are given in Table 26.12.

- **Pathogenesis:** Diabetic nephropathy is caused by the metabolic and hemodynamic changes. Pathogenesis is complex, involving glycosylation of proteins in glomerular basement membrane, hormonally influenced transforming growth factor-β cytokine release, deposition in mesangial matrix and alteration of glomerular hemodynamics. Advanced glycated end (AGE) products bind to collagen fibers in glomerular basement membrane (GBM) and impair the interaction of laminin with GBM resulting in increased capillary permeability and albuminuria. Increased width of GBM is the earliest and most common renal manifestation. In diffuse glomerulosclerosis and nodular glomerulosclerosis, there is increased synthesis of type IV collagen in glomerular basement membrane and deposition in mesangial matrix.
- **Clinical features:** Diabetic nephropathy manifests as slowly progressive albuminuria with worsening hypertension and renal inefficiency.
- **Laboratory diagnosis:** Diagnosis is based on clinical history, physical examination, blood biochemistry, urine analysis and urine albumin/creatinine ration.
- **Treatment:** Patients are treated by strict blood glucose control, angiotensin-converting enzyme (ACE) inhibitors administration, controlling blood pressure and serum lipids.

Diabetic Glomerulopathy

Diffuse thickening of the glomerular basement membrane is the earliest morphologic change. Diffuse increase in mesangial matrix is demonstrated in the early phase which progresses to nodular mesangial sclerosis (Kimmelstiel-Wilson disease) in the later stage of the renal disease. These morphologic changes in glomeruli take a decade or longer to develop and gradually worsen.

- **Diffuse glomerulosclerosis:** Accumulation of basement-like stroma in the mesangium parallels the diffuse thickening of the glomerular basement membrane. These morphologic changes gradually advance until the entire glomerulus is sclerotic. Focal

Table 26.12 Renal lesions in diabetic nephropathy

Renal Lesions	Comments
Glomerular lesions	
Diabetic glomerulosclerosis	Widening of mesangial areas occurs due to accumulation of basement like stroma in the mesangial region, increased number of mesangial cells in 2–5 years after the onset of diabetes mellitus
Nodular glomerulosclerosis (also known as Kimmelstiel-Wilson disease)	Sclerotic nodules are formed in the peripheral region of intercapillary region of glomeruli demonstrated by PAS staining
Bowman capsule lesion	
Capsular drop	Accumulation of plasma proteins inside Bowman's capsule is known as capsular drop
Glomerular subendothelial region lesion	
Fibrin cap	Fibrin cap is formed by plasma proteins deposition in subendothelial region
Papillary necrosis	Diabetic glomerulosclerosis leads to ischemia of papillae (papillary necrosis). Necrotic papillae may detach and pass in the urine as tissue fragments. Undetached papillae may resolve by fibrosis
Renal arterioles lesion	
Hyaline arteriolosclerosis of renal arterioles	Hyaline arteriolosclerosis of renal arterioles is the main cause of renal microangiopathy
Tubulointerstitial tissue lesions	
Armanni-Ebstein lesions	Deposition of glycogen in renal tubules is known as Armanni-Ebstein lesions in uncontrolled diabetes mellitus patients
Acute pyelonephritis	▪ Acute pyelonephritis occurs most frequently secondary to infection by bacteria or fungi and characterized by pus in the tubule and multiple grayish white areas of small microabscesses ▪ Microscopic urine analysis is the presence of white blood cell (WBC) casts

effacement of foot processes is common. Diffuse glomerulosclerosis associated with long-standing diabetes mellitus can be demonstrated by periodic acid–Schiff (PAS) stain. Diffuse glomerulosclerosis in diabetic nephropathy is shown in Fig. 26.11.

- **Nodular glomerulosclerosis:** Nodular glomerulosclerosis is also known as 'Kimmelstiel-Wilson disease'. Sclerotic nodules are formed in the mesangial region as a result of nonenzymatic glycation of proteins and increased synthesis of type IX collagen between capillary loops. The nodules have a hypocellular matrix core which may be homogenous or exhibit lamellations with peripherally arranged mesangial nuclei. The nodules vary in size that can be demonstrated by periodic acid–Schiff and silver methenamine stains. The periphery of the nodules may demonstrate mesangiolysis resulting in the formation of capillary microaneurysms due to the detachment of anchoring points of the glomerular basement membrane to the mesangium. Intercapillary glomerulosclerosis leads to renal ischemia, tubular atrophy, marked interstitial fibrosis, increased mesangial region, arteriolosclerosis and ultimately small contracted kidney. Nodular glomerulosclerosis (Kimmelstiel-Wilson disease) in diabetic nephropathy is shown in Fig. 26.12. Microaneurysm in glomerulus in diabetic nephropathy is shown in Fig. 26.13.

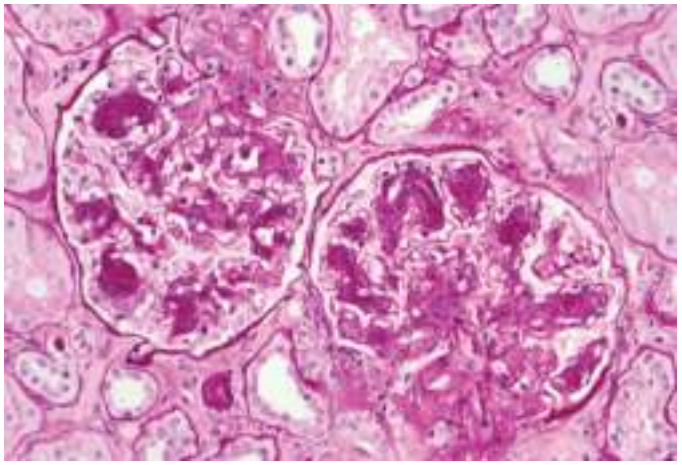


Fig. 26.11: Diffuse glomerulosclerosis in diabetic nephropathy. It shows widening of mesangial areas due to accumulation of basement like stroma in the mesangial region. Electron microscopy is required in the early stages to detect the glomerular basement membrane thickening but it becomes appreciable by light microscopy when the thickness is increased more than four times (X40).

Diabetic Renal Papillary Necrosis

Renal papillary necrosis is caused by diabetes mellitus and urinary tract infection. Renal papillae are considered to be anatomically vulnerable to ischemic changes (papillary necrosis). Renal papillary necrosis is shown in Fig. 26.14.

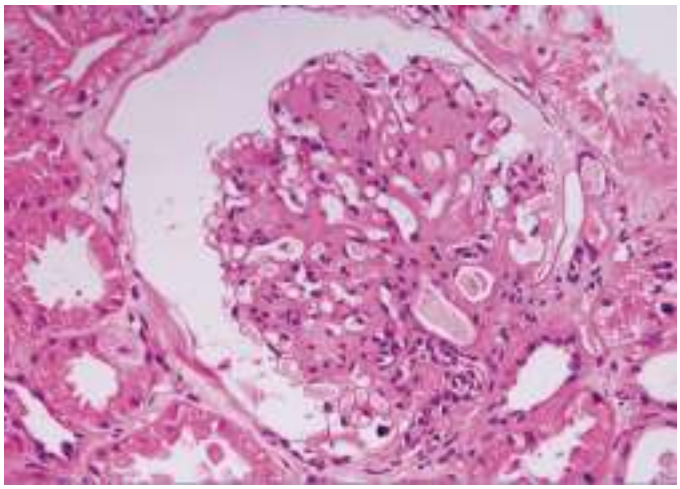


Fig. 26.12: Nodular glomerulosclerosis (also known as Kimmelstiel-Wilson disease) in diabetic nephropathy. Histologic examination shows sclerotic nodules in the peripheral region of intercapillary region of glomeruli. The nodules are formed due to accumulation of trapped proteins and synthesis of type IV collagen in mesangial matrix. The nodules can be demonstrated by periodic acid–Schiff and silver methenamine stains (X40).

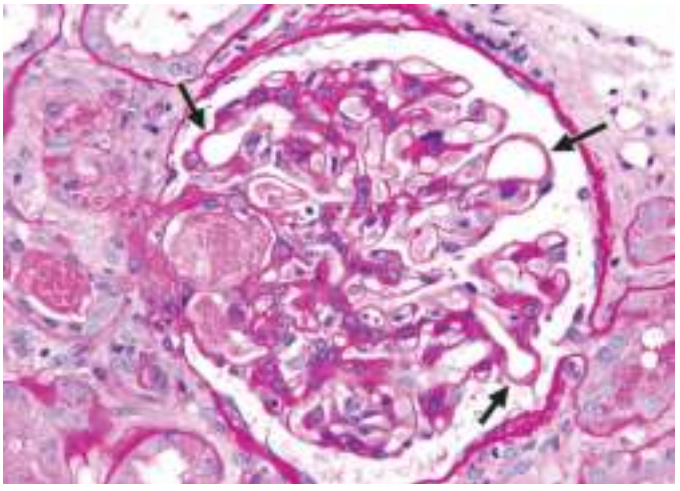


Fig. 26.13: Microaneurysm in glomerulus in diabetic glomerulopathy. Microaneurysm is demonstrated in glomerulus (arrows).

- Necrotic renal papillae may detach and pass in the urine as tissue fragments. Undetached renal papillae may resolve by fibrosis.
- Renal papillary necrosis can be seen in the setting of diabetic nephropathy, chronic analgesic abuse, infectious pyelonephritis and sickle cell anemia.

Diabetic Renal Hyaline Arteriolosclerosis

Hyaline arteriolosclerosis typically involves both the afferent and efferent arterioles. Some authors consider this finding to be most specific for diabetic nephropathy. Renal arterioles exhibit varying degrees of mild intimal fibrosis.

- Hyalinosis lesions result from insudation or exudation of plasma proteins from the blood vessels. It is the main cause of renal microangiopathy in diabetic patients. Blood vessels exhibit homogenous eosinophilic appearance and also show two characteristic patterns: fibrin cap and capsular drop.
- As the disease advances, segmental and global glomerular sclerosis develops. Hyaline arteriolosclerosis in diabetic nephropathy is shown in Fig. 26.15.

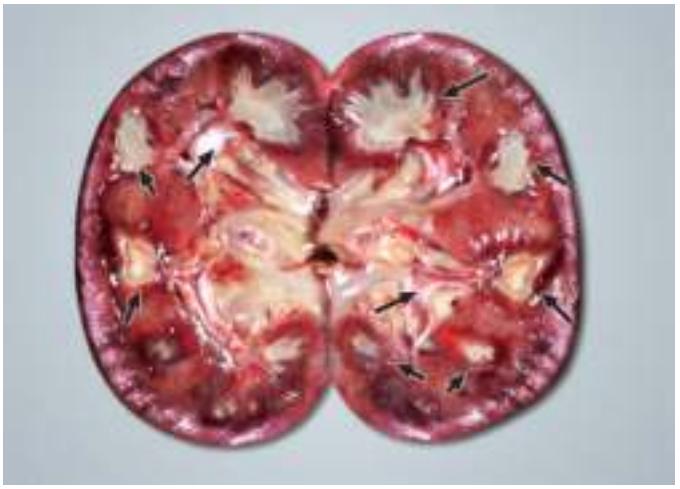


Fig. 26.14: Renal papillary necrosis. Renal papillary necrosis is typically thought to be caused by diabetes mellitus and urinary tract infection (arrows).

Diabetic Capsular Drop in Bowman's Capsule

Capsular drop is produced by accumulation of plasma proteins between parietal cells and Bowman's capsule. The capsular drop lesion is characteristic but not specific for diabetic nephropathy and also has been described in other nondiabetic diseases. Capsular drop in diabetic nephropathy is shown in Fig. 26.16.

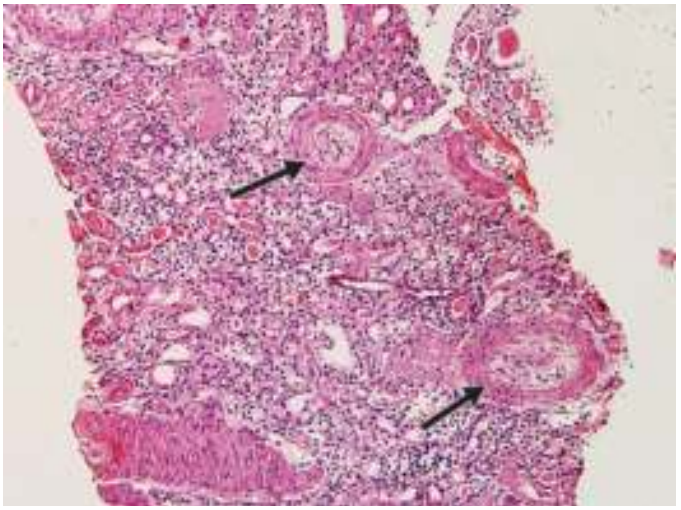


Fig. 26.15: Hyaline arteriolosclerosis in diabetic nephropathy. Histologic examination shows hyalinization of arterioles in diabetic nephropathy (arrows) (X10).

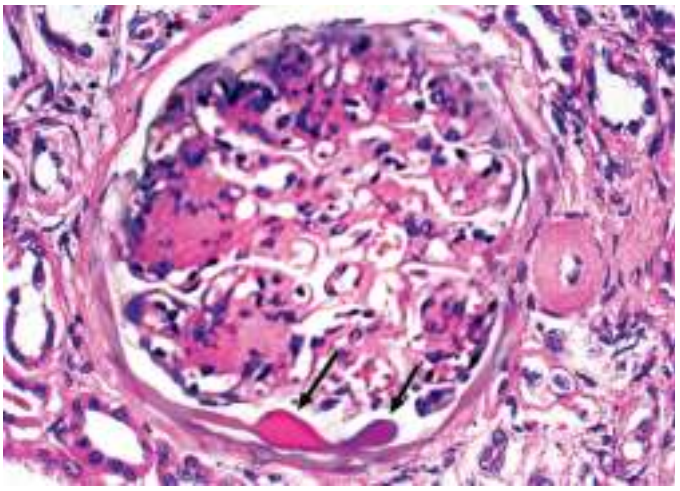


Fig. 26.16: Capsular drop in glomerulus. Accumulation of plasma proteins inside Bowman's capsule is known as capsular drop (arrows).

Diabetic Fibrin Cap in Glomerular Subendothelial Region

Accumulation of plasma proteins in the subendothelial region of glomeruli is known as fibrin cap. Fibrin cap essentially represents segmental sclerosis and is a misnomer as the lesion consists of primarily hyaline and not fibrin.

Diabetic Tubulointerstitial Disease

The earliest change in diabetic nephropathy involving the tubules is thickening of the tubular basement membrane. Tubular basement thickening can be demonstrated by periodic acid–Schiff (PAS) stain. As the disease advances, there is increase in the degrees of intestinal fibrosis and tubular atrophy. Nonspecific chronic inflammation may be demonstrated with the areas of fibrosis.

- **Armanni-Ebstein lesions:** Armanni-Ebstein lesions occur due to increased permeability of renal tubules and deposition of glycogen in renal tubules and are seen in uncontrolled hyperglycemia, which can occur in either type of diabetes mellitus.
- **Acute pyelonephritis:** It is most frequently secondary to infection by bacteria or fungi and characterized by pus in the tubule and by abscess formation. Microscopic urine analysis reveals presence of white blood cell casts. Acute pyelonephritis may progress to chronic pyelonephritis. The kidneys show multiple grayish white areas of small microabscesses in the renal parenchyma. Renal gross morphologic changes in acute pyelonephritis are shown in Fig. 26.17. Renal parenchyma is infiltrated by polymorphonuclear cells. Histologic changes in acute pyelonephritis are shown in Fig. 26.18.



Fig. 26.17: Renal gross morphologic changes in acute pyelonephritis. Acute pyelonephritis is often a focal disease, and much of the kidney often appears normal. The kidney shows multiple grayish white areas of small microabscesses in the renal parenchyma.

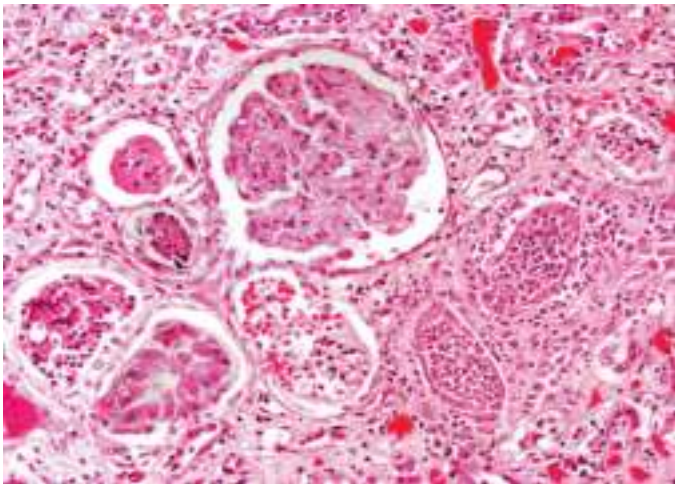


Fig. 26.18: Histologic changes in acute pyelonephritis. Renal parenchyma is infiltrated by polymorphonuclear cells (100X).

Diabetic Ocular Complications

Diabetes mellitus is a metabolic disease defined by elevated blood glucose. Ocular complications can occur in uncontrolled diabetes mellitus that can be reversed by using biodegradable polymers and implantable ocular devices.

- Diabetic ocular complications include: retinopathy, macular edema, retinal ganglion cells neuropathy, ischemic optic neuropathy, glaucoma, keratopathy, corneal edema, corneal nerve alterations, and cataract.
- Proliferative diabetic retinopathy is characterized by retinal exudates, edema, hemorrhages, and microaneurysms of small vessels leading to blindness. Diabetic ocular complications are given in Table 26.13.

Table 26.13 Diabetic ocular complications

Diabetic nonproliferative and proliferative retinopathy
Macular edema
Glaucoma
Cataract
Retinal ganglion cells (RGCs) neuropathy
Ischemic optic neuropathy
Diabetic keratopathy (corneal edema, corneal nerve alterations)

Diabetic Retinopathy

Diabetic retinopathy is a progressive disease that can cause blindness as a result of retinal detachment. It is related to the duration of the disease, occurring in most patients with diabetes mellitus after 10 years. Vascular changes in the inner retina in diabetic patients are given in Table 26.14. Pathologic changes in diabetic retinopathy are given in Table 26.15.

- **Diabetic retinopathy types:** Diabetic retinopathy can be divided into two types: nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). NPDR can be further divided into three stages (e.g. early prediabetes stage, mid-point diabetes mellitus stage and late stage of diabetes mellitus) before progressing to PDR. NPDR usually progresses to PDR leading to vision impairment. Annual ophthalmic examination is mandatory in diabetic patients.
- **Diabetic retinopathy pathogenesis:** Retina has dual blood supply: retinal arteriole supplies inner two-thirds of inner retina and choroid arteriole nourishes pigmented epithelial cells and the outer one-third of the retina.
  - Initially, reduction in retinal perfusion triggers a series of adverse metabolic reactions such as increased expression of angiogenic factors and tissue modelling factors such as VEGF, IL-6, IL-1 and tumor necrosis factor-α, polyol accumulation, oxidative stress and activation of protein kinase C.
  - Moreover, hyperglycemia directly impairs retinal mitochondria leading to increased reactive oxygen species (ROS), inflammation and DNA damage.
  - Together with microvascular changes, reduced retinal perfusion, thickening of the basement membrane, retinal pericyte loss ultimately resulting in neovascularization.
  - Pericyte death, breakdown of vascular endothelial tight junctions, and basement membrane thickening damages blood retinal barrier (BRB) and cause

Table 26.14 Vascular changes in the inner retina in diabetic patients

Biochemical Changes (Metabolic Stress)	
↑Glucose transport	↑Hexosamine biosynthetic pathway (HBP)
↑Redox imbalance	↑Glucose autooxidation and degradation
↑Oxidative stress (mitochondrial reactive oxygen species)	↑Advanced glycated end products (AGEs) synthesis (AGEs/RAGEs interaction)
↑Na^+/K^+ ATPase activity	↑Hexokinase II driven glycolytic overload
↑PKC (protein kinase C)	↑Cyclooxygenase (COX) and lipoxygenase (LOX) enzyme activities
↑Polyol pathway (sorbitol-aldose reductase pathway)	
Growth Factors Synthesis	
↑VEGF (vascular endothelial growth factor)	↑FGF-β (fibroblastic growth factor-β)
↑IGF-1 (insulin growth factor 1)	↑TGF-β (transforming growth factor-β)
↑PDGF (platelet-derived growth factor)	↑EGF (epidermal growth factor)
↑HGF (hepatocyte growth factor)	↑NGF (nerve growth factor)
↑ET-1 (endothelin 1)	↓Antiangiogenic factors
Functional Changes	
Blood flow alteration	Vascular permeability changes
Intercellular junctions' disruption	
Structural Changes	
Reduced oxygen transport	Local swelling of retina
Pericyte loss	Occlusion of precapillary arterioles
Endothelial dropout	Acellular capillaries
Basement membrane thickening	Focal saccular venous bulges due to significant retinal ischemia
Diabetic Retinopathy Consequences	
Extracellular matrix synthesis (ECM)	Severe hemorrhage in retina and vitreous
Endothelial cell migration and proliferation	Retinal tear/detachment and bleeding into vitreous cause visual loss
Angiogenesis	

Table 26.15 Pathologic changes in diabetic retinopathy (DR)

Diabetic Retinopathy	Retinal Findings on Fundus Examination	Pathology
Nonproliferative diabetic retinopathy (NPDR)		
Mild nonproliferative DR	Microaneurysms	Outpouchings of the weakened capillary walls or endothelial buds attempt to revascularize the ischemic retina
Moderate nonproliferative DR	▪ Dot/blot hemorrhages ▪ Hard exudate ▪ Cotton-wool spots	▪ Weakened blood retinal barrier (BRB) results in rupture into deeper retinal layers ▪ Disrupted blood–brain barrier (BRB) results in leakage of lipoproteins and other proteins leading to formation of yellow lipid deposits with sharp margins ▪ Occlusion of the precapillary arterioles results in nerve fiber infarcts
Severe nonproliferative DR	▪ Flame hemorrhages ▪ Venous beading	▪ Retinal hemorrhages occur in the more superficial nerve layer ▪ Focal areas of saccular venous bulges due to significant retinal ischemia
Proliferative diabetic retinopathy (PDR)		
Proliferative retinopathy	Neovascularization	Localized retinal ischemia causes upregulation of VEGF causing fine, irregular, and easily friable neovascularization in the disc, macula, and/or retina leading to traction retinal detachment and vitreous hemorrhage.

Pathophysiology of diabetic retinopathy: Pericyte death, breakdown of endothelial tight junctions, and basement membrane thickening damages blood retinal barrier cause leakage of fluid into the retinal tissues resulting in macular edema in patients with moderate-to-severe nonproliferative retinopathy and proliferative retinopathy.

leakage of fluid into the retinal tissues resulting in macular edema in patients with moderate-to-severe nonproliferative retinopathy and proliferative retinopathy.

- **Clinical features:** Diabetic retinopathy affects both eyes. Patient presents with increase in number of floaters, blurring vision, black and dark areas in fields of vision, poor night vision and blindness.

Pathology Pearls: Nonproliferative and Proliferative Diabetic Retinopathy

Nonproliferative Diabetic Retinopathy (NPDR)

- Nonproliferative diabetic retinopathy (NPDR) is an early mild stage, in which microvascular changes manifest as microaneurysms on the retina.
- Microaneurysms may rupture and forming soft cotton wool exudates (microinfarcts) and later hard exudates as a result of deposits of protein that have leaked from damaged capillaries.
- In moderate NPDR, two or less quadrants of retina demonstrate intraretinal hemorrhages, hard exudates, 'cotton wool spots' and venous beading without hampering vision.
- The microvascular changes are reversible within two to three weeks without hampering vision. In severe NPDR, all four quadrants demonstrate intraretinal hemorrhages; and venous beading is visible in more than two quadrants.

Proliferative Diabetic Retinopathy (PDR)

- The microvascular changes lead to reduction in retinal perfusion. In response to the retinal perfusion reduction and retinal hemorrhages, new blood vessels are formed (neovascularization) in the retina. The neovascularization marks a critical distinction between NPDR and PDR.
- Newly formed blood vessels contain red blood cells hence hamper absorption of light and thus obscure the vision. Newly formed blood vessels can grow into the vitreous and if left untreated can result in retinal tearing and detachment.
- Additionally, newly formed blood vessels are fragile and susceptible to breakage resulting in vitreous hemorrhage. Vitreous hemorrhage can be surgically removed. Bleeding into the vitreous hampers vision.
- Retinal blood vessels possess a blood retinal barrier that prevents plasma, growth factors and other inflammatory mediators from entering the immunologically quiescent eye.
- Therefore, vitreous hemorrhage triggers additional neovascularization and inflammation that perpetuates proliferative diabetic retinopathy. Proliferative retinopathy is treated with laser surgery (photocoagulation).

Diabetic Macular Edema

Macula is located in part of the retina that contains the highest number of cones, which provides the ability to observe color details. Diabetes mellitus-related macular edema happens when fluid builds up under the macula, the center of retina. Macular edema results in central vision loss. When the newly formed blood vessels rupture, the fluid accumulates in macular region causing thickening of the retina resulting in blurry vision. These retinal findings can be viewed with the ophthalmoscope.

Diabetic Glaucoma

In severe diabetic retinopathy, neovascularization can cause adhesions (synechiae) between iris and cornea or iris and lens resulting in improper drainage of aqueous humor through the trabecular meshwork and Schlemm's canal and increased intraocular pressure known as glaucoma. Glaucoma can damage nerves and

blood vessels, producing changes in vision and leading to glaucoma (open-angle and closed-angle).

- **Open-angle glaucoma:** Open-angle glaucoma is usually painless and asymptomatic, which becomes painful on significant progression of glaucoma. These patients are treated with medications.
- **Closed-angle glaucoma:** Closed-angle glaucoma presents with sudden ocular pain, redness, nausea, and intraocular pressure spikes. These patients generally require medical emergency care.

Diabetic Cataract

Cataract is one of the main causes of vision impairment in diabetic patients. It is 15–20 times more common in diabetic patients than nondiabetic counterparts.

- Aqueous humor is produced by the ciliary processes and present in the posterior and anterior chambers of the eye. Since the lens and cornea have no blood supply, the aqueous humor carries nutrients and oxygen to these structures. Aqueous humor also maintains intraocular pressure.
- Hyperglycemia in the aqueous humor results in cataract due to accumulation of sorbitol that induces osmotic damage to the crystalline lens.
- Posterior capsular opacification (PCO) is a common finding following cataract surgery in diabetic patients. In some cases, diabetic retinopathy progresses following extracapsular cataract extraction. As with diabetic retinopathy, there are concerns that cataract surgery in diabetic patients may exacerbate the progression of macular edema.

Diabetic Retinal Ganglion Cells Neuropathy

Retinal ganglion cells (RGCs) are present near the inner surface of the retina. RGCs receive visual information from photoreceptors via two intermediate neuron types: bipolar cells and amacrine cells.

- RGCs together form the optic nerve, optic chiasma, and optic nerve that transmits visual information to the brain.
- Hyperglycemia causes stress to the retina that triggers apoptosis of RGCs in diabetic patients. The stress to retina occurs due to ischemia, oxidative stress, reduction of trophic factors, increased intraocular pressure, neuroinflammation and aldose reductase inhibition. RGCs neuropathy can also occur even during the early stages of diabetes mellitus.

Ischemic Optic Neuropathy

The reduced retinal perfusion and microaneurysms can reduce the oxygen supply to optic nerve in diabetic patients. Prolonged interruption of oxygen supply to optic nerve triggers optic nerve apoptosis resulting in permanent vision loss. The ischemia and optic neuropathy trigger release of additional apoptotic factors that can adversely affect the other areas of retina. Ischemia also triggers release of hypoxia-inducible factors that promote angiogenesis and inflammation.

Diabetic Keratopathy

Diabetic keratopathy results in impaired vision as a result of decreased wound healing, corneal edema and an altered epithelial basement membrane. Corneal repair is most often difficult in diabetic patients. As the diabetic keratopathy progresses, central region of cornea thickens followed by reduced corneal sub-basal nerve density, increased corneal sensitivity and corneal erosion syndrome and later reduced tear meniscus parameters, and less nerve fiber and branch length.

- Cornea suffers from dysfunctional wound healing/repair mechanisms. Some authors observed that presence of diminished corneal peripheral sensation suggests some kind of neuropathy.
- Other studies revealed that abnormal adhesions of the corneal epithelium to the underlying basement membrane can lead to prolonged and recurrent defects in cornea.
- Weakening of the epithelial barrier in diabetic patients increases risk for corneal infections and stromal fibrosis resulting in vision loss.

Diabetic Neuropathy

Diabetic neuropathy is a serious diabetic complication that may affect about 50% of diabetic patients. Development of diabetic neuropathy can be prevented by regulating blood glucose levels. Hyperglycemia can cause neuropathy throughout the body. Depending on the affected nerves, diabetic neuropathy can range from numbness and pain in the legs and feet to symptoms related to gastrointestinal tract, urogenital system and cranial nerves. Classification of diabetic neuropathies is given in Table 26.16.

Diabetic Peripheral Neuropathy

Peripheral neuropathy is also called distal symmetric peripheral neuropathy. It is most common type of diabetic neuropathy that affects feet and legs followed by hands and arms. Signs and symptoms of peripheral neuropathy are often worse at night.

- **Pathophysiology:** The pathophysiology of diabetic peripheral neuropathy results from hyperglycemia induced vascular disease occluding tiny blood vessels that nourish peripheral nerves (vasa nervorum) in lower limbs, vascular endothelial dysfunction, deficiency of myoinositol-altering myelin synthesis and diminished sodium-potassium

Table 26.16 Classification of diabetic neuropathies

Nerves Involved	Comments
Somatic nerves	
Polyneuropathies (bilateral sensory nerves)	▪ Paresthesis including tingling, numbness ▪ Decreased pain, light touch, temperature, two-point discrimination and vibratory sensations ▪ Impaired ankle and knee jerk reflexes
Mononeuropathies (involvement of a mixed nerve trunk)	▪ Impaired sensory nerves associated with pain ▪ Impaired motor nerve associated with skeletal muscle weakness
Amyotrophy	Skeletal muscle weakness, wasting and severe pain in the pelvic girdles and thigh
Autonomic nervous system	
Impaired vasomotor function	Postural hypertension
Impaired vasomotor function	Postural hypertension
Impaired gastro-intestinal tract functions	▪ Gastroparesis ▪ Diarrhea (postprandial and nocturnal)
Impaired genito-urinary tract functions	▪ Paralytic urinary bladder ▪ Incomplete voiding ▪ Erectile ejaculation ▪ Retrograde ejaculation
Cranial nerves	
Cranial nerves (III, IV, and V)	▪ Extraocular muscles affected ▪ Impaired pupillary response

adenine triphosphatase (ATPase) activity, and chronic hyperosmolarity causing edema of nerve trunks and adverse effects of sorbitol and fructose. Osmotic damage to Schwann cells affects both motor (skeletal muscle weakness) and sensory (paresthesia) nerves of peripheral nerves in type 1 and type 2 diabetes mellitus (70%).

- **Clinical features:** Loss of sensation can cause tingling, numbness, burning pain that usually begins at the tips of the toes or fingers and gradually spreads upwards in hands and arms. Patient presents with numbness, tingling, sharp burning pain and neuropathic ulcers at pressure points on the plantar surfaces of both feet.

Diabetic Autonomic Nervous System Neuropathy

Autonomous nervous system controls heart, gastrointestinal tract, genitourinary system, heart and eyes. Diabetes mellitus can affect nerves in any of these regions. Signs and symptoms are related to the involvement of autonomic nervous system.

- **Clinical features:** Patient can present with gastroparesis (delayed gastric emptying), impotence, urinary bladder dysfunction, cardiac arrhythmias and inability of eyes to adjust from light to dark.
- **Urinary tract infection:** Urine remains in the dysfunctional urinary bladder after incomplete voiding that leads to bacterial growth in stagnant urine. The bacteria travel up to the kidney and cause acute pyelonephritis.

Diabetic Proximal Neuropathy (Diabetic Radiculopathy)

Proximal neuropathy is also called diabetic radiculopathy that affects nerves in the thighs, hips, buttocks or legs. Disease can also affect abdominal and chest regions. Signs and symptoms are most often unilateral, but may spread to other side. Patient presents with severe pain in hip, thigh, buttock, and legs, weakness and shrinkage of thigh muscles and difficulty in rising from a sitting posture.

Diabetic Mononeuropathy (Focal Neuropathy)

Mononeuropathy refers to damage to a specific nerve. There are two types of mononeuropathy: involving cranial nerves and peripheral nerves. Cranial nerves (III, IV, and V) are most commonly affected. Patient presents with difficulty in focusing (i.e. double vision), aching behind one eye, unilateral facial palsy (bell's palsy), tingling or numbness in hands or fingers except little finger and weakness of hand.

Recurrent Infections in Diabetic Patients

Diabetes mellitus increases the susceptibility to various types of infections. Hyperglycemia in tissues and body fluids provides favorable growth conditions for bacteria and fungi. Diabetes mellitus impairs the function of neutrophils. Angiopathy caused by hyperglycemia leads to tissue ischemia, and such ischemic tissues are more prone to infections.

- The most common sites of infection in diabetic patients are the skin (*Staphylococcus aureus*), vulvovaginal region (*Candida albicans*) and urinary tract.
- Diabetic patient presents with well-demarcated yellow plaques over anterior surface of the leg/dorsum of ankles, known as necrobiosis lipoidica diabeticorum due to increased synthesis of fat at insulin injection site. Patient may develop xanthomas (collections of lipid-laden macrophages in the dermis).
- Chronic otitis externa (*Pseudomonas aeruginosa*) and rhinocerebral mucormycosis are two head and neck infections seen almost exclusively in patients with diabetes mellitus. Rhinocerebral mucormycosis spreads to face, orbit, skull, and frontal lobe of brain. Mucormycosis has pauciseptate, broad ribbon-like hyphae observed on histologic evaluation.

Diabetes Mellitus-related Nonalcoholic Fatty Liver Disease

Diabetes mellitus increases the risk for nonalcoholic fatty liver disease (NAFLD), a condition in which excess of triglycerides build up in the hepatocytes. In some cases, NAFLD may progress from steatosis to steatohepatitis with evidence of inflammation and hepatocyte injury, cirrhosis (hepatic fibrosis) and ultimately hepatocellular failure. Most patients of NAFLD remain asymptomatic, however the liver functions are impaired. Maintenance of body weight can prevent and treat NAFLD. It is essential to regulate blood glucose in type 2 diabetes mellitus.

Pathology Pearls: Diabetes Mellitus-related Nonalcoholic Fatty Liver Disease (NAFLD)

Gross Morphology

- Mild fatty change in liver may not affect the gross appearance. Severe fatty change results in enlargement of liver.
- Liver becomes progressively yellow on cut surface until it may weigh 3–6 kg. This uniform change is consistent with fatty metamorphosis (fatty change).

Frozen Section Technique

In paraffin-embedded sections, fat is dissolved during processing of tissue in histokinette. Fat can be demonstrated by frozen section technique with the fat stains such as Sudan III, Sudan IV, Sudan black, Oil red O and osmic acid.

Light Microscopy

- Initially, hepatocytes show small fat vacuoles around nucleus (mild fatty change or microvesicular) in hematoxylin and eosin-stained sections.
- Progressive accumulation of fat vacuoles in the cytoplasm coalesces to create spaces that displace the nucleus toward periphery (severe fatty change).
- Occasionally contiguous cells rupture, and the enclosed fat globules unite to produce so-called fatty cysts. Distribution of lipid vacuoles may be diffuse, focal or zonal, midzonal or peripheral depending on severity and etiology.

Diabetic Mastopathy/Lymphocytic Mastitis

Diabetic mastopathy is a fibrous painless breast mass with a prominent lymphocytic infiltrate most often occurring in patients with long-standing diabetes mellitus. Diagnostic criteria of diabetic mastopathy include: (a) prominent keloidal fibrosis, (b) prominent circumscribed lymphocytic infiltrate (B cells) around perivascular, periductal, perilobular regions that may infiltrate lobular epithelium forming lymphoepithelial lesions, and (c) epithelioid stromal cells.

- **Pathophysiology:** Diabetic mastopathy can occur as a result of hyperglycemia that leads to stromal matrix expansion and accumulation of advanced glycosylation end products (AGEs) and B cell inflammatory response. It can also occur as a result of immunologic response to exogenous insulin.
- **Clinical features:** Diabetic mastopathy is associated with insulin-dependent diabetes mellitus and autoimmune disorders. Patient presents with firm, palpable, nontender, mobile lumps or diffuse nodularity at presentation common.
- **Laboratory diagnosis:** Ultrasonographic study shows irregular shaped breast lump(s), indistinct margins, parallel orientation to chest wall, hypoechogenicity with posterior shadowing. Diagnosis is confirmed by needle core biopsy or surgical excision with histologic examination.

LABORATORY DIAGNOSIS OF DIABETES MELLITUS

Diabetes mellitus (DM) refers to a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin actions in peripheral tissues or both.

- Type 1 diabetes mellitus (DM) occurs due to autoimmune destruction of β cells of islets of Langerhans of pancreas leading to absolute insulin deficiency. Type 2 diabetes mellitus (DM) may range from predominantly insulin resistance with relative insulin deficiency to a predominantly defect with insulin resistance in peripheral tissues. Other types of DM include gestational pregnancy-associated diabetes mellitus and monogenic diabetes mellitus.
- Diabetes mellitus may be diagnosed based on blood glucose criteria, either fasting blood glucose or two-hour blood glucose values during 100 g oral glucose tolerance test (OGTT) or glycosylated hemoglobin (HbA1c) criteria. Diagnostic criteria of diabetes mellitus are given in Table 26.17.

Table 26.17 Diagnostic criteria of diabetes mellitus

Laboratory Test	Diagnostic Criteria
1. Fasting blood glucose (8 hours) on two separate tests	≥126 mg/dl (7 mmol/L)*
2. Two-hour blood glucose during oral glucose tolerance test (OGTT)	≥200 mg/dl (11.1 mmol/L)
3. Glycated hemoglobin A1c (HbA1c)	≥6.5
4. Random blood glucose associated with classic symptoms of diabetes mellitus	≥200 mg/dl (11.1 mmol/L)

**Fasting blood glucose value in normal persons (less than 100 mg/dl), prediabetes (100–125 mg/dl) and diabetes mellitus (≥126 mg/dl).*

Pathology Pearls: Metabolic Alterations in Diabetes Mellitus

- **Hyperlipidemia:** Lack of insulin decreases capillary lipoprotein lipase activity in peripheral blood. It leads to accumulation of chylomicrons and VLDL in blood, which may precipitate acute pancreatitis and eruptive xanthoma in the skin (called the hyperchylomicronemia syndrome). Hyperlipidemia accelerates atherosclerosis at an early age. The risk of cerebrovascular disease is high and frequency to develop foot gangrene is about 100 times increased.
- **Skeletal muscle wasting:** In diabetes mellitus, increased protein degradation results in skeletal muscle wasting.
- **Dilutional hyponatremia:** Serum sodium is decreased in diabetes mellitus. Glucose overrides sodium in controlling the osmotic gradient. Hence, water shifts out of the intracellular fluid compartment into the extracellular compartment resulting in dilution hyponatremia.
- **Hyperkalemia:** Serum K^+ is increased in diabetes mellitus, which occurs due to transcellular shift as excess H^+ ions enter cells in exchange of potassium.
- **Metabolic acidosis:** Metabolic acidosis in diabetes mellitus occurs due to diabetic ketoacidosis and lactic acidosis.
- **Severe volume depletion:** Diabetes mellitus produces severe volume depletion and coma due to loss of sodium and water with osmosis diuresis.
- **Prerenal azotemia:** Prerenal azotemia in diabetes mellitus occurs due to volume depletion resulting in accumulation of waste products in body.

Fasting Blood Glucose

Fasting blood glucose measures the amount of glucose in the blood without food intake, which is a good indicator of glucose metabolism. Fasting blood glucose is defined as no caloric intake for at least 8 hours. Normal fasting blood glucose value is less than 100 mg/dl (<5.6 mmol). Fasting blood glucose value in prediabetes ranges between 100 and 125 mg/dl (5.6–6.9 mmol/L). Fasting blood glucose value in diabetes mellitus is 126 mg/dl or higher (7 mmol/L or higher) on two separate tests.

Random Blood Glucose

A random blood glucose analysis is done to measure the amount of glucose circulating in a person's blood. It is useful for persons who need a speedy diagnosis such as those with type 1 diabetes mellitus who require supplement insulin as a matter of emergency. Patients with type 1 diabetes mellitus present with classic symptoms of hyperglycemia or has hyperglycemic crisis (postprandial random blood sugar 200 mg/dl).

Oral Glucose Tolerance Test

Persons with diabetes mellitus can sometimes demonstrate normal results of fasting or random blood glucose levels yet still have diabetes mellitus.

- If clinicians still suspect that a person has diabetes mellitus, they may recommend an oral glucose tolerance test (OGTT).
- OGTT also requires a person not to eat or drink for eight hours. After giving the first blood sample, person drinks a liquid containing 75 g of glucose. The clinician then takes additional blood samples hourly over the next two hours.
- For an oral glucose tolerance test, blood glucose <140 mg/dl is considered normal.
- Blood glucose values between 140 and 199 mg/dl indicates prediabetes. Prediabetes stage means that a person's blood glucose levels are higher than usual, but clinicians do not consider that they have diabetes mellitus. Clinicians sometimes call this impaired glucose tolerance (IGT) or impaired fasting blood glucose. Persons with prediabetes stage are at higher risk of developing diabetes mellitus. Lifestyle modifications, such as exercise, weight loss and other medications can help to reduce this risk.
- Blood glucose value of ≥200 mg/dl indicates diabetes mellitus. Oral glucose tolerance test in diagnosing diabetes mellitus (samples are drawn after 100 g glucose drink) is given in Table 26.18.

Clinical Pearls: Procedural Alert of Glucose Tolerance Test

- Glucose tolerance test (GTT) is contraindicated in patients with a recent history of surgery, myocardial infarction or labor and delivery. Because these conditions can produce invalid values.
- Glucose tolerance test should be postponed in common illness, i.e. flu or severe cold.
- Reactions during glucose tolerance test should be recorded and reported. Patient may develop weakness, unconsciousness and sweating during the second and third hours of the test. In these cases, GTT should be aborted.
- GTT is declared invalid in case the patient vomits out glucose. Therefore, test can be repeated in three days.

Glycosylated Hemoglobin (HbA1c) for Routine Glycemic Control

The glycosylated hemoglobin (HbA1c) has strong predictive value for diabetic complications and HbA1c analysis should be performed routinely in all diabetic patients. Clinicians should interpret when using HbA1c to monitor patients with hemoglobin variants or conditions that affect red blood cells (RBCs).

- HbA1c analysis is the primary assessment diagnostic tool for glycemic control at every three months including self-monitoring of blood glucose to determine whether glycemic targets have been reached and maintained.
- Hemoglobin is a protein in red blood cells that carries oxygen to organs/tissues and transports carbon

Table 26.18 Two-hour oral glucose tolerance test in diagnosing diabetes mellitus except during pregnancy

Oral Glucose Tolerance Test (OGTT)	Fasting Blood Glucose Level	Two-hour Blood Glucose Level
Normal glucose tolerance	<100 mg/dl (5.5. mmol/L)	<140 mg/dl (7.8. mmol/L)
Impaired glucose tolerance (prediabetes)	100–125 mg/dl (5.6 to 6.9 mmol/L)	140 mg/L (7.8 mmol/dl) to 199 mg/L (11.1 mmol/L)
Diabetes mellitus	>126 mg/dl (7.0 mmol/L)	≥200 mg/dl (11.1 mmol/L)

Blood glucose levels are applicable except during pregnancy. Blood samples are drawn fasting and two hours after a glucose drink.

dioxide from organs/tissues. Glucose molecule in the blood is normally bound to hemoglobin molecule. This means the hemoglobin has become glycosylated, and is also referred to as HbA1c.

- As a person's blood glucose becomes higher, more of hemoglobin becomes glycosylated. The glucose molecule remains bound to the hemoglobin for the life span of the red blood cell or about two to three months.
- Glycosylated hemoglobin (HbA1c) is analyzed to monitor the long-term control of diabetes mellitus. The level of glycosylated hemoglobin is increased in the red blood cells of persons with poorly controlled diabetes mellitus including type 2 diabetes mellitus in pediatric patients.
- Normal range for glycosylated hemoglobin (HbA1c) level is between 4% and 5.6%. Glycosylated hemoglobin level in prediabetes stage ranges between 5.7% and 6.4%. The percentage of glycosylated hemoglobin is increased depending upon the degree of hyperglycemia. Glycosylated hemoglobin level >6.5 is diagnostic of diabetes mellitus, which is directly related to the average concentration of glucose in the blood.
- HbA1c analysis is not recommended in postpartum period due to increased red blood cells count related to pregnancy and/or blood loss at delivery, that can affect HbA1c results. Oral glucose tolerance test (OGTT) is more sensitive test than HbA1c analysis during postpartum period.

Monitoring Long-term Diabetic Complications

Monitoring Diabetic Cardiovascular Diseases

Persons with diabetes mellitus usually have cardiovascular diseases related to risk factors including dyslipidemia as a result of accumulation of chylomicrons and VLDL in blood. Lipid profile should be performed to treat and monitor dyslipidemia.

Monitoring Diabetic Nephropathy

Screening for diabetic nephropathy using urinary albumin analysis and estimated glomerular filtration rate (GFR) testing at least once in a year in patients who have had type 1 diabetes mellitus for more than five years and those with type 2 diabetes mellitus.

- **Urine analysis:** Diabetic nephropathy patient may show proteins, glucose, ketone bodies, pus cells, hyaline casts, fatty casts and oval fat bodies (lipid-containing renal tubular epithelial cells). Free fat droplets, fatty casts, and oval fat bodies; when examined by polarized light, the lipids in casts are seen as being doubly refractile or birefringent and they display a symmetric "Maltese-cross" pattern.
- **Serum proteins:** Total serum proteins are decreased, with reversal of albumin/globulin ratio (normal 2:1) in diabetic nephropathy.
- **Blood urea (20–40 mg/dl):** Blood urea is increased in diabetic nephropathy. Never use anticoagulant containing NH_4^+ salt as it increases urea concentration.
- **Serum creatinine (normal 0.6–1.5 mg%):** Serum creatinine is increased in diabetic nephropathy.
- **Renal biopsy:** Renal biopsy should consist of at least five glomeruli to comment. Formalin-fixed paraffin-embedded sections stained with hematoxylin and eosin, and periodic acid–Schiff stain should be examined under light microscope.

Monitoring Autoimmune Disorders in Diabetic Patients

Consider screening patients with type 1 diabetes mellitus for autoimmune disorders such as autoimmune thyroiditis and celiac disease soon after the diagnosis, as autoimmune disorders occur more frequently in patients with type 1 diabetes mellitus.

Diabetes Mellitus-related Autoantibodies Analysis

Classic differentiation between type 1 and type 2 diabetes mellitus may not always be appropriate on overlapping clinical features.

- Diabetes-related autoantibodies analysis such as islet cell antibodies (ICAs), insulin antibodies (IAs) and glutamic acid decarboxylase antibodies (GADAs, i.e. GAD 65) can be helpful in establishing an autoimmune etiology in persons with previously diagnosed type 1 diabetes mellitus and to exclude the diagnosis of type 2 diabetes mellitus.
- Routine screening for type 1 diabetes mellitus risk with autoantibody testing is recommended only

in the setting of a research trial or in first-degree family members of a proband with type 1 diabetes mellitus.

Molecular Genetic Analysis

Molecular genetic testing is recommended in patients suspected of having monogenic diabetes syndromes, such as maturity-onset diabetes mellitus of the young (MODY) and neonatal diabetes mellitus (NDM) to verify genetic etiology, establish treatment and identify affected family members.

Pathology Pearls: Molecular Genetic Defects of Pancreatic β Cell Functions

- Chromosome 12: HNF-1α (MODY 3)
- Chromosome 7: Glucokinase (MODY 2)
- Chromosome 20: HNF-4α (MODY 1)
- Chromosome 13: Insulin promoter factor 1 (IPF-1, also known as PDX1 (pancreatic and duodenal homeobox 1 [MODY 4])
- Chromosome 17: HNF-1β (MODY 5)
- Chromosome 2: Neuro-D1 (MODY 6)
- Mitochondrial DNA defect: Mitochondrial diabetes mellitus

DISORDERS OF THYROID GLAND

ANATOMY OF THYROID GLAND

The thyroid gland is one of the largest endocrine glands. It consists of two lateral lobes connected in the middle by broad isthmus just below the larynx. It weighs 20–25 g in adults. It is composed of two types of secretory element: the follicular and parafollicular cells. Fibrovascular stroma is present in between the thyroid follicles. The follicular cells are responsible for the synthesis of thyroid hormones, and the parafollicular cells elaborate calcitonin.

- The genes responsible for the development of thyroid follicular cells include TTF-1, TTF-2 (thyroid transcription factor 1 and 2), PAX8, TSH and TSHR.
- Homeobox expressed embryonic stem cells 1 (HESX1) is transcriptional repressor essential in the early development of thyroid gland. The factors that control thyroid embryogenesis, agenesis or dysgenesis are poorly understood.
- Although the thyroid gland is capable of performing independent function, the thyroid hormone synthesis is regulated by the hypothalamic-pituitary-thyroid axis. Hypothalamic tripeptide TSH-releasing hormone (TRH) stimulates pituitary gland to synthesize TSH. TSH in turn stimulates the thyroid follicular cells to synthesize prohormone (T_4) and its active form triiodothyronine (T_3). Because thyroid hormones have a profound influence on cellular metabolic process, which play a key role in the normal growth and development.

Structure ot Thyroid Gland

Thyroid gland is composed of thyroid follicles lined by cuboidal epithelium and containing colloid. Fibrovascular stroma is present in between the thyroid follicles.

- **Thyroid follicular cells:** These cells synthesize thyroxine (T_4) and triiodothyronine (T_3), which are needed to keep the body functioning at its normal rate. The thyroid gland needs a regular supply of iodine (which is added to table salt and found in fish and milk) in order to produce thyroid hormones.
- **Parafollicular cells or C cells of thyroid gland:** These cells synthesize thyrocalcitonin (TC), which lowers the blood level of calcium ions by transporting calcium in the bones. Calcitonin secretion is controlled by calcium ion level in the blood.

PHYSIOLOGY OF THYROID GLAND

Thyroid gland responds to many stimuli as in a constant state of adaptation. Thyroid follicular epithelium proliferates and becomes more active during puberty, pregnancy and physiologic stress. When the stress abates, involution occurs and the thyroid follicular cells resume their normal size and architecture.

REGULATION OF THYROID HORMONES

- Low blood levels of T_3 and T_4 or low basal metabolic rate stimulate release of TRH by hypothalamus.
- TRH, carried by hypophyseal portal veins to anterior pituitary gland, stimulates release of TSH by thyrotrophs.
- TSH released into blood stimulates the thyroid follicular cells.
- Thyroid hormones (T_4 and T_3) are synthesized from iodine and tyrosine within thyroglobulin (TBG) and transported in the blood bound to plasma proteins, mostly thyroid-binding protein (TBG).
- Elevated T_3 and T_4 inhibit release of TRH and TSH by negative feedback mechanism, i.e. inhibiting synthesis of TRH by hypothalamus and TSH synthesis by anterior pituitary gland.

SYNTHESIS OF THYROID HORMONES

Synthesis of thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) depend on sufficient quantities

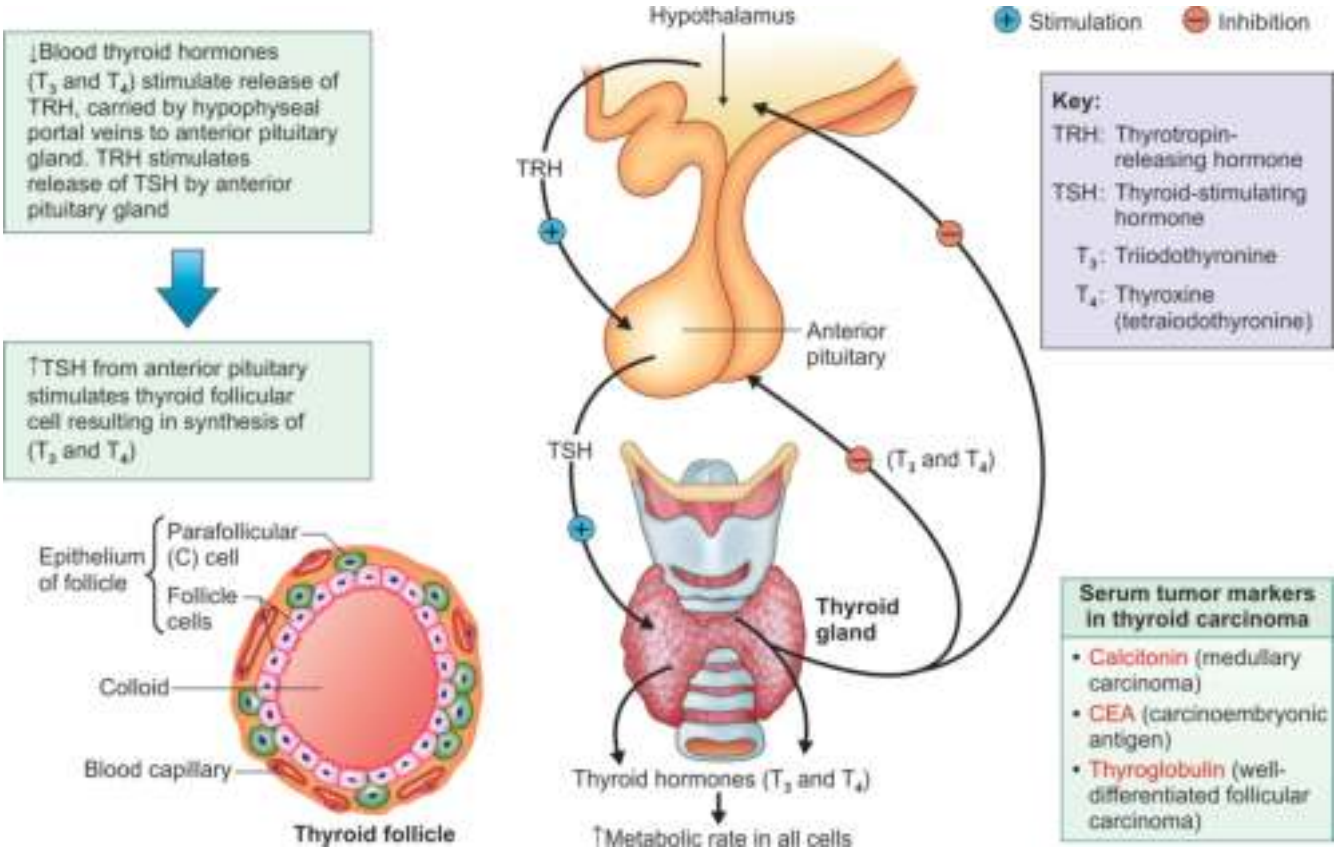


Fig. 26.19: Regulation of thyroid hormones synthesis.

of iodine from dietary sources. Thyroid stimulating hormone (TSH) regulates the rate of extraction of iodine from the bloodstream, synthesis of T_4 and T_3, release from storage as thyroglobulin and secreted into the bloodstream.

Feedback mechanisms regulate TSH synthesis by pituitary gland. Serum T_4 and T_3 are bound to thyroid-binding globulin (TBG). Regulation of thyroid hormones synthesis is shown in Fig. 26.19.

Pathology Pearls: Thyroid Hormones Synthesis and Regulation

- Thyroid transcription factors (TTF-1, TTF-2) and PAX8 participate in thyroid gland development and functioning.
- TTF-1 is a multifunctional nuclear protein that mediates DNA replication fork arrest and regulates RNA polymerase 1 transcription on chromatin. Its biological functions include activation, silencing of transcription, and transcription termination.
- TTF-2 regulates transcription of thyroglobulin (TG) and thyroid peroxidase (TPO). Thyroid hormone receptor inhibits cAMP-response element binding protein (CREB) transcriptional activity.
- Thyroid gland is regulated by hypothalamus and anterior pituitary gland located in the brain. The hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary gland to release thyroid-stimulating hormone (TSH).
- Iodine and tyrosine are essential for synthesis of thyroid hormones. Iodine requirement is 1 mg per week. Iodine consumed through diet is converted into iodide and absorbed by gastrointestinal tract. Sodium iodide is added to common salt to prevent iodine deficiency.

THYROID HORMONES SYNTHESIS: STEPS

- **Thyroglobulin synthesis by thyroid follicular cells:** Endoplasmic reticulum and Golgi appratus in the thyroid follicular cells synthesize and secrete thyroglobulin continuously and stored in the thyroid follicle. Thyroglobulin molecule contains 140 molecules of amino acid tyrosine.
- **Iodide trapping:** TSH participates in trapping of iodide in thyroid follicular cells. Iodide is actively transported from blood into thyroid follicular cells against electrochemical gradient along with sodium by sodium/iodide symporter. Normally, concentration of iodide is 30 times in thyroid gland than in the blood. Concentration of iodide increases 200 times more in thyroid follicular cells during hyperactivity of thyroid gland.
- **Oxidation of iodide:** Peroxidase enzyme converts iodide to organically bound iodine in thyroid

follicular cells. The iodide ions lose electrons to form iodine molecules.

- **Transport of iodide into thyroid follicular cavity:** Iodide is transported from the thyroid follicular cells into the follicular cavity by an iodide-chloride pump called pendrin.
- **Organification of thyroglobulin:** Iodide transported from the thyroid follicular cells into the follicular cavity and binds with thyroglobulin. This process is called the organification of thyroglobulin.
- **Iodination of tyrosine:** Thyroid stimulating hormone (TSH) participates in incorporation of iodide into tyrosine resulting in formation of monoiodotyrosine (MIT) and diiodotyrosine (DIT). Combination of iodide with tyrosine already present in thyroglobulin is known as iodination, which takes place in thyroglobulin. Iodination process is accelerated by the enzyme iodinase, which is synthesized by thyroid follicular cells. Iodination occurs in several stages: (a) tyrosine is oxidized first into MIT and later into DIT, and (b) MIT and DIT are called the iodotyrosine residues.
- **Coupling reactions:** Iodotyrosine residues (MIT and DIT) get coupled with one another and give rise to different thyroid hormones T_3 and T_4. One molecule of MIT and one molecule of DIT combine to form triiodothyronine (T_3). Two molecules of DIT combine to form tetraiodothyronine (T_4). Thyroid hormones (T_4, T_3) are stored as colloid in thyroid follicles.
- **Endocytosis of thyroglobulin colloid into thyroid follicular cells:** Thyroglobulin colloid containing T4 and T_3 is endocytosed and combine with lysosomes leading to formation of phagolysosome.
- **Proteolysis of thyroglobulin colloid in lysosome:** Proteolysis of thyroglobulin colloid containing T_4 and T_3 occurs by lysosomal enzyme and leading to release of T_4 and T_3 in thyroid follicular cells. This step is TSH-mediated process. Thyroid hormones T_4 and T_3 are exocytosed into the blood circulation.

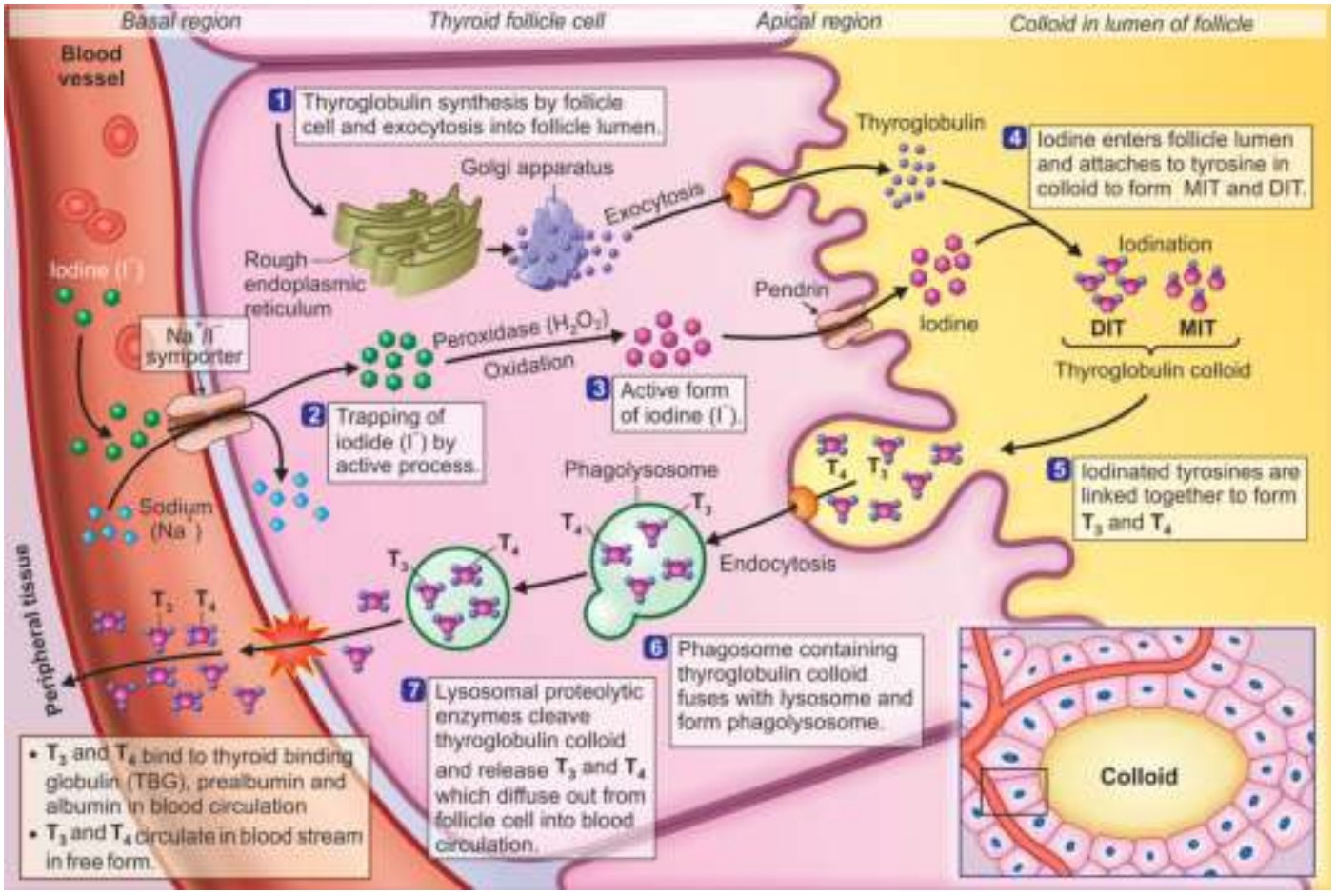


Fig. 26.20: Mechanism of synthesis of thyroid hormones. Steps in synthesis of thyroid hormones include thyroglobulin synthesis, iodide trapping, oxidation of iodide, transport of iodine into follicular cavity, organification of thyroglobulin, iodination of tyrosine, coupling reactions (iodotyrosine residues get coupled with one another and give rise to different thyroid hormones T_3 and T_4, endocytosis of thyroglobulin colloid into thyroid follicular cell, proteolysis of thyroglobulin colloid in lysosome, and exocytosis of T_4 and T_3 and binding to TBG in blood and also circulate in free forms. Note: Thyroid transcription factors 1 and 2 (TTF-1, TTF-2) and PAX8 participate in thyroid gland development and functioning.

- **Exocytosis of T_4 and T_3 and binding to TBG in blood stream:** Thyroid hormones (T_4, T_3) bind to thyroid binding globulin (TBG), prealbumin and albumin. One-third of TBG binding sites are normally occupied. Thyroid hormones (T_4, T_3) circulate and act on peripheral tissues. Mechanism of synthesis of thyroid hormones is shown in Fig. 26.20.

THYROID HORMONES IN CIRCULATION AND TISSUES

- **Synthesis of thyroid hormones in circulation:** Thyroid gland synthesizes T_4 (80 μg) and triiodothyronine T_3 (6 μg) daily.
- **Conversion of T_4 to T_3 in various tissues:** Only a small fraction of thyroid hormones circulates in free form. Outer ring of 5-deiodinase enzyme converts T_4 to T_3 mainly in liver, kidneys (80%) and dendritic cells of nervous system (some conversion).
- **Mechanism of actions of T_3 on various tissues:** T_3 is metabolically active hormone. It acts on nuclear receptors thus increases messenger RNA and protein synthesis.

THYROID HORMONES: ACTIONS

- **Central nervous system:** Thyroid hormones are essential for axonal, dendritic development and myelinization of CNS especially in early first year of postnatal life.

  Deficiency of thyroid hormones during intrauterine life results in mental retardation due to nonmyelinization of central nervous system and causes cretinism (thyroid dwarf) in children.
- **Maturation of growing epiphyseal region and skull:** Thyroid hormones enhance synthesis of growth hormones and insulin-like growth factor; which is necessary for maturation of growing epiphyseal region (linear growth), skull; tooth eruption and dentition.
- **Peripheral vasodilatation:** Thyroid hormones enhance synthesis of β-adrenergic receptors and cause vasodilatation.
- **Basal metabolic rate:** Thyroid hormones increase basal metabolic rate resulting in increased oxygen consumption.
- **Regulation of metabolism:** Thyroid hormones regulate carbohydrate, fat and protein metabolism.
- **Maintenance of water and electrolyte balance:** Thyroid hormones maintain water and electrolyte balance.
- **Hematopoiesis:** Thyroid hormones play an important role in hematopoiesis.

THYROIDITIS

HASHIMOTO'S THYROIDITIS

Hashimoto's thyroiditis is an autoimmune disease. It is the most common cause of hypothyroidism. Autoantibodies such as antithyroid peroxidase antibody, antithyroglobulin antibody and anti-TSH antibody are implicated in its pathogenesis. Infiltrates of lymphocytes with germinal center formation are seen in Hashimoto's thyroiditis (Figs 26.21 to 26.23).

Age Group and Incidence

Hashimoto's thyroiditis occurs more often in women (30–50 years) than men. Its incidence is increased in HLA-DR5, HLA-B5 and autoimmune disorders, i.e. systemic lupus erythematosus, rheumatoid arthritis, pernicious anemia, Sjögren's syndrome, adult-onset type 1 diabetes mellitus, chronic active hepatitis and adrenalitis.

Pathogenesis of Hashimoto's Thyroiditis

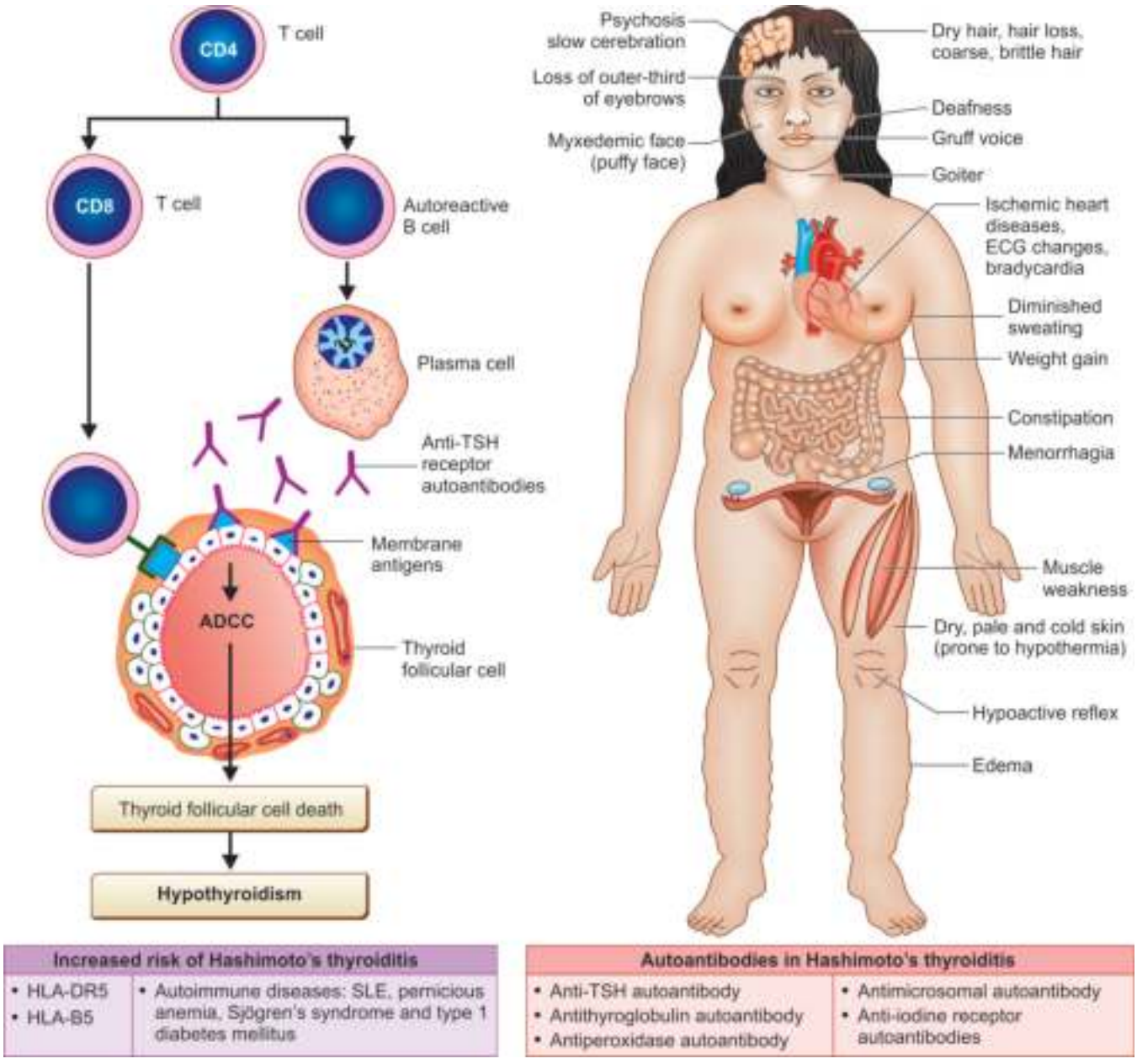
Type II Hypersensitivity Reaction-mediated Disorder

Loss of activity of T-suppressor cells in Hashimoto's thyroiditis results in formation of autoantibodies (IgG or IgM) such as anti-TSH receptor autoantibody, antithyroglobulin autoantibody, antiperoxidase autoantibody (peroxidase enzyme located in the apical region of follicular cells), anti-microsomal autoantibody and anti-iodine receptor autoantibody.

- Anti-TSH receptor autoantibody inhibits intracellular iodine transport resulting in decreased synthesis of thyroid hormones.
- Fc portion of IgG or IgM binds to Fc receptors of cytotoxic leukocytes, i.e. natural killer cells (most important), macrophages, neutrophils and eosinophils which destroy sensitized cells. The cytotoxic leukocytes release hydrolytic enzymes and oxygen-derived free radicals resulting in tissue damage.

Type IV Hypersensitivity Reaction-mediated Disorder

Type IV hypersensitivity reaction plays key role in pathogenesis of Hashimoto's thyroiditis. (a) CD8+ cytotoxic T cells destroy thyroid follicular cells and induce apoptosis through the Fas–FasL system. (b) CD4+ helper T cells synthesize cytokines that cause chemotaxis of macrophages. These macrophages cause destruction of thyroid gland. Initially, patient develops hyperthyroidism due to release of thyroid hormones, and eventually hypothyroidism as a result of depleted thyroid hormones.



Increased risk of Hashimoto's thyroiditis	
• HLA-DR5 • HLA-B5	• Autoimmune diseases: SLE, pernicious anemia, Sjögren's syndrome and type 1 diabetes mellitus

Autoantibodies in Hashimoto's thyroiditis	
• Anti-TSH autoantibody • Antithyroglobulin autoantibody • Antiperoxidase autoantibody	• Antimicrosomal autoantibody • Anti-iodine receptor autoantibodies

Fig. 26.21: Pathogenesis of Hashimoto's thyroiditis.

Surgical Pathology: Hashimoto's Thyroiditis

Gross Morphology

Thyroid gland is diffusely enlarged and firm, weighing 60–200 g. Cut surface is fleshy, pale tan, exhibiting a vaguely nodular pattern.

Light Microscopy

Thyroid gland displays following histologic features in Hashimoto's thyroiditis:

- **Mononuclear inflammatory infiltrate:** It comprises lymphocytes, plasma cells and macrophages. The inflammatory infiltrates are focally arranged in the form of lymphoid follicles, often with germinal centers.
- **Destruction of thyroid follicles:** Extensive destruction and atrophy of the thyroid follicles occur due to heavy inflammatory infiltrate of CD8+ cytotoxic T cells, CD4+ helper T cells, recruited macrophages and cell-mediated cytotoxicity by antithyroid antibodies. The remaining thyroid follicles vary in size and shape.
- **Oxyphil metaplasia:** The persistent thyroid follicular epithelium is transformed into so-called Hürthle cells or oncocytes or Askanazy cells (oxyphilic metaplasia). Hürthle cells have an abundant brightly eosinophilic granular cytoplasm packed with mitochondria and lysosomes. Hürthle cells produce a little or absence of T_3, T_4 and thyroglobulin.
- **Interlobular fibrosis:** Variable interlobular fibrosis in the thyroid is seen in long-standing cases of Hashimoto's thyroiditis.

- Fibrosing variant of Hashimoto's thyroiditis shows extensive dense fibrosis within the capsule of thyroid gland with atrophy of thyroid follicles. It should be differentiated from Riedel's thyroiditis.
- It is worth mentioning that Riedel's thyroiditis invades the surrounding structures with extensive fibrosis and scant thyroid tissue.

Clinical Features

Early in the course of Hashimoto's thyroiditis, there may be transient hyperthyroidism due to excessive release of thyroid hormones from damaged thyroid follicles, but later hypothyroidism occurs due to depletion of thyroid hormones in 40–50% of cases.

- Patient presents with painless diffuse enlargement of thyroid gland, sometimes appear lobulated mass mimicking a tumor.
- Due to destruction of the thyroid follicles over the years, thyroid gland undergoes marked atrophy, which is often not palpable.





Fig. 26.22A and B: Hashimoto's thyroiditis. Cut surface is fleshy, pale tan, exhibiting a vaguely nodular pattern. (*Courtesy:* Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru.)

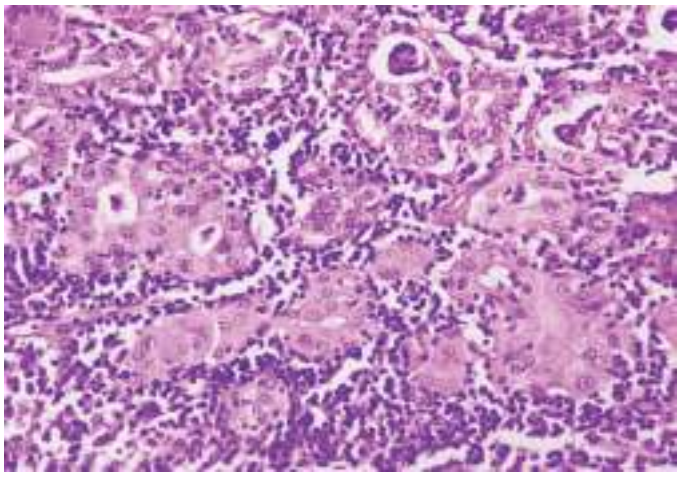


Fig. 26.23: Hashimoto's thyroiditis. Thyroid follicles show destruction due to infiltration by chronic inflammatory cells. Hürthle cell change in follicular cells is seen (400X).

Risk for Development of Malignancy

There is increased risk for subsequent development of primary B cell non-Hodgkin's lymphoma, papillary thyroid carcinoma and Hürthle cell neoplasms.

Laboratory Diagnosis

Diagnostic tests include detection of circulating auto-antibodies against the tissues involved anti-microsomal in 90% of cases, anti-thyroglobulin in 50% of cases, and antiperoxidase are analyzed in Hashimoto's thyroiditis.

Presence of antibody in the lesion (biopsy) is demonstrated by immunofluorescence microscopy. TSH is markedly increased in these patients.

SUBACUTE PAINLESS LYMPHOCYTIC THYROIDITIS

Subacute painless lymphocytic thyroiditis is an autoimmune disorder developing during postpartum period. Thyroid gland lacks germinal follicles.

Clinical Features

Patient presents with abrupt onset of thyrotoxicosis due to gland destruction, painless enlarged thyroid gland. It progresses to primary hypothyroidism in 40–50% of cases. It lacks immunological markers as demonstrated in Hashimoto's thyroiditis.

SUBACUTE GRANULOMATOUS THYROIDITIS

Subacute granulomatous thyroiditis most often affects women of 40–50 years age group. It is also known as de Quervain granulomatous thyroiditis, or giant cell thyroiditis. It is associated with HLA-B35. It has self-limited course of several weeks' duration. Subacute granulomatous thyroiditis is characterized by granulomatous inflammation with multinucleated giant cells resulting in focal destruction of thyroid gland (Fig. 26.24).

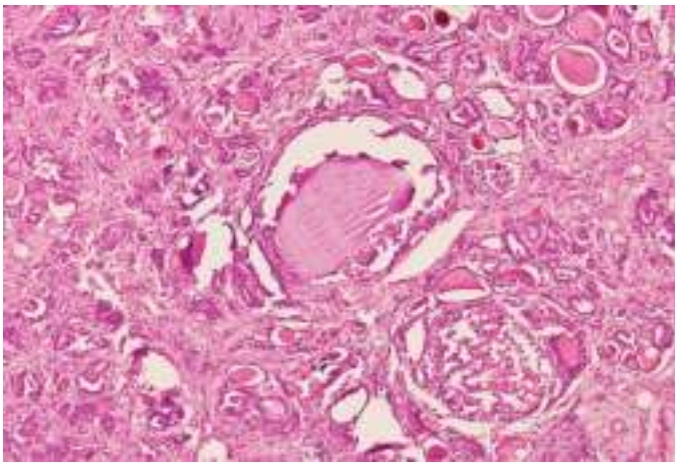


Fig. 26.24: Subacute granulomatous thyroiditis. Histologic examination shows destruction of thyroid follicular cells with colloid depletion by inflammatory cells, mumerous noncaseating granulomas and multinucleated giant cells surrounding thyroid follicles (400X).

Pathogenesis

Subacute granulomatous thyroiditis typically occurs after upper respiratory tract infections caused by influenza virus, adenovirus, echovirus, and coxsackievirus. Thyroid gland destruction initially results in hyperthyroidism, i.e. increased serum T_4 and decreased TSH. Permanent hypothyroidism is uncommon.

Surgical Pathology: Subacute Granulomatous Thyroiditis
Gross Morphology
The thyroid gland is enlarged to 40 g and weighing 60 g, and the cut surface is firm and pale.
Light Microscopy
▪ Initially, thyroid gland shows acute inflammation with microabscesses. It is followed by infiltration by lymphocytes, plasma cells, and macrophages throughout the thyroid gland. ▪ Destruction of thyroid follicles allows the release of colloid, which elicits a conspicuous granulomatous reaction.

Clinical Features

Initially, the patient may develop transient hyperthyroidism with decreased uptake of ^{123}I followed by hypothyroidism in late stage. Patient presents with painful, tender and enlarged thyroid gland often preceded by an upper respiratory tract infection.

RIEDEL'S THYROIDITIS

In Riedel's thyroiditis, the thyroid gland is replaced by fibrous tissue and can clinically mimic carcinoma, which may cause tracheal obstruction. Extension of fibrous tissue occurs in soft tissues of the neck, progressing in retroperitoneum, mediastinum and orbit. Surgical treatment of Riedel's thyroiditis is required to relieve tracheal or esophageal obstruction.

Surgical Pathology: Riedel's Thyroiditis
Gross Morphology
▪ Part or the entire thyroid is hard and is described as woody thyroid. ▪ Cut surface shows tan-gray woody and a vascular areas.
Light Microscopy
▪ Thyroid gland lacks normal lobular pattern. ▪ Thyroid follicles are obliterated or compressed by extensive fibrosis, which also invades surround tissue. ▪ Lymphocytes, plasma cells, and eosinophils are also trapped.

Clinical Features

Riedel's thyroiditis most often affects only one lobe of thyroid gland. Patient presents with inspiratory stridor due to tracheal obstruction, dysphagia (esophagus compression) and hoarseness due to involvement of recurrent laryngeal nerves. Patient may develop hypothyroidism.

ACUTE BACTERIAL THYROIDITIS

Acute thyroiditis is caused by *Staphylococcus aureus*. Thyroid gland destruction initially results in hyperthyroidism due to increased serum T_4 and decreased TSH. Permanent hypothyroidism is uncommon. ^{123}I uptake is decreased.

Clinical Features

Patient presents with fever and tender thyroid gland with cervical lymphadenopathy. Patient is treated by course of antibiotics.

HYPOTHYROIDISM

Impaired activity of any component of the hypothalamic-pituitary-thyroid axis can result in decreased thyroid function. The most common causes of hypothyroidism include faulty embryogenesis, Hashimoto's thyroiditis or reduction in functioning thyroid tissue due to prior surgery, radiation damage, and thyroid malignancy. Hypothyroidism manifests as cretinism in children and myxedema in adults. Hypothyroidism in adults most often affects middle-aged persons. Females are affected eight times more than males.

CONGENITAL HYPOTHYROIDISM

Congenital hypothyroidism is the most common congenital endocrine disorder with an incidence of about 1 in 3500 births. About 85% of cases occur due to faulty embryogenesis. A small number of cases presenting with goiter occur as a result of enzyme defects in thyroid hormone biosynthesis (dyshormonogenesis). Clinically, congenital hypothyroidism often manifests in the first few days of life.

- The genes responsible for the development of thyroid follicular cells include TTF-1, TTF-2 (thyroid transcription factors 1 and 2), PAX8, TSH and TSHR. Mutations of these genes are responsible for 5% of hypothyroidism patients. TTF-1, TTF-2 and PAX8 mutations give rise to nonsyndromic congenital hypothyroidism. On the other hand, mutations of TSH and TSHR are responsible for syndromic congenital hypothyroidism.
- Mutations in sodium iodide symporter (NIS), pendrin (PDS also known as SLC264A), thyroid peroxidase (TPO), thyroid oxidase 2 (THOX2, also known as DUOX2, LNOX2) and thyroglobulin (TG) affect organification of iodide and have been linked to congenital hypothyroidism.
- Depending on the etiology, three types of congenital hypothyroidism can be distinguished: (a) central congenital hypothyroidism due to defect at hypothalamus or pituitary gland, (b) primary hypothyroidism due to impairment of thyroid gland itself, and (c) peripheral hypothyroidism as a result of resistance to thyroid hormones. Gene mutations responsible for hypothyroidism in children and adults are given in Table 26.19.

CRETINISM (THYROID DWARF)

Cretinism results from deficiency of thyroid hormones during fetal development and during postnatal life. Cretinism denotes physical and mental insufficiency secondary to congenital hypothyroidism in children. Cretinism may be endemic, sporadic, or familial, which affects both girls and boys.

- **Physiologic state:** Thyroid hormones are essential for brain development, i.e. axonal and dendrite development especially myelination of central nervous system (formation of myelin sheath around a nerve) in postnatal period in the first two years of life. Thyroid hormones are also necessary for

Table 26.19 Gene mutations responsible for hypothyroidism in children and adults

Characteristics	Gene Locus on Chromosome	Protein Function	Gene Mutation and its Defective Protein	Comments
Central congenital hypothyroidism due to defect at hypothalamus and pituitary gland				
Thyroid-stimulating hormone (TSH)	▪ TSHα-chain on 6p14.3 ▪ TSHβ-chain on 1p22	Synthesis of biologically active TSH	Synthesis of biologically inactive TSH	Homozygous or compound heterozygous patients
Thyroid-stimulating hormone receptor (TSHR)	14q31	Normal synthesis of protein with normal structure and function associated with normal cyclic AMP response to TSH	Abnormal synthesis of protein with normal structure and function associated with defective cyclic AMP response to TSH	Homozygous or compound heterozygous patients
Thyroid dyshormonogenesis				
Sodium/iodide symporter (NIS)	19p12-13.2 with 15 exons	Normal uptake of iodide by thyroid gland	Abnormal uptake of iodide by thyroid gland	Homozygous or compound heterozygous patients
Pendrin (expressed in thyroid, inner ear and kidney)	7q22-31	Normal iodide transport and hence iodide organification	Abnormal iodide transport and hence iodide organification	Pendred syndrome has triad of deafness, goiter (euthyroid or subclinical hypothyroid influenced by dietary iodide uptake) and a partial organification defect
Thyroid peroxidase (TPO)	2p25 with 17 exons	Normal synthesis of iodination of thyroglobulin (TG)	Defects in the synthesis of iodination of thyroglobulin (TG)	Autosomal recessive patients develop goitrous congenital hypothyroidism
Thyroid oxidase 2 (THOX2) expressed in airway epithelium and skin	15q15.3	Generates H_2O_2 that is used by thyroid peroxidase (TPO) in the oxidation of iodine prior to iodination of the tyrosyl residue of thyroglobulin	H_2O_2 not synthesized so not available by thyroid peroxidase (TPO) in the oxidation of iodine prior to iodination of the tyrosyl residue of thyroglobulin	Homozygotes and compound heterozygous patients develop severe and permanent congenital hypothyroidism

Contd...

Table 26.19 Gene mutations responsible for hypothyroidism in children and adults (*Contd...*)

Characteristics	Gene Locus on Chromosome	Protein Function	Gene Mutation and its Defective Protein	Comments
Resistance to thyroid hormone receptor				
Thyroid hormone receptor (TR) genes	▪ TRα-chain on 17 ▪ TRβ-chain on 3	TR protein has a central DNA binding-domain	Defective TR protein without DNA binding-domain	*TRβ gene mutation is associated with thyroid hormone resistant congenital hypothyroidism
Thyroid hormone cell transporter	**MCT8 gene on Xq13.2	Protein encoded by MCT8 gene transports thyroid hormone from extracellular compartments across the cell membrane	Defective protein produced by MCT8 unable to transport thyroid hormone from extracellular compartments across the cell membrane	Milder phenotype with elevated level of serum T_3

**TRβ gene mutation is also demonstrated in pituitary adenomas, **MCT8—monocarboxylate transporter gene.*

linear growth, i.e. maturation of growing epiphyseal region of bones, closure of fontanelle skull and tooth eruption elicitation (Fig. 26.25).

- **Pathologic state:** Deficiency of thyroid hormones causes defective development of brain due to nonmyelination of nervous system and maturation of the growing fetus leading to stunted growth called cretinism (thyroid dwarf).

Predisposing Factors

Most important cause of cretinism is maternal hypothyroidism before development of fetal thyroid gland.

- Transplacental transfer of antithyroid antibodies from a mother suffering from autoimmune thyroid disease may produce cretinism.
- Cretinism may also be caused due to deficiency of iodine and enzymes required for the synthesis of thyroid hormones.
- Other causes of cretinism include maldevelopment of the thyroid gland and failure of the fetal thyroid to descend from its origin at the base of the tongue.

Clinical Features

Initial clinical manifestations of congenital hypothyroidism appear in the early weeks of life such as sluggishness, slow reflexes due to severe mental retardation, large protuberant abdomen often with umbilical herniation flattened broad nose, thick protuberant tongue, hoarse husky voice, coarse dry skin, periorbital edema, eyes seem widely spaced and low body temperature.

- Later on, impairment of physical growth with retarded bone development results in dwarfism. There is delay in closure of fontanelle, eruption of deciduous teeth and refractory anemia.

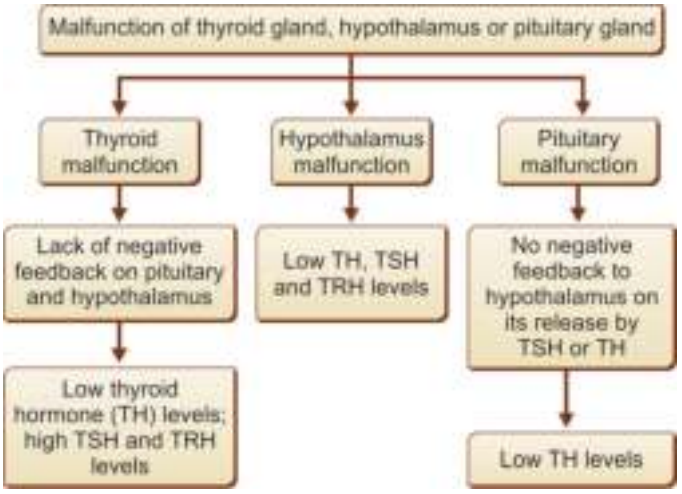


Fig. 26.25: Pathogenesis of hypothyroidism.

- Mental retardation, stunted growth, and characteristic facies become evident. If thyroid hormone replacement therapy is not promptly provided, congenital hypothyroidism results in mentally retarded thyroid dwarf.

Laboratory Diagnosis

The diagnosis of congenital hypothyroidism in infants is confirmed by decreased serum free T_4 and increased TSH. If maternal antibody transfer is suspected, then tests for antithyroid antibody testing can be performed in both the mother and child.

Pathology Pearls: Thyroid Profile in Hypothyroidism

- ↑ TSH
- ↓ T_4
- ↓ T_3
- Possible antibodies to thyroglobulin or thyroid peroxidases

Therapeutic Correlation

Hypothyroid newborns attain normal development, if early hormonal therapy is started. If hormonal therapy is delayed by 6–12 months, then mental retardation becomes permanent due to brain damage.

MYXEDEMA (HYPOTHYROIDISM) IN ADULTS

Hypothyroidism manifests as myxedema in adults primarily after age of 40 years, which is more common in women than men. Decreased synthesis of thyroid hormones, triiodothyronine (T_3) and/or thyroxine (T_4) slows down cellular metabolic processes leads to hypothyroidism. Hypothyroidism in adults is classified as primary or secondary.

Predisposing Factors

Congenital causes of myxedema include congenital absence of thyroid gland or inborn errors of metabolism.

Acquired causes of myxedema include iodine deficiency (endemic goiter), autoimmune thyroiditis, i.e. Hashimoto's thyroiditis, post-radiotherapy to hyperthyroidism, post-surgical thyroidectomy, antithyroid drugs (carbimazole), and primary tumors of thyroid.

Classification

- **Primary hypothyroidism (myxedema):** Primary hypothyroidism most often occurs due to autoimmune mechanism. Approximately 75% of these patients have circulating autoantibodies to thyroid antigens, suggesting that these cases represent the end stage of autoimmune thyroiditis. Nongoitrous hypothyroidism may also result from antibodies that block TSH itself or the TSH receptor, without activating the thyroid gland. Some cases are associated with Hashimoto's disease, amyloidosis, sarcoidosis and irradiation.
- **Secondary hypothyroidism (myxedema):** Secondary hypothyroidism normal thyroid functions are impaired in these patients due to: (a) hypothalamus fails to release thyrotropin-releasing hormone (TRH); (b) anterior pituitary gland fails to secrete thyroid stimulating hormone (TSH); (c) thyroid gland is not able to synthesize thyroid hormones due to iodine deficiency (usually dietary); and (d) patient receiving antithyroid drugs for the treatment of hyperthyroidism (Table 26.20).

Clinical Features

Early Clinical Manifestations in Myxedema

Patient presents with insidious onset symptoms due to decreased metabolic rate such as fatigue, cold intolerance, unexplained weight gain, low pitch voice, progressive constipation (decreased GIT motility), anorexia, and skeletal muscle cramps.

Late Clinical Manifestations in Myxedema

With progression of disease, the patient develops systemic manifestations:

- **Periorbital puffiness and non-pitting edema:** Patient develops periorbital puffiness and non-pitting edema legs due to subcutaneous accumulation of mucopolysaccharides and hyaluronic acid.
- **Tongue:** Tongue becomes dry and thick causing hoarseness, slow and slurred speech.
- **Hair loss:** There is hair loss in axillary region, pubic region and lateral aspect (outer-third) of the eyebrows.
- **Skin manifestations:** Skin becomes dry, inelastic, coarse and yellow due to less conversion of carotene into retinoic acid.
- **Congestive heart failure:** Patient develops flabby congestive heart, bradycardia, and poor peripheral circulation, pleural and pericardial effusions.
- **Serum lipid level:** Serum cholesterol concentration is increased due to decreased synthesis of low-density lipoprotein (LDL) receptors.
- **CNS manifestations:** Patient develops nervous system manifestations such as ataxia (loss of coordination), delayed recovery of 'Achilles reflex'

Table 26.20 Differences between primary and secondary hypothyroidism

Parameters	Primary Hypothyroidism	Secondary Hypothyroidism
Etiology	Hashimoto's thyroiditis	Pituitary gland disorder
Serum TSH	Increased	Increased
Free T_4	Decreased	Decreased
Thyrotropin-releasing hormone stimulation test	Increased response	No response
Autoantibodies	▪ Anti-TSH receptor autoantibody ▪ Anti-thyroglobulin autoantibody ▪ Anti-peroxidase autoantibody ▪ Anti-microsomal autoantibody ▪ Anti-iodine receptor autoantibody	▪ Absent ▪ Absent ▪ Absent ▪ Absent ▪ Absent

(increase in relaxation phase of deep tendon reflexes), behavioral changes ranging from slight mental slowness to severe impairment (dementia).

- **Infertility:** There is impaired fertility in these patients. Women with hypothyroidism suffer from ovulatory failure, progesterone deficiency, decreased libido and irregular and excessive menstrual bleeding (menorrhagia). Erectile dysfunction and oligospermia are common symptoms of hypothyroidism in men.

Laboratory Diagnosis

Increased TSH level and T4 level establish hypothyroidism. Hypercholesterolemia occurs due to decreased synthesis of low-density lipoprotein (LDL) receptors on tissues in patients with hypothyroidism.

Treatment

Patient is administered levothyroxine in small increments every 6–8 weeks to bring serum TSH to the normal range (euthyroid state).

HYPERTHYROIDISM

Hyperthyroidism is a clinical syndrome characterized by hypermetabolic state due to elevation of free serum thyroxine (T4) and/or free triiodothyronine (T3). It can be caused by inherited or acquired factors. It can result from follicular epithelial hyperplasia, hyperpituitarism, various forms of thyroiditis, metastatic tumors and various drugs.

TOXIC NODULAR GOITER (PLUMMER'S DISEASE)

Increased synthesis of thyroid hormone creates a metabolic imbalance, i.e. increased metabolic rate called hyperthyroidism or thyrotoxicosis. Thyrotoxicosis describes excess of thyroid hormones regardless of etiology. Excess thyroid hormones can be due to various thyroid disorders, i.e. Graves' disease (most common) and toxic nodular goiter (Table 26.21 and Fig. 26.26).

Clinical Features

Patient presents with weight loss, increased appetite, recent onset of heat intolerance, warm, moist, flushed skin especially hot sweaty palms, nervousness, fine tremors, bounding peripheral pulses, cardiac arrhythmias (atrial fibrillation), dyspnea on exertion, and menstrual abnormalities, e.g. amenorrhea or oligomenorrhea. Males develop impaired fertility, decreased libido and gynecomastia.

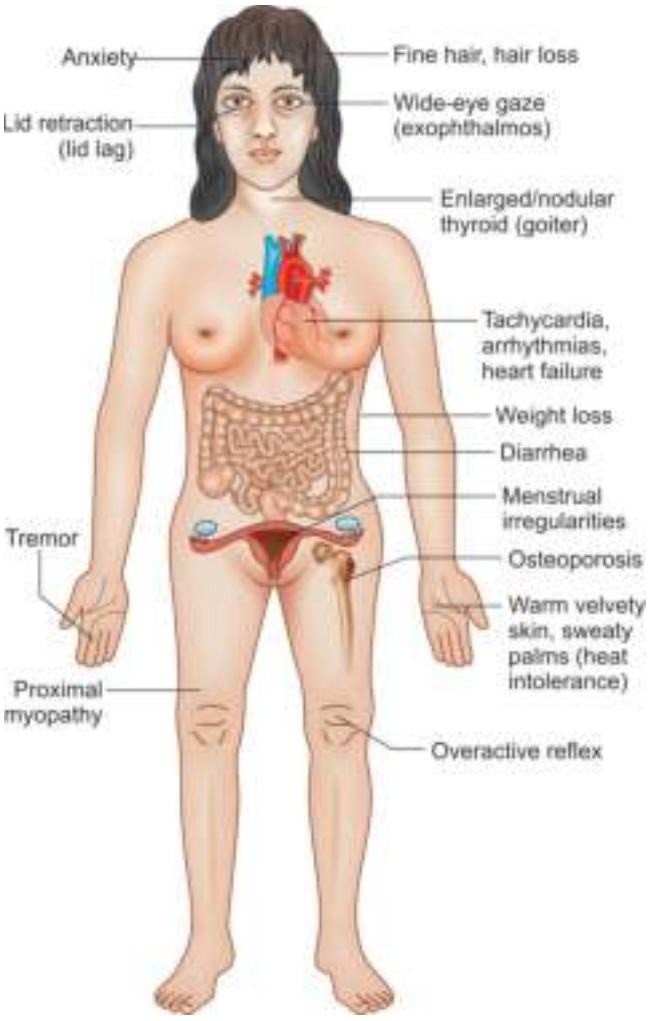


Fig. 26.26: Clinical features of thyrotoxicosis.

GRAVES' DISEASE

Graves' disease is an autoimmune disease associated with decreased TSH activity. TSH receptors are stimulated by thyroid-stimulating immunoglobulin, an IgG autoantibody, not by TSH. Thyroid stimulating immunoglobulin (TSI) mimics the action of TSH, but is not regulated by natural negative feedback controls. Thyroid growth immunoglobulin (TGI) autoantibody stimulates thyroid follicular glandular

Table 26.21 Comparison of Graves' disease and toxic nodular goiter

Parameters	Graves' Disease	Toxic Nodular Goiter
Sex predilection	Women > Men	Women = Men (equally affected)
Eye signs	Exophthalmos most severe common finding	Exophthalmos less severe
Heart	Tachycardia and fibrillation	Angina
Weight	May lose weight	Often profound

hyperplasia resulting in thyroid enlargement. Patient develops diffuse toxic goiter, hyperthyroidism, and exophthalmos. Onset is initiated by infection, iodide excess and postpartum state.

Epidemiology

Graves' disease occurs more often in women than men of young age group. The incidence is increased in HLA-DR3 and HLA-B8 positive individuals. CTL4 and PTPN22 gene mutations increase the risk for Graves' disease. Thyroid acropatchy is associated with swelling of digits and clubbing in patient with Graves' disease.

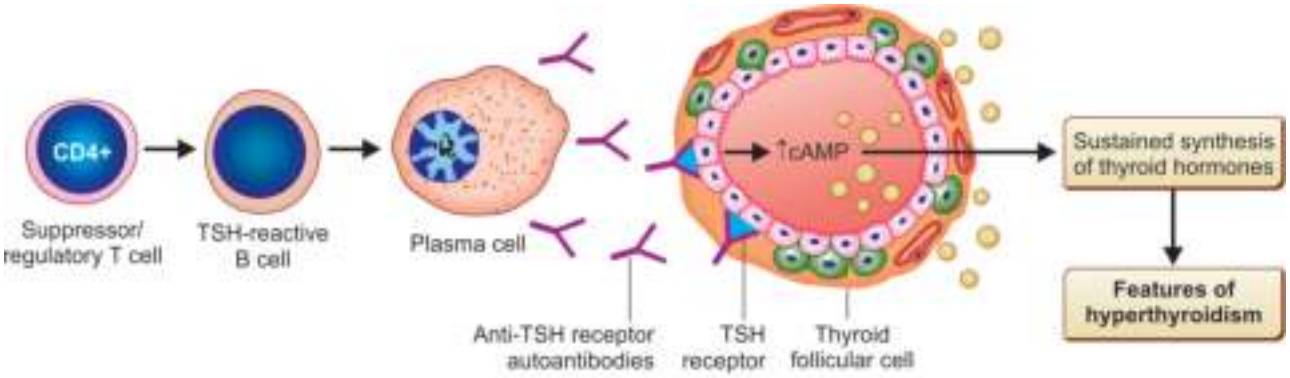
Pathogenesis

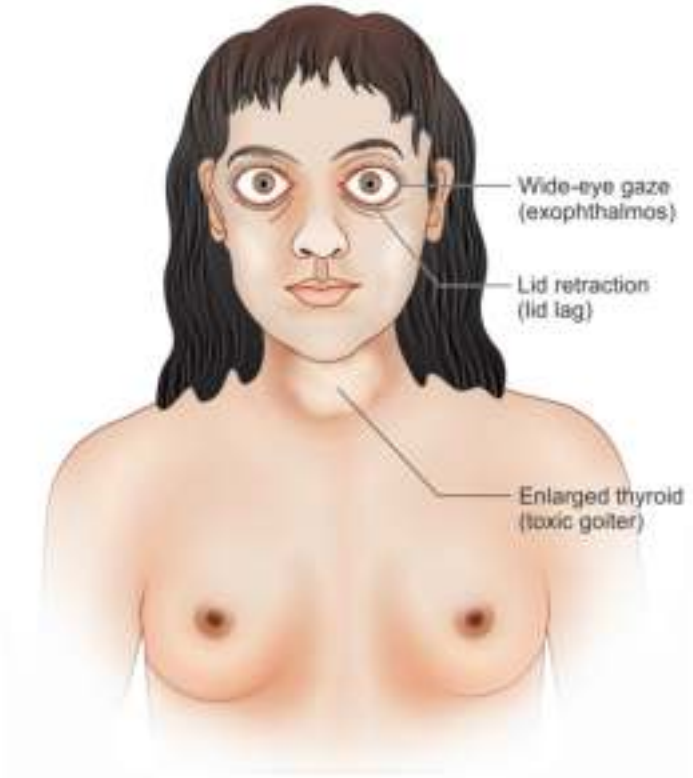
Type 2 hypersensitivity reaction plays an important role in its pathogenesis. CD4+ suppressor/regulatory T cells induce B cells resulting in synthesis of thyroid-stimulating immunoglobulin (TSI), an IgG antibody that reacts with TSH receptors and activates adenyl cyclase resulting in increased production of thyroid hormones.

- Synthesis of thyroid growth immunoglobulin (TGI) stimulates thyroid follicular glandular hyperplasia and thyroid enlargement.
- In addition to TSI and TGI, anti-microsomal and anti-thyroglobulin autoantibodies are also present (Figs 26.27 and 26.28).

Clinical Features

Patient presents with nontender diffuse symmetric thyroid gland enlargement with features of thyrotoxicosis. Graves' disease is associated with exophthalmos in 60–90%, dermatopathy and constitutional symptoms.





Risk factors for Graves' disease

- Young women
- HLA-DR3 and HLA-B8
- CTL4 or PTPN22 gene mutation

Clinical manifestations of Graves' disease

- Exophthalmos (lid lag, upper lid retraction, weakness of extraocular muscles).
- Dermatopathy
- Constitutional symptoms (weight loss, warm skin, fine tremors, increased heart rate).

Demonstration of autoantibodies in blood in Graves' disease

- TGI (thyroid growth immunoglobulin) stimulates glandular hyperplasia and enlargement.
- TSI (thyroid-stimulating immunoglobulin) mimics TSH and reacts with TSH receptors resulting in sustained synthesis of thyroid hormones.
- Anti-microsomal autoantibodies.
- Anti-thyroglobulin autoantibodies.

Fig. 26.27: Pathogenesis of Graves' disease.

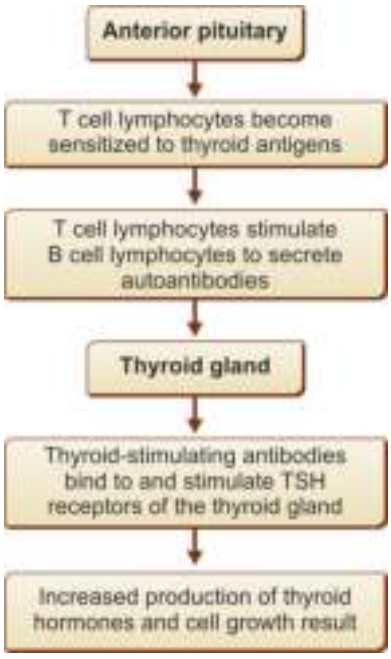


Fig. 26.28: Pathogenesis of Graves' disease.

Thyroid gland and retrobulbar ocular muscles are infiltrated with B cells.

- **Exophthalmos:** Extraocular muscles and retro-orbital tissues are infiltrated by chronic inflammatory cells.
  - There is deposition of hydrophilic mucopolysaccharides, fibrosis and contractures of extraocular muscles resulting in incoordination of eye movements.
  - Patient presents with proptosis, lid lag, upper lid retraction, weakness of eye muscles, diplopia, and periorbital edema.
- **Dermatopathy:** Skin over dorsum of legs or feet becomes edematous and thick orange peeling. Skin may also show discrete plaque-like lesions in 10–15% of cases. Pretibial dermal swelling occurs due to excessive deposition of glycosaminoglycans in 1–2% of cases.
- **Constitutional symptoms:** Constitutional symptoms due to Graves' disease include weight loss, warm skin, fine tremors particularly when hands are stretched, heat intolerance, diarrhea, anxiety, lid stare due to increased sympathetic stimulation of eyelid muscles; cardiac symptoms (e.g. sinus tachycardia, increased risk for atrial fibrillation, systolic hypertension, high-output heart failure due to increased synthesis of α-adrenergic receptors in the heart).

Surgical Pathology: Graves' Disease

Gross Morphology

- The thyroid gland is diffusely enlarged weighing 80–90 g.
- Cut surface reveals soft, red brown, meaty appearance closely resembling cross-section of normal muscle.
- Preoperative administration of iodine causes accumulation of colloid and alters the gross appearance.

Light Microscopy

- Marked thyroid follicular glandular hyperplasia with micropapillary infoldings, thin colloid with peripheral scalloping of the colloid and lymphocytic infiltration in stroma between thyroid follicles are prominent features.
- Peripheral scalloping occurs due to increased proteolysis of colloid and rapid uptake of thyroglobulin by the hyperfunctioning thyroid follicular cells (Figs 26.29 and 26.30).

Laboratory Diagnosis

The best screening test for Graves' disease is free T_4 elevated in Graves' disease and TSH greatly decreased in Graves' disease. Blood sugar is raised



Fig. 26.29: Graves' disease. Cut surface shows a hyperemic, meaty, shiny appearance. Graves' disease is an autoimmune disorder that leads to hyperthyroidism due to circulating autoantibodies, fine fremors of hands, bulging of eyes and dermatopathy.

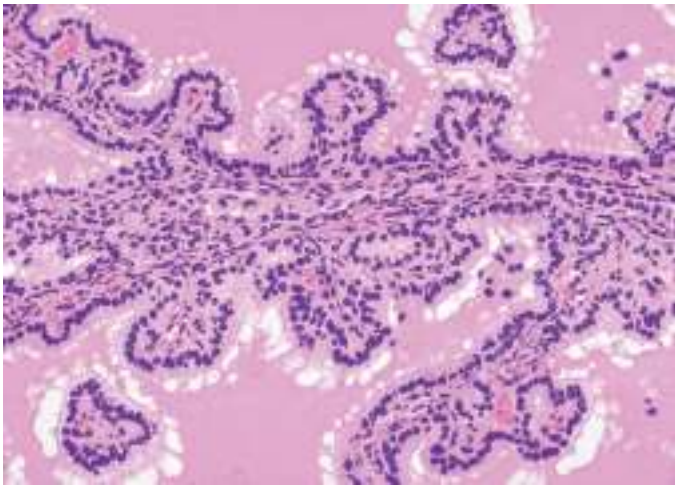


Fig. 26.30: Graves' disease. Histologic examination shows resorption induced scalloping of the margins of the colloid. Antibody-mediated hyperplastic papillary epithelial infoldings result from the lining cells becoming columnar in shape and increased in number (400X).

due to glycogenolysis. Peripheral blood smear examination shows absolute lymphocytosis. Autoantibodies such as thyroid growth immunoglobulin (TGI), thyroid-stimulating immunoglobulin (TSI), antimicrosomal antibody and anti-thyroglobulin antibody should be analyzed in Graves' disease.

Pathology Pearls: Thyroid Profile in Hyperthyroidism

- ↓ TSH
- ↑ T_4
- ↑ T_3
- ↑ ^{131}I uptake

Treatment

Administration of β-blockers decreases adrenergic effects. Thionamides decrease synthesis of thyroid hormones.

HYPERTHYROIDISM: OTHER CAUSES

- **Plummer's disease:** This order is a combination of hyperthyroidism, nodular goiter, and absence of exophthalmos. The 'hot' nodules can be adenomas or non-neoplastic areas of nodular hyperplasia.
- **Pituitary hyperfunction:** It can cause excessive synthesis of TSH resulting in secondary hyperthyroidism.
- **Struma ovarii:** It is variant of ovarian teratoma composed of thyroid tissue. It can be hyperfunctional. Surgical excision of tumor brings thyroid hormones level to normal range.

Differences between primary and secondary hyperthyroidism are given in Table 26.22 and clinical manifestations of hypothyroid and hyperthyroid states are given in Table 26.23.

Table 26.22 Differences between primary and secondary hyperthyroidism

Parameters	Primary Hyperthyroidism	Secondary Hyperthyroidism
Etiology	Graves' disease, toxic adenoma, toxic multinodular goiter	Pituitary gland
Serum TSH	Low	Normal
Free T_4	Increased	Increased
Autoantibodies	▪ TGI (thyroid growth immunoglobulin) ▪ TSI (thyroid-stimulating immunoglobulin) ▪ Anti-microsomal autoantibody ▪ Anti-thyroglobulin autoantibody	▪ Absent ▪ Absent ▪ Absent ▪ Absent

Table 26.23 Clinical manifestations of hypothyroid and hyperthyroid states

Level of Organization	Hypothyroidism	Hyperthyroidism
Basal metabolic rate	Decreased	Increased
Sensitivity to catecholamines	Decreased	Increased
Clinical features	▪ Myxedema features ▪ Deep voice ▪ Impaired growth in children	▪ Exophthalmos in Graves' disease ▪ Lid lag ▪ Accelerated growth in children
General behavior	▪ Mental retardation (infant) ▪ Physical and mental sluggishness ▪ Somnolence	▪ Restlessness ▪ Hyperkinesis ▪ Wakefulness
Cardiovascular system	▪ Decreased cardiac output ▪ Bradycardia	▪ Increased cardiac output ▪ Tachycardia and palpitation
Gastrointestinal tract	▪ Constipation ▪ Loss of appetite	▪ Diarrhea ▪ Increased appetite
Respiratory system	Hypoventilation	Breathlessness
Muscle tone and reflexes	Decreased	Increased with tremor and twitching
Temperature tolerance	Cold intolerance	Heat intolerance
Skin and hair	▪ Decreased sweating ▪ Coarse and dry skin and hair	▪ Increased sweating ▪ Thin silky skin and hair
Weight	Gain	Loss
Blood cholesterol	Increased	Decreased

GOITER

Persistent thyroid gland enlargement from excess colloid is known as goiter. Physiologic enlargement is not uncommon in puberty and pregnancy. Other causes of goiter include iodine deficiency either endemic or sporadic, Hashimoto's thyroiditis, goitrogenic drugs, food and dyshormonogenesis, i.e. enzyme deficiencies. Classification of goiter, morphology and functional activity due to iodine deficiency are given in Table 26.24.

SIMPLE NONTOXIC GOITER

Simple nontoxic goiter occurs due to increased thyroglobulin levels resulting in decreased synthesis of T_3 and T_4. Patient presents with diffuse symmetric enlargement of thyroid. Females are more affected than males.

Predisposing Factors

- **Dietary factors:** Diet deficient in iodine and water rich in Ca^{++} and fluoride play an important role in the pathogenesis of nontoxic goiter. Goitrogens are present in vegetables such as cabbage, turnips, cassava (e.g. containing thiocyanate inhibiting iodine transport), brussels, sprouts, i.e. young shoot of a plant, turnips, Brassica plants, Cruciferae plants and soya beans.
- **Goitrogenic drugs:** Most commonly used goitrogenic drug is lithium being prescribed in the management of manic-depressive states.Therapeutic administration of drugs (e.g. salicylates, cyanates, thiocyanates, resorcinol, lithium chloride and cobalt chloride) may result into nontoxic goiter.
- **Peroxidase enzyme deficiency:** Deficiency of peroxidase enzyme inhibits conversion of iodide to organically bound iodine process and patient develops goiter. Dehalogenase deficiency inhibits the conversion of MIT and DIT to form T_3 and T_4, and results in goiter.

Table 26.24 Classification of goiter, morphology, and functional activity due to iodine deficiency

Parameters	Goiters
Based on morphology	▪ Diffuse goiter is also known as simple or colloid goiter without thyroid hormone dysfunction ▪ Nodular goiter occurs in the late stage of simple goiter and results to irregular thyroid enlargement either consisting of single nodule or multiple nodules. Most nodular goiters become hyperplastic and do not take up radioactive iodine ('*cold nodules*')
Based on functional activity	▪ Nontoxic goiter is also termed simple, colloid, or multinodular goiter. It refers to an enlargement of the thyroid that is not associated with functional, inflammatory, or neoplastic alterations. It occurs due to increased thyroglobulin levels resulting in decreased synthesis of T_3 and T_4. Patient presents with diffuse symmetric enlargement of thyroid. Females are more affected than males ▪ Toxic goiter is associated with hyperthyroidism as seen in Graves' disease
Based on iodine deficiency	▪ Endemic goiter occurs in iodine deficient geographical areas. Without sufficient iodine, thyroid hormones are not produced and the pituitary continuously secretes thyroid stimulating hormone (TSH) ▪ Sporadic goiter occurs in non-iodine-deficient geographical areas. It is caused by enzyme deficiency, goitrogens, puberty, and pregnancy. Females are more affected than males

Pathogenesis

Goiter is caused by absolute or relative deficiency of thyroid hormones. There are two stages, i.e. (a) hyperplasia/hypertrophy of thyroid follicles and (b) involution stage. Enlarged thyroid gland weighs 100–150 g. Initial diffuse thyroid enlargement is followed by multinodular goiter.

- **Hyperplastic stage:** Absolute or relative deficiency of thyroid hormones increase synthesis of TSH by anterior pituitary gland. TSH acts on thyroid follicles, which may undergo hyperplasia/hypertrophy with formation of new thyroid follicles, which attempt to increase thyroid hormones synthesis. Hyperplastic stage is followed by involution stage.
- **Involution stage:** Accumulation of abundant colloid leads to flattening of the epithelium of thyroid follicles. Thyroid gland fails to sustain synthesis of thyroid hormones.

Clinical Features

Patient presents with thyroid enlargement, respiratory distress and dysphagia (Fig. 26.31).

Surgical Pathology: Simple Nontoxic Goiter

Gross Morphology

- During involution stage, cut surface of goiter specimen shows numerous cystic nodules filled with gelatinous glistening excess of colloid.
- Areas of hemorrhages are seen into many of these cystic nodules (Fig. 26.32).

Light Microscopy

- During hyperplastic stage, thyroid follicular cells show hyperplasia and hypertrophy of thyroid follicular cells, with regeneration of new thyroid follicles.
- During involution stage, abundant colloid accumulation in the thyroid follicles results in flattening of epithelium of thyroid follicles (Fig. 26.33).



Fig. 26.31: Diffuse goiter. Diffuse enlargement of thyroid gland in male. (*Courtesy*: Dr. Prasad S Dange.)



Fig. 26.32: Thyroid nodular goiter with cystic degeneration. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

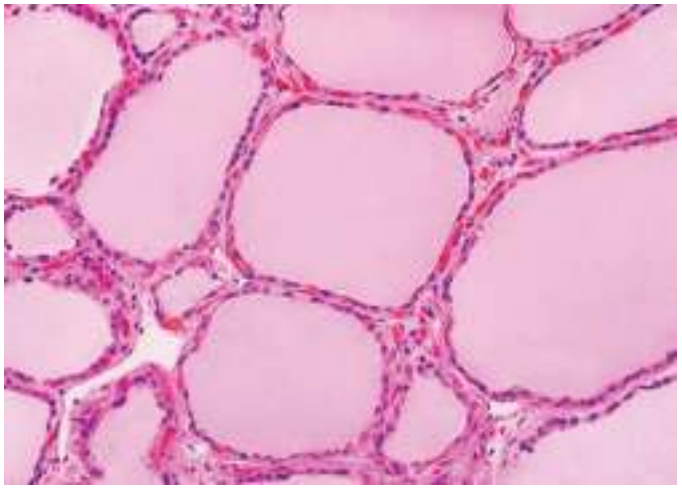


Fig. 26.33: Simple colloid goiter. It is also called euthyroid goiter. The thyroid follicles are filled with an abundant amount of colloid in their lumens and lined by flattened squamous follicular cells (400X).

MULTINODULAR GOITER

Multinodular goiter (MNG) is most often caused by dietary iodine deficiency. Nodules are both monoclonal and polyclonal. Serum T_3 and T_4 are usually within normal limits. Histologic examination shows cystic inactive thyroid follicles and areas of follicular hyperplasia with focal areas of hemorrhage, calcification, macrophages and foreign body giant cells.

- Nearly all cases of long-standing simple nontoxic goiter result in multinodular goiter. It commonly affects older age group. Women are more affected than men (8:1). Dominant nodule in multinodular goiter has risk for malignancy in 2–5% of cases.
- There is uneven proliferation of thyroid follicles, nodules, scar formation, cysts, degeneration and calcification. Enlarged thyroid gland weighs up to 500–1000 g.
- Patient presents with neck mass, compression of esophagus, i.e. dysphagia, compression of trachea, i.e. dyspnea, and compression of great blood vessels of neck (Fig. 26.34).

Pathogenesis

Absolute or relative deficiency of thyroid hormones stimulates increased synthesis of TSH. It results in hyperplasia and hypertrophy of thyroid follicles, with formation of new follicles and colloid accumulation.

- Uneven accumulation of colloid throughout thyroid follicles leads to tension and stress within thyroid gland resulting in rupture of thyroid follicles and blood vessels.
- Fibrosis further adds to tension within thyroid gland. Sometimes areas of dystrophic calcification are seen in multinodular goiter.



Fig. 26.34: Multinodular goiter obstructing superior vena cava. Dilated veins are seen due to superior vena cava (SVC) syndrome. (*Courtesy*: Dr. Prasad S Dange.)

Clinical Features

- Patient presents with inspiratory stridor (compression of trachea), dysphagia (compression of esophagus). Hoarseness of voice may result from compression of recurrent laryngeal nerve. Compression of jugular vein leads to dizziness or syncope known as 'Pemberton's sign', when the patient raises arms above head.
- Hemorrhage into colloid nodule causes sudden painful enlargement of thyroid. Features of hypothyroidism or toxic nodular goiter (TSH-independent 'hot' nodule) may be present. Dominant nodule in multinodular goiter has 2–5% risk for malignancy.

Surgical Pathology: Multinodular Goiter

Gross Morphology

- Most goiters weigh >40 g (some <25 g).
- Cut surface is heterogenous with areas of scarring and calcification.
- Condensation about themselves creates appearance of complete encapsulation so-called adenomatous goiter and multiple colloid adenomatous goiters (Fig. 26.35).

Light Microscopy

Histologic examination reveals variable sized thyroid follicles, random irregular scarring with focal areas of hemorrhage, calcification, microcysts formation; macrophages and foreign body giant cells (Fig. 26.36).

NEOPLASTIC DISORDERS OF THYROID GLAND

GENERAL CONSIDERATIONS

Classification of Thyroid Neoplasms

Thyroid neoplasms are classified into primary (benign or malignant) and secondary. Thyroid adenoma is a benign neoplasm. Primary malignant neoplasms of thyroid are papillary carcinoma, follicular carcinoma, medullary carcinoma and undifferentiated carcinoma. Papillary thyroid carcinoma accounts for 50% of all thyroid cancers. Malignant tumors of thyroid gland are more common in women than men over 40 years of age.



Fig. 26.35: Multinodular goiter in thyroid gland. Cut surface shows multiple nodules filled with colloid material.

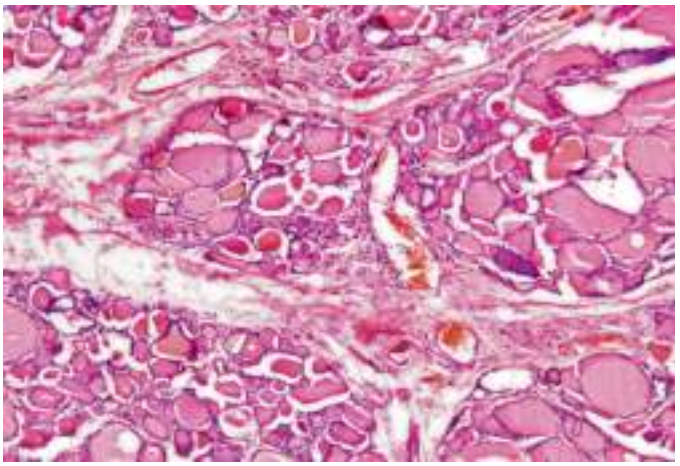


Fig. 26.36: Multinodular goiter. Histologic examination shows variable sized thyroid follicles, random irregular scarring with focal areas of hemorrhage and calcification (400X). (*Courtesy:* Department of Pathology, Dr. DY Patil Medical College, Pune, Maharashtra.)

- Fixation of growth or cervical lymphadenopathy and distant metastases indicates thyroid malignancy.
- Thyroid nodule is well circumscribed grey white solitary nodule delineated from surrounding normal thyroid tissue. Majority of thyroid nodules are neoplastic in nature. Toxic, i.e. hyperfunctioning nodule concentrates iodine. Such thyroid nodules may be benign or malignant tumors.
- Recent WHO classification of thyroid neoplasms has divided the thyroid neoplasms into several new categories that allow for better understanding of the cell of origin, pathologic features (cytopathology and histopathology), molecular classification and biologic behavior.
- Follicular cell-derived neoplasms constitute majority of thyroid neoplasms, which can be benign, low-risk and malignant neoplasms. Benign follicular cell-derived neoplasms include thyroid follicular nodular disease (TFND) in the clinical settings of multinodular goiter showing multifocal hyperplastic/neoplastic lesions, follicular thyroid adenoma, follicular thyroid adenoma with papillary architecture, and oncocytic thyroid adenoma. Low-risk follicular cell-derived neoplasms include non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), follicular tumor of uncertain malignant potential (FTUMP), well differentiated thyroid tumor of uncertain malignant potential (WDUMP) and hyalinizing trabecular tumor (HTT). Malignant thyroid follicular cell-derived neoplasms are stratified based on molecular profiles and aggressive biologic behavior.

- Papillary thyroid carcinoma (PTC) can be categorized into minimally invasive, encapsulated angioinvasive and widely invasive subtypes. Papillary thyroid carcinomas with many morphologic subtypes represent the BRAF-like malignancies, whereas encapsulated follicular variant of papillary carcinoma and follicular thyroid carcinoma represent RAS-like malignancies.
- Anaplastic thyroid carcinoma remains the most undifferentiated form. Squamous cell carcinoma of thyroid is considered subtype of anaplastic carcinoma.
- Medullary thyroid carcinoma (MTC) is derived from parafollicular cells (C cells residing in upper lobes of thyroid). Tumor is composed of both parafollicular cells and any follicular cell-derived malignancy. A grading system for medullary thyroid carcinoma is based on mitotic count, tumor necrosis and Ki-67 labeling proliferative index.
- Mucoepidermoid carcinoma and secretory carcinoma of the salivary gland type are now included in revised 2024 WHO classification of thyroid neoplasms (Table 26.25) as salivary gland carcinomas of thyroid.

Table 26.25 Revised 2024 WHO classification of thyroid neoplasms

Epithelial Thyroid Neoplasms

- **Follicular cell-derived neoplasms**
  - Benign thyroid follicular neoplasms include thyroid follicular disease (TFD), follicular thyroid adenoma, follicular thyroid adenoma with papillary architecture (often hyperfunctional), oncocytic adenoma.
  - Low-risk thyroid follicular neoplasms include non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), follicular thyroid tumor of uncertain malignant potential (FT-UMP), well differentiated thyroid tumor of uncertain malignant potential (WD-UMP), hyalinizing trabecular thyroid tumor (HTT).
  - Malignant thyroid follicular neoplasms include follicular thyroid carcinoma (minimal invasive, encapsulated, angioinvasive, widely invasive histologic subtypes).
- **Papillary thyroid carcinoma (PTC)**
  - Histologic subtypes of papillary thyroid carcinoma (infiltrative, follicular, tall cell, columnar cell, hobnail, solid, diffuse sclerosing, Warthin-like, oncocytic histologic subtypes)
  - Invasive encapsulated follicular variant of papillary thyroid carcinoma (minimal invasive, encapsulated angioinvasive, widely invasive histologic subtypes)
- **Oncocytic thyroid carcinoma**
  - Oncocytic thyroid carcinoma includes minimal invasive, encapsulated angioinvasive and widely invasive
  - Oncocytic follicular cell-derived carcinoma of thyroid is composed of >75% oncocytic cells, that lack characteristic nuclear features of papillary thyroid carcinoma and high-grade features (necrosis and mitotes ≥5/cu mm)
- **Differentiated high grade-thyroid carcinoma (DHGTC)**
  - Histologic subtypes of differentiated high-grade thyroid carcinoma (papillary, follicular or solid, invasive, any nuclear cytology with any one of features such as necrosis or mitotic count ≥5/cu mm)
- **Poorly differentiated thyroid carcinoma (PDTC)**
  - Poorly differentiated thyroid carcinoma (solid, trabecular or insular growth patterns)
  - Poorly differentiated thyroid carcinoma (invasive pattern)
  - Poorly differentiated thyroid carcinoma with at least one of features (necrosis or mitotic count ≥3/cu mm or convoluted nuclei)

Contd...

- **Anaplastic thyroid carcinoma**
  - Anaplastic thyroid carcinoma with anaplastic features
  - Anaplastic thyroid carcinoma undifferentiated phenotype
- **Other thyroid epithelial neoplasms**
  - Salivary gland type carcinoma
  - Mucoepidermoid carcinoma
  - Sclerosing mucoepidermoid carcinoma with eosinophilia
  - Mucinous carcinoma
  - Thymic neoplasm
  - Ectopic thymoma
  - Intrathyroid epithelial thymoma/CASTLE (carcinoma showing thymus like differentiation)
  - Spindle epithelial tumor with thymoma-like differentiation

Neural Crest Origin Parafollicular Cell-derived Thyroid Neoplasms

- Medullary thyroid carcinoma (MTC): MTC low grade, MTC high grade (necrosis, mitoses ≥5/cu mm or Ki-67 proliferative index ≥5%)
- Note: Grading system for medullary thyroid carcinomas is based on mitotic count, tumor necrosis and Ki-67 labeling index

Non-epithelial Thyroid Neoplasms

- Peripheral nerve sheath neoplasms include schwannoma, malignant peripheral nerve sheath tumor (MPNST)
- Vascular neoplasms include hemangioma, lymphangioma, angiosarcoma
- Smooth muscle neoplasms include leiomyoma, leiomyosarcoma
- Solitary fibrous neoplasm
- Histiocytic neoplasms include Langerhans cell histiocytosis, Rosai-Dorfman disease, follicular dendritic cell sarcoma
- Hematolymphoid neoplasms include primary thyroid lymphoma, follicular dendritic cell lymphoma
- Germ cell neoplasms include benign teratoma, immature teratoma, malignant teratoma

Secondary Thydoid Neoplasms

Metastatic neoplasms to thyroid gland include squamous cell carcinoma of larynx, renal cell carcinoma, lung carcinoma, breast carcinoma, colon carcinoma, malignant melanoma, and lymphoma

Risk Factors

Risk factors for thyroid carcinomas are iodine deficiency, radiation exposure, high doses of X-rays and hereditary.

Gene mutations associated with thyroid carcinoma are given in Table 26.26. Molecular pathogenesis of thyroid carcinomas is shown in Fig. 26.37.

Table 26.26 Gene mutations associated with thyroid carcinoma

Category	Proto-oncogene	Mechanism	Associated Cancers
Growth factors			
Hepatocyte growth factor (HGF)	HGF	Overexpression	▪ Thyroid carcinoma ▪ Hepatocellular carcinoma
Growth factor receptors			
Receptors for neurotrophic factors	RET	Point mutation	*Multiple endocrine neoplasia:* MEN IIa, MEN IIb familial thyroid medullary carcinoma
Mitogenic signaling pathway inhibitors (tumor suppressor genes)			
APC gene	Adenomatous polyposis protein (locus 5p21). Mutation by deletion or nonsense	APC inhibits Wnt signaling activator pathway, thus prevents nuclear transcription. It also degrades catenin	*Sporadic cancers:* Thyroid carcinoma, colon carcinoma, gastric carcinoma, pancreas carcinoma and melanoma

Low doses of ionization radiation (radiotherapy) in head and neck region can cause or enhance DNA mutations and chromosomal abnormalities.

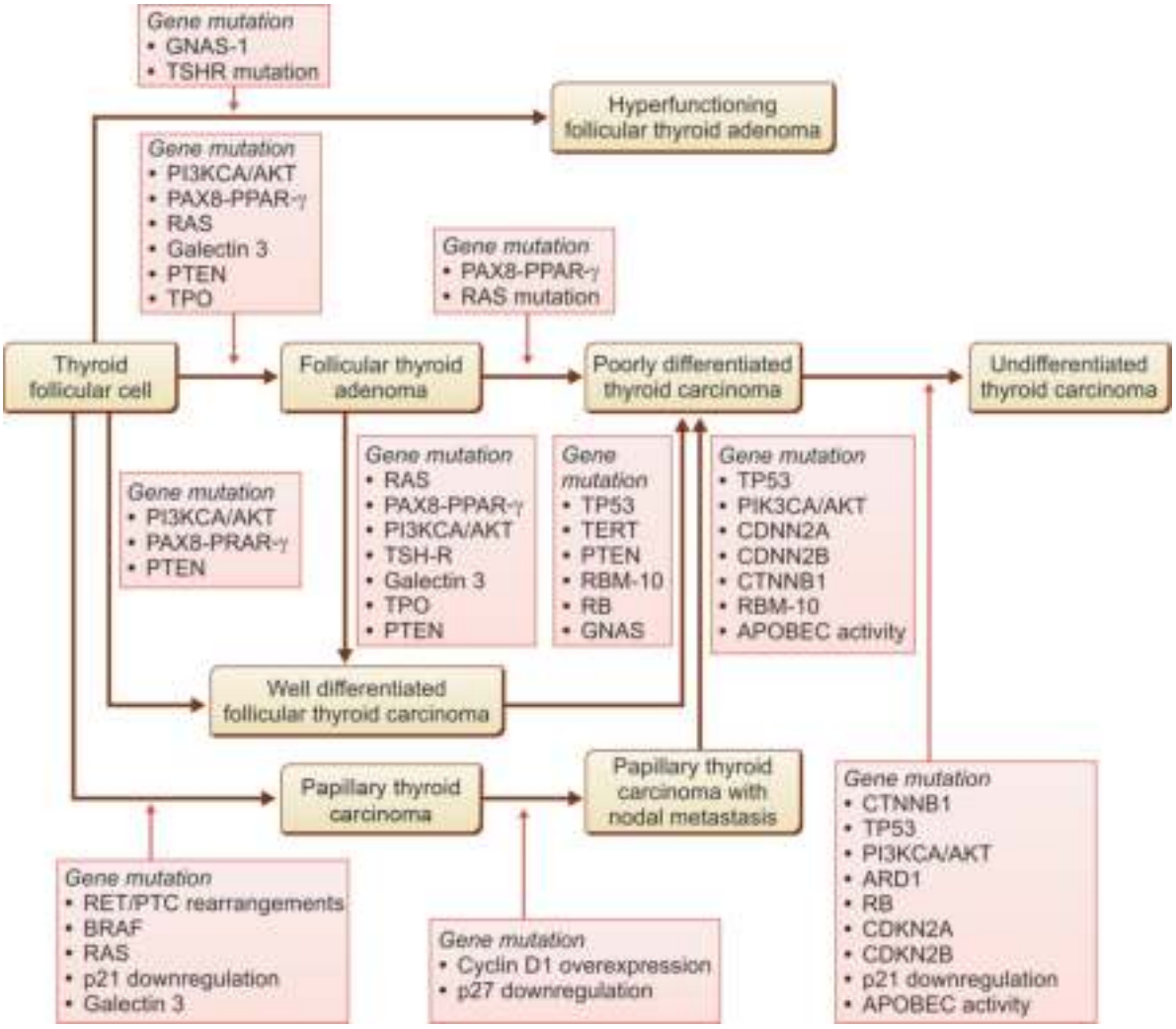


Fig. 26.37: Molecular pathogenesis of thyroid carcinomas.

Clinical Course

Papillary thyroid carcinoma is least aggressive neoplasm with slow metastases. Follicular thyroid carcinoma is less common but spreading early to lymph nodes and distant organs. Medullary thyroid carcinoma synthesizes thyrocalcitonin, histaminase, prostaglandins and ACTH.

Prognosis

Prognosis of thyroid carcinomas is good in children in >70% of cases with 10 years survival. Rapid growth suggests malignancy.

FOLLICULAR THYROID ADENOMA

Follicular thyroid adenoma is the most common benign encapsulated tumor of thyroid gland, which shows follicular cell differentiation that consists of thyroid follicles lined by cuboidal epithelial cells.

- Tumor lacks evidence of capsular and vascular invasion, and also lacks of papillary thyroid carcinoma nuclear features. Tumor is firm or rubbery, homogenous, round or oval and surrounded by a thin fibrous capsule. Most patients with follicular thyroid adenoma are clinically and biochemically euthyroid.
- About 1% of follicular thyroid adenomas attaining 3 cm size can become functional as a result of a monoclonal expansion of thyroid follicular cells and mutation in TSH receptor gene that result in increased thyroid hormone secretion independent of TSH and patients develop hyperthyroidism.
- On radioiodine thyroid scintigraphy, functioning follicular thyroid adenomas avidly concentrate radioiodine and may suppress iodine uptake in the rest of thyroid gland. About 20% of nonfunctioning follicular thyroid adenomas possess proto-oncogene N-RAS, H-RAS and K-RAS mutations that may predispose them to progression to follicular thyroid carcinoma.

Laboratory Diagnosis

The diagnostic evaluation of a patient with a thyroid solitary nodule consists of fine needle aspiration cytology (FNAC), an ultrasound imaging of the neck and a screening serum TSH.

- Fine needle aspiration cytology of thyroid nodule reveals abundant follicular epithelial cells in sheets with crowding and overlapping of cells, microfollicles and scant/no colloid.
- Limitation of fine needle aspiration cytology is the inability to distinguish a follicular thyroid adenoma from follicular thyroid carcinoma. Histologic examination is essential to establish diagnosis of follicular thyroid adenoma.

Clinical Features

Most patents with a follicular thyroid adenoma present with a solitary palpable nodule in normal thyroid gland identified on clinical examination and on ultrasound imaging study. However, tumor may occur in association with thyroiditis and/or nodular hyperplasia.

- Most patients are euthyroid and asymptomatic. Patients with large tumors may present with breathlessness, hoarseness of voice and dysphagia related to compression of structures such as trachea, recurrent laryngeal nerve and esophagus respectively. Patients with functional follicular thyroid adenoma may manifest with hyperthyroidism.
- Secondary changes may occur in large follicular thyroid adenoma, i.e. intratumoral hemorrhage, edema, cystic degeneration, fibrosis, dystrophic calcification and bone formation. Patients may complain of multiple nodules in thyroid and neck pain as a result of sudden tumor enlargement from intratumoral hemorrhage or cystic degeneration.

Treatment

Thyroid lobectomy or isthmusectomy is a definitive treatment for patients with follicular thyroid adenoma. Functional follicular thyroid adenoma with low serum TSH and high T3 and T4 levels analyzed by iodine-123 thyroid scan should be treated by levothyroxine drug for three to six months to reduce size of thyroid nodule before surgery.

Surgical Pathology: Follicular Thyroid Adenoma

Gross Morphology

- Follicular thyroid adenoma is solitary, encapsulated and of variable size (1–10 cm). Tumor resembles multinodular goiter due to secondary changes of intratumoral hemorrhage and cystic degeneration.
- Cut surface reveals solid, fleshy, tam to brown tumor that bulges when fresh tumor compresses adjacent thyroid (Fig. 26.38).

Light Microscopy

The diagnostic histologic criteria of follicular thyroid adenoma include: (a) follicular thyroid adenoma is surrounded by a complete thin capsule without evidence of capsular and vascular invasion, (b) tumor exhibits distinct architecture of thyroid follicles inside and outside capsule, (c) tumor shows uniform histologic features inside capsule, (d) tumor causes compression of surrounding tissue, and there is lack of multinodularity in the remaining thyroid gland, (e) tumor is composed of closely packed thyroid follicles lined by cuboidal to low columnar cells arranged in trabeculae or solid sheets with histologic patterns such as normofollicular (simple), macrofollicular, microfollicular, and trabecular/

solid. Commonly secondary changes in follicular thyroid adenoma include hemorrhage, hemosiderin deposition, edema, necrosis and cystic changes (Fig. 26.39).

- **Normofollicular (simple) thyroid adenoma:** Tumor is composed of uniform normal looking thyroid follicles, which resemble normal thyroid follicles.
- **Macrofollicular (colloid) thyroid adenoma:** Tumor is composed of large thyroid follicles containing colloid.
- **Microfollicular (fetal) thyroid adenoma:** Tumor is composed of closely packed small thyroid follicles with scant colloid arranged in edematous fibrovascular stroma. It is most common histologic pattern of follicular thyroid adenoma.
- **Trabecular/solid (embryonal) thyroid adenoma:** Tumor is composed of thyroid follicles lined by epithelium arranged in solid/trabecular pattern or branching cords.



Fig. 26.38: Gross morphology of thyroid follicular adenoma. Tumor is well circumscribed. Cut surface of tumor is gray-white. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

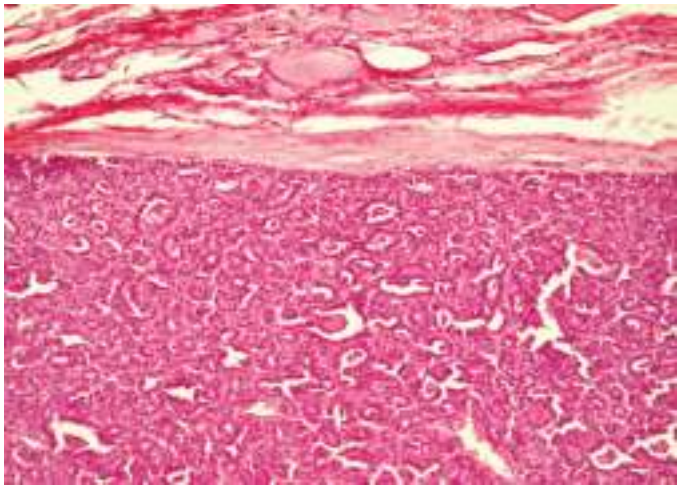


Fig. 26.39: Thyroid follicular adenoma. Tumor is completely surrounded by capsule. Uniform histopathological pattern of thyroid adenoma cells is seen. Tumor has compressed surrounding parenchyma.

HYALINIZING TRABECULAR TUMOR

Hyalinizing trabecular tumor (HTT) is an uncommon neoplasm of the thyroid follicular derivation, with morphology that is similar with papillary thyroid carcinoma. The tumor contains hyaline material that often confuses it for medullary carcinoma of thyroid, because hyaline mimics amyloid material.

- Therefore, the tumor is misdiagnosed as solid variant of papillary thyroid carcinoma, hyalinizing papillary thyroid carcinoma and hyalinizing trabecular adenoma like variant of medullary thyroid carcinoma, because its cytological features resemble papillary thyroid carcinoma and hyaline material resembles amyloid material.
- Both hyalinizing trabecular tumor and papillary carcinoma share significantly RET/PTC fusion gene. BRAF or NRAS mutations present in papillary thyroid carcinoma, but absent in hyalinizing trabecular tumor of thyroid gland.

Clinical Features

Hyalinizing trabecular tumor most often occurs in the females than males between fourth to seventh decades of age. Patients have no obvious symptoms. Clinical examination reveals mass in the neck region.

Surgical Pathology: Hyalinizing Trabecular Tumor
Light Microscopy
▪ Tumor is composed of follicular cells without atypia arranged in trabecular pattern with marked hyalinized vascular stroma. ▪ These features along with invasion of capsule and blood vessels are termed hyalinizing trabecular neoplasm.
Immunohistochemistry Technique
▪ Tumor cells show positivity for MiB1. ▪ MiB1 stains membrane and cytoplasm of tumor cells.

PAPILLARY THYROID CARCINOMA

Papillary thyroid carcinoma (PTC) is the commonest thyroid carcinoma. It accounts for 70–80% of all thyroid carcinomas.

- It most often affects young women 20–30 years of age group. Tumor is usually multifocal in origin within the gland and least aggressive thyroid cancer.
- Papillary thyroid carcinomas with many morphological subtypes (infiltrative, follicular, tall columnar cells, hobnail, solid, diffuse sclerosing, oncocytic and warthin-like) with minimally invasive, encapsulated angioinvasive and widely invasive; represent BRAF mutation malignancies, whereas invasive encapsulated follicular thyroid carcinoma represent the RAS mutation malignancies.

- Tumor most often remains localized to the thyroid and adjacent tissues for many years. It has a slow tendency to metastasize to regional lymph nodes, but distant metastases are rare. Prognosis is excellent even when adjacent lymph nodes are involved.

Diagnostic Features

Histologic features of papillary thyroid carcinoma are presence of true papillae, psammoma bodies, dark red colloid, crowded nuclei, optically clear chromatin exhibiting 'Orphan Annie appearance', irregular nuclear contours, nuclear groove and intranuclear cytoplasmic inclusions.

Radionuclide Scan

Papillary thyroid carcinoma is almost always nonfunctional. It invariably produces a cold nodule on radionuclide scanning.

Risk Factors

Hashimoto's thyroiditis and radiation exposure to the neck increase risk for papillary thyroid carcinoma in 5–10% of cases. Ionizing radiations pose a potential worldwide threat in this nuclear age. Low doses of ionizing radiation can cause DNA mutations and chromosomal abnormalities resulting in thyroid carcinoma. Large doses of radiation can inhibit cell division. Ionizing radiation also can enhance the effects of genetic abnormalities.

Molecular Genetic Alterations

Possible molecular genetic alterations associated with papillary thyroid carcinoma are rearrangement of RET/PTC, BRAF mutation and RAS mutation. Molecular pathogenesis of papillary thyroid carcinoma is shown in Figs 26.40 and 26.41.

- **RET-PTC fusion oncogene:** In some instances, papillary thyroid carcinoma may be associated with changes in chromosome 10. Either there is paracentric inversion of chromosome 10, or a reciprocal translocation between chromosomes 10 and 17.
  - These chromosomal changes activate the tyrosine kinase domain of RET. These novel fusion genes are referred to as RET-PTC oncogene.
  - Mutations in B-type RAS kinase (BRAF) are also common and predictive of tumor behavior and progression.
- **Hepatocyte growth factor (HGF):** Many cancer stem cells acquire the ability to synthesize the same growth factors to which they are responsive, generating an autocrine loop. Increased baseline activity of HGF oncogene product is associated with hepatocellular carcinoma and thyroid carcinoma.

Clinical Features

- The patient presents with slow growing painless occult (<1.5 cm in diameter) to palpable solitary or multifocal thyroid nodule. In some cases, papillary thyroid carcinoma is not palpable, i.e. occult, presenting with lymphadenopathy nearby thyroid gland called Delphian node containing metastases in 50–70% of cases. Distant metastases are uncommon, i.e. lungs and bone in 10% of cases.

Surgical Pathology: Papillary Thyroid Carcinoma

Gross Morphology

- Tumor may be small microscopic occult lesion <1 cm to large 10 cm nodule. Multifocal areas are observed in 20–75% of cases.
- On cut surface, tumor is usually solid grey white and sometimes cystic mass.
- Tumor is clearly invading the capsule.
- Approximately in <10% of cases, tumor is surrounded by a complete capsule (Fig. 26.42).

Light Microscopy

- Tumor is composed of fibrovascular stroma lined by tumor cells exhibiting branching tree-like pattern.
- Tumor cells contain optically clear ground glass nuclei giving (Orphan Annie) appearance. The nuclei show longitudinal nuclear groove and intranuclear pseudoinclusions due to cytoplasmic invagination into the nuclei.
- Fibrovascular stroma is edematous and infiltrated by lymphocytes, foamy histiocytes and hemosiderin.
- Fibrovascular stroma shows laminated calcific spherules called 'psamomma bodies' formed due to necrosis of cells with dystrophic calcification in 50% of cases. Their presence is strong indicator of papillary thyroid carcinoma (Figs 26.43 to 26.45).

Papillary Thyroid Carcinoma: Histologic Variants

- **Follicular variant of papillary thyroid carcinoma:** It consists of neoplastic follicular component and psammoma bodies. Prognosis is similar to conventional papillary carcinoma of thyroid.
- **Encapsulated variant of papillary thyroid carcinoma:** It is well encapsulated tumor associated with lymph node metastases but no distant metastases. Prognosis of this variant of papillary thyroid carcinoma is excellent.
- **Diffuse sclerosing variant of papillary thyroid carcinoma:** It is characterized by diffuse dense fibrous stroma, numerous psammoma bodies, squamous metaplasia and inflammation. It is associated with lymph node and distant lung metastases. Prognosis is poor than conventional papillary thyroid carcinoma.
- **Tall cell variant of papillary thyroid carcinoma:** It consists of tumor cells with abundant eosinophilic cytoplasm and lymphocytic infiltrate. It has worse prognosis than conventional papillary thyroid carcinoma.

- **Columnar variant of papillary thyroid carcinoma:** Tumor is composed of tall columnar cells with nuclear stratification arranged in papillary pseudoglandular formation with CDX2 immunoreactivity in 50% of tumors.

Immunohistochemistry Technique

A panel of immunomarkers for thyroid follicular cells in papillary thyroid carcinoma includes CK AE1/AE3, CK7, TTF-1, thyroglobulin and PAX8. A proportion of cases demonstrates BRAF V600E (VE antibody) and RET–PTC.

Markers	Expression
▪ CK AE1/AE3	▪ Positive
▪ CK7	▪ Positive
▪ TTF-1	▪ Positive
▪ Thyroglobulin	▪ Positive
▪ PAX8	▪ Positive
▪ BRAF V600E	▪ Positive
▪ RET–PTC	▪ Positive

Clinical Course

Patient with papillary thyroid carcinoma without evidence of metastasis has an excellent prognosis especially in persons younger than 45 years of age. Prognosis is good with 10-year survival rate in 80–90% of cases. Cure rate is nearly 100% in young patient

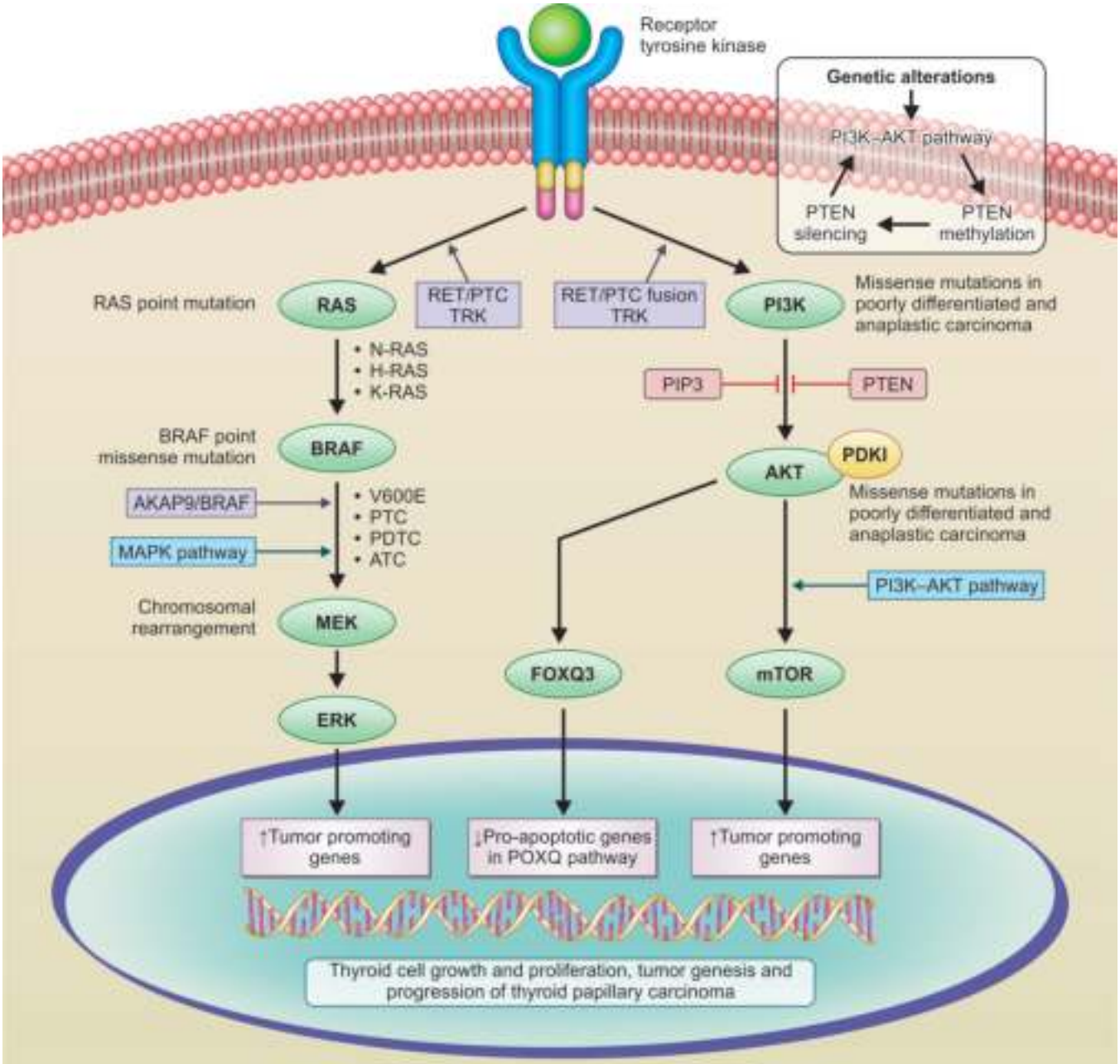


Fig. 26.40: Molecular pathogenesis of papillary thyroid carcinoma.

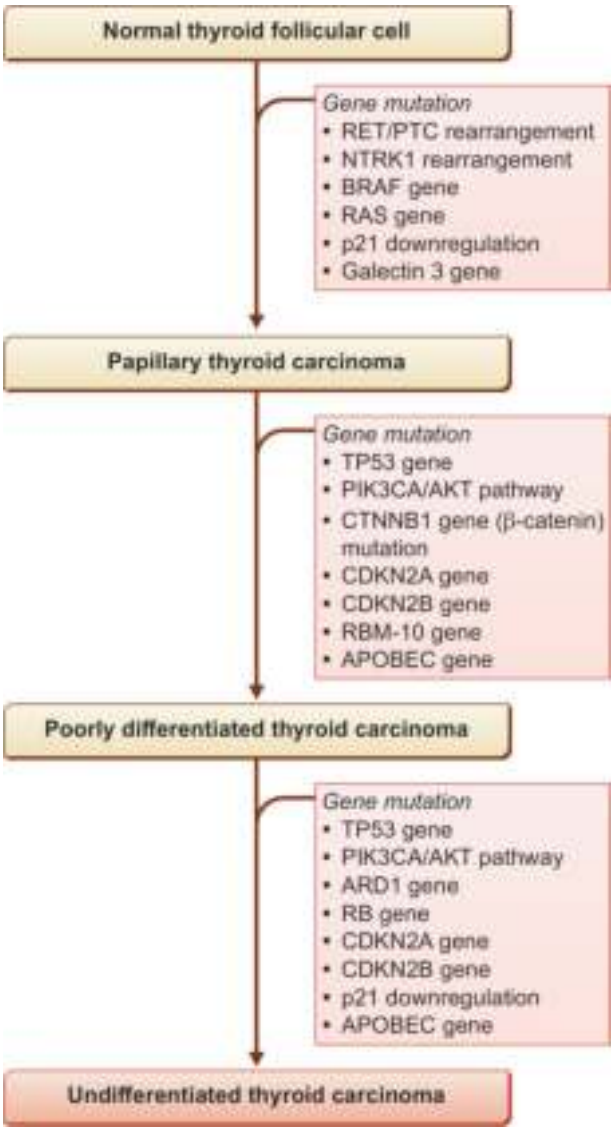


Fig. 26.41: Molecular pathogenesis of papillary thyroid carcinoma.



Fig. 26.42: Papillary thyroid carcinoma. On cut surface, tumor is solid grey white with small cystic spaces. (*Courtesy:* Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

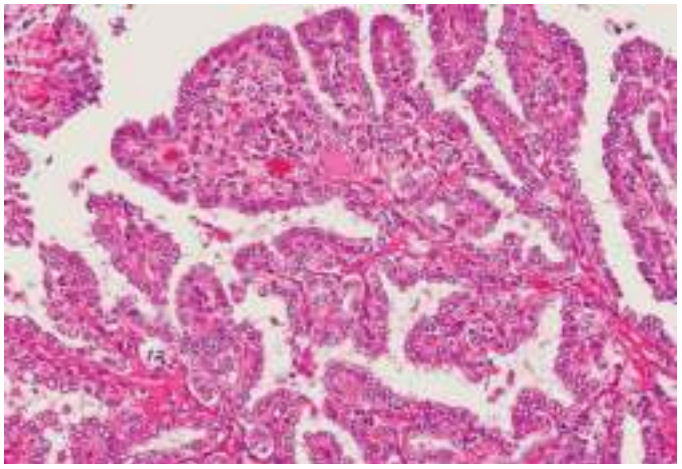


Fig. 26.43: Papillary thyroid carcinoma. Histologic examination shows fibrovascular stroma lined by neoplastic cells exhibiting branching tree-like pattern (100X).

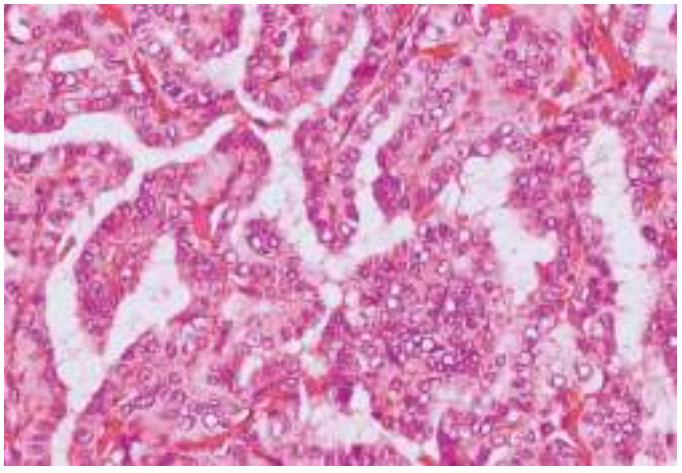


Fig. 26.44: Papillary thyroid carcinoma. Tumor is composed of fibrovascular core lined by neoplastic cells with optically clear nuclei exhibiting overcrowding. Fibrovascular stroma shows psammoma bodies and inflammatory cells (200X).

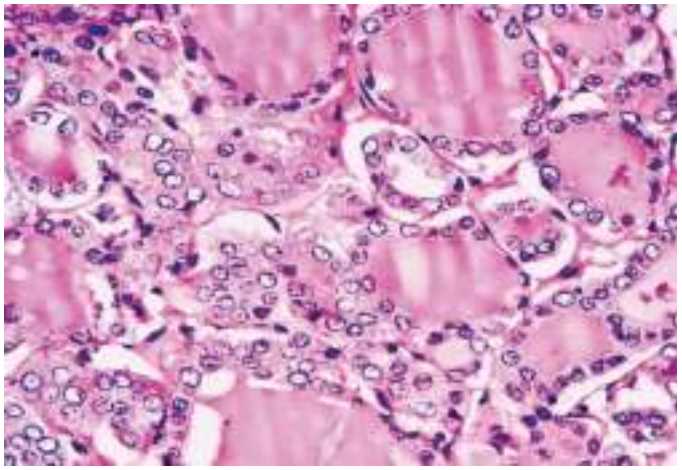


Fig. 26.45: Papillary thyroid carcinoma (follicular variant). Tumor is composed of neoplastic cells with optically clear nuclei exhibiting nuclear grooves (400X).

Table 26.27 Prognostic factors of papillary thyroid carcinoma

Parameters	Good Prognosis	Poor Prognosis
Sex predilection	Female patients	Male patients
Age group	<40 years of age especially children	>40 years of age
Location	Unicentric (unifocal) tumor	Multicentric (multifocal) tumor
Tumor differentiation	Well differentiated carcinoma	Poorly differentiated carcinoma
Extension of tumor	Tumor is confined within thyroid gland	Tumor is extending outside thyroid gland
Aneuploidy	Absent	Present

Table 26.28 Histologic variants of papillary thyroid carcinoma and biologic behavior, when compared with conventional/classic variant

Biologic Behavior	Histologic Variants of Papillary Carcinoma of Thyroid Gland	
Excellent prognosis (less aggressive histologic variants)	▪ Conventional/classic variant ▪ Follicular variant ▪ Warthin-like variant	▪ Papillary microcarcinoma ▪ Encapsulated variant
Poor prognosis (highly aggressive histologic variants)	▪ Solid trabecular variant ▪ Diffuse sclerosing variant	▪ Tall cell variant ▪ Hobnail variant
Variable (less highly aggressive histologic variants)	▪ Columnar cell variant	
Unknown biological behavior	▪ Unknown biological behavior ▪ Cribriform-morular variant ▪ Papillary carcinoma with fibromatosis/ fasciitis like stroma	▪ Oncogenic variant ▪ Spindle cell variant ▪ Clear cell variant

with small focus of cancer. Relapses occur 20–35 years after treatment. Prognostic factors of papillary thyroid carcinoma are given in Table 26.27. Histologic variants of papillary thyroid carcinoma and biologic behavior, when compared with conventional/classic variant are given in Table 26.28.

FOLLICULAR THYROID CARCINOMA

Follicular thyroid carcinoma (FTC) accounts for 6–10% of thyroid carcinomas and occurs in women over 40–60 years, who have dietary iodine deficiency and irradiation exposure. Tumor has hematogenous dissemination but usually lacks lymphatic spread. Three types of FTC include: (a) minimally invasive with capsular invasion only, (b) encapsulated angioinvasive with limited capsular invasion (<4 vessels) has better prognosis, and (c) widely angioinvasive (>4 vessels) with extensive invasion of thyroid and extrathyroid tissue. Extensive sampling of tumor capsule is recommended. Radionuclide scan reveals solitary 'cold' nodule.

Diagnostic Criteria

Follicular thyroid carcinoma is distinguished from a follicular thyroid adenoma on the basis of thick irregular capsule, capsular invasion and lymphovascular invasion, extrathyroidal tumor extension, lymph node metastases or system metastases (lungs, bones, liver, brain and skin).

- Diagnosis of follicular thyroid carcinoma relies on capsular and/or lymphovascular invasion by atypical cells of the tumor. Capsular invasion is defined as follicular thyroid carcinoma extension through the entire capsule. A follicular neoplasm with tumor invasion into but not through entire capsule is considered a follicular thyroid adenoma. Vascular invasion is defined as follicular thyroid carcinoma penetration into a wall of large caliber blood vessel with identifiable vascular endothelial lining within or outside the capsule. Vascular invasion is the most reliable sign of follicular thyroid carcinoma.
- Follicular thyroid carcinoma can be minimally invasive (less aggressive tumor with a disease-free survival in young persons), and invasive (most aggressive tumor metastasizing to lungs, bones, liver, brain and skin) histologic variants based on morphologic criteria. Minimally invasive follicular thyroid carcinoma is an encapsulated tumor with microscopic invasion of tumor capsule without vascular invasion. Invasive follicular thyroid carcinoma is defined by capsular invasion and vascular invasion and/or extension

beyond the thick tumor capsule into the adjacent thyroid parenchyma. It has been suggested that minimally invasive follicular thyroid carcinoma may be precursor to its invasive counterpart.

Pathogenesis

Pathogenesis of follicular thyroid carcinoma may be linked to iodine deficiency, radiation exposure and various oncogenic activation and/or microRNA (miR-192, miR-197 and miR-346) activation.

- About 80% of follicular thyroid carcinomas contain RAS gene mutations or a paired box 8/peroxisome proliferator-activated receptor gamma (PAX8-PPAR-γ) gene rearrangement.
- The RAS genes code for multiple G proteins that are involved in intracellular signaling through the RAF-MEK-MAPK kinase signaling pathway. Point mutations in RAS (N-RAS, H-RAS and K-RAS) may be implicated in the evolution of follicular thyroid adenoma to follicular thyroid carcinoma, producing continuously activated signaling proteins leading to uncontrolled growth. Other gene mutations can be linked to follicular thyroid carcinoma, which include TP53, c-Myc, c-Fos and the thyrotropin (TSH) receptor.
- PAX8-PPAR-γ gene rearrangement has been implicated in many cases of follicular thyroid adenoma and follicular thyroid carcinoma. PAX8 gene codes for nuclear protein product of thyroid specific transcription factor that is involved in differentiation of follicular cell. Mutation in PAX8 leads to its fusion with PPAR-γ, which causes to impaired function of PPAR-γ resulting in loss of growth inhibitory regulation.
- Advances in molecular genetic testing for gene mutations help in the evolution of a follicular thyroid adenoma to follicular thyroid carcinoma. Molecular pathogenesis of follicular thyroid carcinoma is shown in Fig. 26.46.

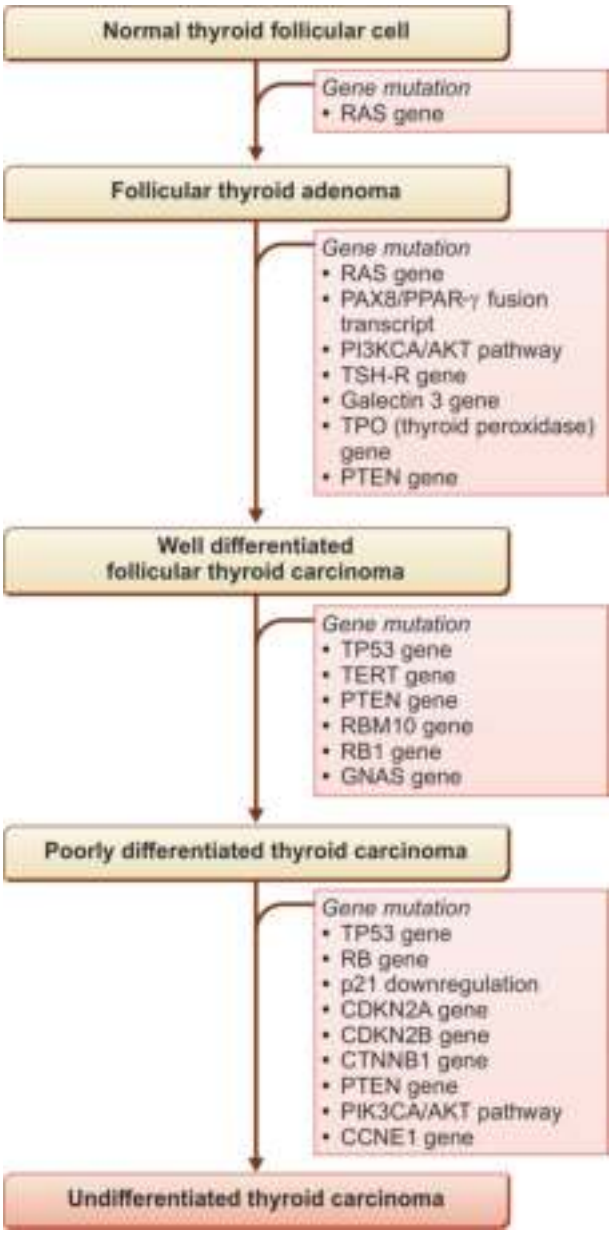


Fig. 26.46: Molecular pathogenesis of follicular thyroid carcinoma.

Clinical Features

Most patients with a follicular thyroid carcinoma present with a solitary palpable nodule in thyroid gland identified on clinical examination and on ultrasound imaging study. However, tumor may occur in association with thyroiditis and/or nodular hyperplasia and radiation exposure.

- Most patients are euthyroid and asymptomatic. Patients with large tumors may present with breathlessness (compression of trachea), hoarseness of voice (compression of recurrent laryngeal nerve) or dysphagia (compression of esophagus). Patients with functional follicular thyroid adenoma may manifest with hyperthyroidism.
- Follicular thyroid carcinoma is usually unifocal and <10% of patients have lymph node metastases via lymphatic route. Tumor has a propensity for distant metastasis via hematogenous route to lungs, bones liver, brain and skin.
- Secondary changes may occur in large follicular thyroid adenoma, i.e. intratumoral hemorrhage, edema, cystic degeneration, fibrosis, dystrophic calcification and bone formation. Patients may complain of multiple nodules in thyroid and neck pain as a result of sudden tumor enlargement from intratumoral hemorrhage or cystic degeneration.

Laboratory Diagnosis

Fine needle aspiration cytology (FNAC) of follicular thyroid neoplasm reveals abundant follicular epithelial cells in sheets with crowding and overlapping of cells, microfollicles and scant/no colloid. Limitation of fine needle aspiration cytology is the inability to distinguish follicular thyroid adenoma from follicular thyroid carcinoma.

- Ultrasonographic features alone cannot distinguish a follicular thyroid adenoma from a follicular thyroid carcinoma. In a patient with a follicular thyroid neoplasm, ultrasound demonstration of thyroid nodular disease in the contralateral thyroid lobe has important implications for operative management.
- Patient with follicular thyroid neoplasm should undergo diagnostic thyroid lobectomy and isthmusectomy for histologic examination, which is essential to distinguish follicular thyroid carcinoma from follicular thyroid adenoma. The assessment of capsular invasion and vascular invasion is essential for diagnosis of follicular thyroid carcinoma. The use of immunohistochemical techniques to identify PAX8-PPAR-γ gene rearrangement is helpful in diagnosing follicular thyroid carcinoma.
- Molecular genetic testing of RAS gene mutations justifies a recommendation for surgery in all patients with RAS gene mutations. Detection of circulating tumor cells in the blood using reverse transcriptase polymerase chain reaction (RT-PCR) for TSH receptor and thyroglobulin messenger RNA may help in distinguishing follicular thyroid carcinoma from follicular thyroid adenoma. TSH receptor and thyroglobulin mRNA transcripts found in the peripheral blood is sensitive and specific diagnostic test for follicular thyroid carcinoma. Detection of carcinoembryonic antigen (CEA) in the peripheral blood is useful in differentiating follicular thyroid carcinoma from follicular thyroid adenoma.

Treatment

Patients with invasive follicular thyroid carcinoma are treated by complete thyroidectomy with tumor-free margins, postoperative radioactive iodine ablation, whole body scanning, and thyrotropin suppressive doses of thyroid hormones. Less than 10% of patients with follicular thyroid carcinoma develop lymph node metastases and hence compartment-oriented neck dissection is reserved for patients with macroscopic disease.

- Regular follow-up of patients is essential which includes clinical history, physical examination, cervical ultrasonography and analysis of serum TSH, thyroglobulin and antithyroglobulin antibody levels.
- Computed tomography (CT) and magnetic resonance imaging (MRI) studies are reserved for patients with an elevated serum thyroglobulin level, negative ultrasonography, and distant metastases to lung and bone (osteolytic lesions in vertebral bodies followed by the pelvis, femur, skull and ribs), and less commonly to liver, brain and skin.
- Microscopic metastases are treated with high doses of radioactive iodine. Isolated macroscopic metastasis should be treated by surgical excision. The overall 10-year survival for patients with minimally invasive follicular thyroid carcinoma is 98% compared with 80% in patients with invasive follicular thyroid carcinoma.
- Comparison of papillary carcinoma and follicular carcinoma of thyroid gland is given in Table 26.29. Histologic features differentiating solitary follicular thyroid adenoma and follicular thyroid carcinoma are given in Table 26.30.

Table 26.29 Comparison of papillary thyroid carcinoma and follicular thyroid carcinoma

Parameters	Papillary Thyroid Carcinoma	Follicular Thyroid Carcinoma
Role of radiation	Present	Absent
Encapsulation	Rare	Common
Invasion	Strongly invasive, invades in small islands	Variably invasive in large islands with pushing borders
Multiple tumor foci	Common	Rare
Blood vessel invasion	Rare	Common
Cellular pattern	Papillae, follicles, solid cell group	Follicles, trabeculae, no papillae, pseudopapillary infoldings may occur
Psammoma bodies	Present in 50% in fibrovascular stroma	Absent, but calcified colloid may occur
Nuclear features	Ground glass (**Orphan Annie**) appearance, grooves, pseudoinclusions, often overlapping	Normochromatic or hyperchromatic, no grooves, no overlap

Table 26.30 Histologic features differentiating follicular thyroid adenoma from follicular thyroid carcinoma

Follicular Thyroid Adenoma	Follicular Thyroid Carcinoma
Complete but delicate capsule	Dense circumferential fibrosis of capsule
Entrapped or sequestered thyroid in near capsule	Transcapsular '**mushrooming**' invasion
Juxtaposed or prolapsed thyroid tissue near vessels, but with intact endothelium	Adherence of thyroid tissue to vascular endothelium with associated fibrosis

Surgical Pathology: Follicular Thyroid Carcinoma

Gross Morphology

- It is difficult to differentiate between well circumscribed follicular thyroid carcinoma and follicular thyroid adenoma. Some follicular thyroid carcinomas bulge from the confines of its capsule. Neoplastic cells show invasion of capsule and blood vessels, thus distinguished from follicular thyroid adenoma.
- Minimally invasive follicular thyroid carcinoma is seen grossly as a well-defined, unifocal encapsulated soft tumor.
- Cut surface of tumor is grey white soft with areas of hemorrhage, necrosis and cyst formation (Fig. 26.47).

Light Microscopy

- Tumor is composed of variable sized thyroid follicles lined by tumor cells with normochromatic/hyperchromatic nuclei with oxyphil/clear cytoplasm arranged in cords and invading capsule.
- Minimally invasive follicular thyroid carcinoma, which shows only capsular invasion without vascular invasion has better prognosis (Fig. 26.48).

Follicular Thyroid Carcinoma: Histologic Variants

- **Encapsulated variant of follicular thyroid carcinoma:** It is well encapsulated with minimal capsular invasion or invasion of blood vessels and distant metastases in 15% of cases.
- **Langhans' struma variant of follicular thyroid carcinoma:** It is sluggish tumor compressing respiratory airway.
- **Insular type of follicular thyroid carcinoma:** It is rare poorly differentiated variant of follicular carcinoma. Its phenotype is similar to the pancreatic insulinoma. It is more aggressive tumor, which shows positivity for thyroglobulin, but negativity for calcitonin.
  - Tumor is composed of broad trabeculae of small primitive cells with evenly distributed chromatin with inconspicuous nucleoli and scant cytoplasm.
  - Tumor cells are arranged around the blood vessels with numerous mitosis and extrathyroid involvement of lymph nodes (Fig. 26.49).

Immunohistochemistry Technique

- Thyroglobulin (Tg) and TTF-1 can be used as a tumor marker for well differentiated follicular thyroid carcinoma.
- Follicular thyroid carcinoma with or without metastases shows significant overexpression of *Ki-67* proliferative marker.



Fig. 26.47: Follicular thyroid carcinoma. Cut surface of tumor is grey white with areas of hemorrhage and necrosis and cyst formation. (*Courtesy:* Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

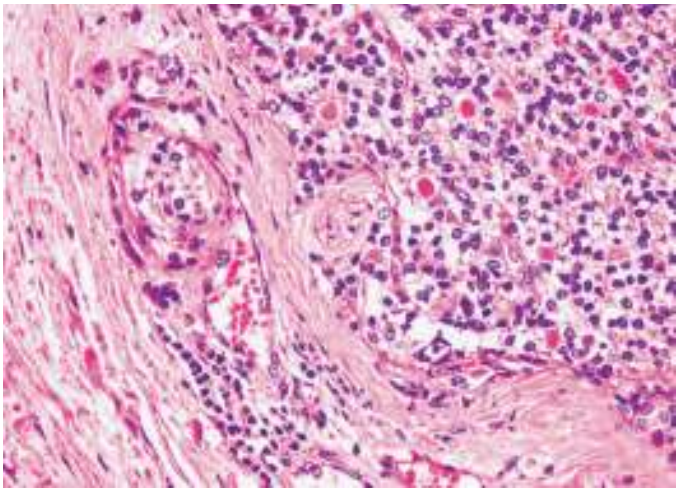


Fig. 26.48: Follicular thyroid carcinoma: Tumor is composed of variable sized thyroid follicles lined by tumor cells with normochromatic/hyperchromatic nuclei with oxyphil/clear cytoplasm. These are arranged in cords and invading capsule (400X).

Markers	Expression
▪ Thyroglobulin	▪ Positive
▪ TTF-1	▪ Positive
▪ Ki-67 proliferation index	▪ Positive

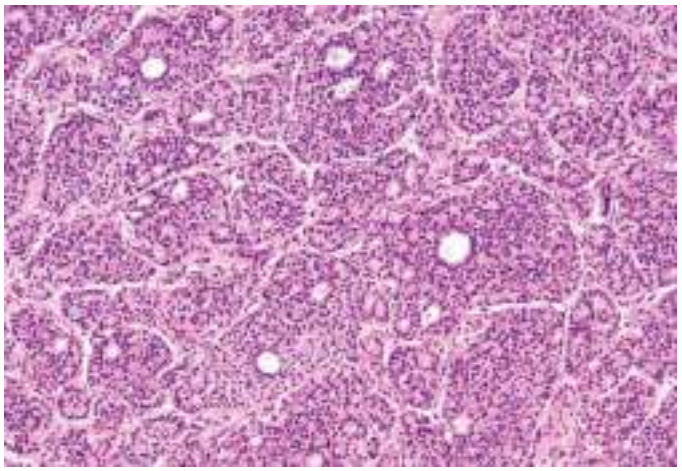


Fig. 26.49: Follicular thyroid carcinoma (insular variant). Tumor shows broad trabeculae of small cells with evenly distributed chromatin with inconspicuous nucleoli and scanty cytoplasm. Tumor cells are arranged around blood vessels with numerous mitosis (200X).

Routes of Metastasis

Tumor mainly spreads by hematogenous route to liver, lungs and bones. Lymphatic spread to regional lymph nodes may also occur.

ONCOCYTIC THYROID NEOPLASMS

Oncocytic thyroid neoplasms are derived from follicular cell composed of >75% of oncocytic tumor cells. Oncocytes (also known as Ashkenazy cells and oxyphil cells) are large polygonal cells found in thyroid and parathyroid glands, which have an abundance of eosinophilic cytoplasm secondary to increased dysfunctional mitochondria content. It is worth mentioning that oncocytic metaplasia occurs due to various cellular stress responses in benign adenomatous nodules, Hashimoto's thyroiditis, papillary thyroid carcinoma (PTC) and poorly differentiated thyroid carcinoma.

- Oncocytic thyroid neoplasms are categorized into benign and malignant. Oncocytic thyroid adenoma is a benign tumor without recurrence after surgical excision. Oncocytic thyroid carcinoma previously known as 'Hurthle cell carcinoma' accounts for 1–2% of all thyroid carcinomas, and is more common in older men with median age of 55 years, which is associated with worse prognosis in older persons, tumor size >4 cm and extensive lymphovascular invasion and metastasis to lymph nodes and distant organs.
- According to the recent 2022 World Health Organization (WHO) classification of thyroid neoplasms, oncocytic thyroid carcinoma is a distinct entity, which indicates oncocytic follicular cell-derived neoplasm composed of >75% of oncocytic tumor cells, which lacks the characteristic nuclear features of papillary thyroid carcinoma (PTC). High-grade follicular cell-derived oncocytic thyroid carcinomas comprise both differentiated oncocytic carcinoma (necrosis and ≥5 mitoses/cu mm) and poorly differentiated oncocytic thyroid carcinoma.

Clinical Features

Patient with oncocytic thyroid adenoma presents with solitary palpable nodule in normal thyroid gland. Patient with large size oncocytic thyroid carcinoma presents with breathlessness (compression of trachea), hoarseness of voice (compression of recurrent laryngeal nerve), dysphagia (compression of esophagus) and metastatic manifestations.

Laboratory Diagnosis

A definite diagnosis of oncocytic thyroid adenoma and oncocytic thyroid carcinoma is confirmed after complete surgical excision and histologic examination. Fine needle aspiration cytology (FNAC), ultrasonography, and occasional computed tomography (CT) do not always provide a definite diagnosis. On histologic examination, oncocytic thyroid adenoma is benign tumor if there is no evidence of capsular or vascular invasion.

- Oncocytic thyroid carcinoma is an aggressive neoplasm than conventional follicular thyroid carcinoma, with extrathyroidal extension, capsular invasion, lymphovascular invasion, and high risk of metastasis, local recurrence after surgical excision, and associated with poor prognosis.
- Typical mutations of thyroid cancer genes including RAS, TSHR, TP53, PTEN, BRAF, PAX8-PPAR-γ, and MEN1 are uncommon in oncocytic thyroid carcinoma. Molecular pathogenesis of oncocytic thyroid carcinoma is shown in Fig. 26.50.

Treatment

Oncocytic thyroid adenoma is treated by thyroid lobectomy. Oncocytic thyroid carcinoma tends to be aggressive with a high risk of metastasis and low survival and is treated by total thyroidectomy or radiation ablation. Oncocytic thyroid carcinoma is likely to be radioiodine-resistant compared to conventional follicular thyroid carcinoma. Prognosis depends on the size of the tumor, extent of extrathyroidal invasion, vascular invasion, cancer stage at diagnosis and degree of dissemination to distant organs.

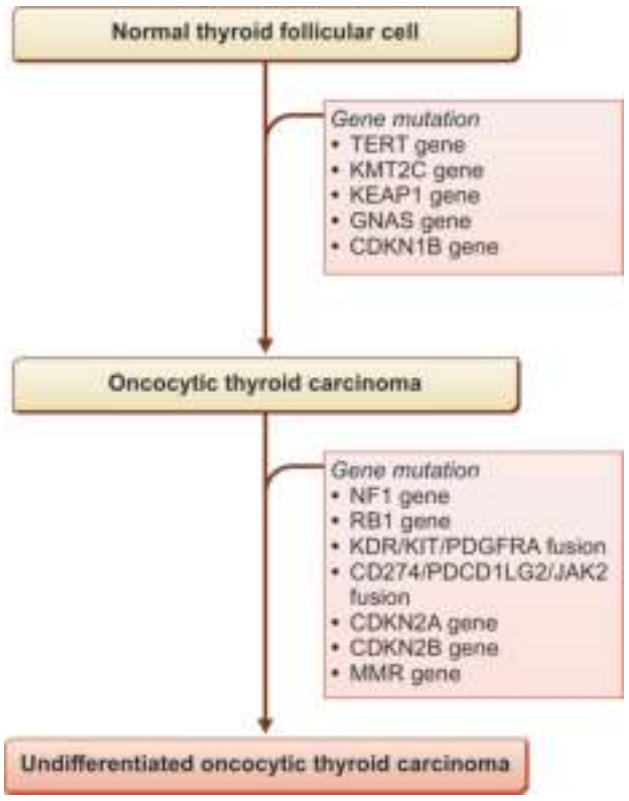


Fig. 26.50: Molecular pathogenesis of oncocytic thyroid carcinoma.

Surgical Pathology: Oncocytic Thyroid Neoplasms

Gross Morphology

- Most oncocytic thyroid neoplasms are >2 cm in dimeter. Tumor is prone to hemorrhage and infarction especially after fine needle aspiration or core biopsy.
- Oncocytic thyroid adenoma is encapsulated solitary, solid, and brown to mahogany color. Tumor may be lobulated with central scarring.
- Invasive oncocytic thyroid carcinoma has irregular borders, may have satellite nodule/multinodular appearance.

Light Microscopy

- **Oncocytic thyroid adenoma:** Histologically, oncocytic thyroid adenoma is benign tumor if there is no evidence of capsular or vascular invasion.
  - Tumor is composed of >75% of oncocytic large tumor cells with distinct cell borders, deeply eosinophilic and granular cytoplasm, large nucleus with prominent nucleus and complete loss of cell polarity.
  - Oncocytic tumor cells are arranged in follicular, trabeculae, solid or papillary growth patterns. Presence random nuclear cytologic atypia is common but not evidence of malignancy.
- **Oncocytic thyroid carcinoma:** Histologically, oncocytic thyroid carcinoma is malignant if there is evidence of capsular or vascular invasion.
  - Tumor tends to have thick capsule than that of oncocytic thyroid adenoma. Tumor is composed of large polygonal tumor cells with abundant eosinophilic granular cytoplasm, large round nuclei, vascular chromatin and prominent nucleoli and mitoses, arranged in solid and trabecular architecture rather than follicular pattern of growth and separated by delicate vasculatures.
  - High-grade differentiated oncocytic thyroid carcinoma shows necrosis and ≥5 mitoses/cu mm. Poorly differentiated oncocytic thyroid carcinoma measures >4 cm in size and is composed of small oncocytic tumor cells, numerous mitoses and tumor necrosis.

MEDULLARY THYROID CARCINOMA

Medullary thyroid carcinoma (MTC) is derived from the parafollicular cells (C cells residing in upper lateral lobes of thyroid gland), i.e. neural crest origin. Parafollicular cells synthesize calcitonin, which is tumor marker for screening medullary thyroid carcinoma. Altered calcitonin contributes to amyloid deposition within the tumor in majority of patients and demonstrated by Congo red stain. Paraneoplastic syndrome and carcinoid syndrome can occur in medullary thyroid carcinoma, which can synthesize hormones such as corticotropin, histamine, serotonin, prostaglandins, somatostatin, gastrin-releasing peptides (bombesin) and melanin.

Sporadic and Familial Medullary Thyroid Carcinoma

About 75–80% of medullary thyroid carcinomas are sporadic tumors (usually unilateral), and the remainder is familial tumors (multicentric and bilateral) as a part of multiple endocrine neoplasia, MEN IIa (pheochromocytoma and parathyroid adenoma), MEN IIb (i.e. pheochromocytoma and multiple mucosal neuromas) and familial medullary thyroid carcinoma (FMTC). RET mutations in neural crest tissue in the thyroid gland can lead to development of medullary thyroid carcinoma. Prophylactic thyroidectomy is recommended for patients with germline RET mutations that run family members at high risk to develop medullary thyroid carcinoma.

Molecular Genetic Alterations

Germline mutations of RET proto-oncogene located on chromosome 11 codes for transmembrane receptor tyrosine kinase are associated with MEN IIa, MEN IIb and FMTC.

- About 40–50% of sporadic thyroid carcinomas have acquired RET mutations located on chromosome 11, involving sequencing of exons 10, 11 and 13 through 16.

- Sporadic medullary thyroid carcinoma has a peak incidence in the fifth to sixth decade of life, whereas those medullary thyroid carcinomas associated with MEN IIa, MEN IIb and FMTC peak around the second or third decade of life. Molecular pathogenesis of medullary thyroid carcinoma (sporadic and hereditary types) is given in Fig. 26.51.

Clinical Features

About 70% of patients can manifest with cervical lymphadenopathy and a few of patients have compression symptoms like dysphagia, hoarseness of voice or respiratory difficulty. Liver, bone, lung and brain are common sites for metastases in a minority of patients.

Laboratory Diagnosis

Fine needle aspiration cytology (FNAC) of medullary thyroid carcinoma is potentially helpful in establishing the diagnosis. Diagnosis is confirmed on histologic examination of thyroid lobectomy specimen. Histologic examination reveals tumor is composed nests of round or ovoid cells and fibrovascular core stroma.

- Once the diagnosis of medullary thyroid carcinoma is made on histologic examination, serum calcitonin and carcinoembryonic antigen (CEA) should be analyzed to assess secretory activity of tumor and preoperative levels can be compared to postoperative levels to confirm the biologic response after surgical removal of tumor. The postoperative serum calcitonin and CEA doubling time provoke a sensitive tumor marker for progression of disease.
- Ultrasonography and computed tomography (CT) are performed to evaluate the involvement of cervical lymph nodes and liver by medullary thyroid carcinoma. Magnetic resonance imaging (MRI) is indicated in patients suspected of having metastatic disease. This can include patients with lymph node metastatic disease and serum calcitonin levels >400 ng/ml.
- Molecular genetic testing for mutation in RET gene mapped on chromosome 11, involving sequencing of exons 10, 11 and 13 through 16 is essential for all patients with parafollicular cell hyperplasia or sporadic medullary thyroid carcinoma. Screening for pheochromocytoma and hyperparathyroidism by using plasma metanephrines and serum calcium respectively is recommended for a patient with unknown RET gene mutational status and confirmed germline RET gene mutational status.

Treatment

Total thyroidectomy is indicated for all patients with medullary thyroid carcinoma (MTC). Bilateral neck dissection is indicated for MTC with more than one cm in diameter as well as for bilateral disease. Total prophylactic thyroidectomy is performed by the age of five years or when RET mutation codon 609, 611, 618, 630, 634. In MEN IIb with RET mutation codon 883, 918 or compound heterozygotes, total prophylactic thyroidectomy is performed by age of one year. Various receptor tyrosine kinase (RTK) inhibitors are administered to treat patients with medullary thyroid carcinoma such as cabozantinib an oral multikinase inhibitor, which inhibits RET, EGFR and VEGFR.

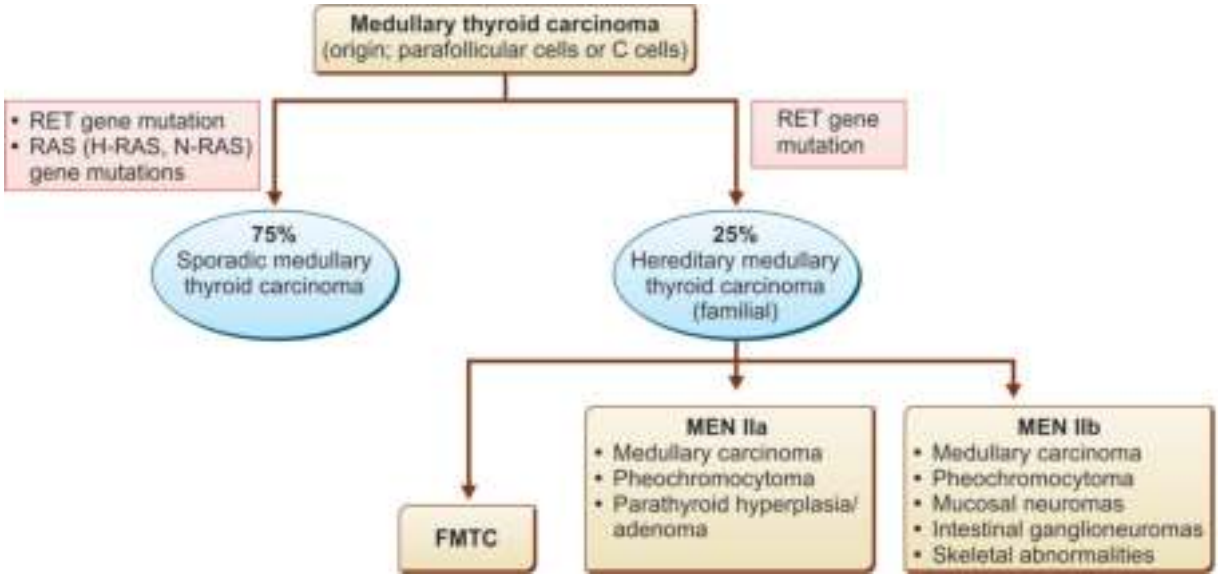


Fig. 26.51: Molecular pathogenesis of medullary thyroid carcinoma (sporadic and hereditary types).

Pathology Pearls: Sporadic and Familial/Hereditary Medullary Thyroid Carcinoma

Sporadic Medullary Thyroid Carcinoma (75%)

Patient presents with solitary tumor located in mid-portion or upper half of thyroid gland corresponding to a greater concentration of parafollicular 'C' cells in this region. Hormones, e.g. calcitonin, prostaglandins, serotonin, or vasoactive intestinal peptide (VIP) synthesized by the tumor increase gastrointestinal secretion and motility responsible for diarrhea in 33% of cases.

Familial Medullary Thyroid Carcinoma (25%)

Familial medullary thyroid carcinoma has a better prognosis than sporadic type. It is associated with pheochromocytoma, and completely curable when detected before it causes symptoms. It is associated with autosomal dominant MEN IIa (Sipple syndrome), MEN IIb and inherited medullary thyroid carcinoma without associated endocrinopathies. Patient with multiple endocrine neoplasia syndromes has gene mutations that make them susceptible to neoplasia or hyperplasia in multiple organs.

- **MEN IIa syndrome (Sipple syndrome):** It is also known as Sipple syndrome. It comprises medullary thyroid carcinoma, parathyroid adenoma or hyperplasia (hyperparathyroidism (33%) and bilateral pheochromocytomas. Hirschsprung's disease (congenital megacolon) and a variety of neural crest tumors (e.g. glioma). Patients with MEN IIb have mutations in RET oncogene, a transmembrane receptor of the tyrosine kinase family. When a diagnosis of pheochromocytoma is made, demonstration of mutation in RET oncogene would justify prophylactic thyroidectomy keeping in view of fatal outcome of medullary thyroid carcinoma.
- **MEN IIb syndrome (Gorlin syndrome):** It is associated with medullary thyroid carcinoma, bilateral pheochromocytomas, and multiple mucosal ganglioneuromas in lips/tongue. In contrast to MEN IIa, it does not induce hyperparathyroidism. It is linked to different mutations in the RET oncogene compared with MEN IIa. Poor prognostic factors in these patients include age >50, male, and distant metastases. Inherited medullary thyroid carcinoma without associated endocrinopathies is the least aggressive with peak incidence in the ages of 40 and 50.

Clinical Features

Patient presents with symptoms related to paraneoplastic syndrome and carcinoid syndrome due to secretory products of medullary thyroid carcinoma such as corticotropin, histamine, serotonin, prostaglandins. Somatostatin, gastrin-releasing peptides and melanin. Patient develops atypical Cushing's syndrome (ACTH) and watery diarrhea due to secretion of vasoactive intestinal peptide in 33% of cases.

Surgical Pathology: Medullary Thyroid Carcinoma

Gross Morphology

- Tumor is well circumscribed, solid, firm with grey white to yellow brown. Foci of hemorrhage necrosis are seen in large tumors.
- Tumor diameter 1 cm or less is referred to as medullary microcarcinoma (Fig. 26.52).

Light Microscopy

- Tumor is composed of round to polyhedral granular cells arranged in trabecular, glandular, carcinoid-like patterns.
- The tumor cells are separated by a distinctly fibrovascular stroma containing amyloid material representing deposition of procalcitonin.
- Amyloid material may elicit a florid foreign body type of giant cell reaction. This material is eosinophilic with the hematoxylin and eosin stain and takes up the Congo red stain.
- The stroma may be scanty, hemorrhagic or ossified or edematous (Fig. 26.53).

Immunohistochemistry Technique

- Tumor cells show positivity for calcitonin, creatine kinase, neuron-specific enolase, chromogranin, synaptophysin, TTF-1 and carcinoembryonic antigen.
- Carcinoembryonic antigen (CEA) is an oncofetal glycoprotein presents in large quantities in medullary carcinoma of thyroid. Monoclonal antibodies offer a greater degree of tumor specificity.

Markers	Expression
Calcitonin	Positive
Neuron-specific enolase (NSE)	Positive
Chromogranin	Positive
Synaptophysin	Positive
Thyroid transcription factor 1 (TTF-1)	Positive
Creatine kinase	Positive
Carcinoembryonic antigen (CEA)	Positive

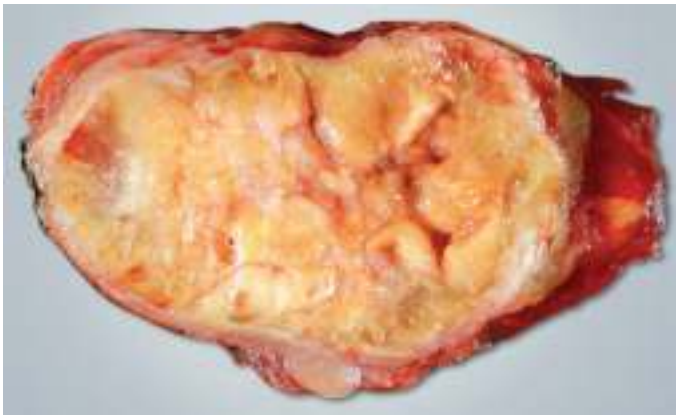


Fig. 26.52: Gross morphology of medullary thyroid carcinoma. Tumor is well circumscribed, fleshy, solid, grey white to yellow brown, firm inconsistency, foci of hemorrhage necrosis are seen. (*Courtesy:* Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

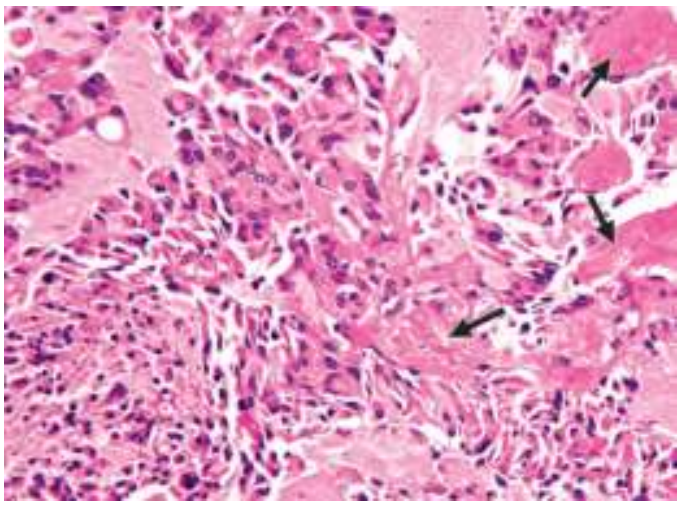


Fig. 26.53: Medullary thyroid carcinoma. The tumor is composed of round to polyhedral granular cells arranged in trabecular, glandular, carcinoid-like patterns. The tumor cells are separated by a distinct fibrovascular stroma containing amyloid material (arrows) representing deposition of procalcitonin (400X).

Mode of Spread

Medullary thyroid carcinoma extends by direct invasion into soft tissues and metastasizes via lymphatic route to the regional lymph nodes, e.g. cervical and mediastinal regions, and distant organs such as lungs, liver, bone, brain, and adrenal medulla by hematogenous route.

Surgical Correlation

Prior to thyroid surgery in MEN IIa and MEN IIb, 24-hour urinary catecholamine estimations are done to confirm pheochromocytomas. Prior to thyroidectomy, pheochromocytoma must be surgically excised first to prevent the risk for severe hypertensive episodes.

Residual Disease Following Surgery or Recurrence

Residual disease of medullary thyroid carcinoma can be detected by measuring calcitonin. Comparison of papillary, follicular and medullary thyroid carcinomas is given in Table 26.31.

Table 26.31 Comparison of papillary, follicular and medullary thyroid carcinomas

Parameters	Papillary Thyroid Carcinoma	Follicular Thyroid Carcinoma	Medullary Thyroid Carcinoma
Incidence	70–80%	17%	6%
Age group	20–30 years	50–60 years	Middle age
Etiology	Irradiation	Endemic goiter	Sporadic or familial
Encapsulation	Rare	Common	Circumscribed
Gross morphology	On cut surface, tumor is usually solid grey white and sometimes cystic mass	Cut surface is grey white soft with areas of hemorrhage (tan), necrosis and cyst formation	Circumscribed, solid, firm with grey white to yellow brown
Cellular pattern	Papillae, follicles, solid cell group	Follicles, trabeculae, no papillae, pseudopapillary in foldings may occur	Round to polyhedral granular cells arranged in trabecular, glandular with carcinoid-like patterns and separated by fibrovascular stroma containing amyloid material representing deposition of procalcitonin
Psammoma bodies	Psammoma bodies are present in fibrovascular stroma (50%)	Psammoma bodies are absent, but calcified colloid may occur	Psammoma bodies are absent
Nuclear features	Ground glass (**Orphan Annie**) appearance, grooves, pseudo-inclusions, often overlapping	Normochromatic or hyperchromatic, no grooves, no overlap	Normochromatic or hyperchromatic, no grooves, no overlap
Amyloid material in tumor	Absent	Absent	Present
Invasion	Strongly invasive, invades in small islands	Variably invasive in large islands with pushing borders	Invades locally
Multiple tumor foci	Common	Rare	Rare
Blood vessel invasion	Rare	Common	Common
Metastases	Lymph nodes	Lungs, liver, bones	Lungs, liver, bones, brain, and adrenal medulla
Prognosis	Excellent	Good	Poor

POORLY DIFFERENTIATED THYROID CARCINOMA

Poorly differentiated thyroid carcinoma is a follicular cell-derived neoplasm, which occupies an intermediate position between well differentiated follicular thyroid carcinoma or papillary thyroid carcinoma and anaplastic thyroid carcinoma in respect of morphology and clinical behavior. Response to radioiodine treatment is poor in poorly differentiated thyroid carcinoma.

Molecular Genetic Alterations

Poorly differentiated thyroid carcinoma has multiple gene mutations load intermediate between papillary thyroid carcinoma and anaplastic thyroid carcinoma. Micro-RNA profile is also different from well differentiated and anaplastic thyroid carcinomas.

Surgical Pathology: Poorly Differentiated Thyroid Carcinoma

Gross Morphology

Tumor size is large with pushing margins and may be partially encapsulated. Cut surface is grayish white and pale areas of necrosis.

Light Microscopy

- Poorly differentiated thyroid carcinoma originates from follicular epithelial cells, which lies between well-differentiated follicular and undifferentiated thyroid carcinomas.
- Diagnostic criteria for poorly differentiated thyroid carcinoma include: (a) presence of at least one of the three growth patterns (i.e. solid, trabecular, insular), (b) absence of the conventional nuclear features of papillary thyroid carcinoma, (c) presence of at least one of the following features: convoluted nuclei, mitotic activity ≥3 per 10 high power fields, and (d) tumor necrosis.
- Tumor with insular growth pattern is composed of uniform small cells with round or convoluted hyperchromatic arranged in solid nests, atypical mitoses, and necrosis. Tumor may contain microfollicles.

ANAPLASTIC THYROID CARCINOMA

Anaplastic thyroid carcinoma is the least common malignancy. It mostly occurs in older patients associated with very poor prognosis. Invasion beyond capsule, blood vessel involvement and foci of infarct necrosis highlight the aggressive rapid growth of anaplastic thyroid carcinoma.

Etiopathogenesis

Anaplastic thyroid carcinoma occurs as a result of anaplastic transformation of a pre-existing well differentiated papillary thyroid carcinoma, and follicular thyroid carcinoma, i.e. insular variant of oncocytic carcinoma. Approximately 50% of cases with multinodular goiter develop anaplastic thyroid carcinoma.

Clinical Features

Rapidly growing tumor in neck region invades esophagus (dysphagia symptom) or trachea (dysphonia symptom) and vocal cord paralysis. Extrathyroid extension is encountered at the time of initial presentation in most cases. Presence of cervical lymph node metastases is responsible for high recurrence.

Molecular Genetic Alterations

TP53 tumor suppressor gene is often demonstrated anaplastic thyroid carcinoma, which may also harbor activating mutation of β-catenin.

Surgical Pathology: Anaplastic Thyroid Carcinoma

Gross Morphology

The tumor is solid with areas of hemorrhage and necrosis.

Light Microscopy

- The tumor does not make thyroid follicles, papillae, or even trabeculae, but it still retains epithelial (squamoid) appearance on morphologic and immunohistochemical grounds.
- Presence of admixture of follicular and papillary element may represent residual of previously existing low-grade thyroid carcinoma (Fig. 26.54).

Histologic Variants: Anaplastic Carcinoma

- **Small cell carcinoma:** Tumor is composed of compact, closely packed cuboidal to polygonal cells growing in cords or clusters and numerous mitoses; separated by a fibrous stroma. It should be differentiated from malignant lymphoma.
- **Carcinosarcoma:** Tumor is composed of malignant epithelial and mesenchymal component.
- **Giant cell carcinoma:** Tumor is composed of highly anaplastic cells having multiple or multilobate nuclei. Other cells may assume spindle shape or elongated with numerous mitoses. Bizarre mitoses and giant cells are characteristic.

Immunohistochemistry Technique

Anaplastic thyroid carcinoma shows positivity with cytokeratins and PAX8 (50%). It is usually negative for TTF-1. PAX8 is useful marker to establish the thyroid origin of tumor.

Markers	Expression
▪ Cytokeratins	▪ Positive
▪ PAX8	▪ Positive (50%)
▪ TTF-1	▪ Negative

SECONDARY THYROID TUMORS

Thyroid gland is a vascular organ, which harbors metastases at a higher frequency than many other organs. The tumors metastasize to thyroid gland either by direct extension from adjacent structures or via hematogenous route.

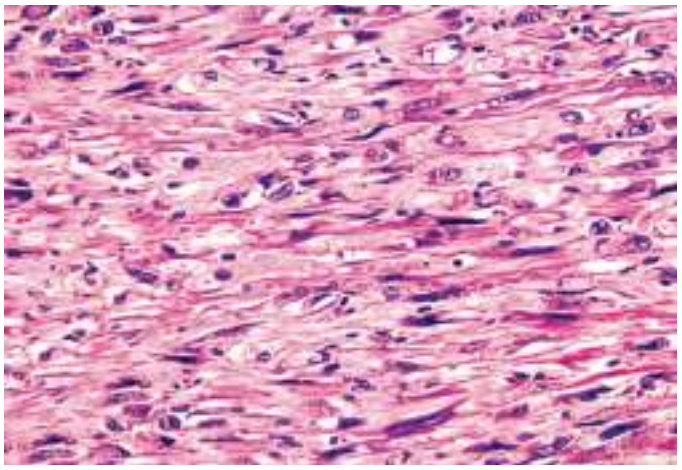


Fig. 26.54: Anaplastic thyroid carcinoma. Tumor is composed of compact closely packed tumor cells arranged in clusters and separated by fibrous stroma. Numerous mitoses are present (400X).

- Squamous cell carcinoma of larynx metastasizes to thyroid gland by direct invasion.
- Most common tumors metastasizing to thyroid gland via hematogenous route are renal cell carcinoma, lung carcinoma, breast carcinoma, colon carcinoma and malignant melanoma.
- Fine needle aspiration cytology (FNAC), histologic examination and immunohistochemistry are useful for planning adjuvant therapy for primary tumor.

THYROID GLAND ANOMALIES

THYROGLOSSAL DUCT CYST

During its normal development, the thyroid gland descends from the base of the tongue to its final position in the neck.

- Thyroglossal duct cyst, a remnant of the thyroglossal duct is the most common thyroid anomaly, which may occur anywhere along the path of descent. It is midline cystic swelling close to or within the hyoid bone.
- Patient presents with dysphonia, sore throat and midline swelling evident during adolescence and pregnancy.
- Patient does not develop endocrine complications. Surgical excision of the proximal duct along with hyoid bone cures it. Thyroglossal duct cyst versus branchial cleft cyst is given in Table 26.32.

ECTOPIC THYROID TISSUE

Ectopic thyroid tissue may be found anywhere along the course of thyroglossal duct.

LINGUAL THYROID

Lingual thyroid occurs due to failure of descent of thyroid gland from the base of the tongue. Patient presents with

Table 26.32 Thyroglossal duct cyst versus branchial cleft cyst

Parameters	Thyroglossal Duct Cyst	Branchial Cleft Cyst
Origin	Thyroglossal duct	Usually second branchial cleft or pouch
Location	▪ Midline location ▪ Usually near hyoid bone with connection through fibrous remnant of thyroglossal duct	▪ Lateral location ▪ Usually along anterior border of sternocleidomastoid muscle near angle of mandible ▪ May be associated with cutaneous or mucosal sinus or fistula
Cyst contents	▪ Mucoid or gelatinous material ▪ Purulent exudate if infected	▪ Serous, mucoid, or pasty ▪ Purulent exudate if infected
Microscopic features	▪ Variable lining • Ciliated respiratory epithelium • Stratified squamous epithelium • Cuboidal epithelium • Granulation tissue secondary to inflammation ▪ Variable acute or chronic inflammation in wall ▪ About half contain thyroid follicles in wall	▪ Usually stratified squamous epithelium • Ciliated respiratory epithelium may be seen • Granulation tissue secondary to inflammation ▪ Lymphoid tissue in wall ▪ Variable acute or chronic inflammation in wall
Age	Usually children to young adults	Usually children to young adults
Gender ratio	Even (males and females equally affected)	Even (males and females equally affected)
Neoplasia	▪ Occurs in 1% or less ▪ Usually papillary carcinoma	▪ Extremely rare ▪ Putative cases usually metastatic squamous cell carcinoma from primary in head or neck

dysphagia for solids and swelling in the base of tongue. ^{123}I is a diagnostic tool to locate the lesion. Lingual thyroid is usually hypofunctional. Lingual thyroid is ablated with thyroxine. Surgical excision is indicated, if lesion causing obstructive symptoms. Forms of thyroid dysgenesis are given in Table 26.33. Differential diagnosis of thyroid tissue with abnormal location in neck is given in Table 26.34.

THYROID DISORDERS: DIAGNOSTIC APPROACH

THYROID FUNCTION TESTS

In many patients with thyroid disease, thyroid gland produces excessive amounts of thyroid T_3 and T_4 hormones (hyperthyroidism) or insufficient amounts of thyroid hormone (hypothyroidism).

Table 26.33 Forms of thyroid dysgenesis

Type of Dysgenesis	Features	Thyroid Function Tests
Ectopic thyroid	Abnormal location of thyroid follicular tissue due to defective descent	Hypothyroidism
Thyroid agenesis	Absence of thyroid follicular tissue in ectopic or orthotropic location	Hypothyroidism
Thyroid hemigenesis	Absence of one of the lateral lobes of thyroid gland	Euthyroid state
Thyroid hypoplasia	Hypoplastic thyroid gland in orthotropic location	Hypothyroidism

Table 26.34 Differential diagnosis of thyroid tissue with abnormal location in neck

Specific Entity	Clinical Examination	Light Microscopic Features
Ectopic non-neoplastic thyroid tissue		
Ectopic thyroid tissue due to abnormal descent of median anlage	▪ Most commonly located in the base of tongue (lingual thyroid) ▪ Present along midline descent tract with well or ill-defined borders and presence of thyroid tissue	▪ Thyroid follicles varying from normofollicular and/or microfollicular patterns lacking features of papillary carcinoma ▪ Thyroid follicles may be admixed with skeletal muscle or other soft tissue
Thyroglossal duct cyst	▪ Thyroglossal duct cyst ranges 1–5 cm with smooth outer surface ▪ Thyroglossal cyst contains gelatinous or mucoid content without thyroid tissue	▪ Cyst lining variable ranging ciliated columnar, and/or cuboidal epithelium or granulation tissue ▪ Foci of thyroid follicles may be present in wall in 50% of cases ▪ Variable admixture of acute and chronic inflammatory cells
Benign lymph node inclusions	Inclusions in lymph node median to jugular vein not grossly visible	Histology lacks features of papillary carcinoma
Parasitic thyroid nodule	Swelling separate from thyroid gland; may be attached by a thin fibrous strand with thyroid nodular hyperplasia	Thyroid nodular hyperplasia seen without evidence of lymph node
Thyroid neoplasms		
Papillary thyroid carcinoma metastasizing to other areas	Present within one or more lymph nodes revealing cystic change	▪ Papillary and/or follicular architecture ▪ Psammoma bodies may be demonstrated ▪ Neoplastic cells are optically clear with **Orphan Annie** appearance
Thyroid neoplasm arising in ectopic non-neoplastic tissue	▪ Usually occurs in the thyroglossal duct cyst ▪ Rarely occurs in lingual thyroid	▪ Papillary carcinoma most common followed by squamous cell carcinoma ▪ Follicular carcinoma and anaplastic carcinoma very rare ▪ Medullary carcinoma unusual
Teratoma	Pediatric cervical teratoma may involve thyroid gland, adult cervical teratoma rarely involves thyroid gland	Teratomas occurring in the cervical region rarely contain thyroid tissue as a component

- The pituitary TSH stimulates the thyroid gland to synthesize and release thyroid hormones. When thyroid hormone levels decrease, the TSH rises and *vice versa*.
- Basically, a normal TSH excludes primary thyroid disease. TSH assay is able to differentiate hypothyroid and hyperthyroid patients from normal individuals. Thyroid carcinoma rarely occurs in a hyperfunctioning nodule.

Serum TSH, T_4 and T_3 Levels

The pituitary TSH stimulates the thyroid gland to make and release the thyroid hormone. When thyroid hormone levels (T_4 and T_3) decrease, the TSH rises and *vice versa*.

Increased serum TSH level is suggestive of hypothyroidism. Decreased serum TSH level suggests hyperthyroidism. Thyroid profile in normal persons and thyroid function tests are given in Tables 26.35 and 26.36.

Thyroid Hormone Binding Protein (TBP)

Antithyroglobulin and antimicrosomal proteins are thyroid hormone binding proteins. Free portion of TBP is active at tissue level. Increased serum level of TBP occurs due to pregnancy, contraceptive pills and increased estrogen level. Increased serum TBP results in increased synthesis of T_4 and T_3 in the body.

Table 26.35 Thyroid profile in normal persons

Parameters	Reference Range
Total serum T_4	5.0–12 µg/dl
Free serum T_4	0.7–1.9 ng/dl
Total serum T_3	80–180 ng/dl
Free serum T_3	2.3–4.2 pg/ml
Radioactive iodine uptake	10–30%

IMAGING TECHNIQUES

Ultrasonography

Ultrasonography detects 2–3 mm thyroid lesion. Cystic nodules in thyroid are seldom malignant. Approximately 80% of patients with thyroid carcinoma do not show cystic change.

Table 26.36 Thyroid function tests in various disorders

Parameters	Total T_4 (µg/dl)	Total T_3 (µg/dl)	TSH [Total T_4 (µl/ml)]	% Radioactive Iodine Uptake by Thyroid Gland in 24 Hours (µg/dl)	Comments
Normal range	5–12	95–190	0.3–5	10–30	
Thyrotoxicosis					
Primary hyperthyroidism (untreated cases)	↑	↑	↓	↑	Thyroid stimulating immunoglobulin increased in Graves' disease
Secondary hyperthyroidism	↑	↑	↑	↑	
Thyrotoxicosis due to treated cases with overdose of T_4	↑	↓	↓	↓	
Thyrotoxicosis due to treated cases with overdose of T_3	↓	↑	↓	Normal or ↑	
Hypothyroidism					
Primary hypothyroidism untreated cases	↓	↓	↑	↓	
Secondary hypothyroidism untreated cases due to pituitary failure	↓	↓	↓	↓	
Euthyroid					
Euthyroid patient treated with T_4	Normal	Varies	Normal or ↓	↓	
Patient on iodine therapy	Normal	Normal	Normal	↓	Correct dosage determined by clinical effects
Euthyroid sick syndrome	Normal or ↓	Normal	Varies	↓	No clinical evidence of hypothyroidism

Solitary thyroid nodules in adult women occur as a cyst in goiter in 60% or a follicular thyroid adenoma in 25% of cases. Approximately 85–90% of cases are euthyroid. Presence of irregular poorly defined margins and microcalcifications are suggestive of thyroid carcinomas.

Computed Tomography (CT) Scan

CT scan defines extent of thyroid lesion, metastases, tracheal and retrosternal involvement.

Radioactive Iodine Uptake and Thyroid Imaging

^{131}I, ^{123}I or ^{99}Tc scanning play major role in evaluation of metastatic disease and in treatment of well-differentiated thyroid carcinoma. Patient is advised to stop levothyroxine drug prior to radionuclide scan.

Although its sensitivity is variable, its specificity in follow-up of thyroid cancer is very high, i.e. 96–100%. Most solitary thyroid nodules are cold, i.e. 95%. Most thyroid carcinomas arise in cold nodules. Risk for thyroid carcinoma in a cold nodule is 10–15%; while negligible in a hot nodule.

Scintigraphy

Scintigraphy is of minimal use in evaluation of solitary thyroid nodules; but useful in diagnosis of recurrent thyroid swellings, retrosternal goiters and thyroid carcinomas.

FINE NEEDLE ASPIRATION CYTOLOGY

Fine needle aspiration cytology (FNAC) plays an important role in diagnosing colloid goiter, Hashimoto's thyroiditis (Fig. 26.55), papillary thyroid carcinoma (Fig. 26.56) and medullary thyroid carcinoma (Fig. 26.57).

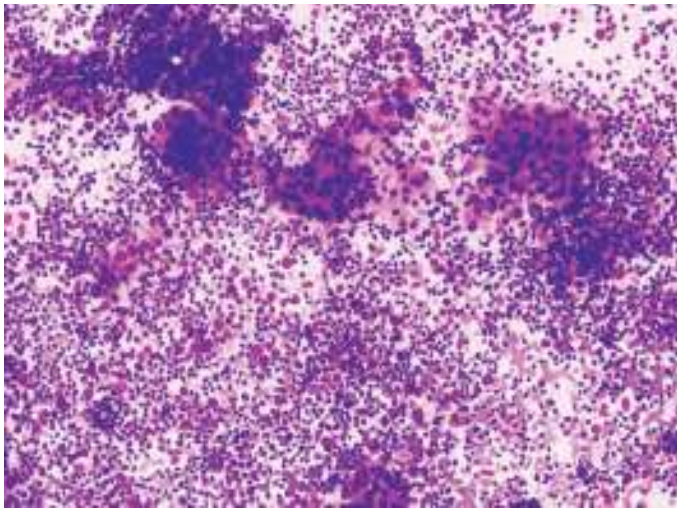


Fig. 26.55: Hashimoto's thyroiditis. FNAC smear shows lymphocytes and plasma cells infiltrating thyroid follicles, Hurhle cell change, multinucleated giant cells and epithelioid cells cluster (400X).

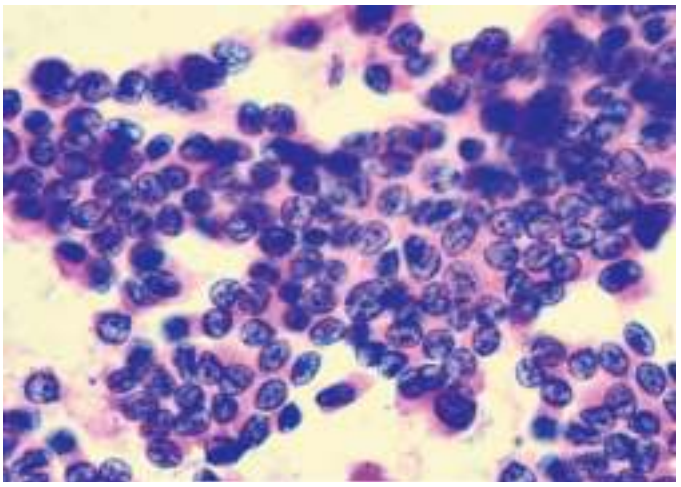


Fig. 26.56: Papillary thyroid carcinoma. FNAC obtained smear shows tumor cells arranged in sheets and forming syncytial aggregates with distinct cell borders, dense cytoplasm, nuclear crowding and overlapping, intranuclear cytoplasmic inclusions and nuclear grooves (400X).

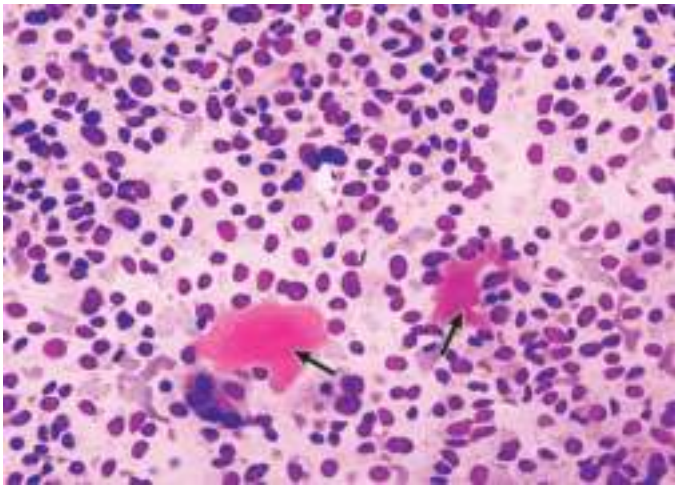


Fig. 26.57: Medullary thyroid carcinoma. Fine needle aspiration cytology obtained smear shows many neoplastic cells and amyloid material deposits. Amyloid material deposits are shown by arrows (400X).

HISTOPATHOLOGIC EXAMINATION

Thyroid biopsy and histologic examination are confirmatory. The specimens are processed, stained by hematoxylin and eosin and examined under microscope. Monoclonal antibodies used in diagnosis of thyroid carcinomas are given in Table 26.37.

MOLECULAR GENETIC TESTING

Mutation of the RET proto-oncogene is also responsible for familial medullary thyroid carcinoma and MEN IIb. Prophylactic thyroidectomy is performed in these cases. Genetic alterations in histologic variants of thyroid carcinoma is given in Table 26.38.

Table 26.37 Monoclonal antibodies used in diagnosis of thyroid carcinomas

Antibody	Specificity	Immunoreactivity	Interpretation
Thyroglobulin	Thyroid follicular differentiation	Follicular adenoma/follicular carcinoma, papillary carcinoma, poorly differentiated carcinoma of thyroid	Anaplastic carcinoma is negative for thyroglobulin. This marker is invaluable for confirming thyroid origin of metastatic tumor
Calcitonin	Parafollicular cells (C cells)	Medullary thyroid carcinoma	Calcitonin immunoreactivity in metastatic medullary carcinoma of thyroid
Chromogranin	Pan-neuroendocrine tumor marker	Medullary thyroid carcinoma, paraganglioma. Intrathyroid–parathyroid tumor, metastatic neuroendocrine tumor	Chromogranin supports a diagnosis of medullary carcinoma of thyroid
Synaptophysin	Pan-neuroendocrine most sensitive and specific marker	Similar to chromogranin (medullary carcinoma, paraganglioma. Intrathyroid–parathyroid tumor, metastatic neuroendocrine tumor)	Similar to chromogranin supports a diagnosis of medullary carcinoma of thyroid
Thyroid transcription factor 1 (TTF-1)	TTF-1 is a transcriptional factor	Follicular carcinoma (100%), papillary carcinoma (100%), medullary carcinoma (100%), anaplastic carcinoma (0–50%) of thyroid	TTF-1 is also positive in lung cancers (adenocarcinoma, small cell carcinoma and carcinoid tumor)
Cytokeratin	Epithelial marker	Follicular epithelial tumors, intrathyroid–parathyroid tumors	Neuroendocrine tumors positive
Leukocyte common antigen (LCA)	Leukocyte-specific marker	Lymphoma, leukemia	Useful in work up of undifferentiated thyroid tumors
S-100 protein	Expressed in normal thyroid follicular cells and especially Hürthle cells	Many thyroid tumors are positive for S-100 protein	Not used in diagnosis of thyroid carcinoma
Parathormone	Specific for parathyroid cells	Intrathyroid–parathyroid tumors	Most useful in diagnosis of parathyroid origin tumors

SERUM TUMOR MARKERS

Serum Calcitonin

Plasma calcitonin, the product of the parafollicular cells, is the most sensitive marker for diagnosis and monitoring of medullary thyroid carcinoma. It should be measured when medullary thyroid carcinoma is suspected in a patient with a positive family history.

Serum Thyroglobulin

Thyroglobulin is a reliable marker of diagnosing papillary thyroid carcinoma. It shows positivity for tumor cells in primary site including recurrent disease or secondary sites such as lymph nodes and distant metastases.

Serum Carcinoembryonic Antigen

Medullary thyroid carcinoma (MTC) originates from parafollicular cell and synthesizes excess of calcitonin (neuroendocrine peptide) and carcinoembryonic antigen. CEA is an excellent marker for residual MTC disease.

Table 26.38 Genetic alterations in histologic variants of thyroid carcinoma

Histologic Variants
Papillary thyroid carcinoma
▪ RET tyrosine kinase receptor (10q) arrangement ▪ BRAF V600E mutation
Follicular thyroid carcinoma
▪ RAS family of oncogene mutation ▪ t(2,3) fusion of PAX8 and PPAR-γ
Medullary thyroid carcinoma
RET proto-oncogene mutation (MEN II)
Anaplastic thyroid carcinoma
▪ TP53 mutation ▪ BRAF V600E mutation

24 HOURS URINARY CATECHOLAMINES ASSAY

In patients with neuroblastoma (most common) and pheochromocytoma, adrenal gland synthesizes excess of catecholamines (dopamine, norepinephrine, and epinephrine), which are degraded to inactive metabolic products in brain and liver, excreted in the urine. In MEN II syndrome (medullary thyroid carcinoma and pheochromocytoma), 24 hour-urine catecholamines assay is done to exclude pheochromocytoma prior to medullary thyroid carcinoma surgery.

DISORDERS OF PARATHYROID GLANDS

ANATOMY OF PARATHYROID GLANDS

There are two each superior and inferior parathyroid glands embedded on the posterior surfaces of the lateral lobes of the thyroid gland. Parathyroid glands are derived from fourth pharyngeal and third pharyngeal pouches, respectively.

Parathyroid glands consist of principal/chief and oxyphil cells arranged in cords; supported by a network of reticulin fibers. Numerous sinusoids lie in close relationship to the cells.

PHYSIOLOGY OF PARATHYROID GLANDS

Principal/chief cells synthesize parathormone, which regulates the homeostasis of calcium and phosphate ions by increasing blood calcium level and decreasing phosphate level. Secretion of parathormone (PTH) is regulated by calcium level in the blood. Secretion of parathormone is not under control of pituitary gland.

CALCIUM METABOLISM: REGULATION

Calcium metabolism is regulated by parathormone and clacitonin.

- **Parathormone (PTH):** Decreased plasma concentration of ionized calcium stimulates principal cells to synthesize parathormone. Parathormone maintains ionized calcium level in blood by release of calcium due to bone resorption, and retarding renal excretion of calcium in the urine. Thus, serum calcium level is increased. Parathormone decreases phosphorus reabsorption in the proximal renal tubule and increases the synthesis of 1α-hydroxylase in proximal renal tubules.

  The 1α-hyroxylase enzyme converts 25-hydroxycholecalciferol (vitamin D_2) to 1, 25-dihydroxycholecalciferol (vitamin D_3). Decreased serum calcium and increased phosphate levels stimulate the synthesis of parathormone (Fig. 26.58).
- **Calcitonin:** Increased plasma calcium concentration stimulates thyroid parafollicular cells (C cells) to synthesize calcitonin.
  - Calcitonin promotes deposition of calcium into bone matrix. With respect to regulation of blood Ca^{++}, calcitonin and parathormone are antagonists.
  - Total body calcium in adults is approximately 1.0 kg. It is present in cellular compartment of bones, i.e. 995 g and skeletal muscles, i.e. 4–5 g. Calcium is circulating in blood in three forms.

Calcium in Blood

- **Albumin bound calcium (45%):** It is physiologically inactive form.
- **Calcium complexed with citrates and phosphates (5%):** It is diffusible, non-ionized, but physiologically inactive.
- **Free ionized calcium (50%):** It is diffusible metabolically active form. Normal free ionized calcium maintains normal parathormone levels.

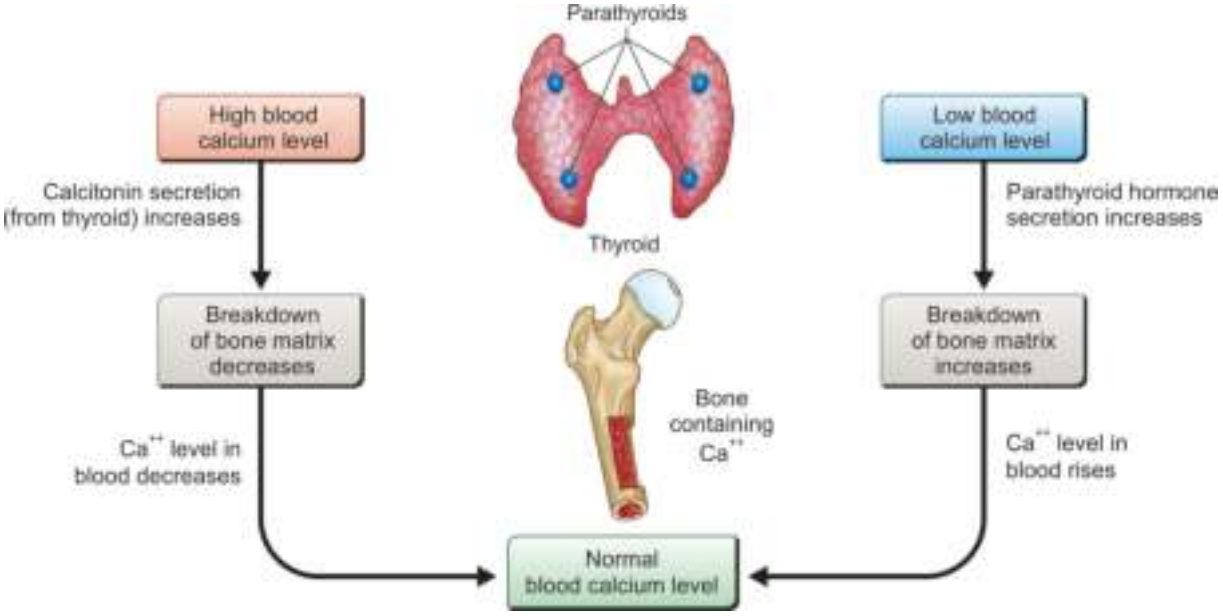


Fig. 26.58: Regulation of calcium by parathormone and calcitonin.

Calcium Absorption

Gastric hydrochloric acid makes calcium in ionic form and hence gets easily absorbed by whole length of small intestine.

- Calcium absorption is increased in younger and normal secretion of bile.
- While absorption is decreased due to aging process, phosphates in diet and decreased bile secretion, which results in increased fatty acids in the gut which form calcium salts.

Calcium Functions

Calcium participates in bone calcification, excitation/contraction of muscles, stabilization of cell membranes, release of neurotransmitters, secretion of material from glands, synthesis of nucleic acids and proteins, promotion of mitosis in thymus, bone marrow, activation, regulation of enzymes and blood coagulation.

VITAMIN D METABOLISM: REGULATION

Vitamin D is normal requisite for normal mineralization of bone and cartilage. It maintains normal blood levels of calcium and phosphates.

Vitamin D collaborates with parathormone in mobilizing calcium from bone and stimulates parathormone-dependent reabsorption of calcium in distal renal tubules. It stimulates intestinal absorption of calcium independent phosphorus.

Vitamin D Sources

Preformed vitamin D in the diet (20%) consists of cholecalciferol derived from sea fish and ergocalciferol, i.e. vitamin D_2 in plants, and grains. Endogenous synthesis of vitamin D in the skin occurs by photoconversion of 7-dehydrocholesterol via sunlight to vitamin D_3, i.e. cholecalciferol. Dietary source of vitamin D is reabsorbed by small intestine.

Vitamin D Transport

Vitamin D binds with vitamin D binding proteins to form complex is carried to liver.

Vitamin D Metabolism in Organs

- **Liver:** In liver, first hydroxylation of vitamin D conversion to 25-hydroxyvitamin D occurs in the liver cytochrome 450 enzyme system. This conversion is augmented by calcium. 25-hydroxyvitamin D and 5–10 times more active than parent vitamin D.
- **Kidney:** In kidneys, second hydroxylation of vitamin D occurs by 1α-hydroxylase enzyme synthesized in proximal renal tubule cells. The enzyme converts 25-hydroxycholecalciferol (vitamin D_2) to calcitriol (vitamin D_3).

Vitamin D Storage Sites

Vitamin D is stored in adipose tissue and liver.

Vitamin D_3 (Calcitriol): Functions

Vitamin D attaches to nuclear receptors in target tissues. It increases reabsorption of calcium in the duodenum, and phosphorus in jejunum and ileum.

- Vitamin D increases bone mineralization by alkaline phosphatase derived from osteoclasts. Alkaline phosphatase participates in bone mineralization by hydrolysis of inhibitors (pyrophosphate), which prevent calcium phosphate mineralization.
- Vitamin D increases the production of osteoclasts from macrophage stem cells. Parathormone and vitamin D_3 act on distal convoluted tubules, hence Ca^{++} absorption occurs.

Vitamin D Deficiency: Causes

- Causes of vitamin D include low dietary intake, decreased synthesis of vitamin D, malabsorption of fats, and deranged metabolism of vitamin D in chronic renal failure.
- Depending on severity, duration, stresses on bones and age of the patients, vitamin D deficiency causes failure of bone mineralization resulting in excessive formation of mineralized matrix and abnormally wide osteoid seams.

HYPERPARATHYROIDISM

Hyperparathyroidism can be primary as a result of autonomous proliferation of chief cells or may be secondary, in which case it is a compensatory mechanism. Disorders of calcium homeostasis are given in Table 26.39.

PRIMARY HYPERPARATHYROIDISM

Primary hyperparathyroidism is most common cause of hypercalcemia. It occurs in postmenopausal women. More than 50% of patients are asymptomatic. It is diagnosed when an asymptomatic patient has increased serum calcium levels in routine laboratory tests. Women are more affected than men over 40 years of age. Pathophysiology of hyperparathyroidism is shown in Fig. 26.59.

Etiopathogenesis

Primary hyperparathyroidism occurs due to parathyroid adenoma in 80% of cases, hyperplasia in 20% or carcinoma (rarely). Primary hyperparathyroidism can occur as a part of MEN I and MEN IIa. Parathormone like hormone may be synthesized by bronchogenic squamous cell carcinoma or renal cell carcinoma.

Table 26.39 Disorders of calcium homeostasis

Disorder	Parathormone (PTH)	Calcium	Phosphate
Primary hyperparathyroidism due to parathyroid adenoma and hyperplasia	Increased	Increased	Decreased
Secondary hyperparathyroidism due to chronic renal failure or vitamin D deficiency	Increased	Decreased	Increased
Tertiary hyperparathyroidism due to autonomous transformation of secondary hyperparathyroidism	Increased	Increased	Increased
Primary hypoparathyroidism	Decreased	Decreased	Increased
Pseudohypoparathyroidism	Increased	Decreased	Increased
Vitamin D deficiency	Increased	Decreased	Decreased

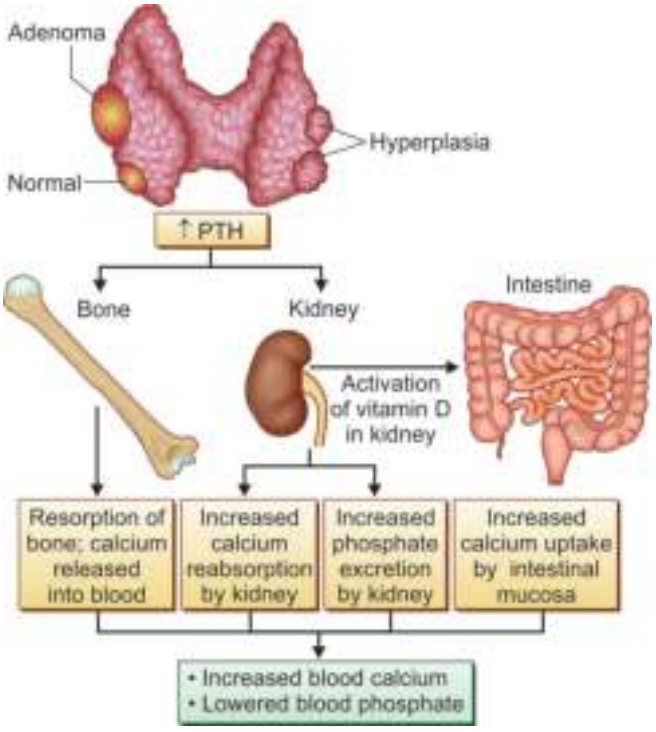


Fig. 26.59: Pathophysiology of hyperparathyroidism.

Clinical Features

Patient develops symptoms due to increased bone resorption, i.e. osteitis fibrosa cystica by osteoclasts resulting in increased serum calcium levels. Skeletal manifestations are less common. Patient presents with bone pains and pathologic fractures due to generalized demineralization of skeleton. Clinical manifestations of hyperparathyroidism is shown in Fig. 26.60.

Primary Hyperparathyroidism: Bone Lesions

- Osteoclastic bone resorption results in osteitis fibrosa cystica also known as brown tumor in jaw and long bones.
- When osteitis fibrosa cystica is generalized, it is called von Recklinghausen disease of bone. The lesion is expansile and multilocular with solid and cystic areas. Cystic areas have a brown color due to presence of abundant hemosiderin.
- Radiologic examination reveals decrease in bone densities, i.e. osteoporosis, bone deformities, chondrocalcinosis, i.e. pseudogout, and fractures. Subperiosteal bone resorption occurs along margins of middle phalanges on radial aspect of fingers and tooth sockets. X-ray skull shows salt and pepper appearance.
- Histologic examination of tissue section reveals loss of osseous tissue, poorly calcified immature bone formation, numerous osteoclasts associated with cystic areas and hemosiderin-laden macrophages.

Metastatic Calcification

Metastatic calcification occurs in various tissues such as kidney, lungs, cornea, blood vessels, fundal glands of stomach and heart. Blood vessels show metastatic calcification in media and internal elastic lamina.

- **Renal changes:** Calcium stones are most common presentation. Metastatic calcification occurs in tubular epithelial cells and basement membranes in acute cases, while in chronic cases, calcification occurs in whole nephron including collecting ducts known as nephrocalcinosis with formation of calcific casts, foci of fibrosis, nephron atrophy and chronic inflammatory cells infiltration, thereby formation of calcium oxalate calculi which may cause hydronephrosis and chronic pyelonephritis.
- **Peptic ulcer disease:** Hypercalcemia predisposes to duodenal peptic ulcer. Calcium stimulates gastrin, which increases gastric acid secretion.
- **Acute pancreatitis:** Calcium activates phospholipase that causes acute pancreatitis. Patient may develop constipation. Serum amylase is analyzed in these patients.
- **Diastolic hypertension:** Diastolic hypertension occurs due to hypercalcemia. Electrocardiogram shows shortening of QT interval.
- **Ocular changes:** Patient develops band keratopathy in the limbus of the eye due to metastatic calcification.
- **Central nervous system changes:** Patient may develop psychosis, confusion, anxiety and coma.

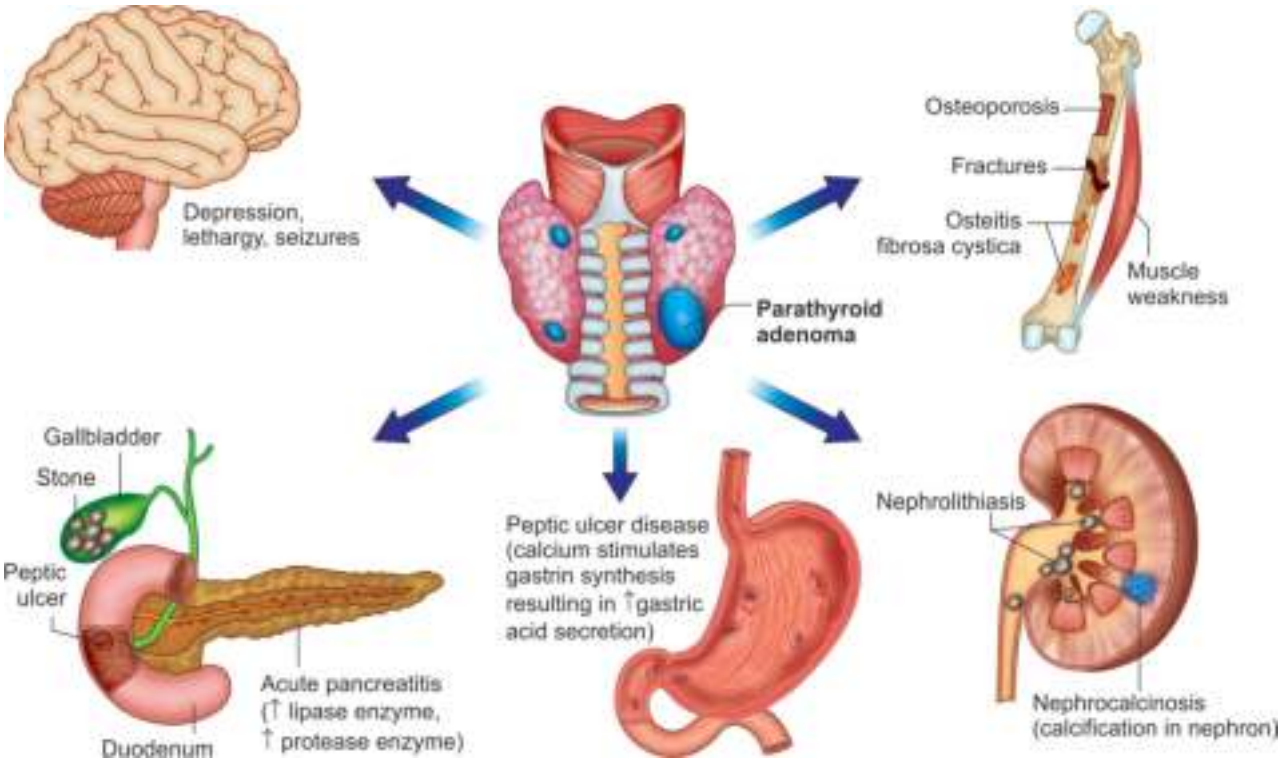


Fig. 26.60: Clinical manifestations of hyperparathyroidism.

Laboratory Diagnosis

Serum parathormone (PTH) analysis is the best initial screening test. Patient with bone metastases has increased serum calcium level with normal PTH.

- Technetium 99m sestamibi radionuclide scan is done to localize parathyroid adenoma. Serum calcium, phosphorus and alkaline phosphatase are increased.
- Urine analysis shows increased excretion of calcium. Decreased tubular reabsorption of phosphorus and increased excretion of phosphorus result in decreased serum phosphorus levels.

SECONDARY HYPERPARATHYROIDISM

Decreased concentration of serum ionized calcium due to renal retention of phosphate causes compensatory hyperplasia of parathyroid glands resulting in secondary hyperparathyroidism.

Etiopathogenesis

Chronic kidney disease and intestinal malabsorption are important causes of secondary hyperparathyroidism. Diabetic glomerulosclerosis is a major cause of renal insufficiency. Uncommon causes of secondary hyperparathyroidism are Fanconi syndrome, and renal tubular acidosis. Advanced renal disease contributes to reduction in serum calcium. Other causes are rickets and vitamin D deficiency.

- **Physiologic state:** Normally, 1α-hydroxylase enzyme synthesized in the proximal renal tubule cells converts 25-hydroxycholecalciferol (vitamin D_2) to 1, 25-dihydroxycholecalciferol (vitamin D_3) also called calcitriol.
- **Pathologic state:** In chronic kidney disease, kidneys fail to convert 25-hydroxycholecalciferol (D_2) to 1, 25-dihydroxycholecalciferol (D_3). Deficiency of vitamin D_3 impairs reabsorption of calcium in the duodenum, and phosphorus in jejunum and ileum. Chronic kidney disease also impairs reabsorption of calcium by distal tubules. Decreased serum calcium level stimulates parathormone.
  - Bone changes in chronic kidney disease are known as renal osteodystrophy. Bone changes such as diffuse osteoclastic bone disease and metastatic calcification are similar to those seen in primary hyperparathyroidism. Patient may develop tertiary hyperparathyroidism. Parathyroid glands become autonomous regardless of calcium level. It may bring serum calcium into normal or increased range.
  - Blood vessels show metastatic calcification in media and internal elastic lamina, which is more severe in secondary hyperparathyroidism. Some patients develop ischemic skeletal muscle pains in extremities and even gangrene. Stomach, heart, lungs and eyes also show ischemic changes.

Laboratory Diagnosis

Radioimmunoassay determination of circulating parathormone is roughly proportionate to the severity of kidney disease. Serum calcium is decreased. There is increased serum levels of phosphorus, alkaline phosphatase and parathormone.

TERTIARY HYPERPARATHYROIDISM

Persistent parathyroid hyperfunction occurs in spite of correction of hypocalcemia and pre-existing secondary hyperparathyroidism. Most often, the cause is development of an adenoma in a previously hyperplastic parathyroid gland.

α-HYDROXYLASE DEFICIENCY (VITAMIN D-RESISTANT RICKETS)

Patient with vitamin D-resistant rickets has increased secretion of parathormone. But congenital deficiency of 1α-hydroxylase in renal tubular cells impairs hydroxylation of 25-hydroxycholecalciferol. Kidneys are not able to reabsorb calcium, in spite of increased plasma parathormone levels.

HYPOPARATHYROIDISM

Hypoparathyroidism occurs due to deficiency of parathormone resulting in decreased plasma calcium level.

- Most common cause of hypoparathyroidism is accidental surgical excision of parathyroid glands during thyroidectomy. Autoimmune hypothyroidism is common cause of hypoparathyroidism.
- DiGeorge syndrome is characterized by congenital thymic hypoplasia as a result of failure of descent of third and fourth pharyngeal pouches between 2nd and 10th week of embryogenesis. When this midline development does not occur properly, the parathyroid glands are not formed.
- Hypomagnesemia occurs due to diarrhea, aminoglycosides, diuretics and alcoholism. Magnesium is a cofactor for adenyl cyclase. Cyclic adenosine monophosphate (cAMP) is required for parathormone activation.
- Patient with hypoparathyroidism may develop tetany, cataracts, loss of hair, abnormal nail growth, increased bone density, convulsions, papilledema, gastrointestinal disturbances, and calcification of blood vessels of basal ganglia of nervous system. Increased phosphorus level drives calcium into the brain tissue.

TETANY (TETANIC SEIZURE)

Tetany is a classical sign and symptom of low serum-free ionized calcium levels in blood (hypocalcemia) that involves involuntary contraction of skeletal muscles that leads to painful muscle cramps, spasms of larynx and sensory disturbances. Hypocalcemia can result from a decreased level of parathormone (PTH) in the settings of hypoparathyroidism, surgical removal of parathyroid gland and DiGeorge syndrome.

- Maintaining stable normal ionized calcium level in blood plays an important role in stabilizing the resting membrane potential of neurons, thereby preventing their spontaneous activation. Blood calcium level is regulated by parathormone, which is released into the blood circulation, when serum levels are too low.
- Low serum-free ionized calcium in blood causes increased neuromuscular hyperexcitability of nerves and skeletal muscles by decreasing the threshold needed for the activation of neurons. As a result, neurons become unstable and fine spontaneous action potentials that trigger the involuntary contraction of the skeletal muscles, which eventually leads to tetany.
- The main goal of treatment of tetany is to normalize calcium levels in blood using calcium supplements and, if appropriate, vitamin D and magnesium supplementation.

Clinical Features

Patient presents with quick ipsilateral facial muscle contraction on tapping the facial nerve at the external auditory meatus (Chvostek's sign) or carpopedal spasm—interphalangeal joint extension, metaphalangeal joint flexion, and thumb adduction elicited by occluding the brachial artery of arm with a blood pressure cuff (Trousseau's sign).

Laboratory Diagnosis

Tetany is most often suspected in persons with muscle spasms, paresthesias affecting the hands and feet and numbness around mouth. In some cases, Trousseau's sign and Chvostek's sign can be indicative of hypocalcemia-related tetany. A tetany diagnosis is most often confirmed by evidence of low calcium levels in blood. In addition, blood levels of parathormone (PTH), vitamin D (1,25-hydroxyvitamin D_3), magnesium and phosphorus may help in identifying underlying cause of tetany in an individual.

Table 26.40 Revised 2024 WHO classification of parathyroid tumors

Histologic Diagnostic Criteria	Immunohistochemistry Technique
Parathyroid adenoma	
▪ Parathyroid adenoma encapsuled ▪ Capsule lacks tumor cells infiltration ▪ No atypical features of tumor cells	▪ Parafibromin is encoded by CDC73 gene ▪ Parafibromin positive/negative: no risk of recurrence of tumor ▪ Parafibromin negative: need for CDC73 gene sequencing ▪ FGP9.5 positive ▪ APC positive ▪ Galectin 3 negative ▪ Ki-67 labeling index (<1%)
Atypical parathyroid adenoma	
▪ Cellular nests in a thickened connective tissue ▪ Capsule shows tumor cells infiltration ▪ Adherence to adjacent structures without frank invasion ▪ Band-like fibrosis ▪ Trabecular growth ▪ Increased mitotic activity (>5 per 10 mm^2) ▪ Atypical mitotic figures ▪ Coagulative necrosis ▪ Ki-67 labeling index (>5%) ▪ Other immunohistochemical aberrancies ▪ Low-risk of tumor recurrence	▪ Parafibromin is encoded by CDC73 gene ▪ Parafibromin positive: very low-risk of tumor recurrence, and no need for CDC73 gene sequencing ▪ Parafibromin negative: low-risk of tumor recurrence and need for CDC73 gene sequencing ▪ FGP9.5 positive/negative ▪ APC positive/negative ▪ Galectin 3 positive/negative ▪ Ki-67 upregulation/downregulation
Parathyroid carcinoma	
▪ Angioinvasion ▪ Lymphatic invasion ▪ Perineural invasion ▪ Unequivocal invasion into adjacent structures ▪ Histologically confirmed metastasis ▪ Parafibromin (PFIB) positive/negative on immunohistochemistry ▪ High-risk of tumor recurrence	▪ Parafibromin is encoded by CDC73 gene ▪ Parafibromin positive/negative: high-risk of recurrence and need for CDC73 gene sequencing ▪ FGP9.5 positive ▪ APC negative ▪ Galectin 3 positive ▪ Ki-67 upregulation

Breast carcinoma, renal cell carcinoma, lung carcinoma and melanoma can metastasize to parathyroid glands.

PSEUDOHYPOPARATHYROIDISM

Pseudohypoparathyroidism is characterized by end-organ unresponsiveness to parathormone.

Molecular Genetic Alterations

Pseudohypoparathyroidism is related to gene mutation, whose product couples hormone receptors to the stimulation of adenylyl cyclase. Gene mutation impairs the synthesis of cAMP by renal tubular epithelium resulting in inadequate resorption of calcium from the glomerular filtrate.

Clinical Features

Patient develops **Albright hereditary osteodystrophy**, characterized by short stature, obesity, mental retardation, subcutaneous calcification, and a number of congenital anomalies of bone.

NEOPLASTIC DISORDERS OF PARATHYROID GLANDS

Tumors of parathyroid glands include parathyroid adenoma, parathyroid carcinoma and secondary tumors. Revised 2024 WHO classification of parathyroid tumors is given in Table 26.40.

PARATHYROID ADENOMA

Parathyroid adenoma usually involves right inferior parathyroid gland. It is the cause of primary hyperparathyroidism in 85% of cases. Tumor arises sporadically or as a part of multiple endocrine neoplasia (MEN I and MEN IIa, 20% of cases).

In a small number of cases of sporadic adenoma, molecular genetic analysis has identified rearrangement and overexpression of the cyclin D proto-oncogene.

Surgical Pathology: Parathyroid Adenoma

Gross Morphology

Tumor appears as well circumscribed, encapsulated, reddish brown, solitary mass, measuring 1–3 cm in diameter.

Light Microscopy

- Tumor is composed of sheets of chief cells in a rich capillary network with no intervening adipose tissue.
- A rim of normal parathyroid tissue is usually evident outside the tumor capsule and distinguishes adenoma from parathyroid hyperplasia.

PARATHYROID CARCINOMA

Parathyroid carcinoma is a rare malignant neoplasm. Most patients present with hyperparathyroidism and hypercalcemia (blood calcium >16 mg/dl with secondary nephrolithiasis, renal insufficiency, osteopenia and/or brown tumor of bone, weakness, fatigue and depression. A palpable neck mass may be present.

Surgical Pathology: Parathyroid Carcinoma

Gross Morphology

- Tumor is usually large, poorly circumscribed and adherent to surrounding structures especially thyroid gland. It ranges from 1.5 to 6 cm or larger with average weight 6.7 kg.
- Cut section is usually firm, pinkish tan with lobular appearance.

Light Microscopy

- Tumor exhibits nodular and solid patterns. Tumor is composed of uniform chief cells with mild to moderate nuclear atypia; and increased mitoses. Broad fibrous bands can be present.
- One of the following features is essential for definitive diagnosis, which include vascular invasion, lymphatic invasion, perineural invasion, invasion of adjacent structures, organs and distant metastasis.

Management

The tumor is difficult to separate from the surrounding structure during surgery. En bloc surgical resection with ipsilateral thyroid lobe is the recommended treatment.

DISORDERS OF ADRENAL GLANDS

ANATOMY AND PHYSIOLOGY

Each adrenal gland consists of cortex and medulla.

ADRENAL CORTEX

Adrenal cortex consists of zona glomerulosa, zona fasciculata and zona reticularis. Adrenal cortex participates in synthesis of glucocorticoids, mineralocorticoids and sex hormones. Critical enzymes in the biosynthetic pathways of adrenal cortex include 11β-hydroxylase and 21-hydroxylase.

Zona Glomerulosa

Zona glomerulosa secretes mineralocorticoids mainly aldosterone and deoxycorticosterone. Aldosterone acts on distal convoluted tubules, which results in absorption of Na^+, Cl^- and water; and hence controls electrolyte and water balance. In exchange K^+ enters the tubules.

Zona Fasciculata

Zona fasciculata synthesizes cortisol, corticosterone and cortisone, which participate in gluconeogenesis in the liver resulting in increased blood glucose level. These hormones cause breakdown of fat, i.e. lipolysis and proteins, i.e. proteolysis and inhibit cellular uptake and utilization of amino acids. These hormones are anti-inflammatory and immunosuppressants.

Zona Reticularis

Zona reticularis synthesizes sex hormones, i.e. androgens and estrogens and glucocorticoids. Their main actions on gonads result in development of secondary sexual characteristics, particularly those of the male, i.e. growth of axillary hairs, pubic hairs and facial hairs during puberty. Concentrations of these hormones in adults are so low that their effects are usually insignificant (Figs 26.61 to 26.63).

ADRENAL MEDULLA

Adrenal medulla synthesizes catecholamines, i.e. adrenaline and noradrenaline, also called **emergency hormones** or hormones of fight or flight in response to stress.

Adrenaline (Epinephrine)

Adrenaline mainly acts on skeletal muscles, cardiac muscles, smooth muscles, blood vessels and fat cells. Adrenaline converts liver glycogen into glucose, thus blood glucose is increased. It raises blood pressure; accelerates heart rate and force of heartbeat. Adrenaline causes constriction of capillaries of skin and visceral smooth muscle; dilation of arterioles of heart and skeletal muscles; increase in oxygen consumption; erection of hairs, i.e. piloerection; dilation of pupils and initiation of stress responses.

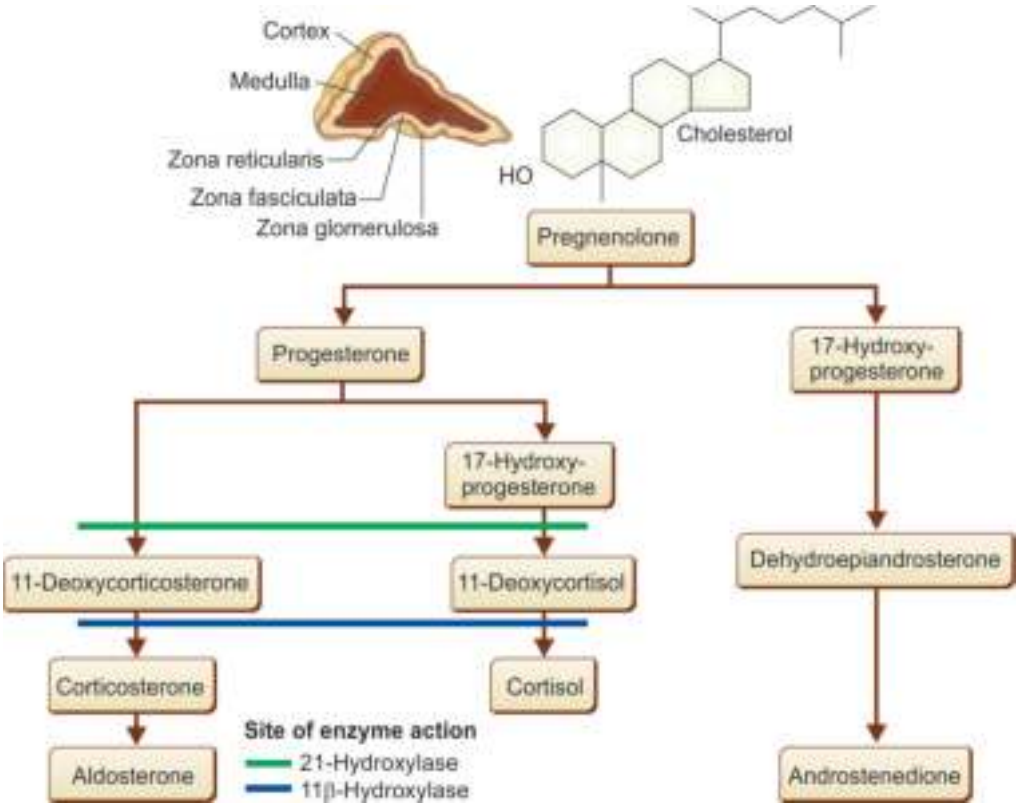


Fig. 26.61: The adrenal gland shows the medulla and the three layers of the cortex. Outer layer of the cortex (zona glomerulosa) is primarily responsible for mineralocorticoid production, and the middle layer (zona fasciculata) and the inner layer (zona reticularis) produce the glucocorticoids and the adrenal androgens.

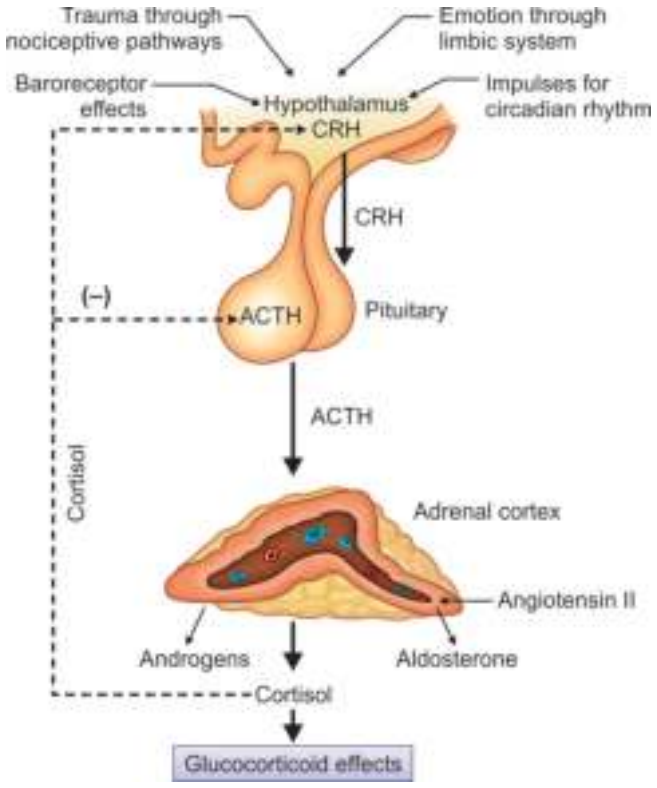


Fig. 26.62: Regulation of glucocorticoids synthesis.

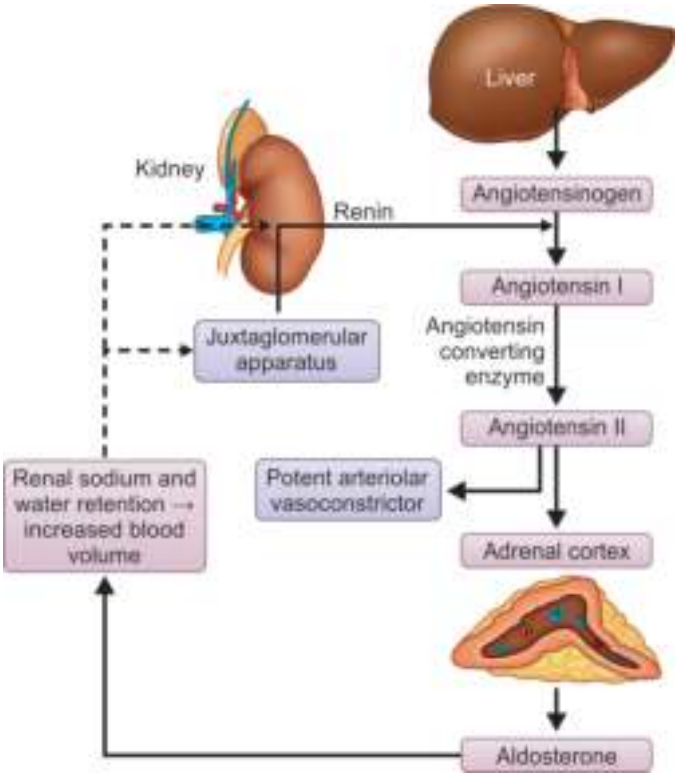


Fig. 26.63: Renin-angiotensin-aldosterone system.

Noradrenaline (Norepinephrine)

Noradrenaline also mainly acts on skeletal muscles, cardiac muscles, smooth muscles, blood vessels and fat cells. It converts liver glycogen into glucose, resulting in increased blood glucose. The functions of noradrenaline are similar to adrenaline.

ADRENAL CORTEX HYPERFUNCTIONING

Excessive synthesis of corticosteroids occurs due to adrenal cortical adenoma and carcinoma. Cushing's syndrome and Conn's syndrome are disorders of adrenal cortex due to excess of corticosteroids and aldosterone respectively.

Cushing's disease is reserved for excess synthesis of ACTH by pituitary corticotroph tumors.

CUSHING'S SYNDROME

Cushing's syndrome results from excess of circulating glucocorticoids especially cortisol.

- Cushing disease is reserved for excess of adrenocorticotropic hormone by pituitary corticotroph tumors. Cushing's disease due to pituitary disorders is five times more common than Cushing's syndrome.
- Cushing's syndrome most often occurs in women between 20 and 45 years of age than men. Causes of Cushing's syndrome are given in Table 26.41.

Etiopathogenesis

- **Therapeutic administration of corticosteroids:** Most common cause of Cushing's syndrome is the prolonged administration of corticosteroids in the treatment of immunological and inflammatory diseases. Long-term administration of corticosteroids causes increased bone resorption and decreased bone formation, thereby resulting in osteoporosis. Approximately 20% of patients with Cushing's syndrome suffer from compression fractures of the vertebrae.
- **Adrenal gland disorders:** Adrenal cortical adenoma, carcinoma and hyperplasia synthesize excess of corticosteroids resulting in Cushing's syndrome.
- **Pituitary disorders:** Pituitary corticotroph microadenoma, adenoma and hyperplasia synthesize excess of corticosteroids resulting in Cushing's disease. Cushing's disease is reserved for excess synthesis of ACTH by pituitary corticotroph tumors.
- **Respiratory system:** Small cell lung carcinoma and bronchial carcinoid tumor synthesize ectopic corticosteroids resulting in atypical Cushing's syndrome.

Clinical Features

Clinical manifestations of Cushing's syndrome depend on the degree and duration of excessive corticosteroid levels, as well as on the levels of adrenal androgens and mineralocorticoids.

Patient presents with redistribution of subcutaneous fat with round moon face, dorsal buffalo hump and diabetes mellitus (Fig. 26.64).

Physical Examination

Physical examination reveals relatively thin extremities caused by muscle wasting (muscle weakness); skin atrophy with easy bruising and purplish striae, especially over the abdomen, and hirsutism.

Other clinical manifestations include osteoporosis, amenorrhea, hypertension and psychiatric dysfunction.

Diagnostic Approach

In addition to dexamethasone suppression test, ACTH determinations are useful diagnostic measures in determining the cause of hypercorticism.

ACTH is increased in pituitary hypercorticism and ectopic ACTH production. ACTH is low in hypercorticism of adrenal origin. Half of patients exhibit absolute lymphopenia. Dexamethasone suppression test in Cushing's syndrome is shown in Fig. 26.65.

HYPERALDOSTERONISM

Hyperaldosteronism is a disorder in which the adrenal gland releases too much aldosterone hormone into the blood. Urine aldosterone is elevated in both primary and secondary aldosteronism. There is decreased

Table 26.41 Causes of Cushing's syndrome

Etiology	Disorders	ACTH Dependent or Independent
Therapeutic administration of corticosteroids	Corticosteroid administration in immunological and inflammatory disorders	–
Adrenal causes	Adrenal hyperplasia, adrenal cortical adenoma, adrenal cortical carcinoma	ACTH independent
Pituitary gland causes	Corticotrope microadenoma, corticotrope adenoma, corticotrope hyperplasia	ACTH dependent
Respiratory system	Small cell lung carcinoma, bronchial carcinoid tumor	ACTH dependent

Cushing's disease is reserved for excess synthesis of ACTH by pituitary corticotroph tumors.

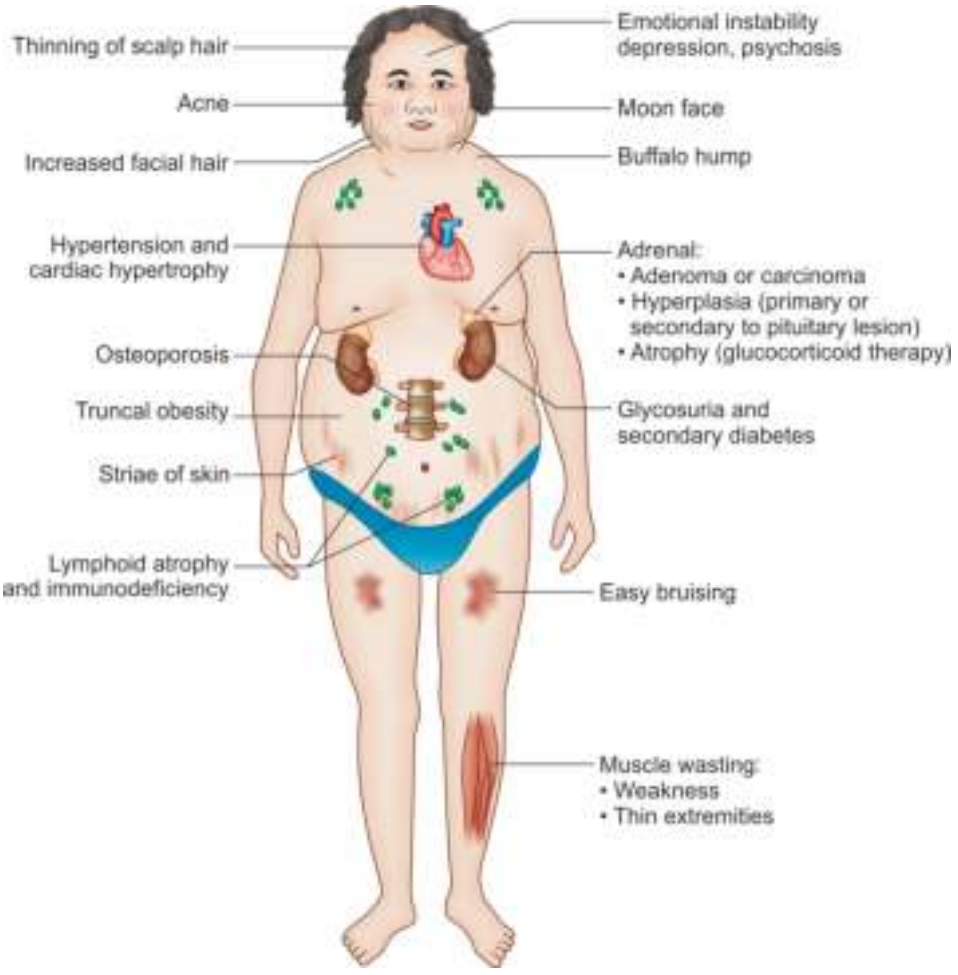


Fig. 26.64: Clinical manifestations of Cushing's syndrome.

plasma renin level in primary aldosteronism. Secondary aldosteronism has increased blood renin level.

Primary Aldosteronism

Primary aldosteronism is also known as Conn's syndrome, which reflects inappropriate secretion of aldosterone by an adrenal adenoma in 75% of cases or hyperplastic adrenal glands. Rarely, it may be caused by adrenocortical carcinoma.

- Patient presents with asymptomatic diastolic hypertension, persistent hypokalemia with alkalosis and slightly elevated serum sodium. Skeletal muscle weakness and fatigue are produced by the effects of potassium depletion in skeletal muscle. Decreased serum renin level occurs due to negative feedback of increased blood pressure.
- The diagnosis of primary aldosteronism can be confirmed by demonstration of increased aldosterone, lack of response of aldosterone to sodium loading, and decreased serum renin.

Secondary Aldosteronism

Secondary aldosteronism occurs due to renal ischemia, renal tumors, and edema. The cause of secondary aldosteronism is stimulation of the renin-angiotensin-aldosterone system. Serum renin is increased in contrast to primary aldosteronism.

Renin synthesized in the juxtaglomerular apparatus of the kidney promotes the conversion of angiotensinogen to angiotensin I, which is converted catalytically by angiotensin-converting enzyme (mainly in the lungs) to angiotensin II. The release of aldosterone is facilitated by angiotensin II.

Glucocorticoid-remediable Aldosteronism

Glucocorticoid-remediable aldosteronism (GRA) is an autosomal dominant disorder characterized by ectopic production of aldosterone by zona fasciculata of adrenal cortex.

- **Physiologic state:** Aldosterone-synthase gene and 11β-hydroxylase gene are present on chromosome 8.

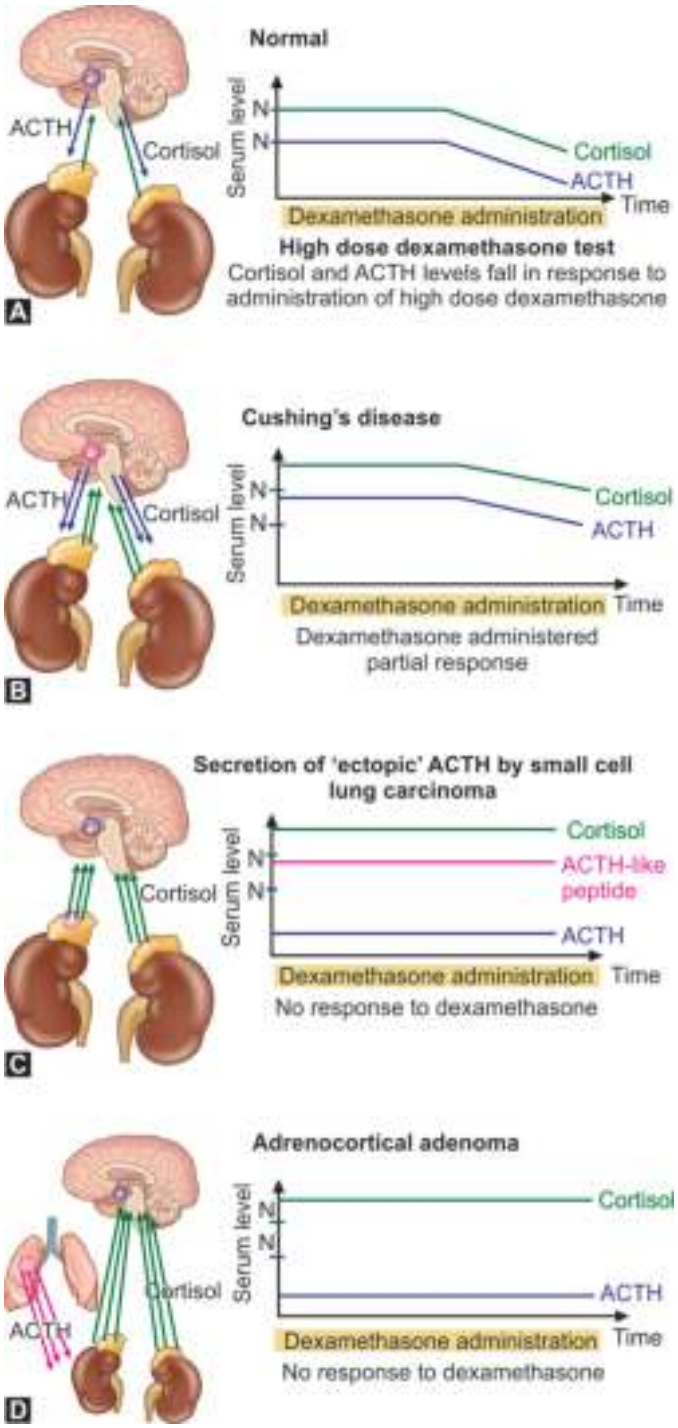


Fig. 26.65A to D: Dexamethasone suppression test in Cushing's syndrome has many causes. Cushing's disease is caused by a pituitary adenoma. The dexamethasone suppression test can help to identify the underlying problem. The principles of the test are shown here.

Aldosterone-synthase acts on zona glomerulosa to synthesize aldosterone hormones. 11β-Hydroxylase gene controls the synthesis of cortisol by zona fasciculata of adrenal glands.

- **Pathologic state:** Mutations of aldosterone-synthase gene and 11β-hydroxylase gene present on chromosome 8 form hybrid genes resulting in ectopic production of aldosterone by zona fasciculata of adrenal cortex, which leads to expansion of blood volume resulting in hypertension.

ADRENOCORTICAL INSUFFICIENCY

Hypocorticism (adrenocortical insufficiency) can be due to primary adrenal cause or secondary to hypothalamic or pituitary dysfunction. Deficiency of glucocorticoids, primarily cortisol, is often associated with mineralocorticoid deficiency.

Examples of hypocorticism are Addison's disease and Waterhouse-Friderichsen syndrome.

ADDISON'S DISEASE

Primary chronic adrenal insufficiency also known as Addison's disease most often reflects autoimmune destruction of the adrenal gland in 70% of cases. Until recently the most frequent cause of Addison's disease was tuberculosis.

Addison's disease may be caused due to metastatic tumor, and various infections. Atrophic adrenal gland fails to produce glucocorticoids, mineralocorticoids, and androgens.

Clinical Features

More than 90% of the adrenal gland must be destroyed before the symptoms of chronic adrenal insufficiency appear.

Patient with Addison's disease presents with weakness, weight loss, gastrointestinal symptoms, hypotension, electrolyte disturbances such as decreased serum sodium, bicarbonate, chloride and increased serum potassium, and hyperpigmentation due to compensatory hypothalamic production of proopiomelanocortin, the precursor peptide of both corticotropin and melanocyte-stimulating factor. Clinical manifestations of Addison's disease are shown in Fig. 26.66.

WATERHOUSE-FRIDERICHSEN SYNDROME

Waterhouse-Friderichsen syndrome is life-threatening disorder associated with bilateral adrenal hemorrhage. Sometimes it occurs due to dissemination of meningococcal infection, most often in association with meningococcal meningitis.

Clinical Features

Patient presents with vascular collapse due to hemorrhagic necrosis of the adrenal cortex. It is often associated with disseminated intravascular coagulation (DIC).

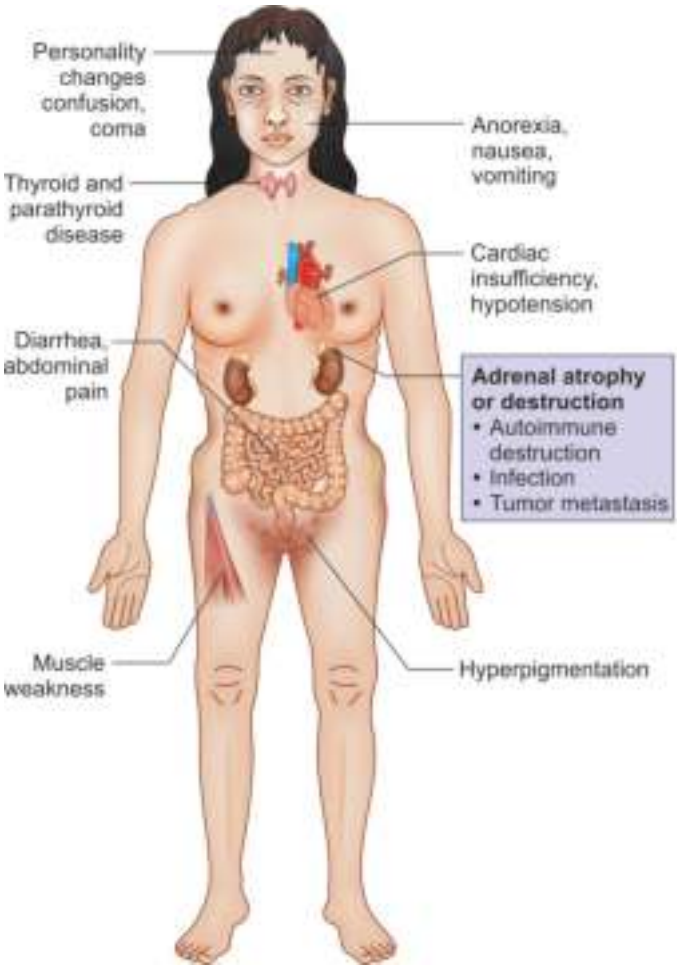


Fig. 26.66: Clinical manifestations of Addison's disease.

ADRENOGENITAL SYNDROME

Congenital deficiency of 21-hydroxylase is most common cause of adrenogenital syndrome. It is characterized by decreased synthesis of cortisol, and mineralocorticoids, and an increase in androgenic steroid sex hormones resulting in salt-losing hypotension and virilization, i.e. masculinization. Compensatory increased ACTH leads to resultant adrenal hyperplasia.

- Adrenogenital syndrome may also be caused by functional adrenocortical hormone producing adenomas, common component of MEN I, Carney complex, and McCune-Albright syndrome.
- Adrenogenital syndrome can occur in the settings of sporadic adrenocortical carcinoma, Li-Fraumeni and Beckwith-Wiedemann syndromes.

Clinical Features

Patient with adrenogenital syndrome presents with virilization, ambiguous genitalia such as hypertrophic clitoris and partial fusion of labioscrotal folds in female infants.

Male infants show normal external genitalia, but develop precocious puberty. Eventually, high levels of adrenal androgens lead to premature closure of the epiphyses and stunted growth. Clinical manifestations in adrenogenital syndrome in male and female are given in Table 26.42.

NEOPLASTIC DISORDERS OF ADRENAL MEDULLA

PHEOCHROMOCYTOMA

Pheochromocytoma is a rare tumor derived from chromaffin cells of the adrenal medulla, which releases excess of epinephrine and norepinephrine. There is increased levels of their metabolites such as vanilylmandelic acid (VMA) and metanephrin. Tumor arising from extra-adrenal chromaffin cells is called paraganglioma.

- Pheochromocytoma has most often benign course in 90% of cases. The 10% rule of pheochromocytoma includes 10% malignant, 10% familial, 10% bilateral, 10% children involvement and 10% extra-adrenal.
- Classic triad of pheochromocytoma includes episodic headaches, sweating and tachycardia is present in 30% of cases. Other common clinical features include paroxysmal hypertension, palpitations, anxiety and postural hypotension. Additional features may be present in hereditary syndromes associated with pheochromocytoma.

Association of Pheochromocytoma in Various Syndromes

Pheochromocytoma can be part of MEN IIa (Sipple syndrome) or MEN IIb. It can also be associated with neurofibromatosis or with von Hippel-Lindau disease.

Table 26.42 Clinical manifestations in adrenogenital syndrome in male and female

Patient	Findings
Pre-pubertal male	Premature puberty
Post-pubertal male	Asymptomatic or paradoxical oligospermia
Pre-pubertal female	Virilization of external genitalia, which may appear ambiguous
Post-pubertal female	Clitoris—megaly, male-type hirsutism, deepening of the voice, menstrual irregularities

- **MEN IIa syndrome (Sipple syndrome):** It is associated with familial medullary thyroid carcinoma, bilateral pheochromocytomas and primary hyperparathyroidism as a result of parathyroid adenoma or hyperplasia.
- **MEN IIb syndrome:** It is associated with medullary thyroid carcinoma, bilateral pheochromocytomas, and multiple mucosal ganglioneuromas in lips/tongue. In contrast to MEN IIa, it does not induce hyperparathyroidism. MEN IIa is linked to different mutations in the RET oncogene compared with MEN IIa. Poor prognostic factors in these patients include age >50, male, and distant metastases.

Surgical Pathology: Pheochromocytoma

Gross Morphology

- Pheochromocytoma fixed in dichromate solution gives brown discoloration (Fig. 26.67).
- Tumor is well circumscribed and unencapsulated.
- Cut surface reveals solid white to red brown with areas of hemorrhage (Fig. 26.67).

Light Microscopy

- Tumor is composed of large polygonal cells with abundant granular cytoplasm with eccentric nuclei arranged in alveolar, nests, trabecular or solid patterns.
- Nests of tumor cells are separated by thin vascular septa. The tumor cells contain intranuclear inclusions.
- Cytoplasm of tumor cells may show pink hyaline diastase resistant PAS positive globules (Fig. 26.68).

Immunohistochemistry Technique

Tumor cells show positivity for synaptophysin, chromogranin A, neuron-specific enolase (NSE), S-100 and N-CAM (CD56).

Markers	Expression
Synaptophysin	Positive
Chromogranin A	Positive
Neuron-specific enolase (NSE)	Positive
S-100 (calcium binding protein)	Positive
N-CAM (CD56)	Positive

- **Composite pheochromocytoma:** Pheochromocytoma admixed with ganglioneuroma or neuroblastoma or ganglioneuroblastoma or malignant nerve sheath tumor is known as composite pheochromocytoma.

Clinical Features

Approximately 66% of patients are asymptomatic. One-third cases show fluctuating (paroxysmal or episodic) hypertension and tachycardia due to excessive secretion of catecholamines (epinephrine and norepinephrine) by the tumor. Patient is resistant to antihypertensive therapy.

Patient may also develop hyperglycemia, malignant hypertension (e.g. encephalopathy, papilledema, and proteinuria), myocardial infarction, aortic dissection, and convulsions.



Fig. 26.67: Pheochromocytoma—adrenal gland. Tumor is solid white to red brown and hemorrhagic on cut surface and mean diameter of benign tumor is 4–6.5 cm. Classic triad of pheochromocytoma includes paroxysmal hypertension, episodic headaches, sweating and tachycardia in about 30% of patients.

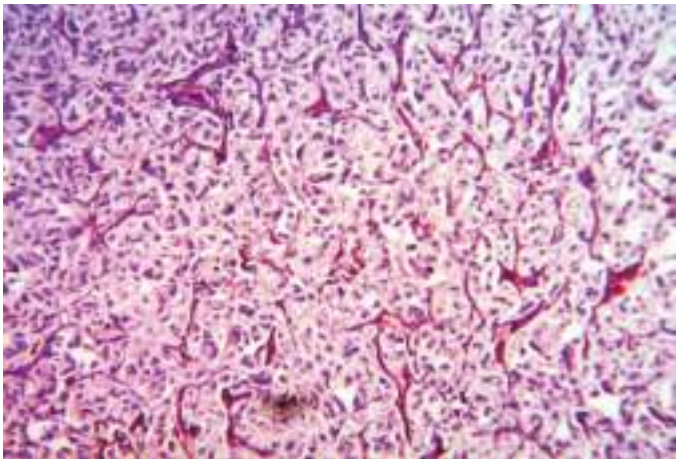


Fig. 26.68: Pheochromocytoma. Tumor is composed of large polygonal cells with abundant granular cytoplasm with eccentric nuclei arranged in alveolar, nests, trabecular or solid patterns. These nests of tumor cells are separated by thin vascular septa (400X).

Molecular Genetic Testing

Pheochromocytoma is linked to mutations in the RET oncogene, a transmembrane receptor of the tyrosine kinase family in sporadic cases in 25–70%.

When a diagnosis of pheochromocytoma is made, demonstration of RET oncogene would justify prophylactic thyroidectomy keeping in view of fatal outcome of medullary thyroid carcinoma.

Urine Analysis

Urinary vanillylmandelic acid (VMA), a norepinephrine metabolite, is markedly elevated in pheochromocytoma.

NEUROBLASTOMA

Neuroblastoma is a highly malignant catecholamine-producing tumor of neural crest origin from adrenal medulla or precursor cells of autonomic nervous system closely related to sympathetic ganglia. Tumor is composed of neoplastic neuroblasts. *In situ* neuroblastoma reported in infancy, has higher frequency of apparent spontaneous regression. Occasionally, tumor converts into a more differentiated form termed ganglioneuroma.

Association of Neuroblastoma in Various Syndromes

Neuroblastoma may be seen in patients with Beck-with-Wiedemann syndrome, neurofibromatosis, Hirschsprung's disease and fetal hydantoin syndrome.

Neuroblastoma Sites

Adrenal medulla is the most common site of neuroblastoma in 50–80% of cases. Posterior mediastinum in paravertebral regions is next common site. Rare sites of tumor include pelvis, lower cervical sympathetic chain, and posterior cranial fossa. In some cases, having heredofamilial basis, both adrenal glands and multiple extra-adrenal sites are involved.

Age Group

Neuroblastoma most often affects children under 5 years with peak incidence is in the first 3 years of life. Adults are rarely affected.

Molecular Genetic Testing in Neuroblastoma

Tumor reveals amplification of the N-Myc oncogene. Multiple copies of the N-Myc oncogene are demonstrated as homogeneously staining regions or as extrachromosomal double minute chromatin bodies. A greater degree of amplification correlates with worse prognosis.

N-Myc Oncogene Amplification

- **Physiologic state:** The 'myc' protein acts as a transcription factor and controls the expression of several genes. It is activated by gene rearrangement (breaking and sealing of chromosomes) or amplification.
- **Pathologic state:** Neuroblastoma reveals amplification of the N-Myc oncogene forming thousands of N-Myc oncogene copies per cell in neuroblastoma (30% cases).
  - Multiple copies of the N-Myc proto-oncogene are demonstrated as homogeneously staining regions (HSRs) or as extrachromosomal double minute chromatin bodies. A greater degree of N-Myc proto-oncogene amplification correlates with worse prognosis.
  - The malignant cells of neuroblastoma sometimes differentiate into benign cells, and this change is reflected by a marked reduction in gene amplification.

NPM-ALK Fusion Oncogene

- **Physiologic state:** ALK (anaplastic lymphoma kinase) gene is located on chromosome 2. ALK gene provides instructions for making a protein is called ALK receptor tyrosine kinase (RTK).
- **Pathologic state:** As a result of the t(2:5) chromosomal translocation ALK gene is fused with the NPM (nucleophosmin) gene, leading to production of a hybrid NPM–ALK protein. Mutant ALK receptors deliver continuous mitogenic signals to the cell, even in the absence of growth factor in the environment. It is associated with anaplastic large cell lymphoma, i.e. most important, rare cases of lung adenocarcinoma, and neuroblastoma. These cancers synthesize excessive ALK receptors.

Surgical Pathology: Neuroblastoma

Gross Morphology

Neuroblastoma grows rapidly to become well-circumscribed to multilobular mass. Cut surface reveals variable amounts of hemorrhage, necrosis and calcification. Calcification is demonstrated on radiological examination (Fig. 26.69).

Light Microscopy

Neoplasms of neuroectodermal origin are comprised of neuroblasts or ganglion cells. These neoplasms can be least differentiated to well differentiated: neuroblastoma → ganglioneuroblastoma → ganglioneuroma.

- **Neuroblastoma** is composed of small round blue immature tumor cells (neuroblasts) forming Homer-Wright pseudorosettes around blood vessels.
  - Tumor cells contain round to ovoid nuclei with stippled 'salt and pepper chromatin', inconspicuous nucleoli, and minimal cytoplasm.
  - Homer-Wright pseudorosettes are circular, ovoid, angular zones of pale staining neuritic cell processes surrounded by tumor cell nuclei (Fig. 26.70).
- **Ganglioneuroblastoma** is composed of mixture of immature neuroblasts and ganglion cells (Schwannian stroma-rich).
- **Ganglioneuroma** is composed only of ganglion cells (Schwannian stroma-dominant).

Immunohistochemistry Technique

Tumor cells of neuroblastoma show positivity for neuron-specific enolase (NSE), neurofilament, chromogranin A, and synaptophysin.

Markers	Expression
Neuron-specific enolase (NSE)	Positive
Neurofilament	Positive
Chromogranin A	Positive
Synaptophysin	Positive

Clinical Features

Patient with neuroblastoma often presents with a large abdominal mass, malaise, fever, pallor and loss of weight. Tumor synthesizes catecholamines (norepinephrine) in 90% of cases, but it rarely causes hypertension.

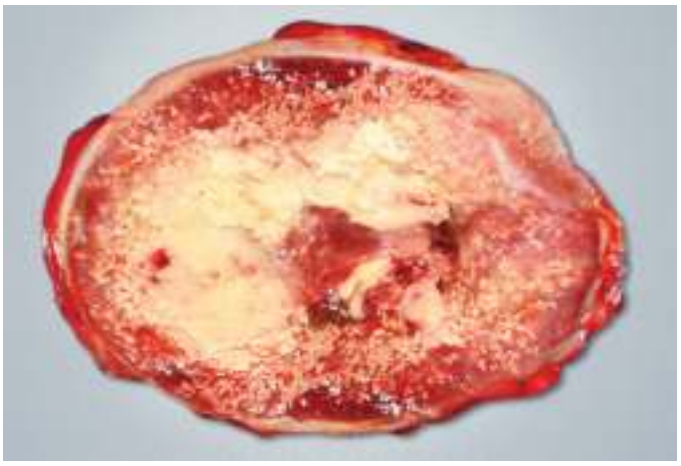


Fig. 26.69: Gross morphology of neuroblastoma of adrenal gland. Tumor is well circumscribed and multilobular. Cut surface reveals variable amounts of hemorrhage, necrosis and calcification in background of solid areas. (*Courtesy:* Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

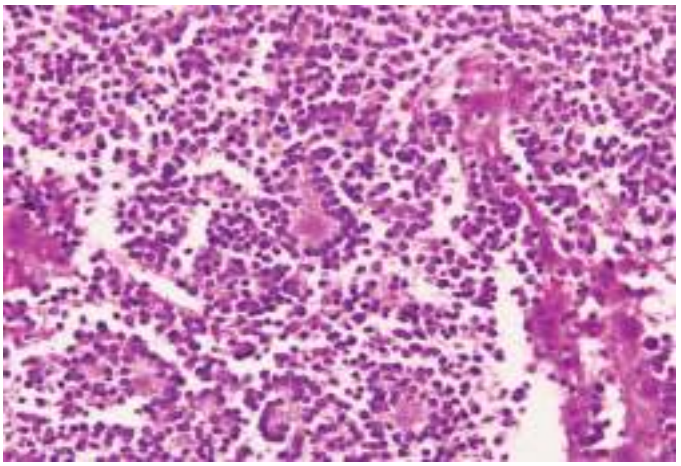


Fig. 26.70: Histology of neuroblastoma of adrenal gland. Tumor is composed of small round blue cells (neuroblasts) and Homer-Wright pseudorosettes. Tumor cells contain round to oval nuclei with stippled salt and pepper chromatic and scanty cytoplasm (400X).

On clinical examination, neuroblastoma has knobby contour in contrast to smooth contour of Wilms' tumor. As the tumor enlarges, it crosses midline.

Metastasis

Neuroblastoma spreads to the liver, bones (skull, orbit), scalp, lymph nodes, subcutaneous tissue, intraspinal extension or kidney. Bone metastases are multiple and sometimes symmetric.

- **Pepper type of neuroblastoma:** It is characterized by primary neuroblastoma and its secondaries especially liver metastases on right side of body.
- **Hutchinson's type of neuroblastoma:** It is characterized by left side primary neuroblastoma and metastases in the orbit and skull.

Urine Analysis

More mature descendants of tumor cells in the adrenal medulla may secrete catecholamines. These catecholamines are metabolized and excreted as urinary vanillylmandelic acid (VMA) in the urine. Urinary catecholamines and catecholamine metabolites are the same as in pheochromocytoma.

Prognostic Factors

Amplification of N-Myc proto-oncogene, gain of 17q, deletion of 1p, and high expression of neuron-specific enolase are associated with poor prognosis. Prognostic factors of neuroblastoma are given in Table 26.43.

Table 26.43 Prognostic factors of neuroblastoma

Features	Good Prognosis	Bad Prognosis
Age group	Less than 2 years	More than 2 years
Site of tumor	Extra-adrenal tumor	Adrenal gland
Stage	Tumor confined to organ	Tumor extending outside organ
DNA ploidy	Hyperdiploid or near triploid	Diploid near diploid or near tetraploid
N-Myc proto-oncogene amplification	Absent	Present
Gain of chromosome 17q	Absent	Present
Loss of chromosome 11q	Absent	Present
Telomerase overexpression	Absent	Present
MRP expression	Absent	Present
TrkB expression	Absent	Present
TrkB expression-A (Trk family of neutropin receptors)	Present (respond to TrkA inhibitor drug with spontaneous regression)	Absent
CD44 expression	Present	Absent

Contd...

Table 26.43 Prognostic factors of neuroblastoma (*Contd...*)

Features	Good Prognosis	Bad Prognosis
Lymphocytes around tumor	Present	Absent
S-100 protein expression in tumor cells	Present	Absent
Ratio of vanillylmandelic acid to homovanillic acid	Ratio >1	Ratio <1
Serum ferritin level	Low level	High level
Serum LDH	<1500 U/ml	>1500 U/ml
Neuron-specific enolase levels (cut of value is 15 ng/ml)	Low	High
Creatine kinase BB (normal cut of less than 1 ng/dl)*	Increased	Highest in stage IV

**Creatine kinase level is highest in stage IV of neuroblastoma.*

NEOPLASTIC DISORDERS OF ADRENAL CORTEX

ADRENOCORTICAL ADENOMA

Adrenocortical adenoma is a benign tumor in adults during 5th–7th decades. Tumor can be functional (a few cases) and nonfunctional (most cases). The patient shows signs and symptoms of Cushing's syndrome is characterized by upper truncal obesity and hypercortisolism.

Surgical Pathology: Adrenocortical Adenoma

Gross Morphology

The surgical specimen reveals a circumscribed tumor of the adrenal cortex.

Light Microscopy

Tumor is composed of nests of clear, lipid-laden epithelial cells.

Immunohistochemistry Technique

Tumor cells show positivity for Ki-67, p53, phosphohistone H3 and steroidogenic factor 1.

Markers	Expression
▪ Ki-67	▪ Positive
▪ p53	▪ Positive
▪ Phosphohistone H3	▪ Positive
▪ Steroidogenic factor 1	▪ Positive

ADRENOCORTICAL CARCINOMA

Adrenocortical carcinoma is a rare neoplasm affecting any age group. It is most often functional tumor associated with clinical manifestations in contrast to nonfunctional adrenocortical adenoma. Tumor has strong tendency to invade adrenal vein, inferior vena cava and lymphatic channels.

- Tumor is associated with Li-Fraumeni syndrome, Beckwith-Wiedemann syndrome and congenital adrenal hyperplasia.
- Lung adenocarcinoma frequently metastasizes to adrenal glands. It is difficult to differentiate from primary adrenocortical carcinoma.

Mode of Spread

- **Lymphatic route:** Tumor metastasizes to para-aortic lymph nodes.
- **Hematogenous route:** Tumor invades blood vessels and spread to lungs and liver. Bone metastases are unusual.

Surgical Pathology: Adrenocortical Carcinoma

Gross Morphology

- Adrenocortical carcinoma is unencapsulated large tumor weighing 200 g and measuring 20 cm.
- Cut surface shows variegated appearance due to hemorrhage, necrosis and cysts.
- Tumor is often soft, friable intratumoral necrosis; often invading major veins (Fig. 26.71).

Light Microscopy

- Adrenocortical carcinoma has various growth patterns of well-differentiated to anaplastic cells.
- Tumor shows capsular invasion marked mitotic activity, tumor giant cells, neutrophils and eosinophils.
- Only definitive criteria of malignancy are distant metastasis and local invasion (Fig. 26.72).

Modified Weiss Diagnostic Criteria of Adrenocortical Carcinoma

- Mitotic figures >5 per 50 high power fields
- Abnormal mitosis
- Cytoplasm (clear cells comprising about 25% of the tumor)
- Necrosis
- Capsular invasion
- Calculate diagnostic criteria:
  - 2 × mitotic rate criteria +
  - 2 × clear cytoplasm criteria + abnormal mitoses + necrosis + capsular invasion (score of ≥ suggests malignancy).

Each criterion is scored 1 when present in the tumor and zero when absent.

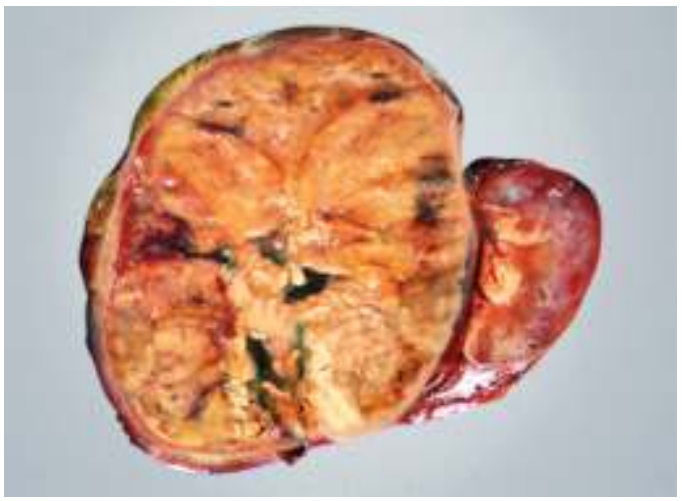


Fig. 26.71: Gross morphology of adrenocortical carcinoma. Tumor is large, fleshy partially necrotic tumor that has completely replaced adrenal gland. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

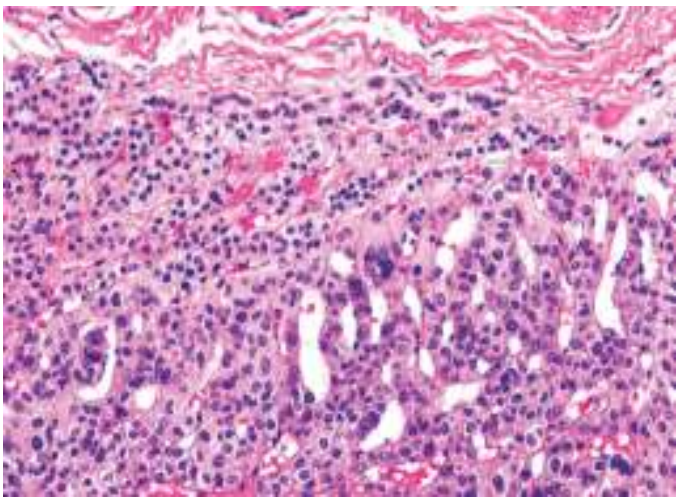


Fig. 26.72: Histology of adrenocortical carcinoma. Histologic examination shows tumor cells containing clear to eosinophilic cytoplasm and hyperchromatic nuclei. There is presence of capsular invasion, marked mitotic activity, tumor giant cells, neutrophils and eosinophils (400X).

DISORDERS OF PITUITARY GLAND

ANATOMY AND PHYSIOLOGY

Pituitary gland consists of adenohypophysis, i.e. anterior pituitary or pars distalis, pars intermedia and neurohypophysis, i.e. posterior pituitary or pars nervosa.

- **Anterior pituitary gland:** Hormones synthesized by anterior pituitary are growth hormone, prolactin, thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), follicle stimulating hormone (FSH) and luteinizing hormone (LH).
- **Pars intermedia:** It is merged with anterior pituitary gland. It synthesizes melanocyte-stimulating hormone (MSH).
- **Posterior pituitary gland:** Hypothalamic neurons synthesize oxytocin and antidiuretic hormone (ADH), i.e. vasopressin, which pass through axons and released at nerve endings and hormones reach the posterior pituitary gland through portal circulatory system and regulate the functions of the posterior pituitary gland. Posterior pituitary gland is under the direct neural regulation of the hypothalamus.

Hormones synthesized by anterior pituitary gland and their actions are given in Table 26.44. Pituitary gland consists of anterior and posterior divisions (Fig. 26.73).

Table 26.44 Hormones synthesized by anterior pituitary gland and their actions

Cells in Pituitary Gland	Hormone Synthesis by Anterior Pituitary Gland	Target Organs	Actions on Target Cells	Net Result
Somatotropes (basophilic cells)	Growth hormone (acidophilic cells)	Skeletal system	Growth promoting and anti-insulin actions	Linear bone growth in children
Corticotropes (basophilic cells)	ACTH	Adrenal glands	Synthesis of aldosterone and corticosteroids	Regulate carbohydrate, fat and proteins
Thyrotropes (basophilic cells)	TSH (basophilic cells)	Thyroid gland	T_3, T_4	Regulation of metabolism of carbohydrate, fat and proteins
Lactotropes (acidophilic cells)	Prolactin (PRL)	Female breasts	Synthesis and ejection of milk	Controls reproduction, osmoregulation, growth and metabolism

Contd...

Table 26.44 Hormones synthesized by anterior pituitary gland and their actions (*Contd...*)

Cells in Pituitary Gland	Hormone Synthesis by Anterior Pituitary Gland	Target Organs	Actions on Target Cells	Net Result
Gonadotropes (basophilic cells)	FSH, LH	▪ Testes ▪ Ovaries	▪ FSH: Spermatogenesis ▪ LH: Acts on Leydig cells to synthesize testosterone ▪ FSH ▪ LH	▪ Increased sperms count ▪ Growth, development, maturation of spermatozoa and development of secondary characters ▪ Stimulates formation of graafian follicles ▪ Triggers ovulation, and formation of corpus luteum
Melanophores	Melanocyte-stimulating hormone (MSH)	Skin	Synthesis of cutaneous pigmentation	Subcutaneous dispersion of melanin granules

Somatostatin inhibits synthesis of growth hormone by anterior pituitary gland.

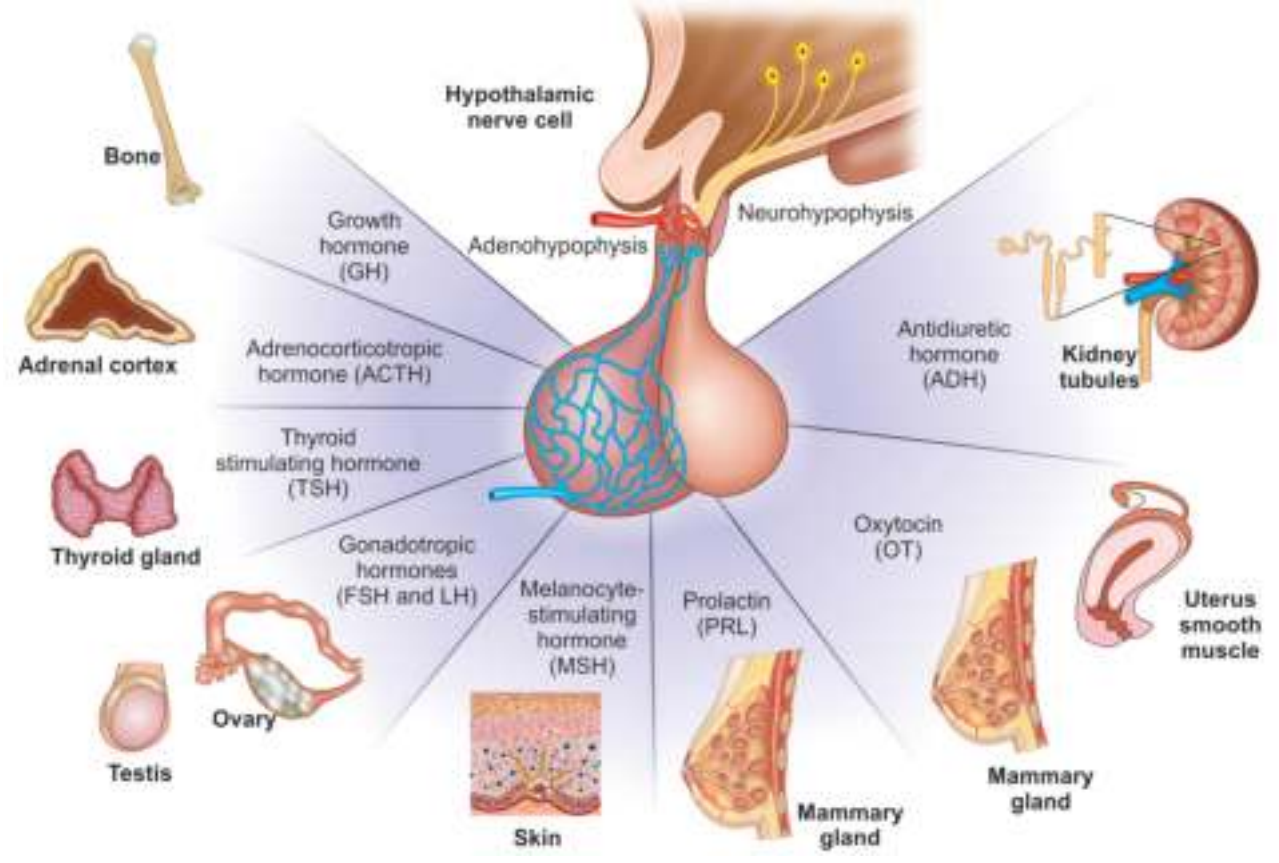


Fig. 26.73: Pituitary gland consists of anterior and posterior divisions.

ANTERIOR PITUITARY GLAND: HYPOFUNCTIONING

Partial or complete forms of hypopituitarism of anterior pituitary gland most often affects children and adults, which may cause dwarfism and delayed puberty. Initially, there is decreased FSH and LH levels resulting in hypogonadism, impotence in men and menstrual disturbances in women.

Growth hormone deficiency leads to short stature, delayed growth and delayed puberty in children. There is subsequent decrease in TSH and ACTH.

PRIMARY HYPOPITUITARISM

Primary hypopituitarism of anterior pituitary gland is caused by nonsecretory adenoma, Sheehan's syndrome (postpartum pituitary necrosis), empty sella syndrome, partial or total hypophysectomy;

TB meningitis, irradiation, hemochromatosis and Hand-Schüller-Christian disease.

Etiopathogenesis

- **Simond's disease:** Patient presents with cachexia (marked wasting) due to destruction of pituitary gland by pituitary tumors usually adenomas in >50% of cases.
- **Sheehan's syndrome:** Sheehan's syndrome refers to infarction of anterior pituitary gland due to postpartum hemorrhage and shock during childbirth.
  - During pregnancy, pituitary gland becomes enlarged, that renders it vulnerable to a reduction in blood flow.
  - Patient presents with adrenal cortical insufficiency, and ovarian failure (gonadotropin deficiency with decreased FSH and LH), pallor, (MSH deficiency), secondary hypothyroidism (decreased TSH), and failure of lactation (decreased prolactin). Clinical manifestations of Sheehan's syndrome are shown in Fig. 26.74.
- **Iatrogenic hypopituitarism:** Pituitary gland may be damaged due to radiation therapy in the head or neck region and intracranial neurosurgical procedures.
- **Traumatic injury to skull:** Basal skull fractures and trauma to the sella turcica may cause damage to the pituitary gland.
- **Infiltrative diseases:** Pituitary gland may be damaged by bacterial or viral infections. Hemochromatosis of pituitary may cause panhypopituitarism. Hand-Schüller-Christian disease most often causes diabetes insipidus, but may also cause hypopituitarism.

Molecular Genetic Alterations

Mutation of Pit1 gene (3p11), PROP1 gene (5q) or HSEX1 gene (3p21) causes hypopituitarism (Table 26.45).

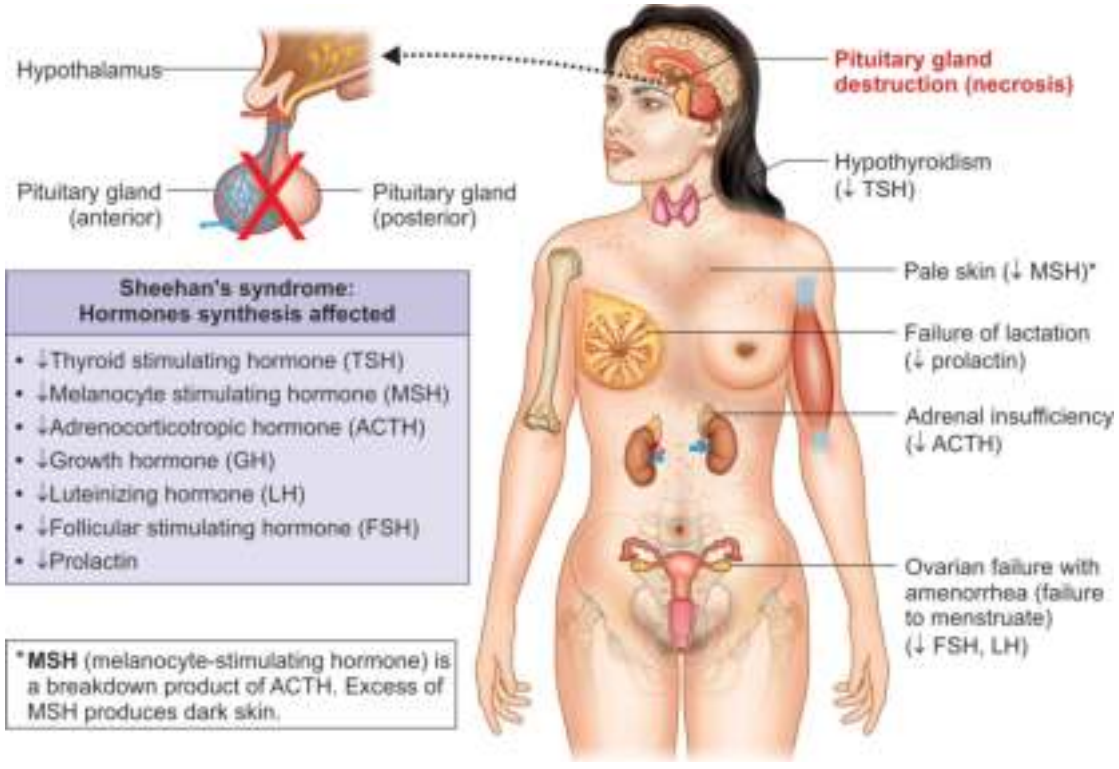


Fig. 26.74: Sheehan's syndrome. Clinical manifestations of Sheehan's syndrome is caused by hemorrhagic destruction of the pituitary gland during pregnancy or postpartum period.

Table 26.45 Molecular genetic alterations in hypopituitarism

Gene Mutations	Deficiency of Hormones
Pit1 gene (3p11)	Growth hormone, prolactin and thyroid stimulating hormone
PROP1 gene (5q) transcription factor	Inactivation of FSH, LH, prolactin and thyroid stimulating hormone
HSEX1 gene (3p21) participates in development of optic nerves and pituitary gland	Growth hormone and ADH

Clinical Manifestations

- **Growth hormone deficiency:** Deficiency of growth hormone in children results in growth retardation, i.e. pituitary dwarfism. In adults, patient develops increased insulin sensitivity associated with hypoglycemia, decreased muscle strength, and anemia.
- **Gonadotropin deficiency:** In preadolescent children, gonadotropin deficiency results in retarded sexual maturation. Adult men present with loss of libido, impotence, inhibition of spermatogenesis, loss of muscular mass, and decreased facial hair. Women develop amenorrhea and vaginal atrophy.
- **TSH deficiency:** Deficiency of TSH results in secondary hypothyroidism. Patient presents with weight gain, constipation, cold intolerance, fatigue and coarse hair.
- **Prolactin deficiency:** Patient presents with lactation dysfunction in women or gynecomastia in men. Serum ACTH, FSH, LH, and TSH levels are analyzed. CT scan and MRI of pituitary gland and target glands are essential diagnostic tools.
- **ACTH deficiency:** Deficiency of ACTH results in secondary adrenal failure. It does not result in hyperpigmentation of the skin, probably because of the lack of both ACTH and β-melanocyte-stimulating hormone; this is in contrast to primary adrenal failure, i.e. Addison's disease, in which ACTH is increased and hyperpigmentation is the rule.

  Patient presents with weakness, fatigue, hypoglycemia, loss of hair in axillae and pubic regions, hypotension, and decreased sodium level.

OTHER CAUSES OF HYPOPITUITARISM

- **Empty sella syndrome:** It is caused by conditions that destroy all or part of the pituitary gland.
- **Nelson syndrome:** Nelson syndrome involves the development of large pituitary adenomas following bilateral adrenalectomy. This is thought to be due to loss of feedback inhibition on growth of pre-existing pituitary microadenoma.
- **Growth hormone insensitivity (Laron syndrome):** It is an autosomal recessive disorder characterized by extreme resistance to growth hormone resulting in short stature and obesity. Biochemical analysis shows high level of serum growth hormone and low level of insulin-like growth factor 1 (IGF-1).
- **Isolated gonadotropin deficiency (Kallmann syndrome):** It occurs due to mutation of KAL1 gene resulting to failure of migration of gonadotropin releasing hormone (GnRH) from its origin in olfactory anlage to their normal location in hypothalamus.

POSTERIOR PITUITARY GLAND: HYPOFUNCTIONING

Hormones are synthesized in the hypothalamus and transported via axons to the posterior pituitary.

- Oxytocin induces uterine contraction during labor and ejection of milk from mammary alveoli.
- Antidiuretic hormone (ADH, vasopressin) promotes water retention through action on the renal collecting ducts. Mutations in genes causing combined pituitary hormone deficiency (CPHD) are given in Table 26.46.

ADH DEFICIENCY

Antidiuretic hormone (ADH) participates in determining the concentration of urine by affecting water reabsorption in the distal renal tubules.

- Low ADH activity results in dilute urine and free water excretion. Decreased synthesis of ADH by posterior pituitary gland results in central diabetes insipidus.
- Kidneys unresponsive to ADH results in nephrogenic diabetes insipidus.

Central Diabetes Insipidus

Central diabetes insipidus may be caused by trauma that damages the posterior lobe of pituitary gland (neurohypophysis) and interrupts the secretion of ADH.

- Approximately 25% cases of central diabetes insipidus occur due to brain tumors such as craniopharyngioma and germ cell tumor. Other causes include inflammatory processes, lipid storage disorders resulting in damage to the neurohypophysis or hypothalamus.
- Patient presents with polyuria, with consequent dehydration and insatiable thirst. Patient drinks water constantly to avoid feeling dehydrated. On clinical examination, patient appears dehydrated.

Nephrogenic Diabetes Insipidus

Kidneys unresponsive to ADH results in nephrogenic diabetes insipidus. Demeclocycline and lithium are potential causes of nephrogenic diabetes insipidus.

SYNDROME OF INAPPROPRIATE ADH SECRETION

Syndrome of inappropriate ADH (SIADH) secretion most often occurs due to ectopic production of ADH by small cell lung carcinoma (most common). It is also reported in patient with carcinomas of the prostate, gastrointestinal tract, and pancreas, thymomas, NHL, Hodgkin's disease, trauma, and infections.

Table 26.46 Mutations in genes causing combined pituitary hormone deficiency (CPHD)

Gene	Chromosome Locus	Normal Functions	Gene Mutations
HESX1 (homeobox expressed in embryonic stem cells)	3p14.3	Transcriptional repressor essential for early determination and differentiation of the pituitary gland, PROP1 gene has opposite action	Missense, insertion and deletion (homozygotes/heterozygotes)
LHX3	▪ 9p34.3 (LHX3) ▪ 1q25.2 (LHX4)	Transcriptional factors essential for early development and maintenance of mature anterior pituitary cells	Missense, nonsense mutations, with frameshift, partial or complete gene deletion
PROP1 gene	5p25.3	Transcriptional factor developing anterior pituitary gland (somatotrophs, lactotrophs and thyrotrophs)	Autosomal recessive disorder
POU1F1	3p11	Transcriptional factor essential for terminal differentiation and expansion of somatotrophs, lactotrophs and thyrotrophs resulting in deficiency growth hormone, prolactin and TSH deficiency	Autosomal dominant, autosomal recessive (missense, nonsense, frameshift, and splicing mutation)
Zinc finger protein GLI2 (GLI2)	2q14	Transcription factor mediates Sonic hedgehog signals	Forebrain defect holoprosencephaly, malformed anterior pituitary and panhypopituitarism

A number of genes have been demonstrated to cause CPHD. Among these are the homeobox expressed in embryonic stem cells 1 (HESX1), LIM homeobox protein 3 (LHX3), LIM homeobox protein 4 (LHX4), prophet of pit-1 (PROP1), POU domain class 1, transcription factor 1 (POU1F1), and zinc finger protein GLI2 (GLI2). Homeobox expressed in embryonic stem cells 1 (HESX1) is transcriptional repressor essential to the early development.

Clinical Features

Syndrome of inappropriate ADH (SIADH) secretion results in retention of water with consequent dilutional hyponatremia, reduced serum osmolality, and inability to dilute the urine. Water intoxication with hyponatremia results in altered mental status, seizures, coma, and sometimes death.

NEOPLASTIC DISORDERS OF PITUITARY GLAND

PITUITARY ADENOMAS

Pituitary adenomas occur in the regions of sella turcica (most common) and extracellular region (rare site) of anterior pituitary gland during 4th to 7th decades. These tumors are classified according to pituitary cell lineage, i.e. lactotroph, somatotroph, corticotroph, gonadotroph and pleurihormonal adenomas. Pituitary adenomas be functional (secretory) or nonfunctional. Secretory adenomas may secrete hormones. Clinical manifestations depend on synthesis of hormones by pituitary adenomas. Frequency of pituitary adenomas is described in Table 26.47.

- **Molecular genetic alterations:** Guanine nucleotide binding protein, α-stimulating (GNAS) is a cytoplasmic signal-transducing protein. Alteration of GNAS is associated with pituitary adenoma and other endocrinal tumors.

Table 26.47 Frequency of adenomas of anterior pituitary gland

Cell Type	Hormone	Frequency (%)
Lactotrope	Prolactin	32
Somatotrope	Growth hormone	21
Corticotrope	ACTH (adrenocorticotropic hormone)	13
Lactotrope/ somatotrope	Mixed PRL/GH	6
Gonadotropes	▪ FSH (follicle-stimulating hormone) ▪ LH (luteinizing hormone)	<4
Thyrotrope	TSH (thyroid-stimulating hormone)	
Nonfunctional tumors	–	25

- **Pathogenesis:** Pituitary adenomas may be part of multiple endocrine neoplasia type 1 (MEN1). Acquired adenomas may occur due to mutation of cyclin D1, CREB, RAS, and pituitary tumor transforming gene (PTTG).
- **Histologic classification:** Pituitary adenomas of anterior lobe have been classified according to the functional properties of cells.
  - **Chromophil adenomas:** Acidophil adenoma synthesizes excess of growth hormone and prolactin. Basophil adenomas synthesize excess of ACTH, TSH, FSH and LH.
  - **Chromophobe adenomas:** These are nonfunctional tumors without synthesis of hormones.

Table 26.48 Revised 2024 WHO classification of pituitary neuroendocrine tumors (PitNETs)

PitNETs Type and Hormones Synthesis	Subtype	Transcription Factors	Low Molecular Weight Cytokeratin
Somatotroph tumors (GH)	▪ Somatotroph tumors with densely distributed granules ▪ Somatotroph tumors with sparsely distributed granules	▪ PIT1 ▪ PIT1	▪ Perinuclear staining ▪ Fibrous bodies (>70%)
Lactotroph tumors (prolactin)	▪ Lactotroph tumors with sparsely distributed granules ▪ Lactotroph tumors with densely distributed granules	▪ PIT1, ER-α ▪ PIT1, ER-α	▪ Weak or negative staining ▪ Weak or negative staining
Mammosomatotroph tumors (GH)	No subtype	PIT1, ER-α	Perinuclear staining
Thyrotroph tumors (TSH-α and TSH-β subunits)	No subtype	PIT1, GATA3	Weak or negative staining
Mature plurihormonal IPT1-lineage tumor (GH++ and variable prolactin, α-TSH or β-TSH)	No subtype	PIT1, ER-α, GATA3	Perinuclear staining
Immature PIT1-lineage tumor	No subtype	PIT1 (ER-α, GATA3)	Focal/variable staining
Acidophil stem cell tumor (deficient hormones synthesis)	No subtype	PIT1, ER-α	Scattered fibrous bodies
Mixed somatotroph and lactotroph tumors	No subtype	PIT1, ER-α	Tumor subtype characteristics
Corticotroph tumors (ACTH and POMC derivatives)	▪ Corticotroph tumors with densely distributed granules ▪ Corticotroph tumors with sparsely distributed granules ▪ Crook cell tumor	▪ TPIT ▪ TPIT ▪ TPIT	▪ Strong diffuse staining ▪ Variable diffuse staining ▪ Perinuclear ring-like cytoplasmic staining
Gonadotroph tumor	No subtype	SF1, ER-α, GATA3	Variable or negative staining
Plurihormonal tumor	No subtype	Multiple combinations	Variable staining
Null cell tumor	No subtype	None	Variable staining

- **Revised 2024 WHO classification:** Revised 2024 WHO classification of pituitary neuroendocrine tumors (PitNETs) is given in Table 26.48.
- **Consequences:** Microadenoma is small <1 cm in diameter. It does not produce symptoms unless it secretes hormones. Macroadenoma is space occupying lesion with clinical effects such as visual disturbances and increased intracranial pressure. Functional adenoma, which synthesizes growth hormone leads to gigantism, acromegaly, Cushing's syndrome, Nelson's syndrome and increased prolactin.
  - **Visual disturbances:** Tumor erodes the sella turcica and impinges on adjacent cranial structures such as the optic chiasm resulting in bitemporal hemianopia and loss of central vision.
  - **Cranial nerve palsy:** Tumor invading the cavernous sinuses leads to oculomotor nerve palsies and headache.
  - **Hyperphagia and loss of temperature regulation:** Tumor invading hypothalamus results in loss of temperature regulation, hyperphagia, and hormonal syndromes because it interferes with the normal hypothalamic input to the pituitary.

Surgical Pathology: Adenomas of Anterior Pituitary Gland

Gross Morphology

Tumors measuring <1 cm are microadenomas, and >1 cm are macroadenomas. Giant adenomas are rare tumors of >5 cm in diameter.

Light Microscopy

- Tumors are composed of large cells with moderately abundant cytoplasm, uniform nuclear morphology and delicate stippled (salt and pepper) chromatin and inconspicuous nucleoli. Tumor cells may appear without definite pattern, i.e. pseudorosettes-rich, papillary or organized fashion.

- Tumor cells may be classified as chromophilic (acidophilic, basophilic) or chromophobic based on tinctorial differences. Cytoplasmic appearance of tumor cells usually correlates with content of hormone containing secretory cells (dense versus sparse granules).
- Crooke's hyaline change occurs due to accumulation of cytokeratin filaments in eosinophilic or chromophobic cells, if prominent, called Crooke's hyaline adenoma as per WHO terminology of neoplasms of the pituitary gland.
- Null cell adenoma is composed of adenohypophyseal cells that does not exhibit any cell type specific differentiation.

LACTOTROPH ADENOMA (PROLACTINOMA)

Lactotroph adenoma (prolactinoma) is most common prolactin-secreting benign tumor of the pituitary gland, which constitutes 30% of pituitary adenomas. Staining is usually chromophobic. Tumor is generally of small size in 90% of cases. Large tumor impinges on adjacent structures, and bitemporal hemianopia from pressure on the optic chiasm is common (Fig. 26.75).

Age and Sex Predilection

Lactotroph adenoma occurs more common in men than women (20–50 years). Increased synthesis of prolactin can be caused by hypothalamic lesions or by medication of methyldopa, or reserpine that interfere with dopamine (prolactin-inhibitory factor) secretion. Lactotroph adenoma can also be associated with estrogen therapy.

Clinical Features

Young women present with amenorrhea, galactorrhea and infertility. There is increased concentration of serum prolactin level. Increased prolactin level inhibits LH, which is essential for ovulation. Men present with decreased libido and erectile dysfunction.

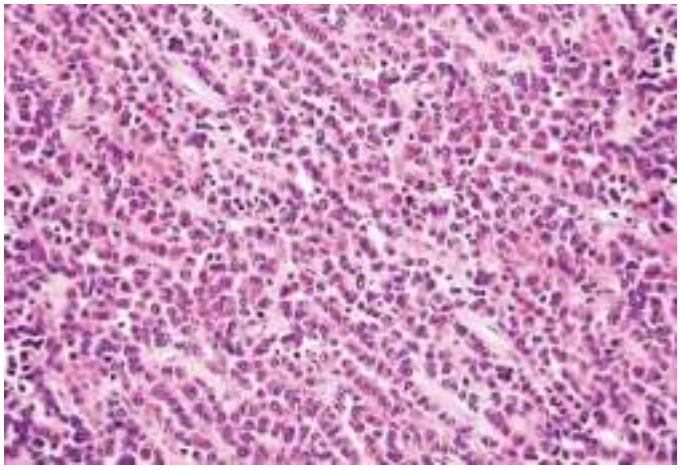


Fig. 26.75: Lactotroph adenoma (prolactinoma). Tumor consists of acidophilic or chromophobe cells (100X).

Management

Functional lactotroph adenoma is treated with dopamine agonists to inhibit prolactin synthesis. Macroadenoma may require surgery or irradiation.

Tumor resected post-therapy usually shows interstitial fibrosis and reduction in size. Tumor cells show low proliferative index and focally positive for prolactin.

SOMATOTROPH ADENOMA

Somatotroph adenoma is the second most common pituitary tumor, which secretes growth hormone, causing acromegaly or gigantism.

- Most cases of acromegaly are diagnosed in the fourth and fifth decades. Gigantism most often affects infants and children.
- Somatotroph adenoma arising before epiphyseal closure in children or adolescents cause gigantism. However, adenoma originating after the fusion of epiphyses of the long bones leads to acromegaly in adults. Expansion of the tumor within the sella turcica may cause local compression effects.
- Patient develops acromegaly due to combined effects of growth hormone, somatomedin C and insulin-like growth factor 1, headache, and symptoms due to compression of optic chiasma. Patient also develops generalized enlargement of viscera.

Growth Hormone

- Growth hormone is synthesized by somatotropes in the anterior pituitary gland, which has growth promoting and anti-insulin actions. Growth hormone is essential for linear bone growth in children.
- Growth hormone increases rate of transport amino acids across cell membranes and utilization of fatty acids. Growth hormone decreases the utilization of carbohydrates. Mechanism of action of growth hormone is given in Table 26.49. Growth promoting and anti-insulin effects of growth hormone are shown in Fig. 26.76.

Clinical Features

Clinical manifestations of acromegaly are shown in Fig. 26.77.

- **Head and neck:** Patient has prominent frontal and orbital ridges, coarsened features, recurrent serous otitis media, macroglossia, prominent jaw and thyroid enlargement.
- **Axilla:** There is increased perspiration in axillae.
- **Cardiovascular system:** Patient presents with hypertension, heart failure, cardiomegaly, edema and breathlessness.
- **Skeletal system:** Patient presents with barrel chest, enlarged hands, paresthesia in hands, broad feet, joint

Table 26.49 Mechanism of action of growth hormone

Actions	Effects
Growth promoting actions	
Growth hormone stimulates liver to synthesize IGF-1 (insulin-like growth factor 1)	▪ IGF-1 increases linear growth of bone and cartilage ▪ IGF-1 participates in increase in size of organs and their functions ▪ IGF-1 increases lean muscle mass ▪ IGF-1 participates in protein synthesis
Anti-insulin actions	
Growth hormone has anti-insulin actions	▪ Growth hormone promotes lipolysis, utilization of fatty acids leading to decrease in adiposity ▪ Growth hormone decreases utilization of glucose leading to increased blood sugar level

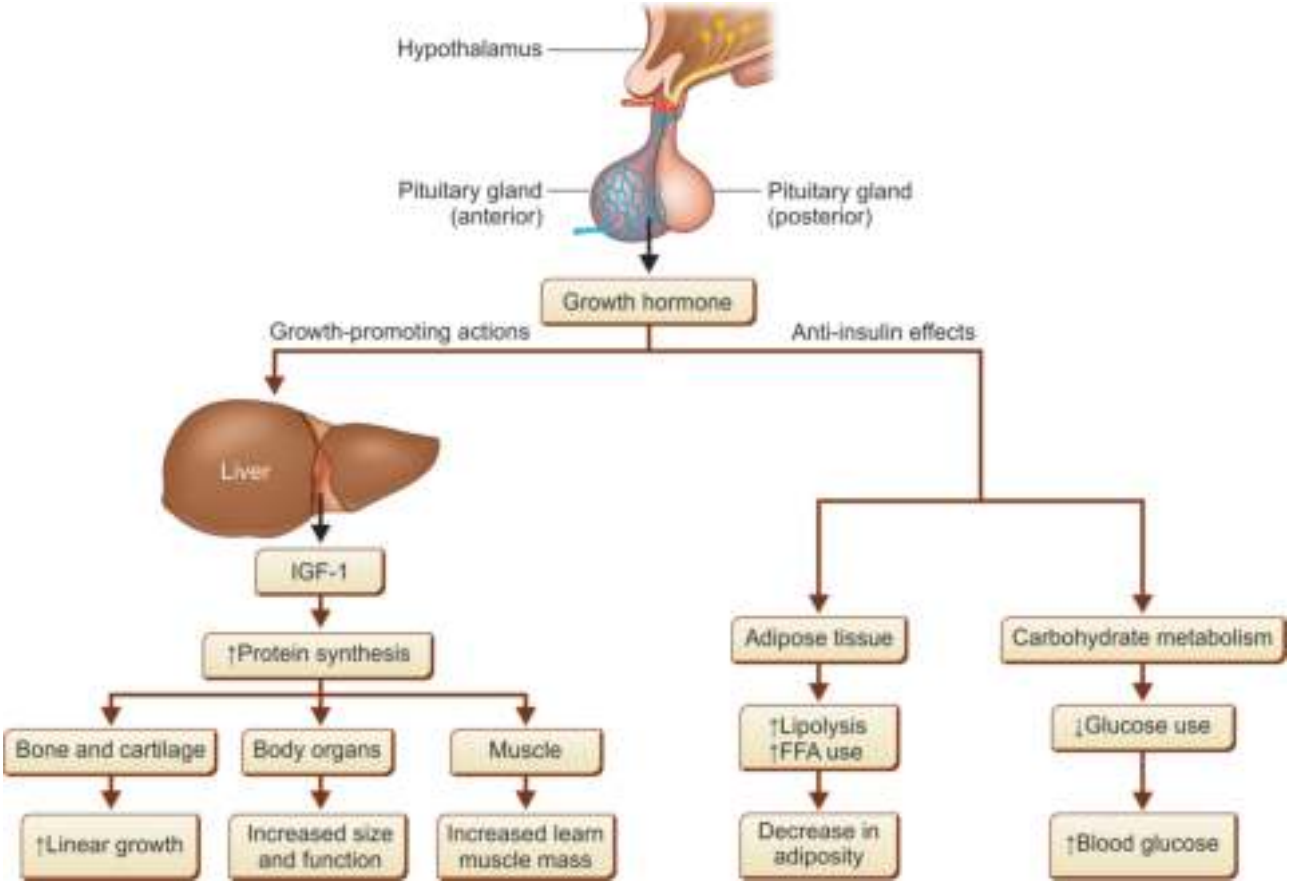


Fig. 26.76: Growth promoting and anti-insulin effects of growth hormone (FFA, free fatty acids; IGF-1, insulin-like growth factor 1).

pain, degenerative arthritis, peripheral neuropathy, and myopathy.

- **Skin:** Patient develops pigmentation and thickened skin.
- **Sexual problems:** Male develops sexual dysfunction. Female presents with menstrual disturbances.

Diagnostic Serum Marker

Growth hormone excess causes elevation in serum concentration of insulin-like growth factor 1 (somatomedin C) by the liver, measurement of which is a reliable indicator of disease activity.

CORTICOTROPH ADENOMA

Corticotroph adenoma secretes excess of ACTH, resulting in Cushing's syndrome of pituitary origin (Cushing's disease). It is five times more frequent than adrenal tumor associated with Cushing's syndrome.

Clinical Features

Patient develops hypercorticism most often of pituitary gland origin and less often of adrenal origin. It is worth mentioning that ectopic ACTH production occurs in small cell lung carcinoma.

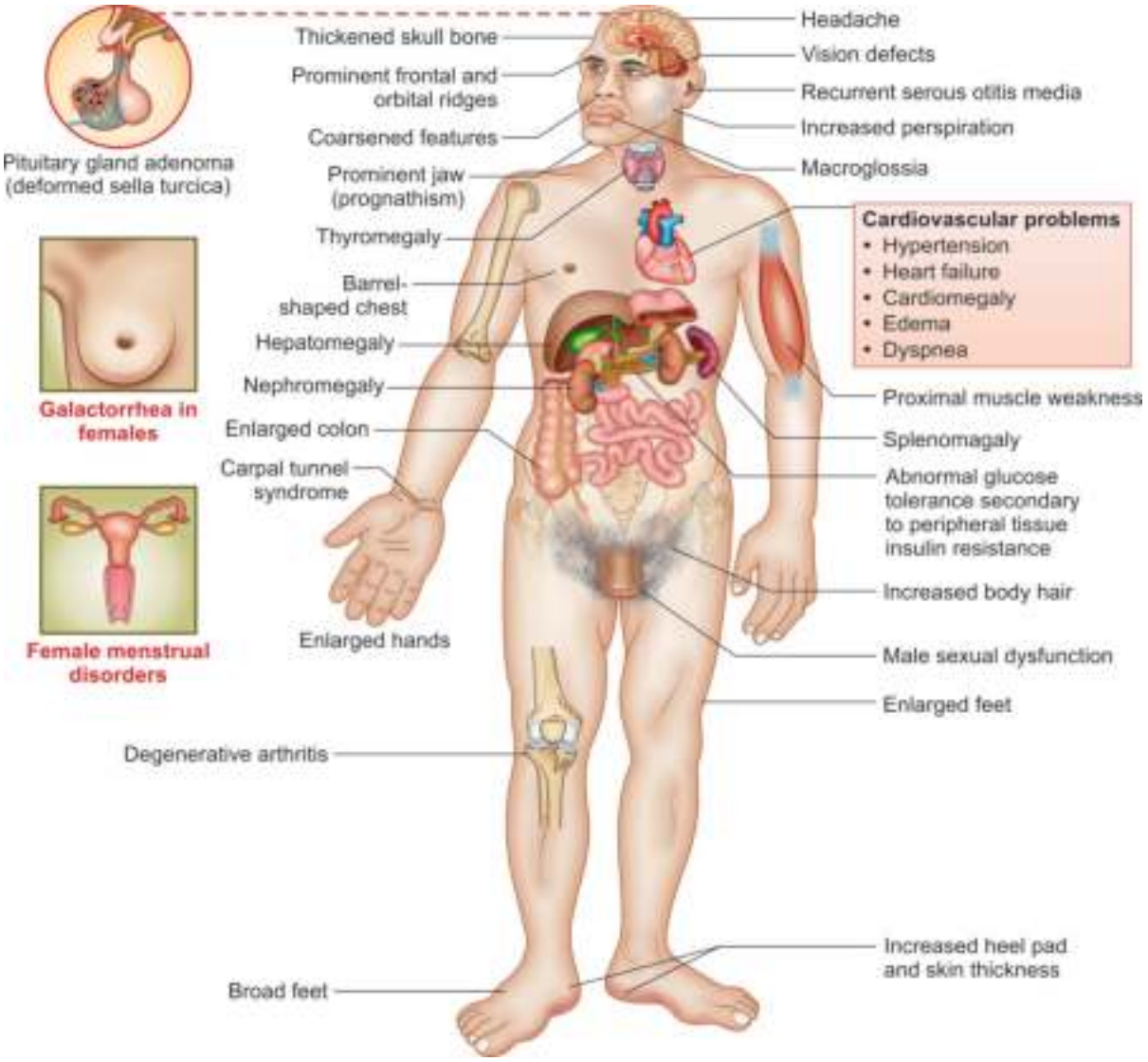


Fig. 26.77: Clinical manifestations of acromegaly. Growth hormone excess leads to enlargement of hands, feet and skull.

Dexamethasone Suppression Test

Dexamethasone suppression test distinguishes between ACTH-dependent and ACTH-independent forms of Cushing's syndrome.

Dexamethasone suppresses pituitary secretion of corticotropin, whereas it has no effect in cases of adrenal hyperplasia or functional adrenal tumors.

MIXED SOMATOTROPH–LACTOTROPH ADENOMA

Tumor consists of two types of cells: somatotropic cells synthesize growth hormone and lactotropic cells secrete prolactin.

Clinical Features

Approximately 50% of tumors with acromegaly, excess synthesis of prolactin causes loss of libido and erectile dysfunction in men.

Management

Patients are treated with dopamine agonists, which inhibit prolactin secretion.

CRANIOPHARYNGIOMA

Craniopharyngioma is an infiltrative epithelial tumor located in the region of the pituitary fossa above the sella turcica. It does not secrete hormones.

Tumor Origin

Tumor is derived from remnants of Rathke's pouch located above the sella turcica, the embryological source of the anterior pituitary gland. It is similar to ameloblastoma of the jaw.

Pathology

Clinical manifestations vary depending on destruction or pressure caused by tumor. Destruction of either/or

both lobes of pituitary gland result in hypopituitarism. It causes local destruction displacing the midline structures in the region of the hypothalamus.

Clinical Features

Tumor compresses the pituitary gland and damages the overlying hypothalamus and optic chiasma, presenting with either hypopituitarism or visual disturbances with bilateral hemianopia (loss of peripheral visual fields) or hydrocephalus and headaches in children between 10 and 20 years. Cranial nerve damage by tumor causes palsies.

- Patient often presents with diabetes insipidus due to ADH deficiency, which is characterized by an inability to concentrate the urine, with consequent chronic water diuresis, thirst, and polydipsia.
- Approximately 25% of cases of central diabetes insipidus are associated with brain tumors, particularly craniopharyngioma.

Surgical Pathology: Craniopharyngioma

Gross Morphology

- Tumor shows solid and cystic areas. It also shows areas of calcification.
- The cyst is filled with thick fluid containing lipid derived from breakdown of the lining epithelium.

Light Microscopy

- Tumor is composed of solid and cystic areas. Cystic areas are lined by nests and cords of squamous or columnar cells in a loose stroma, closely resembling the appearance of the embryonic tooth bud enamel organ.
- It is most often lined by flat or columnar cells resulting in papillary projections (Fig. 26.78).

Immunohistochemistry Technique

β-Catenin gene mutation study is useful in the diagnosis of colorectal adenocarcinoma versus adenocarcinomas of other sites, craniopharyngioma, solid and pseudopapillary tumor of the pancreas, and other neoplasms.

Radiological Analysis

Calcification in craniopharyngioma apparent on radiograph is often prominent; facilitating diagnosis.

Recurrence of Tumor after Surgical Excision

Although craniopharyngioma is benign, local recurrence is often a problem because of inability to completely excision of infiltrative tumors.

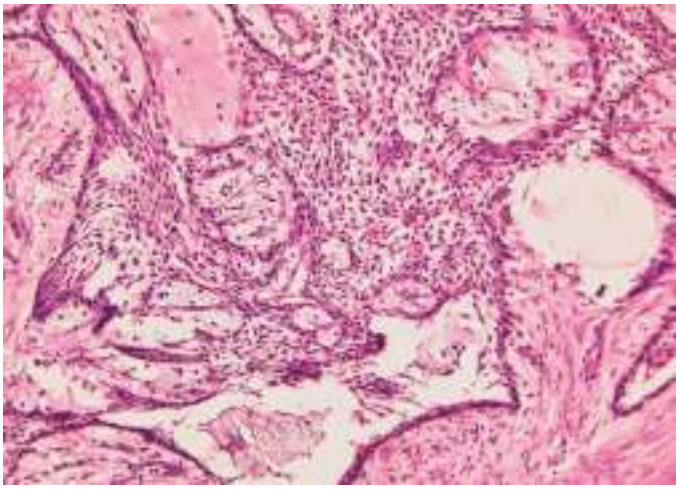


Fig. 26.78: Craniopharyngioma—tumor is composed of solid and cystic areas. Solid areas exhibit solid sheets of well-differentiated nonkeratinizing squamous epithelium (400X).

CHAPTER

27

Bones and Joints

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

DISORDERS OF BONES

- Bones: overview
  - Cells in normal bone
  - Histology
  - Bone diseases

OSTEOMYELITIS (BONE INFECTION)

- Pyogenic osteomyelitis
- Tubercular osteomyelitis

METABOLIC BONE DISEASES

- Metabolic bone disease: overview
  - Rickets
  - Osteomalacia
  - Renal osteodystrophy
  - Calcification in tissues
  - Osteitis fibrosa cystica
  - Osteoporosis
  - Paget's disease of bone (osteitis deformans)
  - Scurvy

BONE TUMORS

- Bone tumors: diagnostic approach
  - Clinical features
  - Physical examination
  - Imaging studies
  - Histologic examination
  - Immunohistochemistry Technique
  - Staging system
  - Grading system
- Chondrogenic tumors
  - Chondromas (enchondroma and periosteal chondroma)
  - Enchondromatosis (Ollier disease)
  - Osteochondroma
  - Chondroblastoma
  - Chondromyxoid fibroma
  - Conventional chondrosarcoma (grades 1, 2, 3)
  - Dedifferentiated chondrosarcoma
  - Mesenchymal chondrosarcoma
  - Clear cell chondrosarcoma
- Osteogenic tumors
  - Osteoma
  - Osteoid osteoma
  - Osteoblastoma
  - Conventional intramedullary osteosarcoma
  - Low-grade central osteosarcoma
  - Telangiectatic osteosarcoma
  - Small cell osteosarcoma
  - Parosteal osteosarcoma
  - Periosteal osteosarcoma
  - High-grade surface osteosarcoma
- Ewing sarcoma/primitive neuroectodermal tumor
  - Ewing sarcoma
- Adamantinoma
- Osteoclastic giant cell-rich bone tumors and cystic lesion
  - Giant cell tumor of bone (osteoclastoma)
  - Aneurysmal bone cyst
- Notochordal tumors
  - Conventional chordoma
- Fibrogenic bone tumors
  - Desmoplastic fibroma of bone
  - Fibrosarcoma of bone
- Fibrohistiocytic tumors of bone
  - Benign fibrous histiocytoma nonossifying fibroma (metaphyseal fibrous defect)
  - Malignant fibrous histiocytoma of bone
- Hematopoietic neoplasms of bone
  - Multiple myeloma
  - Primary bone marrow lymphoma (PBML)
- Bone tumors of undefined neoplastic nature
  - Simple bone cyst
  - Fibrous dysplasia of bone
  - Osteofibrous dysplasia (ossifying fibroma)
  - Langerhans cell histiocytosis
  - Erdheim-Chester disease
  - Chondromesenchymal hamartoma
  - Rosai-Dorfman disease
  - Achondroplasia
  - Osteogenesis imperfecta
  - Osteopetrosis
  - Osteonecrosis (avascular necrosis)
- Secondary bone tumors (metastatic bone disease)

DISORDERS OF JOINTS

- Classification of joints
- Arthritis of probable autoimmune origin
  - Rheumatoid arthritis
  - Ankylosing spondylitis
- Degenerative disease of joints
  - Osteoarthritis
- Arthritis of metabolic origin
  - Gouty arthritis
  - Chondrocalcinosis (pseudogout)
  - Ochronosis of joints
- Infective arthritis
  - Gonococcal arthritis
  - Lyme arthritis
  - Tubercular arthritis
- Miscellaneous disorders of joints
  - Hypertrophic osteoarthropathy
  - Ganglion cyst
  - Tibial pseudoarthrosis

DISORDERS OF BONES

BONES: OVERVIEW

Bone comprises osteocytes, osteoblasts and osteoclasts, which are related to bone formation, bone maintenance, and resorption of the bone matrix.

- Bone also contains fibroblasts, and blood vessels. The joint space of the mature bone and growth plate of the growing long bones contain cartilage cells.
- The bone marrow contains hematopoietic cells and adipocytes. Osteoblast derived proteins of bone matrix are given in Table 27.1. Most common type of collagen found in the bone is type 1 collagen.

CELLS IN NORMAL BONE

- **Osteocytes:** Osteocytes are mature resting cells involved in the maintenance of the basic functional and anatomic units of bone, called osteons. Osteocytes participate in the maintenance of the blood concentration of calcium and phosphate.
- **Osteoblasts:** Osteoblasts are bone forming cells, which synthesize osteoid and participate in its calcification, which can be stimulated by parathormone (PTH), vitamin D, and estrogens.

Table 27.1 Osteoblast derived proteins of bone matrix

Categories	Examples
Collagen fibers	Collagen fibers type 1
Cell adhesion proteins	Osteopontin, thrombopontin and fibronectin
Proteins participating in mineralization	Osteocalcin
Calcium binding proteins	Osteonectin, bone sialoprotein
Enzymes	Alkaline phosphatase, collagenase
Growth factors	Insulin-like growth factor (IGF), TGF-β, platelet-derived growth factor (PDGF)
Cytokines	IL-1, IL-6

- **Osteoclasts:** Osteoclasts participate in resorption of bone, which are closely related to macrophages. Thus, they are derived from the same bone marrow precursors responding to granulocyte macrophage colony stimulating factor (GM-CSF). In contrast to mononuclear macrophages, osteoclasts are multinucleated.

HISTOLOGY

- **Woven bone:** Woven bone is newly formed bone in growing children and composed of haphazardly arranged strands of collagen, appearing like woven cloth when examined microscopically under polarized light.
- **Lamellar bone:** Woven bone gradually transforms into lamellar bone and is composed of parallel arrays of mineralized osteoid, which is stronger and more resistant to stress. Lamellar bone is a typical component of adult bones.

BONE DISEASES

Bones are affected by numerous developmental, metabolic, infectious and neoplastic diseases. Bones are also prone to mechanical trauma. Most common site of infection and tumors is the fastest growing end of the long bones. Genetic disorders may result in the formation of short, deformed, or structurally abnormal bones.

- Patient presents with focal pain in fracture/trauma, osteomyelitis, or osteoid osteoma. Diffuse pain occurs due to bone metastases, osteoporosis, and osteomalacia. Focal or diffuse pain is observed in bone metastases and Paget's disease of bone. Salient features of non-neoplastic bone disorders are given in Table 27.2.
- Avascular necrosis of bone occurs in femoral head, proximal scaphoid, posterior talus, tibia, lunate and navicular bone due to absence of collateral arterial supply.

Table 27.2 Salient features of non-neoplastic bone disorders

Tumor/Lesion	Location	Age	Radiologic Findings	Pathologic Findings	Differential Diagnosis
Congenital/ developmental disorders	Diffuse, sometimes localized; usually symmetrical	<10	Modeling abnormality	Disease-dependent	Very broad
Traumatic disorders	Any part of bone	Any	Fracture lines; dislocation	Hemorrhage; organization; woven bone and chondroid matrix	Osteosarcoma and chondrosarcoma
Circulatory disorders					
Avascular necrosis	Convex ends of long bones	5–40	Wedge-shaped radiodensity: crescent sign; collapse of articular cartilage	Necrotic marrow and bone; subarticular plate fracture	None

Contd...

Table 27.2 Salient features of non-neoplastic bone disorders (*Contd...*)

Tumor/Lesion	Location	Age	Radiologic Findings	Pathologic Findings	Differential Diagnosis
Idiopathic infarction	Medulla of long bones	>20	Serpiginous radio-opacities	Necrotic marrow and bone; calcification and ossification of marrow fat	Enchondroma
Metabolic disorders					
Paget's disease of bone (osteitis deformans)	Any portion of any bone; almost always extending to articular end	>50	▪ *Early:* Bone resorption in wedge-shaped edge ▪ *Later:* Coarse trabeculation: loss of corticomedullary differentiation	Osteoclastic resorption + increased vascularity and marrow fibrosis: *Mosaic* cement lines in middle to late stages	Hyperparathyroidism; myelodysplasia and myelofibrosis; metastatic carcinoma
Infectious disorders					
Hematogenous	Cortex of long bones	2–15	▪ *Early:* ↑Uptake on bone scan; change of marrow signal on MRI ▪ *Later:* Mixed sclerosis and radiolucency	Marrow fibrosis with osteonecrosis and exudate/mixed inflammatory cell infiltrate	Round cell tumors and Langerhans cell histiocytosis
Direct	Any; open trauma or deep ulcer	Varies	Mixed sclerosis and radiolucency	Marrow fibrosis with osteonecrosis and exudate/mixed inflammatory cell infiltrate	Round cell tumors and Langerhans cell histiocytosis

OSTEOMYELITIS (BONE INFECTION)

Osteomyelitis is inflammation of bone marrow caused by bacteria. Infectious organisms may reach the bone through the bloodstream.

- Osteomyelitis primarily affects the metaphyseal region of the long bones of extremities around knee, ankle, and hip joints because of the unique vascular supply in these regions.
- Normally, arterioles entering calcified portion of the growth plate form a loop, and then drain into the medullary cavity without establishing a capillary bed.
- Slow blood flow into vascular loop allows more time for the bacteria to invade the walls of the blood vessels and establish an infective focus within bone marrow.

PYOGENIC OSTEOMYELITIS

Pyogenic osteomyelitis may affect neonates, children, adolescents and adults described as under.

AGE GROUP

Pyogenic Osteomyelitis in Children and Adolescents

- *Staphylococcus aureus* is the most common cause of pyogenic osteomyelitis in children >14 years of age due to penetrating injuries and post-surgical wounds.
- In growing bones of the children, most bone infection begins predominantly in the highly vascularized growth plate (metaphyseal region) of long bones, with greatest blood flow in the nutrient artery.
- Pyogenic osteomyelitis most often involves femur (distal end), tibia (proximal end) and humerus (proximal end). Salmonella infection causing osteomyelitis is frequent in association with sickle cell anemia.

Pyogenic Osteomyelitis in Adults

Pyogenic osteomyelitis occurs usually as a complication of compound fracture or sequelae of surgery. Osteomyelitis is an uncommon complication of closed fracture. Osteomyelitis in adults most often begins in epiphyseal and subchondral locations due to hematogenous dissemination of *Staphylococcus aureus*.

- Hematogenous dissemination of *Escherichia coli*, *Pseudomonas* and *Klebsiella* species occurs in urinary tract infection and intravenous drug addicts.
- *Pseudomonas aeruginosa* infection also occurs through nail puncture in persons wearing rubber footwear.
- Patient may develop osteomyelitis due to extension of infection from contiguous sites (e.g. periodontal abscess).

Pyogenic Osteomyelitis in Neonates

Haemophilus influenzae, *Escherichia coli* and Streptococci group B (β-hemolyticus) are responsible for pyogenic osteomyelitis in neonates.

ANATOMICAL ASPECT

Metaphyseal arteries give terminal branches and forming irregular loops of afferent venous sinusoids at growth plate. Blood flow becomes slow and turbulent in the regions of growth plate in metaphyseal region of bone, predisposing to bacterial seeding.

In addition, bone lining cells (BLCs) in metaphyseal region have little or no phagocytic activity. This region becomes a catch basin for bacteria and forms an abscess.

PATHOGENESIS

Focus of infection in the metaphysis of long bones attracts polymorphonuclear cells. Entire bone marrow cavity space becomes edematous due to exudates. In the acute stage, pyogenic osteomyelitis may resolve with antibiotic therapy.

Chronic pyogenic osteomyelitis results from persistent suppurative acute infection of bone. Sequence of events in the pathogenesis of pyogenic osteomyelitis is shown in Fig. 27.1.

Sequestrum (Necrotic Bone) Formation

Due to rigid structure and limited bone space, inflammatory exudates in bone marrow cavity compress endosteal blood vessels and impair the inflow of blood.

- Pyogenic abscess, limited by growth plate, spreads transversely along 'Volkmann haversian canals' and elevates the periosteum. It will further compress the perforating blood vessels of periosteum in subperiosteal region. Then it extends subperiosteally and may invade shaft of bone in infants of less than one year of age.
- Some metaphyseal arterial branches pass through growth plate, and infection may invade epiphysis and joints.
- Osteolytic enzymes detach dead bone from main bone that floats inside the abscess cavity. The necrotic bone is called a sequestrum (Fig. 27.2).

Involucrum (Reactive Viable Bone Formation)

Involucrum is the formation of reactive viable bone in the periosteum that surrounds necrotic bone (sequestrum) in pyogenic 'osteomyelitis' (Fig. 27.3A).

Brodie Abscess (Chronic Bone Pyogenic Abscess)

Brodie abscess is a chronic bone pyogenic abscess, which consists of dense reactive bone from the periosteum and endosteum that surrounds and contains infective focus (Fig. 27.3B and C).

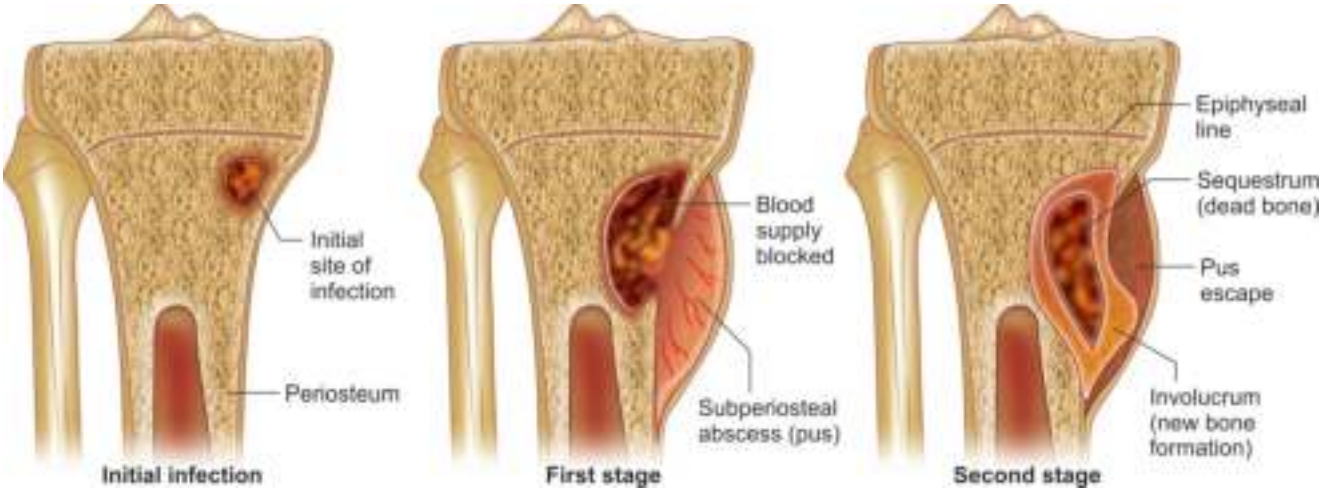


Fig. 27.1: Osteomyelitis shows stages of abscess formation, sequestrum and involucrum.



Fig. 27.2: Acute osteomyelitis. There is a large collection of pus in the medullary cavity of the shaft of the femur (arrows).

Cloacae Cortical Defect in Pyogenic Osteomyelitis

Cloaca is the hole found in the bone during formation of a draining sinus. Pyogenic osteomyelitis may erode periosteum and form discharging sinuses onto the skin surface of the body through holes in bones. Process of cloaca in the bone is influenced by virulence of organism, development of host resistance to administration of antibiotics and fibrotic and sclerotic responses.

RADIOGRAPHIC FINDINGS

Initially, bony lesion is osteolytic in pyogenic osteomyelitis. Sclerosis appears only after the disease has progressed and persisted for >2 months. Early diagnosis of pyogenic osteomyelitis is made by **Indium-labeled leukocytic scintigram**, which shows focal signal increase in the site of infection.



Fig. 27.3A: Chronic osteomyelitis. This specimen shows death of shaft of the tibia. This has separated completely as a sequestrum. New bone—an involucrum has formed around the dead bone. Lacunae open through the involucrum and result in discharging sinuses on the skin surface (arrow).

CLINICAL FEATURES

Patient with pyogenic osteomyelitis of long bone presents with fever, pain and tenderness (most common), swelling in acute cases; later followed by discharging sinuses onto the skin surface, effusions in adjacent joints and limitation of joint movement.

- In acute pyogenic osteomyelitis, peripheral blood shows leukocytosis and blood culture may be positive.
- Systemic manifestations in vertebral involvement are usually milder. Pain may be principal manifestation especially in adults.

COMPLICATIONS

- Pyogenic osteomyelitis fails to resolve in 5–25% of patients, which progresses to chronic osteomyelitis. The weakened bone is prone to fracture complicated by pyogenic osteomyelitis, which fails to heal and resulting in development of pseudoarthrosis.
- Pyogenic osteomyelitis may be complicated by secondary (reactive systemic) amyloidosis and cutaneous squamous cell carcinoma within sinus tract.



Fig. 27.3B: Brodie's abscess in diaphysis of tibia. Radiograph demonstrates a well-defined, vertically oval, intramedullary osteolytic lesion with narrow zone of transition in distal metaphysis of tibia. The lesion does not show any new bone formation. No periosteal cortical break is seen. No periosteal reaction or swelling or calcification seen. These findings are suggestive of infective etiology: Brodie's abscess in diaphysis of left tibia shown by arrow. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)



Fig. 27.3C: Osteomyelitis of skull in 45-year-old patient with history of road traffic accident. Skull radiograph demonstrates diffuse lytic bone destruction with moth-eaten appearance and soft tissue component involving the frontal bone and maxilla. Few punctate shaped bony fragments are noted involving superficial soft tissues in frontal region. Findings are suggestive of osteomyelitis shown by arrow. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

TUBERCULAR OSTEOMYELITIS

Tubercular osteomyelitis occurs most commonly secondary to hematogenous extension from a primary tubercular focus in the lung, which principally targets the vertebral column, called *Pott's disease* in 1–3% of people. Vertebral body destruction may result in impingement on the spinal cord (Fig. 27.4). Tubercular oseomyelitis also targets hip bone, long tubular bones (femur, tibia) and small bones (hands, feet).

However, any bone may be affected in tubercular osteomyelitis. In the long tubular bones, tuberculosis originates in the epiphysis and disseminates to the adjacent joint. This feature differentiates tubercular osteomyelitis from pyogenic osteomyelitis, which starts in the metaphysis but cannot extend to the epiphysis in children. Tubercular dactylitis occurs frequently in young children.

CLINICAL FEATURES

Patient presents with fever, fatigue, bone pain, soft tissue swelling decreased range of motion, difficult ambulation and discharging sinuses (Fig. 27.5).

Surgical Pathology: Tubercular Osteomyelitis

Light Microscopy

- In tubercular osteomyelitis marrow shows fibrosis infiltrated by chronic inflammatory infiltrate composed of lymphocytes, macrophages and plasma cells.
- Caseous necrosis and epithelioid cell granulomas are present.

COMPLICATIONS

Patient may develop many complications such as deformities of bones, pathologic fractures, secondary amyloidosis or squamous cell carcinoma. The skin at the edges of sinus tracts draining the pus from the infected bone may undergo malignant transformation.

LABORATORY DIAGNOSIS

In tubercular osteomyelitis, radiograph demonstrates cortical destruction, juxtacortical abscess formation, and medullary infarction.

- Pus culture and sensitivity performed in patients with tubercular osteomyelitis may demonstrate pyogenic bacteria due to superadded infection.
- Patients with tubercular osteomyelitis do not respond to antibiotic therapy. Biopsy may not demonstrate epithelioid cell granulomas.



Fig. 27.4: Tuberculosis in the spine of a young boy (Pott's fracture of the vertebrae). In spinal tuberculosis, there is destruction of the intervertebral disk space and the adjacent vertebral bodies, collapse of the spinal elements, and anterior wedging leading to the characteristic angulation and gibbus (palpable deformity due to involvement of multiple vertebrae) formation shown by arrow.



Fig. 27.5: Tuberculous psoas abscess. Two lumbar vertebrae involved by tuberculosis with extension of the caseation into the psoas muscle. When such infection spreads along the psoas, the abscess may point in the groin (arrow).

- Therefore, in every case of osteomyelitis with discharging sinuses, blood culture on Lowenstein-Jensen medium should be done. Imaging techniques are employed as diagnostic tools.

METABOLIC BONE DISEASES

Metabolic bone disease refers to osteopenia (diffuse radiolucency of bone). It occurs due to abnormalities in vitamin D metabolism, phosphate deficiency and defects in the mineralization process. Vitamin D deficiency causes rickets in children and osteomalacia in adults. Intramembranous ossification occurs in multiple skeletal healing scenarios. Mesenchymal cells form osteoblasts, resulting in formation of collagenous osteoid matrix. Endochondral ossification occurs in the development of long tubular bones. Growing cartilage at epiphyseal plate gets mineralized, and then resorbed and is replaced by osteoid matrix and then mineralized to form bone.

METABOLIC BONE DISEASE: OVERVIEW

RICKETS

Rickets is the softening and weakening of bones in children usually due to an extreme and prolonged vitamin D deficiency.

Rickets is caused by failure of action of calcitriol (1,25-dihydroxycholecalciferol), the active form of vitamin D in children and due to diet deficient in vitamin D, lack of exposure to sunlight, intestinal malabsorption, renal and liver disease. The bony abnormalities in rickets occur due to decreased bone mineralization (calcification), which results in excessive accumulation of unmineralized matrix (osteoid), wide osteoid seams, increased thickness of the epiphyseal growth plates and other skeletal deformities. During infancy, head and neck are under greatest stress; hence changes are marked in these regions. Clinical features are given in Table 27.3 and Fig. 27.6.

Pathology Pearls: Key Features of Vitamin D

Vitamin D occurs in two forms, i.e. vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol).

- **Sources:** Vitamin D may be derived from endogenous (80%) or exogenous (20%) sources. Endogenous source exists in the form of dehydrocholesterol in the skin. When skin is under exposure to ultraviolet rays, dehydrocholesterol is converted into vitamin D_3 (cholecalciferol).
- **Metabolism:** After absorption, vitamin D combines with α-globulin, and 2-hydroxyvitamin D (vitamin D_2) is formed in the liver. Vitamin D_2 to vitamin D_3 (active form of vitamin D) conversion takes place in kidneys. Low level of serum calcium augments the conversion of vitamin D_2 to vitamin D_3. Vitamin D is stored in adipose tissue.
- **Functions:** Vitamin D maintains normal plasma levels of calcium and phosphorus. Vitamin D enhances calcium absorption by small intestine. It collaborates with parathormone in mobilization of calcium from bone. It stimulates parathormone-dependent absorption of calcium in distal tubular cells parathormone. It is requisite for normal mineralization of epiphyseal cartilage and osteoid.

OSTEOMALACIA

Osteomalacia is softening of bones in adults and characterized by inadequate mineralization, i.e. calcification

Table 27.3 Clinical features of rickets

Terms	Clinical Findings
Craniotabes	There is thinning and softening of occipital and parietal bones. There is late closure of fontanelles
Frontal bossing	Frontal bone is squared due to increased formation of osteoid
Rachitic rosary	Thickening of costochondral junction occurs due to overgrowth of cartilage and osteoid tissue results in a string of beads-like appearance
Harrison's groove	A depression occurs along the line of insertion of the diaphragm into the rib cage due to inward pull of diaphragm
Pigeon-shaped chest	Anterior protrusion of sternum occurs due to inward pull of metaphyseal areas of ribs by respiratory muscles
Pelvis bones	Pelvis bones are deformed
Spine deformity	Lumbar lordosis occurs, when child is ambulatory



Fig. 27.6: Rickets in a two-year-old male child. Radiograph demonstrates irregular fraying and cupping of metacarpals with growth-plate widening. Findings are suggestive of rickets shown by arrow. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

of newly formed bone matrix. The bones are susceptible to fracture, but few with bone deformities.

Etiopathogenesis

Vitamin D deficiency in adults occurs due to malabsorption syndrome (commonest cause), chronic kidney disease (renal osteodystrophy) and paraneoplastic syndrome due to PTH-like protein synthesized by squamous cell lung carcinoma, breast carcinoma, ovarian carcinoma and Fanconi's syndrome. Osteomalacia in Fanconi's syndrome occurs due to renal wastage of phosphate, amino acids and glucose in urine.

Clinical Features

Patient presents with bone pain and tenderness, and weakness of proximal limb muscles.

Radiologic Findings

- Spontaneous incomplete fractures (looser's zones) often occur in the long bones or pelvis. Vertebral bodies become biconcave due to central compression of softened vertebrae by intervertebral discs.
- These bone changes can be reversed by supplementing the diet with adequate amounts of vitamin D and normalization of calcium metabolism.

Surgical Pathology: Osteomalacia

Light Microscopy

- In osteomalacia, the bony trabeculae are normal in thickness and rimmed by broad bony spicules of osteoid (incompletely calcified) lined by osteoclasts.
- Central portion of bony spicules are normally calcified, but their peripheral portion uncalcified.
- On the other hand, the bone spicules in osteoporosis are thin but normally mineralized.

Blood Biochemistry

Blood calcium is decreased. Serum alkaline phosphatase is increased due to increased activity of osteoblasts. These biochemical changes are absent in osteoporosis.

RENAL OSTEODYSTROPHY

Bone changes in chronic renal failure are known as renal osteodystrophy. Patient develops clinical, radiologic or pathologic evidence of bone diseases, i.e. osteoporosis, osteomalacia, bone necrosis, and soft tissue calcification.

Pathogenesis

- **Physiologic state:** Normally, $25(OH)_2D_3$ (inactive form) is converted to the systematically $1,25(OH)_2D_3$ by the renal enzyme 1α-hydroxylase. Vitamin D_3 participates in absorption of calcium by intestine.
- **Pathologic state:** In chronic renal disease, kidneys fail to convert vitamin D_2 to vitamin D_3 (active form).
  - Prolonged hemodialysis inhibits calcification of osteoid leading to osteomalacia. High level of serum phosphates inhibits renal enzymes catalyzing formation of $1,25(OH)_2D_3$. Therapeutic administration of steroid in patients with chronic glomerulonephritis causes osteoporosis and avascular necrosis of bone.
  - Nonavailability of vitamin D_3 results in decreased ionized calcium in serum, which stimulates parathyroid glands to synthesize parathormone.
  - Parathormone causes resorption of bone resulting in osteomalacia. Disordered remodeling of such bones leads to alternating areas of thickened bone (osteosclerosis) and osteoporosis. In renal osteodystrophy, spine shows '**rugger jersey appearance**'.

CALCIFICATION IN TISSUES

Deposition of calcium in tissues is demonstrated by imaging technique and light microscopy.

- Nephrocalcinosis can cause interstitial nephritis further aggravating kidney disease.
- Calcium deposition occurs in joint cartilage (chondrocalcinosis), periarticular region at the junction of metacarpals and proximal phalanges, and intra-articular and periarticular regions of shoulders.
- Calcium deposits in cornea are demonstrated by slit-lamp examination revealing band keratitis.
- Medial calcification occurs in tunica media of small size arteries, aorta and major branches.

OSTEITIS FIBROSA CYSTICA

Osteitis fibrosa cystica is a skeletal disorder caused by an increased synthesis of parathormone in late manifestation of primary hyperparathyroidism as a result of bone resorption by parathormone.

Pathogenesis

- Osteoclasts lining subperiosteum and endosteum invade the cortex. As the disease progresses, trabecular bone resorption occurs resulting in replacement of bone marrow by loose fibrosis and cystic degeneration.
- These areas contain reactive woven bone, and hemosiderin-laden macrophages and often display many giant cells, which are actually osteoclasts.
- The bone lesion has been termed a 'brown tumor of hyperparathyroidism'. This is not a true tumor, but rather a bone repair reaction.

Clinical Features

Patient presents with nephrolithiasis, nephrocalcinosis, calcification in blood vessels, epulis (mouth),

Table 27.4 Clinical manifestations of osteitis fibrosa cystica

Organs	Clinical Manifestations
Kidneys	Nephrolithiasis, nephrocalcinosis
Skull (X-ray)	Salt and pepper appearance
Bone	Cyst formation and fractures
Mouth	Epulis
Vertebrae	Codfishing of vertebrae
Blood vessels	Calcium deposition
Eye	Limbus keratopathy
GIT	Peptic ulcer disease
Pancreas	Pancreatitis
Miscellaneous	Multiple adenomas in endocrines

cod fishing of vertebrae, limbus keratopathy, peptic ulcer, pancreatitis and multiple adenomas in pituitary, thyroid gland, pancreas and adrenal glands. Clinical manifestations of osteitis fibrosa cystica are given in Table 27.4.

Laboratory Diagnosis

Blood tests on patients with osteitis fibrosa cystica generally show high levels of calcium, parathormone and alkaline phosphatase. Laboratory findings in metabolic bone disorders are given in Table 27.5.

OSTEOPOROSIS

Osteoporosis is characterized by decreased bone mass but otherwise normally mineralized bone, which occurs either due to impaired synthesis of bone matrix or increased resorption of bone matrix proteins. Bone loss and eventually fractures (hip, wrist, and vertebrae) are the hallmarks of osteoporosis. Serum calcium and phosphate levels are within normal range.

Types of Osteoporosis

Primary Osteoporosis

- Type 1 primary osteoporosis is more common in women with postmenopausal state due to estrogen deficiency. It occurs due to absolute increase in osteoclast activity.
- Type 2 primary osteoporosis occurs in elderly persons of both sexes, which reflects decreased osteoblastic activity.

Secondary Osteoporosis

Secondary osteoporosis results from a variety of identifiable conditions that may include: hyperparathyroidism, multiple myeloma or metastatic carcinoma, malnutrition, drug therapy (corticosteroids), prolonged immobilization and weightlessness with space travel.

Clinical Features

The affected bones in osteoporosis are unable to bear weight. Patient commonly develops compression fractures of the vertebrae resulting in spinal deformity (kyphosis most common) and shortened stature.

- **Axial osteoporosis:** Patient develops vertebral compression fractures in mid-thoracic or mid-lumbar region progression to thoracic kyphosis. Patient presents with continuous pain in acute phase and intermittent pain in chronic phase.
- **Appendicular osteoporosis:** Patient develops fractures caused by minimal trauma. Common sites of fracture in osteoporosis are head or neck of femur (intertrochanteric or intracapsular), vertebrae of thoracic or lumbar region, Colles fracture in distal radius and fracture of proximal end of humerus.

Laboratory Diagnosis

Radiographic measurement of bone density is the best diagnostic tool. Approximately 40% of bone mass must be lost before a routine radiograph will show signs of osteopenia.

- Radiograph shows diffuse radiolucency of bone. Increased risk for fracture correlates with decreasing bone density.
- Various imaging diagnostic techniques to measure bone density include single-photon absorptiometry, dual-photon absorptiometry, quantitative computed tomography, and dual X-ray absorptiometry.

PAGET'S DISEASE OF BONE (OSTEITIS DEFORMANS)

Paget's disease of bone is an increased thickness of abnormal bone architecture due to increased osteoclastic and osteoblastic activity.

- Paget's disease of bone results from disordered remodeling, in which excessive bone resorption initially results in osteolytic lesions, followed by disorganized and excessive bone formation.

Table 27.5 Laboratory findings in metabolic bone disorders

Disorders	Serum Calcium	Serum Phosphate	Alkaline Phosphatase	Parathormone (PTH)	Vitamin D
Osteoporosis	Normal	Normal	Normal	Normal	Normal
Osteomalacia	Low	High/low	High	Normal	Low/normal
Hyperparathyroidism	High	Low	Normal/high	High	Normal
Renal osteodystrophy	Normal/high	High	High	High	Normal

- There is marked increase in serum alkaline phosphatase (a manifestation of osteoblastic activity) and normal serum calcium and phosphorus levels.

Diagnostic Hallmark in Late Disease

Paget's disease of bone is characterized by abnormal arrangement of lamellar bone containing islands of irregular bone formation, which resembles pieces of a **jig-saw puzzle**, which are separated by prominent cement lines known as **mosaic pattern**.

Pathology Pearls: Morphologic Phases of Paget's Disease of Bone

There are three phases of Paget's disease of bone: osteolytic, mixed and late phases described as under:

- **Osteolytic phase:** Osteolytic phase causes resorption of bone.
- **Mixed phase:** Osteoblastic and osteoclastic phase's lead to new bone formation resulting in mosaic pattern.
- **Osteosclerotic phase:** Osteosclerotic phase is characterized by increased bone density, thickened trabeculae with prominent 'mosaic pattern'.

Age Group

Paget's disease of bone begins after the age of 40 years and mainly affects elderly males.

Predisposing Factors

Paget's disease of bone is of unknown etiology. Viral etiology has been suggested by presence of intranuclear inclusions in osteoclasts demonstrated by electron microscopy. The studies suggest the possible role of a slow virus infection by a paramyxovirus.

Pathogenesis

The majority of patients are first recognized by the presence of an unexpectedly high serum alkaline phosphatase level on biochemical analysis. There are three phases in the development of Paget's disease of bone described as under:

- **Osteoclastic phase:** In this phase, there is excessive resorption of bones.
- **Osteoclastic and osteoblastic phases:** Resorption of bones is followed by bone formation with raised alkaline phosphatase activity.
- **Osteosclerotic phase:** Osteosclerotic phase is followed by production of thick bone without a normal lamellar bone. The bones are weak in osteosclerotic stage.

Bones Involvement

Paget's disease of bone usually involves multiple bones (polyostotic) in 85% of cases or single bone (monostotic) in 15% of cases.

- **Polyostotic lesions:** Polyostotic lesions occur in spine (70%), pelvis (65%), skull (frontal and occipital regions), sacrum, femur, tibia or humerus.
- **Monostotic lesions:** In decreasing order of frequency, bones involved in monostotic lesions include tibia, pelvis, skull (frontal and occipital regions), femur, vertebrae and humerus. Bone changes begin in subchondral ends of tibia, femur and humerus.

Complications

- **Microfractures:** Although the affected bone is thick but it lacks strength, which becomes painful due to microfractures.
- **Anterior bowing of leg bones:** Anterior bowing of tibia and femur is common deformity.
- **Hearing loss:** It occurs due to narrowing of the auditory foramen and compression of the eighth cranial nerve or direct involvement of middle ear bones (malleus, incus, and stapes).
- **Frontal bossing:** It occurs due to head enlargement.
- **Narrowing of foramen magnum:** It occurs due to flattening of base of skull (platybasia) resulting in compression of spinal cord.
- **Osteosarcoma:** Approximately 1% of cases may develop osteosarcoma in a focus of Paget's disease of bone, usually in the femur, humerus, or pelvis.
- **Cardiovascular manifestations:** High output cardiac failure may result from multiple functional arteriovenous shunts within highly vascular early lesions.

SCURVY

Scurvy occurs due to deficiency of vitamin C and is characterized by defective formation of mesenchymal tissue and osteoid matrix; defective wound healing and hemorrhagic phenomena.

Vitamin C is required for hydroxylation of proline and lysine essential for synthesis of reticulin and collagen fibrils from mucopolysaccharide ground substance in wound healing.

Source of Vitamin C

Vitamin C is derived from fruits, vegetables, milk, liver and fish. It is not synthesized endogenously. Vitamin C is stored in the body (normal range 1.5–4.0 g).

Causes of Vitamin C Deficiency

Deficiency may be observed in elderly individuals and alcoholics. Peritoneal and hemodialysis patients and infants on processed milk may develop scurvy.

Clinical Features

Hemorrhages and wound healing defects occur in both children and adults.

Bleeding (Hemorrhages)

- Defective connective tissue also leads to fragile capillaries, resulting in abnormal bleeding.
- Infants and children present with hemorrhages in mucocutaneous and skeletal muscles along fascial planes at mechanical stress points due to loosened endothelial cells from capillaries especially in nail beds, subperiosteal hematomas, bleeding into spaces: joints, retrobulbar, subarachnoid, and intracerebral regions. In adults, ulceration and hemorrhage in gums occur due to loosening of teeth.

Skeletal Changes

- Bone changes in scurvy are secondary to defective osteoid matrix formation. Insufficient production of osteoid matrix results in cartilaginous overgrowth, widening of epiphysis, bowing of the long bones and chest deformity.
- Osteoporosis is seen especially at the metaphyseal ends of bone.

Other Changes

Patient presents with gingival swelling and periodontal infection, impaired wound healing, and anemia.

BONE TUMORS

Conventional approach to the classification of bone tumors is divided into various categories: chondrogenic, osteogenic, myogenic, fibrogenic, fibrohistiocytic, hematopoietic tissue, osteoclast-rich tumors, notochordal tissue, vascular tissue, and miscellaneous tissue of origin, tumors of undefined neoplastic nature and metastatic tumors.

- Primary benign bone tumors such as osteochondroma and osteoid osteoma are seven times more common than malignant tumors.
- Most common primary malignant bone tumors in decreasing frequency are multiple myeloma, osteosarcoma, chondrosarcoma, Ewing sarcoma and giant cell tumor of bone.
- Metastatic bone disease is more common than primary malignant tumors. Bone forming and fibrogenic tumors are more common. Recent 2020 WHO classification of bone tumors is given in Table 27.6. Most common metastatic tumors to bone are given in Table 27.7.

Table 27.6 Recent 2020 WHO classification of bone tumors

Chondrogenic Tumors

- Benign tumors
  - Osteochondroma
  - Chondroma (enchondroma, periosteal chondroma)
  - Osteochondromyxoma
  - Subungual exostosis
  - Bizarre parosteal osteochondromatosis proliferation
  - Synovial chondromatosis
- Intermediate tumors (locally aggressive)
  - Chondromyxoid fibroma
  - Atypical cartilaginous tumor/chondrosarcoma grade 1
- Malignant tumors
  - Chondrosarcoma grade 2
  - Chondrosarcoma grade 3
  - Dedifferentiated chondrosarcoma
  - Mesenchymal chondrosarcoma
  - Clear cell chondrosarcoma

Osteogenic Tumors

- Benign tumors
  - Osteoma
  - Osteoid osteoma
- Intermediate tumors (locally aggressive)
  - Osteoblastoma
- Malignant tumors
  - Low-grade osteosarcoma
  - Conventional osteosarcoma (chondroblastic osteosarcoma, fibroblastic osteosarcoma)
  - Telangiectatic osteosarcoma
  - Small cell osteosarcoma
  - Secondary osteosarcoma
  - Parosteal osteosarcoma
  - Periosteal osteosarcoma
  - High-grade surface osteosarcoma

Fibrogenic Tumors

- Intermediate tumor (locally aggressive)
  - Desmoplastic fibroma of bone
- Malignant tumors
  - Fibrosarcoma of bone

Fibrohistiocytic Tumors

- Benign tumors
  - Benign fibrous histiocytoma/nonossifying fibroma
- Malignant tumors
  - Malignant fibrous histiocytoma

Hematopoietic Neoplasms

- Malignant tumors
  - Plasma cell myeloma
  - Solitary plasmacytoma of bone
  - Primary non-Hodgkin's lymphoma of bone

Contd...

Contd...

Table 27.6 Recent 2020 WHO classification of bone tumors (*Contd...*)

Osteoclastic Giant Cell-rich Tumors

- Benign tumors
  - Giant cell lesion of the small bones
- Intermediate tumors (locally aggressive, rarely metastasizing)
  - Giant cell tumor of bone
  - Aneurysmal bone cyst
- Malignant tumor
  - Giant cell tumor of bone, malignant

Notochordal Tumors

- Low malignant tumor
  - Conventional chordoma
- Malignant tumors
  - Chondroid chordoma (conventional chordoma with chondroid component)
  - Poorly differentiated chordoma
  - Dedifferentiated chordoma

Vascular Tumors

- Benign tumors
  - Hemangioma
- Intermediate tumors (locally aggressive, rarely metastasizing)
  - Epithelioid hemangioma
- Malignant tumors
  - Epithelioid hemangioendothelioma
  - Angiosarcoma

Myogenic Tumors

- Malignant tumors
  - Leiomyosarcoma of bone

Contd...

Lipogenic Tumors

- Malignant tumor
  - Liposarcoma of bone

Tumors of Undefined Neoplastic Nature

- Benign tumors
  - Simple bone cyst
  - Fibrous dysplasia
  - Osteofibrous dysplasia
  - Chondromesenchymal hamartoma
  - Rosai-Dorfman disease
- Intermediate tumors (locally aggressive)
  - Langerhans cell histiocytosis (monostotic, polyostotic) (Letterer-Siwe disease, Hand-Schüller-Christian disease and eosinophilic granuloma)
  - Erdheim-Chester disease

Miscellaneous Tumors

- Ewing sarcoma
- Adamantinoma
- Undifferentiated high-grade pleomorphic osteosarcoma

Secondary Tumors (Metastatic Bone Disease)

- Adult males
  - Prostatic carcinoma
  - Lung carcinoma
- Adult females
  - Breast carcinoma
  - Thyroid carcinoma
  - Renal cell carcinoma
  - Endometrial carcinoma
  - Cervical carcinoma
- Children (males and females)
  - Rhabdomyosarcoma
  - Neuroblastoma
  - Clear cell sarcoma of kidney

Table 27.7 Most common metastatic tumors to bone

Age Group and Gender	Most Common Metastatic Tumors to Bone	Most Common Bone Sites
Adult males	▪ Prostatic carcinoma ▪ Lung carcinoma	▪ Axial skeleton ▪ Proximal femur ▪ Proximal humerus
Adult females	▪ Breast carcinoma ▪ Thyroid carcinoma ▪ Renal cell carcinoma ▪ Endometrial carcinoma ▪ Cervical carcinoma	Appendicular skeleton
Children (males and females)	▪ Rhabdomyosarcoma ▪ Neuroblastoma ▪ Clear cell sarcoma of kidney	Appendicular skeleton

- Clinical behavior of bone tumors under each category is different.
  - Benign bone tumors behave in a nonaggressive fashion with little tendency to recur locally after surgical excision without distant metastasis.
  - Intermediate bone tumors are locally aggressive with metastatic potential.
  - Malignant bone tumors such as osteosarcoma are capable of invasive locally destructive growth with recurrence tendency after surgical excision as well as metastatic potential to distant sites.

Table 27.8 Salient features of common primary malignant tumors of bone

Bone Tumor	Frequency	Age Group and Male to Female Ratio	Location of Tumor	Clinical Behavior	Treatment and Cure Rate
Osteosarcoma	30%	10–20 years (3:2)	Knee joint (distal femur, upper tibia) and jaw	Rapid growth, pain, swelling and distant metastases	Surgery, chemo-therapy (40%)
Chondrosarcoma	15%	40–60 years (2:1)	Pelvis, vertebrae, long bones	Slow growth, vascular invasion	Surgery (75%)
Ewing sarcoma	7%	5–20 years (1:1)	Long bones in diaphyseal region	Fever, pain, widespread metastases	Surgery, chemo-therapy (50%)
Fibrosarcoma and malignant fibrous histiocytoma	20%	30–40 years (3:2)	Long bones in diaphyseal regions (femur, tibia, humerus and pelvis)	Local growth, vascular invasion	Surgery (40%)

BONE TUMORS: DIAGNOSTIC APPROACH

Analytic approach to establish diagnosis of bone tumors includes clinical features, radiologic findings, laboratory findings, gross morphologic study of bone tumors, histologic examination and molecular genetic alteration studies.

CLINICAL FEATURES

Generally small-sized benign bone tumors tend to be asymptomatic and represent incidental finding. Most patients present with pain, swelling, or pathologic fracture.

- Osteoid osteoma presents nocturnal pain in tibia or femur due to synthesis of prostaglandins by the tumor.
- Symptoms may be misleading in Ewing sarcoma and osteomyelitis; as both may present with fever. Patient may develop pathologic fracture in benign and malignant tumors.
- Laboratory investigations such as radiologic studies, serum and urinary findings are important in diagnosis of multiple myeloma.
- Certain bone tumors are relatively specific to particular age.
- The duration of symptoms, rate of growth of tumor, pain associated with the bone mass, history of trauma, personal history, family history of carcinoma and systemic manifestations can help to elucidate the diagnosis. The small size bone lesions tend to be symptomatic and represent as incidental findings.

Age-related Predilection

Certain bone tumors are specific to particular age group. Most common bone tumors according to patient age are described as under. Salient features of common primary malignant tumors of bone are given in Table 27.8.

- **First and second decades:** Bone tumors occurring during this period include nonossifying fibroma, unicameral bone cyst, aneurysmal bone cyst, osteoma, osteochondroma, chondroblastoma, chondromyxoid fibroma, Langerhans cell histiocytosis, conventional intramedullary osteosarcoma (10–20 years) and Ewing sarcoma (5–20 years).
- **Third to fifth decades:** Bone tumors occurring during this period include giant cell tumor, lymphoma, Erdheim-Chester disease and hemangioma.
- **Fourth decade and above:** Bone tumors occurring during this period include metastatic bone disease, multiple myeloma, mesenchymal chondrosarcoma, dedifferentiated chondrosarcoma (40–60 years), chordoma, lymphoma and malignant fibrous histiocytoma. Primary malignant bone tumors and their site of occurrence are shown in Fig. 27.7.

Benign Entities with Multiple Presentations

Certain benign entities that may be multiple at clinical presentations, e.g. brown tumor of hyperparathyroidism, enchondromatosis, fibrous dysplasia, hemangiomatosis, Langerhans cell histiocytosis, nonossifying fibromas (Jaffe-Campanacci syndrome), osteochondromatosis, SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis and osteitis) and Paget's disease of bone (Table 27.9).

Neoplastic and Non-neoplastic Precursor Lesions of Malignancy in Bone

Neoplastic and non-neoplastic precursor lesions of malignancy in bone are given in Table 27.10.

Duration of Complaint-related to Bone Lesions

Benign bone lesions are observed for an extended period over years. On the other hand, malignant bone tumors are noticed within weeks to months.

Rate of Bone Tumor Growth

A rapidly growing bony mass within weeks to months is most likely to be malignant. However, deep-seated bone tumor is noticed late by the patient.

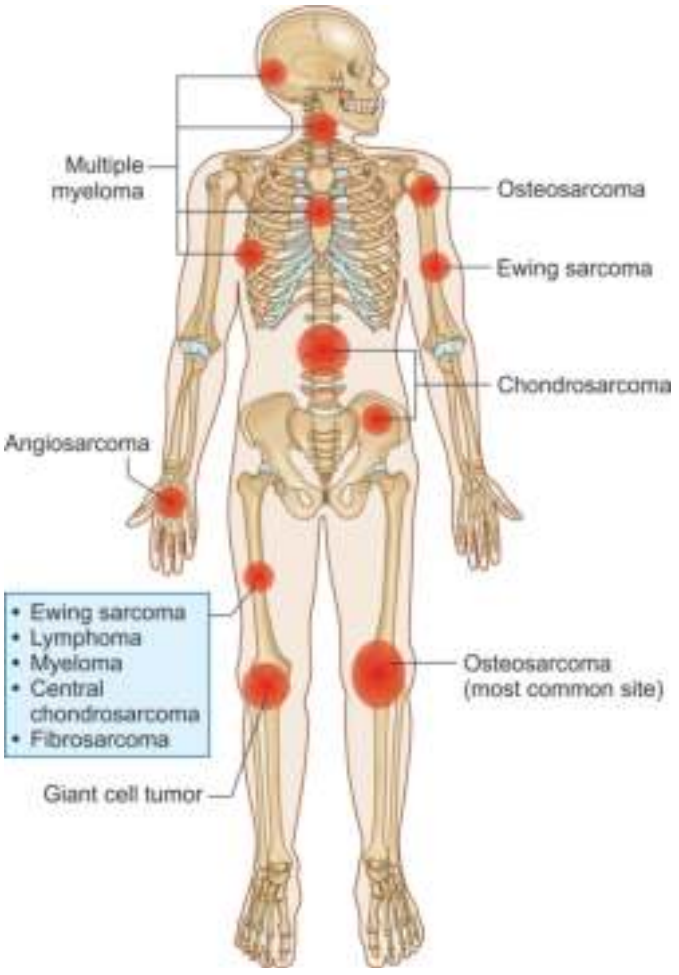


Fig. 27.7: Primary malignant bone tumors and their site of occurrence.

Pain Associated with Bony Mass

Benign bone lesions are usually asymptomatic except osteoid osteoma that is associated with nocturnal pain in tibia or femur due to synthesis of prostaglandins by the tumor. Osteochondroma may produce secondary symptoms because of its encroachment on the surrounding structures. Pain may be noticed due to locally aggressive osteoblastoma, giant cell tumor of bone and malignant bone tumors.

History of Penetrating/Blunt Trauma

Clinician must rule out pyogenic osteomyelitis in a patient with history of penetrating trauma. With a history of blunt trauma, healing of bone fracture must be entertained.

Pathologic Bone Fracture

Many malignant bone tumors may metastasize and weaken the bone resulting in pathologic fracture. Patient may also sustain pathologic fracture complicating primary benign and malignant bone tumors.

Table 27.9 Select benign entities with multiple presentations

Brown tumor of hyperparathyroidism
Enchondromatosis, fibrous dysplasia
Hemangiomatosis
Langerhans cell histiocytosis
Nonossifying fibromas (Jaffe-Campanacci syndrome)
Osteochondromatosis
SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis and osteitis)
Paget's disease of bone

Table 27.10 Neoplastic and non-neoplastic lesions are precursors of malignancy in bone

Non-neoplastic Conditions	Benign Bone Tumors
▪ Paget's disease of bone ▪ Chronic osteomyelitis ▪ Bone infarct ▪ Metallic implant ▪ Radiation osteitis ▪ Osteogenesis imperfecta ▪ Fibrous dysplasia ▪ Bone cyst ▪ Synovial chondromatosis	▪ Osteochondroma ▪ Enchondroma ▪ Chondromyxoid fibroma ▪ Giant cell tumor of bone ▪ Osteoblastoma ▪ Ollier's disease ▪ Maffucci syndrome ▪ Metaphyseal fibrous defect ▪ Osteofibrous dysplasia ▪ Adamantinoma of long bones

Clinical History

Adults with a history of carcinomas of prostatic, breast, lung, renal and thyroid are at risk for development of metastatic bone disease.

- Children with neuroblastoma are prone to bony metastasis in skull. Patient with retinoblastoma is at greater risk for development of osteosarcoma.
- Secondary osteosarcoma and other carcinomas can result from treatment of childhood carcinomas with radiotherapy and/or chemotherapy.
- Patient with history of Li-Fraumeni syndrome may develop osteosarcoma and soft tissue sarcoma. Multiple hereditary exostosis can run in families.

Clinical Features

Patient presents with fever, chills, malaise, night sweats, change in appetite and other symptoms should alert the clinician that an infectious or neoplastic process may be involved.

PHYSICAL EXAMINATION

A thorough physical examination is also important to evaluate bone lesion. The clinician must assess the location and size of tumor mass, quality of overlying skin and warmth over bony lesion, tenderness and firmness of the bony lesion.

- Bone tumor mass >5 cm should raise the suspicion of malignancy.
- Tenderness may reflect a more rapidly growing bone tumor.
- Malignant bone tumors tend to be firmer on clinical examination than benign bone lesions.
- Involvement of lymph node and examination for enlarged liver or spleen should be carried out in these patients. Lymph node involvement is most often demonstrated in rhabdomyosarcoma, epithelioid sarcoma and clear cell sarcoma.

IMAGING STUDIES

Imaging studies are done to evaluate bony lesions by conventional radiography, ultrasonography, isotope scanning, computed tomography and magnetic resonance imaging.

Conventional Radiograph

Initial evaluation of bone or soft tissue lesion is done by conventional plain radiograph. While analyzing bone lesions, one must evaluate whether a mass arising from soft tissue is involving and possibly eroding the bone cortex. Primary bone tumors and their locations according to age group predilection are shown in Fig. 27.8. Conventional plain radiograph demonstrates location of the bone lesion in epiphysis, metaphysis or diaphysis (Table 27.11). Origin of selected bone tumors in long bones, age group and location is given in Table 27.12.

Bone Lesions: Location

- **Epiphyseal bone lesions:** Epiphyseal bone lesions include chondroblastoma (open epiphysis), giant cell tumor of bone (closed epiphysis, subarticular), osteomyelitis and clear cell chondrosarcoma.
- **Metaphyseal bone lesions:** Metaphyseal bone lesions include non-ossifying fibroma (eccentric), unicameral bone cyst (central), enchondroma, osteochondroma, aneurysmal bone cyst, solitary bone cyst, osteoid osteoma, osteoblastoma, osteosarcoma,

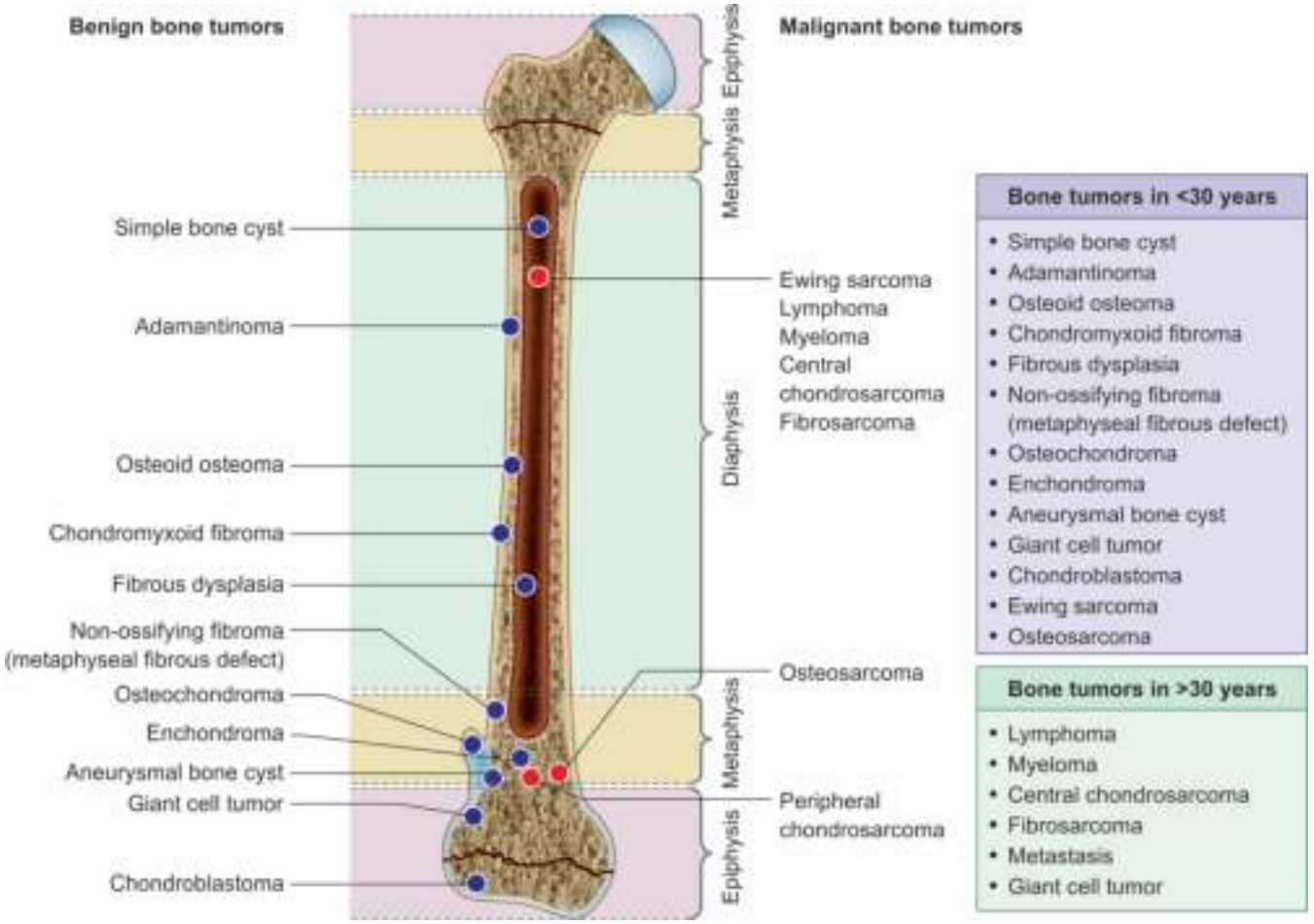


Fig. 27.8: Primary bone tumors and their locations according to age group predilection.

Table 27.11 Location of common tumors and tumor-like lesions within bone

Epiphysis of Bone
▪ Chondroblastoma (open epiphysis) ▪ Giant cell tumor of bone (closed epiphysis, subarticular) ▪ Osteomyelitis ▪ Clear cell chondrosarcoma
Metaphysis of Bone
▪ Nonossifying fibroma (eccentric) ▪ Unicameral bone cyst (central) ▪ Enchondroma ▪ Osteochondroma ▪ Aneurysmal bone cyst ▪ Solitary bone cyst ▪ Osteoid osteoma ▪ Osteoblastoma ▪ Osteosarcoma ▪ Chondrosarcoma ▪ Malignant fibrous histiocytoma ▪ Lymphoma (within medullary cavity)
Diaphysis of Bone
▪ Fibrous dysplasia ▪ Enchondroma ▪ Osteofibrous dysplasia ▪ Adamantinoma ▪ Ewing sarcoma ▪ Periosteal osteosarcoma ▪ Lymphoma, round cell tumor (within medullary cavity) ▪ Multiple myeloma, round cell tumor (within medullary cavity) ▪ Langerhans cell histiocytosis

chondrosarcoma, malignant fibrous histiocytoma, and lymphoma (within medullary cavity).

- **Diaphyseal bone lesions:** Round cell tumors such as Ewing sarcoma, multiple myeloma, and lymphoma arise within medullary cavity in diaphyseal region; however, these may also be demonstrated in the metaphyseal regions. Other tumors arising in diaphyseal region include fibrous dysplasia, enchondroma, osteofibrous dysplasia, adamantinoma, periosteal osteosarcoma and Langerhans cell histiocytosis. It is worth mentioning that osteochondroma and parosteal osteosarcoma arise from the surface region of bone.

Radiographic Features of Bone Tumors

Radiographic appearance of bone tumors has been classified into geographic, moth-eaten and infiltrative appearance on conventional radiograph (Table 27.13). Pyogenic osteomyelitis can present with any of three appearances on radiograph and suggest a neoplastic lesion. Radiographic appearances in bone lesions are given in Table 27.14.

- **Geographic appearance:** The term 'geographic appearance' refers to a lytic area of bone destruction. Presence of lytic area surrounded by a rim of sclerosis indicates a benign bone lesion. Presence of sharp line of demarcation from normal bone also indicates a benign bone lesion. Loss of sharp line of demarcation from normal bone indicates an aggressive process.

Table 27.12 Origin of selected bone tumors in long bones, age group and location

Bone Tumor	Age Group	Location
Epiphyseal region		
Giant cell tumor of bone	20–40 years	Knee joint and distal radius (65%). It is practically never demonstrated in children or person older than 60 years
Chondroblastoma	10–20 years	Epiphysis of long bones around knee joint (most common), skull, ribs, vertebrae, small bones of feet
Chondrosarcoma	40–60 years	Axial skeleton (bones of pelvic girdle, shoulder girdle, trunk, vertebrae), pelvic bones affected in 25–30%
Metaphyseal region		
Osteosarcoma	10–20 years	Long bones of extremities around knee joint in 60% (lower end of femur and upper end of tibia most common sites)
Osteosarcoma arising in Paget's disease of bone	>40 years	Upper end of tibia (most common site), humerus and upper end of femur (occasional site)
Nonossifying fibroma (metaphyseal fibrous defect)	Children during skeletal growth	Distal femur or proximal tibia in 80%
Diaphyseal fibrous defect		
▪ Ewing sarcoma ▪ Malignant lymphoma ▪ Multiple myeloma	▪ 5–15 years ▪ Elderly persons ▪ Elderly persons	Hematopoietic marrow in axial skeleton
Adamantinoma/ameloblastoma	Young adults	Long bone, jaw

Table 27.13 Radiographic features associated with benign and malignant tumors

Bone Tumor	Type of Lesion	Radiographic Appearance
Benign tumor	Well circumscribed lesion	Geographic appearance (lytic area indicative of bone destruction)
Malignant tumor	Ill-defined infiltrative process with loss of cortical integrity	▪ **Moth-eaten appearance** (presence of multiple tiny lucencies admixed with normal appearing bone seen in lymphoma) ▪ **Permeative appearance** (presence of multiple tiny lucencies coursing through bone seen in Ewing sarcoma)

Periostitis appearance (soft tissue mass invading periosteum)

Table 27.14 Radiographic appearances in bone lesions

Bone Lesion	Radiographic Appearance
Osteoid osteoma	Geographic appearance (lytic area of bone destruction surrounded by rim of sclerosis)
Lymphoma	Moth-eaten appearance (presence of multiple tiny lucencies admixed with normal appearing bone)
Ewing sarcoma	Permeative appearance (presence of multiple tiny lucencies coursing through bone; lesion deceptively looks like normal bone; and onion skin appearance)
Multiple myeloma	Multiple punched out lesions
Giant cell tumor of bone	Soap bubble appearance
Conventional osteosarcoma involving periosteum	Sun-ray (sun-burst) appearance forming Codman triangle
Fibrosarcoma	Bear bite lesions
Chondrosarcoma	Ring and arc chondroid matrix mineralization within tumor
Eosinophilic granuloma	Solitary punched out area
Fibrous dysplasia	Ground glass appearance
Aneurysmal bone cyst	Expansile osteolytic lesion with fluid-fluid levels on MRI
Enchondroma	Popcorn calcification with ring and arc appearance
Congenital syphilis involving bone	Rat-bite appearance
Rickets/osteomalacia	Looser's line
Bone medullary infarct	Central lucency with sclerosis having serpiginous border
Neurofibromatosis	Scallops the bone
Osteogenesis imperfecta	Gracile bones

- **Moth-eaten appearance:** The term 'moth-eaten appearance' refers to the presence of multiple tiny lucencies admixed with normal appearing bone. Moth-eaten appearance of bone suggests an aggressive neoplasm such as lymphoma. Moth-eaten appearance is a poorly defined infiltrative process and sometimes characterized by periosteal reaction. An exception to the rule is multiple myeloma, which frequently demonstrates punched out lesions on multiple sites on conventional radiographs.
- **Permeative appearance:** The term 'permeative appearance' refers to the presence of multiple tiny lucencies coursing through the bone, which may deceptively appear a normal bone. Permeative appearance is most often demonstrated in Ewing sarcoma.

Computed Tomography Scan

Computed tomography scan provides the best evaluation of cortical integrity, osseous remodeling and bone matrix mineralization. Thin-section computed tomography (CT) imaging provides fine bone details, which demonstrates the nidus in osteoid osteoma. CT imaging is particularly useful to evaluate to bone lesions that may not be seen on conventional radiograph especially in the pelvis, spine, ribs, scapula and chest wall. CT imaging-guided biopsy is taken to evaluate the lungs for metastasis. It is worth mentioning that lungs are the most common site of metastases via hematogenous route from primary high-grade bone sarcomas.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is the examination of choice for the staging of bone tumors and planning limb salvage and tissue sparing surgical procedures.

- MRI is the best technique to evaluate bone marrow involvement and soft tissue extent of the bone tumor.
- MRI is best technique to demonstrate fluid, necrosis and hemorrhage. It is the best diagnostic tool for accurate delineating the extent of the bone tumor.
- Measurements of the bone tumor are taken before induction of chemotherapy in order to dissect the involved segment of bone after chemotherapy.

Bone Scintigraphy

Bone scintigraphy uses Tc ^{99}m-labeled diphosphonates that bind to hydroxyapatite at the sites of osteoclastic activity resulting in increased uptake of radionuclide at areas of bone turnover. Uptake of radionuclide can occur in both benign and malignant bone tumors, osteomyelitis and fractures. It is most consistently performed to determine the distribution of metastases in the whole skeleton. It does not evaluate the extent of lesion in intraosseous region or soft tissue.

- Increased uptake of radionuclide is demonstrated in fibrous dysplasia, giant cell tumor and Paget's disease of bone.
- Radionuclide uptake is most often absent in multiple myeloma, lymphoma and highly anaplastic tumors.
- Langerhans cell histiocytosis and chondrosarcoma may not show increased radionuclide uptake. Therefore, bone scintigraphy alone cannot be used and hence, it should be correlated with clinical history and conventional radiographs.

Positron Emission Tomography-Computed Tomography (PET-CT) Scan

Positron emission tomography-computed tomography (PET-CT) scan plays most valuable role in evaluation of systemic disease staging, skeletal survey, lung metastasis and response to neoadjuvant therapy.

- PET-CT technique uses fluorodeoxyglucose (FDG), a glucose analog labeled with fluorine 18 that is administered by intravenous route to detect lesions with increased metabolic activity.
- PET-CT is most accurate and sensitive technique to detect bone metastasis. It is commonly performed in staging of Ewing sarcoma. False positive results are obtained in enchondroma, fibrous dysplasia, fracture and osteomyelitis.
- Chondrosarcoma may be mistaken for a benign bone lesion based on the low to moderate radionuclide uptake depending on the grade of the bone lesion.

HISTOLOGIC EXAMINATION

Fundamental to the diagnosis of bone tumors is the histologic examination of formalin-fixed (calcified or decalcified) paraffin-embedded tissue and stained with hematoxylin and eosin in conjunction with clinical and radiologic data.

- Histologic grade is the most important prognostic feature of bone sarcomas.
- Periodic acid–Schiff (PAS) stain is used to demonstrate intracellular glycogen in Ewing sarcoma and clear cell chondrosarcoma. It also demonstrates neutral mucin to outline the basement membrane and stains fungi and parasites.
- Salient features of most common benign bone tumors and tumor-like lesions are given in Table 27.15. Salient features of most common malignant bone tumors are given in Table 27.16.

IMMUNOHISTOCHEMISTRY TECHNIQUE

Immunohistochemistry technique plays a diagnostic role for most mesenchymal tumors. It is based on the binding of a specific cellular or extracellular substance by the antibody, with subsequent visualization of the bound antibody by a color-based detection system. Visualization

Table 27.15 Salient features of most common benign bone tumors and tumor-like lesions

Tumor/Lesion	Locations	Age Predilection	Salient Pathologic Features
Chondrogenic tumors or tumor-like lesions			
Osteochondroma	Metaphysis of long bones	10–30 years	Cartilage-capped bony protrusion
Chondroma	Hands/feet; medulla of long bones	Any group	Variably cellular hyaline cartilage
Chondroblastoma	Epiphysis/apophysis of long bones	10–20 years	Chondroid-like matrix; S-100 positive cells with grooved nuclei
Chondromyxoid fibroma	Metaphysis of long bones	10–30 years	Hypocellular chondromyxoid lobules surrounded by more cellular spindle cell areas

Contd....

Table 27.15 Salient features of most common benign bone tumors and tumor-like lesions (*Contd...*)

Tumor/Lesion	Locations	Age Predilection	Salient Pathologic Features
Osteogenic tumors			
Osteoma	Facial bones	Adults	Mineralized compact bone
Osteoid osteoma	Cortex of long bones	10–30 years	Nidus of immature bone surrounded by sclerotic bone
Osteoblastoma	Vertebrae; cortex of long bones	10–30 years	Identical to osteoid osteoma but larger; often no sclerosis
Fibrous tissue-derived lesions			
Fibrous dysplasia	Ribs, jaw, long bones—medullary	10–30 years	Irregular woven bone within fibroblastic stroma
Osteofibrous dysplasia	Tibial cortex	<20 years	Similar to fibrous dysplasia but with appositional osteoblasts
Desmoplastic fibroma	Long bones, jaw, pelvis	20–30 years	Fibromatosis-like proliferation
Nonossifying fibroma	Long bones	5–15 years	Bland spindle cells in storiform pattern + histiocytes + giant cells
Histiocytic tumors			
Benign fibrous histiocytoma	Long bones, pelvis	>20 years	Identical to nonossifying fibroma but variable
Langerhans cell histiocytosis	Skull, jaw, metaphysis and diaphysis of long bones	5–15 years	Mixed inflammatory cells and eosinophils and S-100/CD1a-positive cells with grooved/multi-lobated nuclei
Erdheim-Chester disease	Long bones	>40 years	Foamy histiocytes and fibrosis
Giant cell tumor of bone	Epiphysis/metaphysis of long bones	20–45 years	Evenly placed giant cells among mononuclear cells with identical nuclei and normal serum calcium/phosphate/blood urea nitrogen/creatinine
Miscellaneous lesions			
Aneurysmal bone cyst	Vertebrae, flat and long bones	10–20 years	Blood-filled spaces separated by fibrous septae and giant cells
Simple bone cyst	Metaphysis of long bones	10–20 years	Fluid-filled cysts lined by connective tissue
Hemangioma	Vertebrae, flat and long bones	20–50 years	Capillary and/or cavernous-sized vessels

Table 27.16 Salient features of most common primary malignant bone tumors

Tumor/Lesion	Locations	Age Predilection	Salient Pathologic Features
Chondrogenic malignant tumors			
Chondrosarcoma	Flat bones, metaphysis and epiphysis of long bones	20–80 years	Variably cellular hyaline cartilage permeating bone
Conventional (not otherwise specified)	Metaphysis	20–80 years	Variably cellular hyaline cartilage permeating bone
Dedifferentiated chondrosarcoma	Metaphysis	>30 years	Conventional chondrosarcoma + high-grade spindle cell sarcoma
Mesenchymal chondrosarcoma	Metaphysis	20–50 years	Undifferentiated small cell tumor + hyaline cartilage
Clear cell chondrosarcoma	Epiphysis	20–70 years	Conventional tumor with abundant large clear tumor cells
Osteogenic malignant tumors			
Osteosarcoma	Metaphysis of long bones, jaw	10–20 years, >40 bimodal	Osteoid formed directly by malignant cells
Conventional osteosarcoma	Medullary region	10–20 years, >40 bimodal	Osteoid formed directly by malignant cells

Contd...

Table 27.16 Salient features of most common primary malignant bone tumors (*Contd...*)

Tumor/Lesion	Locations	Age Predilection	Salient Pathologic Features
Low-grade central osteosarcoma	Medullary region	10–20 years, >40 bimodal	Mildly atypical fibroblastic + thick bone trabeculae
Telangiectatic osteosarcoma	Medullary region	10–20 years, >40 bimodal	Blood-filled spaces + fibrous septae + highly malignant osteoid
Parosteal osteosarcoma	Cortex outside periosteum	10–20 years, >40 bimodal	Mildly atypical fibroblastic proliferation + thick bone trabeculae
Periosteal osteosarcoma	Cortex inside periosteum	10–20 years, >40 bimodal	Abundant cartilage matrix with variable malignant osteoid
Ewing sarcoma (primitive neuroectodermal tumors)			
Ewing sarcoma	Diaphysis of long bones	5–20 years	Small round blue cells ± rosettes, characteristic IHC, translocation
Fibrous/fibrohistiocytic malignant tumors			
Fibrosarcoma	Metaphysis of long bones, may extend to end of bone	20–60 years	Malignant spindle cells in a fascicular pattern
Malignant fibrous histiocytoma	Metaphysis of long bones, may extend to end of bone	20–80 years	Malignant spindle cells in storiform pattern + histiocytic cells (other patterns may be seen)
Hematolymphoid malignant tumors			
Myeloma	Skull, vertebrae, pelvis, long bones	>40 years	Variably atypical (monoclonal) plasma cells
Lymphoma	Any bone	Any age group	Lymphoid proliferations similar to non-bony lesions
Notochord derived tumors			
Chordoma	Base of skull, sacrum	>30 years	Lobules of vacuolated cells embedded in myxoid matrix
Blood vessels derived malignant tumors			
Angiosarcoma and hemangioendothelioma		20–60 years	Anastomosing vascular channels lined by highly atypical cells or vacuolated cells in myxoid background; characteristic immunochemistry staining
Miscellaneous tumors			
Adamantinoma	Cortex of tibia and/or fibula	20–35 years	Epithelial cells + fibroblasts + woven or lamellar bone
Metastatic carcinomas	Any bone	>40 years	IHC confirmatory

of bound antibodies with different reactions include: (a) direct and indirect staining methods using primary and secondary antibody, (b) avidin–biotin method, in which preformed complex reactions with the secondary antibody, (c) labeled streptavidin–biotin method, in which there is a linkage between primary antibody with a streptavidin-peroxidase conjugate, and (d) two-step polymer method.

STAGING SYSTEM

Staging system refers to an assessment of the grade of the malignant bone tumor and the extent to which the disease has spread. It helps the clinician to plan a logical treatment and establish prognosis for the patient. Staging of malignant bone tumors is done by imaging techniques and categorized into local and systemic disease. Local staging is achieved with MRI to determine the intra-compartmental disease and its relation to nerves and blood vessels. Systemic staging is done with CT of thorax and bone scintigraphy; but it is gradually replaced by positron emission tomography-computed tomography (PET-CT). The two major staging systems are discussed here, i.e. American Joint Committee on Cancer Staging System and Enneking Staging System.

American Joint Committee on Cancer Staging System

According to this staging system, malignant bone tumors are classified on the basis of their histologic appearance as grades 1, 2, 3 or 4. Grade 1 or 2 tumor in

this equivalent to stage 1 tumor in the Enneking system; grade 3 or 4 is equivalent to Enneking stage II.

Enneking Staging System

According to Enneking staging system, bone tumors are classified based on their histologic and biologic appearance and their metastatic potential to regional lymph nodes and distant sites, i.e. lung. Enneking staging system of malignant bone tumors is given in Table 27.17. Enneking staging system further classifies tumors based on location of tumor as intra-compartmental (type A) or extra-compartmental (type B).

- **Intra-compartmental (type A) tumors:** These malignant bone tumors are confined to muscle facial planes with better prognosis.
- **Extra-compartmental (type B) tumors:** These malignant bone tumors are located in popliteal space, axilla, pelvis or mid-portion of hands and feet. Tumor measuring >5 cm has a poor prognosis.

Table 27.17 Enneking staging system of malignant bone tumors

Stage	Comments
Stage I	It refers to low-grade bone sarcomas with <25% chance of metastatic potential
Stage II	It refers to high-grade bone sarcomas with >25% chance of metastatic potential
Stage III	It refers to either low- or high-grade bone sarcomas with metastasis to regional lymph nodes and or distant sites

Table 27.18 French Federation of Comprehensive Carcinoma Center Grading System of bone tumors

Criterion	Score
Tumor differentiation according to Table 27.6	
▪ Well differentiated tumor closely resembling normal adult mesenchymal tissue sarcoma closely resembling normal adult mesenchymal tissue	▪ Score 1
▪ Moderately differentiated tumor of well-defined certain histogenetic types	▪ Score 2
▪ Poorly differentiated (undifferentiated) tumors and undefined histogenetic type	▪ Score 3
Mitotic count	
▪ 0–9/10 per high power fields	▪ Score 1
▪ 10–19/10 per high power fields	▪ Score 2
▪ 20/10 per high power fields	▪ Score 3
Tumor necrosis on histologic examination	
▪ No necrosis	▪ Score 0
▪ ≤50% tumor necrosis	▪ Score 1
▪ >50% tumor necrosis	▪ Score 2
Grade on histologic examination	**Sum of the above scores**
▪ Grade 1	▪ Total score 2 or 3
▪ Grade 2	▪ Total score 4 or 5
▪ Grade 3	▪ Total score 6, 7 or 8

Table 27.19 French Federation of Comprehensive Carcinoma Center Grading System of bone sarcomas

Grade	Bone Sarcoma: Histologic Type
Grade 1	▪ Parosteal osteosarcoma ▪ Low-grade intramedullary osteosarcoma ▪ Chondrosarcoma grade 1 ▪ Clear cell chondrosarcoma ▪ Adamantinoma classic type
Grade 2	▪ Periosteal osteosarcoma ▪ Chondrosarcoma grade 2 ▪ Chordoma
Grade 3	▪ Conventional osteosarcoma ▪ Osteosarcoma telangiectatic variant ▪ Osteosarcoma small cell variant ▪ Osteosarcoma secondary ▪ Osteosarcoma high-grade surface variant ▪ Undifferentiated high-grade pleomorphic sarcoma ▪ Ewing sarcoma ▪ Chondrosarcoma grade 3 ▪ Chondrosarcoma dedifferentiated type ▪ Chondrosarcoma mesenchymal type ▪ Chordoma dedifferentiated type ▪ Malignant change in giant cell tumor

GRADING SYSTEM

French Federation of Comprehensive Carcinoma Center Grading System is based on tumor differentiation, mitotic count, tumor necrosis and histologic grade of bone tumors (Tables 27.18 and 27.19). Grade 1 bone tumors have better prognosis than grade 3. Grade 2 bone tumors have intermediate prognosis. French Federation of Comprehensive Carcinoma Center Grading System is based on multivariable analysis of tumor differentiation, mitotic count, tumor necrosis and grade on histologic examination.

CHONDROGENIC TUMORS

Chondrogenic tumors according to revised 2020 WHO classification of bone tumors are given in Table 27.20. Salient features of commonest chondrogenic tumors and tumor-like lesions are given in Table 27.21.

CHONDROMAS (ENCHONDROMA AND PERIOSTEAL CHONDROMA)

Chondromas (enchondroma and periosteal chondroma) are derived from hyaline cartilage, which share many histologic features. However, chondromas differ in the location and clinical features.

Table 27.20 Chondrogenic tumors according to revised 2020 WHO classification of bone tumors

Chondrogenic Tumors
Benign tumors
▪ Osteochondroma ▪ Chondroma (enchondroma, periosteal chondroma) ▪ Osteochondromyxoma ▪ Subungual exostosis ▪ Bizarre parosteal osteochondromatosis proliferation ▪ Synovial chondromatosis
Intermediate tumors (locally aggressive)
▪ Chondromyxoid fibroma ▪ Atypical cartilaginous tumor/chondrosarcoma grade 1
Malignant tumors
▪ Chondrosarcoma grade 2 ▪ Chondrosarcoma grade 3 ▪ Dedifferentiated chondrosarcoma ▪ Mesenchymal chondrosarcoma ▪ Clear cell chondrosarcoma

Origin of Chondroma

Enchondroma arises within the medullary cavity. Parosteal chondroma arises beneath the periosteum; and it is also known as juxtacortical chondroma. Both chondragenic tumors are sporadic and most often solitary lesion.

Incidence, Location and Clinical Features

- **Enchondromas:** Enchondromas constitute 10–25% of benign bone tumors. Most common sites are short tubular bones in 40% of cases. Proximal end of humerus and both ends of femur are next common sites. Patient presents with palpable swelling and pathologic fracture. Enchondroma is tumor of long bones and asymptomatic.
- **Parosteal chondromas:** Parosteal chondromas most often occur in the metaphyseal and diaphyseal regions of long bones especially femur and humerus. Patient presents with painless mass for years. Tumor may be detected as incidental finding on radiologic examination done for unrelated complaints.

Table 27.21 Salient features of commonest chondrogenic tumors and tumor-like lesions

Tumor/Lesion	Locations	Age Group	Salient Pathologic Features
Osteochondroma	Metaphysis of long bones	10–30 years	Cartilaginous capped bony protrusion
Chondroma	Hands and feet bones and medulla of long bones	Any age group	Variably cellular hyaline cartilage
Chondroblastoma	Metaphysis/epiphysis of long bones	10–20 years	Chondroid-like matrix with S-100 protein positive cells with grooved nuclei
Chondromyxoid fibroma	Metaphysis of long bones	10–30 years	Hypocellular chondromyxoid lobules surrounded by more cellular spindle cell areas
Conventional chondrosarcoma (not otherwise specified)	Flat bones, metaphysis of long bones	30–80 years	Tumor is composed of atypical pleomorphic chondrocytes. Lacunae of the chondrocytes contain >2 hyperchromatic nuclei
Dedifferentiated chondro-sarcoma	Flat bones, metaphysis of long bones	30–80 years	Dedifferentiated chondrosarcoma consists of two components. Central zone of tumor is composed of uniform chondrocytes containing hyperchromatic nuclei
Mesenchymal chondrosarcoma	Flat bones, metaphysis of long bones	20–50 years	Tumor is composed of two components: Well differentiated hyaline cartilage and small round blue cells component. Both components of the tumor are haphazardly arranged. Small round blue cells component shows spindled areas, necrosis, hemorrhage and hemangiopericytoma-like vascular spaces
Clear cell chondrosarcoma	Flat bones, metaphysis of long bones	20–70 years	Tumor is composed of large clear cells with prominent clear-to-pale eosinophilic cytoplasmic borders, round-to-oval nuclei and prominent nucleoli. Mitotic activity is low. The growth pattern is at least permeative. Variable amount of woven bone is present. Multinucleated osteoclast-like giant cells may be present

Radiologic Findings

Conventional radiograph shows radiolucent nodule of cartilage with thin central calcification and intact cortex.

Surgical Pathology: Chondromas
Gross Morphology
▪ Most enchondromas measure 3 cm in size. These tumors are pale blue firm cartilage attached to cortical surface. ▪ Parosteal chondromas form well marginated bone surface tumors. Cortex underlying the parosteal enchondroma is indented (Figs 27.9 and 27.10).
Light Microscopy
▪ Histologic features are similar in both enchondroma and parosteal chondroma. Both are avascular tumors with moderate cellularity. ▪ These tumors are composed of well differentiated hyaline cartilage. Chondrocytes contain small round nuclei with condensed chromatin. These are situated within sharp-edged lacunar spaces.
Differential Diagnosis
Chondromas should be differentiated from synovial chondromatosis and periosteal osteosarcoma.
Molecular Genetic Alterations: Ancillary Studies
▪ Both enchondroma and parosteal chondroma frequently harbor heterozygous IHD gene mutation (IHD1 and IHD2). ▪ Clonal chromosomal abnormality such as loss of chromosome 6 material has been demonstrated in parosteal chondroma.

Prognosis and Treatment

Enchondromas are treated by curettage. Local recurrences are uncommon. Parosteal chondroma is treated with intralesional, marginal and en bloc excision. Recurrence rate is low after surgical intervention.



Fig. 27.9: Chondroma. The small cartilaginous tumor resected from the tibia measuring 3 cm in diameter. Cut surface shows gray-blue discoloration.

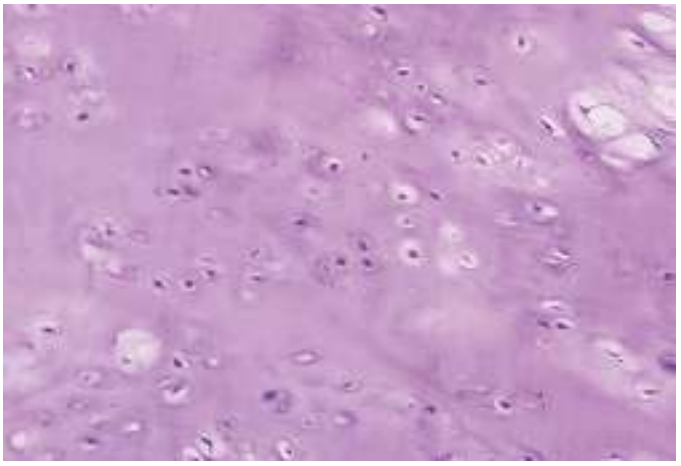


Fig. 27.10: Chondroma. Tumors are composed of well differentiated hyaline cartilage. Chondrocytes contain small round nuclei with condensed chromatin. These are situated within sharp-edged lacunar spaces (100X).

ENCHONDROMATOSIS (OLLIER DISEASE)

Ollier disease is a rare nonhereditary sporadic disorder, where intraosseous benign cartilaginous tumors (enchondroma) develop close to the growth plate cartilage.

- Ollier disease is characterized by multiple enchondromas, which usually develop during childhood. The growth of enchondromas stops after skeletal maturation. The affected extremity becomes shortened and sometimes undergoes bowing due to epiphyseal fusion abnormalities.
- Patient presents with swollen arching extremities resulting in fracture.
- Patients with Ollier disease carry high risk for skeletal, visceral and brain malignancy in 25% of cases. The deformities are managed surgically to preserve the function of the limbs. Gross morphology of multiple enchondromatosis (Ollier disease) is shown in Fig. 27.11.

Radiologic Findings

Conventional radiograph shows streaks of low density presenting through the diaphysis into the epiphyses of the long bones due to ectopic cartilage deposits.

OSTEOCHONDROMA

Osteochondroma is a benign mushroom-shaped exophytic tumor. Tumor is composed of bone capped with cartilage near the growth plate. It is also known as exostosis. Benign osteochondroma has <2 cm thick cartilaginous cap. But presence of irregular cartilaginous cap >2 cm in thickness may indicate tumor with metastatic potential. EXT1 and EXT2 gene mutations play important role in the pathogenesis of osteochondroma.



Fig. 27.11: Multiple enchondromatosis (Ollier disease). The head of the humerus is greatly expanded by the intramedullary chondromas resulting in bony deformities. The residual hyaline cartilage from the growth plate does not undergo enchondral ossification and remains confined within the bones resulting in formation of multiple tumor-like masses of abnormally arranged hyaline cartilage. It has strong tendency to undergo malignant change, i.e. chondrosarcoma.

Incidence and Locations

Multiple osteochondromas occur in various syndromes. Sporadic osteochondroma is solitary and six times more common than those seen in the setting of multiple osteochondromas. Most common sites of osteochondroma are metaphysis of long bones, e.g. femur, proximal tibia and proximal humerus. Pelvic bones and scapula are uncommon sites.

Age and Sex Predilection

Peak incidence of osteochondroma is seen in second decades of life (<25 years) with male predilection.

Clinical Features

Initially, patient is asymptomatic because the osteochondroma starts growing in childhood and becomes clinically painless mass over long duration. It may be an incidental finding on radiograph done for unrelated complaints. Osteochondroma may become painful due to nerve compression or fracture of affected bone.

Surgical Pathology: Osteochondroma

Gross Morphology

- Osteochondroma may be pedunculated with a stalk or sessile/flat or cauliflower shaped. Thin <2 cm thick pale blue cartilaginous cap overlies a stalk composed of hard cancellous bone.
- Cut surface of tumor shows medullary and cortical contiguous with underlying native bone (Fig. 27.12).

Light Microscopy

- Osteochondroma is composed of cartilaginous cap undergoing enchondral ossification with orderly arrangement of chondrocytes with minimal atypia towards epiphyseal growth plate.
  - Chondrocytes contain single small nucleus with fine chromatin in benign osteochondroma.
  - Chondrocytes may contain two nuclei during active bone growth.
- Cancellous bone is present beneath the cartilaginous cap. Intertrabecular spaces are filled with hematopoietic tissue and fat. Occasionally, degenerative changes are demonstrated in the cartilaginous cap (Fig. 27.13).

Electron Microscopy

Chondrocytes show accumulation of glycogen, lipid droplets and dilatation of rough endoplasmic reticulum.

Differential Diagnosis

Osteochondroma should be differentiated from chondrosarcoma arising in osteochondroma, parosteal osteosarcoma and bizarre periosteal osteochondromatosis proliferation (Nora's lesion).

Prognosis and Treatment

Asymptomatic patients with osteochondroma should be followed up. Symptomatic osteochondroma is treated by surgical excision. Malignant transformation in osteochondroma occurs in <1% of sporadic cases; and multiple osteochondroma syndrome in 5–20% of cases.

CHONDROBLASTOMA

Chondroblastoma is a rare benign matrix forming tumor arising from the epiphyseal cartilage of long

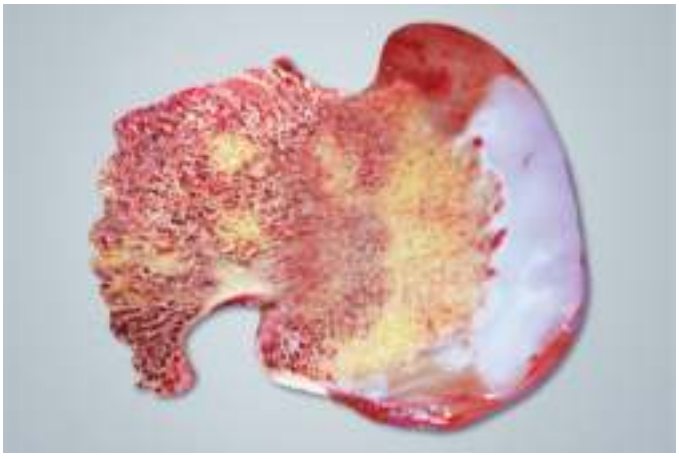


Fig. 27.12: Osteochondroma in long bone. Osteochondroma is pedunculated and cauliflower shaped with a stalk. Thin <2 cm thick pale blue cartilaginous cap overlies a stalk composed of hard cancellous bone. Cut surface of tumor shows medullary and cortical contiguous with underlying native bone.

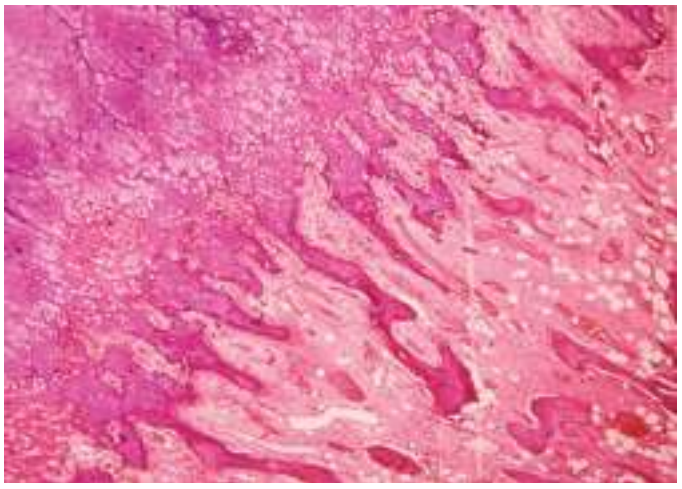


Fig. 27.13: Osteochondroma in long bone. It is composed of cartilaginous cap undergoing enchondral ossification with orderly arrangement of chondrocytes with minimal atypia towards epiphyseal growth plate. Chondrocytes contain single small nucleus with fine chromatin in benign osteochondroma. Chondrocytes may contain two nuclei during active bone growth. Cancellous bone is present beneath the cartilaginous cap. Intertrabecular spaces are filled with hematopoietic tissue and fat (100X).

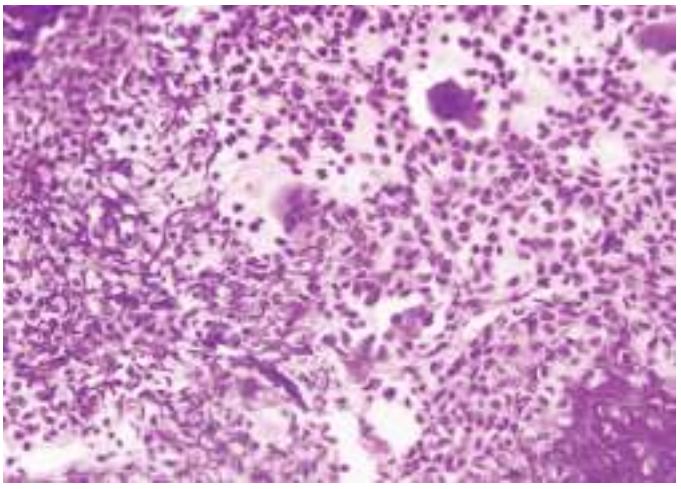


Fig. 27.14: Chondroblastoma. Tumor is a cellular tumor composed of round or polyhedral chondroblasts with well-defined outlines. These cells contain grooved round nuclei and pale cytoplasm. Mitoses and atypia are absent. The tumor cells merge with areas of hyaline cartilage. There is presence of chicken wire mesh-like areas of calcification among tumor cells. Scattered multinucleated giant cells are present among tumor cells (400X).

bones before the closure of epiphysis. Tumor features primitive chondroblasts and cartilaginous amphophilic or eosinophilic matrix.

Incidence and Locations

Chondroblastoma accounts for <1% of bone tumors, which originates in the epiphysis of long bones. Rare sites of chondroblastoma are flat bones of cranium, pelvis, ribs, vertebrae and small bone of feet.

Age and Sex Predilection

Chondroblastoma most often occurs in the first two decade of life with peak incidence in the second decade of life. Males are more affected than females.

Clinical Features

Patient presents with history of pain in the affected bone over months to years associated with limp.

Radiologic Findings

Conventional radiograph shows a well circumscribed osteolytic lesion with multiple areas of calcification.

Surgical Pathology: Chondroblastoma

Gross Morphology

- Curettage fragments of tumor demonstrate granular texture, tan-pink firm tissue with calcifications without typical pale blue hyaline cartilage.
- Focal blue gray areas resemble chondroid cartilage.

Light Microscopy

- Chondroblastoma is a cellular tumor composed of round or polyhedral chondroblasts with well-defined outlines. Chondroblasts contain grooved round nuclei and pale cytoplasm. Mitoses and atypia are absent. The tumor cells merge with areas of hyaline cartilage.
- There is presence of chicken wire mesh-like areas of calcification among tumor cells. Scattered multinucleated giant cells are present among tumor cells.
- In some cases, typical areas of chondroblasts show alternate arrangement of large vascular blood-filled spaces. Such tumors are referred as cystic chondroblastomas (Fig. 27.14).

Differential Diagnosis

Chondroblastoma should be differentiated from giant cell tumor of bone, aneurysmal bone cyst and chondromyxoid fibroma.

Prognosis and Treatment

Curettage of chondroblastoma and bone graft is curative. Recurrence of tumor may occur in <15% of cases.

CHONDROMYXOID FIBROMA

Chondromyxoid fibroma is a benign cartilaginous tumor with aggressive behavior. Tumor is composed of nodules with myxoid matrix and spindle to stellate cells, which rarely exceeds >5 cm in size. Chromosomal abnormalities are frequently observed in the tumor.

Incidence and Locations

Chondromyxoid fibroma accounts for <1% of bone tumors. Most common site of chondromyxoid fibroma

is proximal end of tibia, and other sites are distal femur, small bones of the feet and jaw.

Age Group

Chondromyxoid fibroma occurs in adolescents and young adults during second and third decades.

Clinical Features

Patient presents with pain in distal femur and mass in small bones of feet. Tumor may extend into the soft tissue.

Surgical Pathology: Chondromyxoid Fibroma
Gross Morphology
▪ Tumor is solid, lobulated and pale blue. Presence of translucency suggests cartilaginous origin of tumor. Matrix is softer in consistency than hyaline cartilage. ▪ Margin of the tumor shows sclerotic rim.
Light Microscopy
▪ Tumor exhibits lobular growth pattern separated by bands of fibrous tissue. Tumor is composed of spindled to stellate cells with long cytoplasmic processes. ▪ Tumor contains immature cartilage showing prominent myxoid tissue, among which are scattered chondroblasts alternate with stellate myxoid cells and scattered large pleomorphic cells. ▪ Peripheral portion of lobules shows increased tumor cells and multinucleated cells. Occasional cells show hyperchromatic nuclei but maintaining normal nuclear to cytoplasmic ratio. ▪ Rarely, tumors representing hybrids contain chondromyxoid fibroma and chondroblastoma components (Fig. 27.15).
Immunohistochemistry Technique
Tumor cells show positivity for SOX2.
Differential Diagnosis
Chondromyxoid fibroma should be differentiated from chondroblastoma, enchondroma and chondroblastic osteosarcoma.

Prognosis and Treatment

Chondromyxoid fibroma is treated with curettage and bone grafting. Recurrence occurs in 25% of cases after surgery.

CONVENTIONAL CHONDROSARCOMA (GRADES 1, 2, 3)

Conventional chondrosarcoma is a malignant cartilaginous matrix forming tumor with diverse morphologic features and clinical course. It accounts for 15–20% of all primary malignant bone tumors during fourth to seventh decades. Chondrosarcoma is classified into four categories:

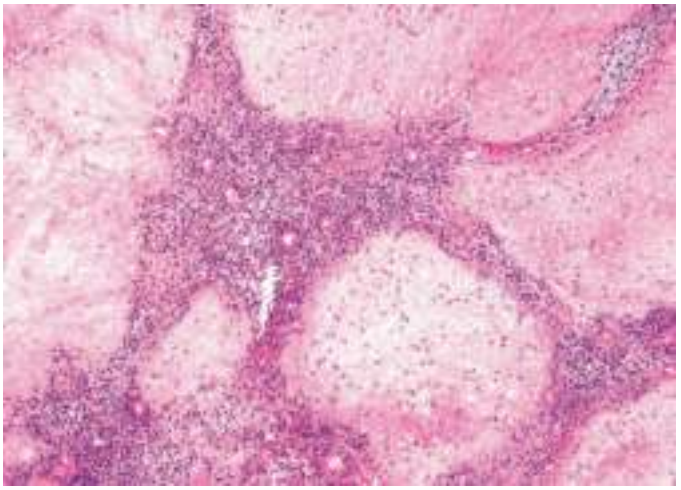


Fig. 27.15: Chondromyxoid fibroma. Tumor is composed of lobules separated by bands of fibrous tissue. The lobules are composed of contain multinucleated giant cells, immature cartilage, myxoid tissue (100X).

- **Primary central chondrosarcoma:** Primary central chondrosarcoma originates in any bone derived from enchondral ossification without a precursor bone lesion (*de novo*). It accounts for 85% of conventional chondrosarcomas. Most common sites of tumor are pelvic girdle (ilium), shoulder girdle (scapula), proximal and distal femur, proximal humerus, ribs and base of skull. Short bones of hands and feet, spine and base of the skull are rarely affected.
- **Secondary central chondrosarcoma:** Secondary central chondrosarcoma originates in a previously existing enchondroma and familial nonhereditary enchondromatosis disorders, e.g. Ollier disease and Maffucci syndrome. It most often affects young persons. Continued progression of known enchondroma should raise the suspicion of malignant transformation.
- **Secondary peripheral chondrosarcoma:** Secondary peripheral chondrosarcoma is a low-grade tumor, which occurs after second decade of life. It is juxtaposed to the cartilaginous cap on osteochondroma. Clinical features combined cartilaginous cap of osteochondroma >1.5–2 cm establishes the diagnosis after taking into consideration of histologic features.
- **Periosteal chondrosarcoma:** Periosteal chondrosarcoma originates from periosteum of distal femur and humerus in the metaphyseal region. It occurs during second to fourth decades of life.

Clinical Features

Patient presents with pain and tenderness in the affected bone. Tumor arising from base of the skull can cause neurological symptoms. Pain may be referred due to involvement of the vertebral or sacral nerve roots by the tumor.

Table 27.22 Radiologic findings in chondrosarcoma variants

Conventional Chondrosarcoma Variants	Comments
Primary central chondrosarcoma	
Conventional radiograph	▪ Fusiform expansion in long bones with cortical thickening and erosion; and scant or absence of periosteal reaction ▪ Radiolucent areas with variable ring-like opacities due to mineralization
Computed tomography scan	Matrix calcification in tumor
Magnetic resonance imaging	Helpful in delineating the extent of tumor, presence of extension into the soft tissues and diagnosing progression towards high-grade chondrosarcoma
Secondary central chondrosarcoma	
Conventional radiograph	Cortical destruction by the tumor and extension into soft tissues
Secondary peripheral chondrosarcoma	
Conventional radiograph	Irregular mineralization
Magnetic resonance imaging	▪ Demonstrates size of the cartilaginous cap ▪ Presence of cartilaginous cap >1.5–2 cm raises the suspicion of malignant transformation of osteochondroma
Parosteal chondrosarcoma	
Conventional radiograph	▪ Demonstrates a large lobular mass >5 cm located on the surface of the long bone delineated towards the soft tissue ▪ Presence of cartilaginous calcification and calcified shell ▪ Bone marrow involvement rare

Radiologic Findings

Radiologic findings in chondrosarcoma variants are given in Table 27.22.

Surgical Pathology: Conventional Chondrosarcoma

Gross Morphology

- Tumor appears as nodular, expansile and pale blue firm cartilage with glistening translucent appearance. Myxoid and viscous liquid change in the matrix is seen. Periosteal chondrosarcoma is attached to the surface of the bone. Gritty calcifications may be seen.
- Resected specimens show permeation through medulla or cortex into the soft tissues (Fig. 27.16).

Light Microscopy

- Tumor is composed of atypical pleomorphic chondrocytes.
- Lacunae of the chondrocytes contain >2 hyperchromatic nuclei. Atypical mitosis is best demonstrated at the edge of the tumor.
- Permeation through viable lamellar bone or haversian canal of bone is the most important diagnostic criteria of chondrosarcoma.
- Myxoid change or chondroid matrix liquefaction is a common feature of chondrosarcoma. Necrosis and mitoses can be demonstrated (Fig. 27.17).

Histologic Grading

Histologic grading of chondrosarcoma is the most important predictor of local recurrence and metastasis. Conventional chondrosarcomas are graded on a scale of I, II and III based on nuclear size, hyperchromasia of the nuclei, mitoses and cellularity of the tumor.

- **Grade 1 chondrosarcoma:** It is a locally aggressive tumor. Metastasis can occur in an occasional case. On histologic examination, grade 1 chondrosarcoma is moderately cellular and well differentiated tumor. Tumor is composed of uniform chondrocytes. Cartilaginous lacunae contain >2 cells with hyperchromatic nuclei. Tumor in long bone is treated by curettage. Five-year survival rate is seen in >80% of cases.
- **Grade 2 chondrosarcoma:** On histologic examination, grade 2 chondrosarcoma is more cellular. Tumor is composed of chondrocytes. Cartilaginous lacunae contain >2 cells with hyperchromatic nuclei and atypical mitoses. It is treated by *en bloc* resection. Patients have poor prognosis.
- **Grade 3 chondrosarcoma:** On histologic examination, grade 3 chondrosarcoma is highly cellular and composed of chondrocytes containing hyperchromatic nuclei and atypical mitoses. It is treated by *en bloc* resection. Patients have very poor prognosis. Histologic grading of conventional chondrosarcoma variants is given in Table 27.23.

Molecular Genetic Alterations: Conventional Chondrosarcoma

- **IDH1 and IDH2 gene mutations:** IDH1 and IDH2 gene mutations are demonstrated in primary central chondrosarcoma (35–70%), secondary central chondrosarcoma (85%) and periosteal chondrosarcoma (100%). IDH1 and IDH2 gene mutations are also demonstrated in Ollier disease and Maffucci syndrome. IDH1 gene mutation is demonstrated by using IDH1 R82H antibody.

- **EXT1 and EXT2 gene mutations:** EXT1 and EXT2 gene mutations play an important role in the pathogenesis of osteochondroma. But their role in the pathogenesis of chondrosarcoma is not known.
- **Single numerical or structural chromosomal aberrations to heavily rearranged karyotype:** These chromosomal alterations are demonstrated in chondrosarcoma.
- **RB1 signaling pathway:** RB1 signaling pathway is affected in high-grade chondrosarcoma in 85% of cases.
- **CDKN2A loss:** CDKN2A loss is demonstrated in conventional chondrosarcoma.
- **CDK4 gene amplification:** CDK4 gene amplification is demonstrated in conventional chondrosarcoma.
- **IHH/PTHLH signal pathway:** IHH/PTHLH signal pathway is active in primary central chondrosarcoma, secondary central chondrosarcoma and secondary peripheral chondrosarcoma. But activity of this signal pathway is decreased in high-grade chondrosarcoma.

Prognosis

Histologic grade of chondrosarcoma is the most important predictor of local recurrence and metastasis. Depending on surgical treatment of periosteal chondrosarcoma, local recurrence occurs in 15–30% of cases. Metastasis is rare in periosteal chondrosarcoma. In primary central chondrosarcoma and secondary central chondrosarcoma, prognosis depends on grade of the tumor and treatment.

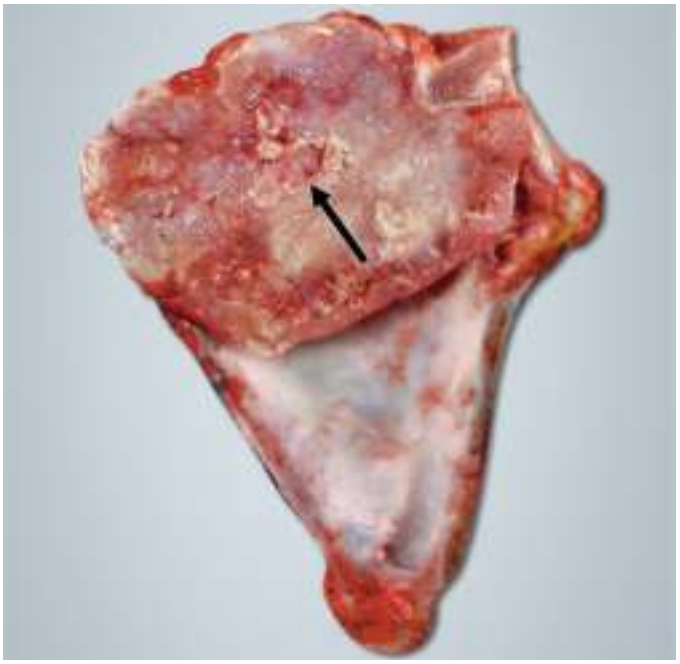


Fig. 27.16: Chondrosarcoma. Tumor is arising in the scapula. It appears as nodular, expansile and pale blue firm cartilage with glistening translucent appearance. Myxoid and viscous liquid change in the matrix is seen (arrow). Periosteal chondrosarcoma is attached to the surface of the bone. Gritty calcifications may be seen.

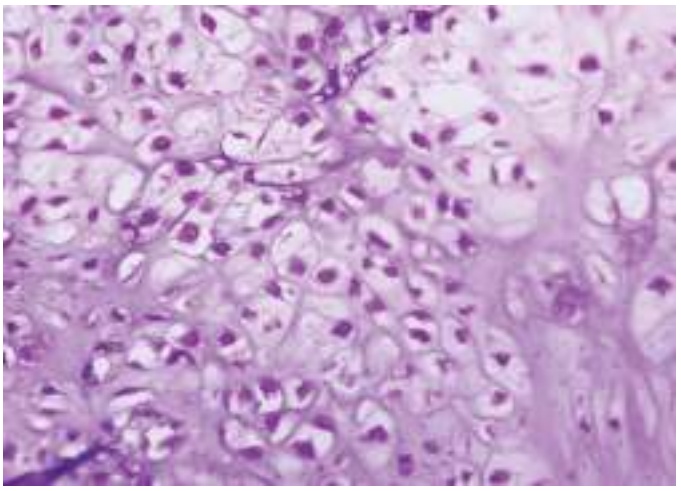


Fig. 27.17: Chondrosarcoma. Tumor is moderately cellular and well differentiated tumor. It is composed of atypical chondrocytes. Cartilaginous lacunae contain >2 cells with hyperchromatic nuclei.

- **Grade 1 chondrosarcoma:** It is locally aggressive tumor. Metastasis may occur in an exceptional case. Five-year survival rate is seen in >80% of cases treated by curettage.
- **Grades 2 and 3 chondrosarcoma:** Five-year survival rate is seen in these cases treated by *en bloc* resection.

DEDIFFERENTIATED CHONDROSARCOMA

Dedifferentiated chondrosarcoma is defined by the presence of a biphasic tumor composed of a conventional chondrosarcoma (most often grade 1) and a high-grade noncartilaginous sarcoma.

Incidence and Locations

Dedifferentiated chondrosarcoma accounts for 10–15% of all chondrosarcomas. Most common sites are pelvic girdle (ilium) and proximal femur in the epiphyseal region, distal femur, proximal humerus and ribs.

Age and Sex Predilection

Dedifferentiated chondrosarcoma has bimodal age distribution. Patient with exostosis may develop chondrosarcoma earlier in life. It occurs during fourth to seventh decades of life. Males and females are equally affected.

Clinical Features

Patient presents with pain, paresthesia and hard mass in the affected bone over 6–10 months.

Radiologic Findings

Radiograph shows a biphasic tumor. Presence of intramedullary component (central zone) with ring or stippled calcification is suggestive of cartilaginous component of tumor. Second component (peripheral

Table 27.23 Histologic grading of conventional chondrosarcoma variants

Parameters	Grade 1 Conventional Chondrosarcoma	Grade 2 Conventional Chondrosarcoma	Grade 3 Conventional Chondrosarcoma
Matrix	Abundant and well developed	Abundant along with myxoid areas	Sparse often chondroid with myxoid areas
Cellularity of tumor	Relatively low cellular tumor	Moderately cellular tumor especially at the periphery of tumor lobules	Highly cellular tumor
Nuclear morphology in atypical chondrocytes	Small and dark nuclei with large nucleoli	Large nuclei with irregular hyperchromatic nuclei and large nucleoli	Pleomorphic hyperchromatic nuclei and large nucleoli
Mitoses	Absent or rare	Scattered mitoses <2/10 high power field	Scattered mitoses >2/10 high power field
Necrosis in tumor	Necrosis absent	Necrosis focal to abundant	Necrosis abundant

zone) of tumor is aggressive with cortical permeation and a large soft tissue mass lacking calcification.

Surgical Pathology: Dedifferentiated Chondrosarcoma

Gross Morphology

- Dedifferentiated chondrosarcoma consists of two components, i.e. central zone and peripheral zone.
- Central zone of tumor is composed of pale blue hyaline cartilage with rubbery to myxoid tissue and soft in consistency.
- Peripheral zone of tumor is composed of fleshy soft tissue component. Areas of hemorrhage and necrosis.

Light Microscopy

- Dedifferentiated chondrosarcoma consists of two components, i.e. central zone and peripheral zone. Both zones are sharply demarcated.
- Central zone of tumor is composed of uniform chondrocytes containing hyperchromatic nuclei (grade 1 conventional chondrosarcoma).
- Peripheral zone of tumor is high-grade component of osteosarcoma, chondrosarcoma or malignant fibrous histiocytoma with features of pleomorphic nuclei, abundant atypical mitoses and necrosis.

Differential Diagnosis

Dedifferentiated chondrosarcoma should be differentiated from chondroblastic osteosarcoma, conventional chondrosarcoma and malignant fibrous histiocytoma.

Prognosis and Treatment

Patient with dedifferentiated chondrosarcoma is treated with wide surgical excision usually with wide amputation and adjuvant chemotherapy. Long-term survival is seen in <10% of cases.

MESENCHYMAL CHONDROSARCOMA

Mesenchymal chondrosarcoma is high-grade bone tumor with biphasic pattern. It is characterized by primitive (undifferentiated) stromal or mesenchymal cells in which islands of well differentiated cartilage is interspersed.

Incidence and Locations

Mesenchymal chondrosarcoma accounts for 1–3% of all cartilaginous bone tumors. Most common sites are pelvis, ribs and jaw. Extraskeletal (extraosseous) chondrosarcoma occurs in 30% of cases.

Age and Sex Predilection

Mesenchymal chondrosarcoma most often affects young persons with peak incidence during second and third decades of life with sex predilection.

Clinical Features

Patient with mesenchymal chondrosarcoma presents with pain and tender mass in the affected bone.

Radiologic Findings

Radiograph shows permeative osteolytic lesion with cortical and soft tissue destruction.

Surgical Pathology: Mesenchymal Chondrosarcoma

Gross Morphology

Tumor is an admixture of firm blue cartilage and soft fleshy pink tissue. Gritty calcifications may be demonstrated.

Light Microscopy

- Tumor is composed of two components, i.e. well differentiated hyaline cartilage and small round blue cells component. Both components of the tumor are haphazardly arranged. Interface may be abrupt or gradual.
- Small round blue cells component shows spindled areas, necrosis, hemorrhage and hemangiopericytoma-like vascular spaces.

Differential Diagnosis

Mesenchymal chondrosarcoma should be differentiated from chondroblastic osteosarcoma, Ewing sarcoma and dedifferentiated sarcoma.

Prognosis and Treatment

Patient with mesenchymal chondrosarcoma is treated with wide local excision and adjuvant chemotherapy in most cases. Recurrence and metastasis may be observed decades after initial diagnosis and treatment.

CLEAR CELL CHONDROSARCOMA

Clear cell chondrosarcoma is a low-grade malignant tumor with low metastatic potential. Tumor is composed of cartilage with characteristic large clear cells.

Incidence and Locations

Clear cell chondrosarcoma accounts for 1–2% of all cartilage forming tumors. Most common site is proximal femur in epiphyseal region. Less common sites of tumor are proximal humerus and distal femur.

Age and Sex Predilection

Clear cell chondrosarcoma most often affects young persons with peak incidence during third decade of life with male predilection.

Clinical Features

Patient with clear cell chondrosarcoma presents with pain and tender mass in the affected bone over years.

Radiologic Findings

Radiograph shows a well-circumscribed radiolucent lesion in the epiphysis of long bone especially proximal femur with sclerotic border and absence of periosteal reaction and soft tissue mass. Stippled calcifications in tumor are demonstrated in 25% of cases.

Surgical Pathology: Clear Cell Chondrosarcoma

Gross Morphology

- Curettage of clear cell chondrosarcoma has gritty calcifications and pale blue cartilage.
- Consistency is softer than normal hyaline cartilage. Cystic areas are demonstrated.

Light Microscopy

- Tumor is composed of large clear tumor cells with prominent clear-to-pale eosinophilic cytoplasmic borders, round-to-oval nuclei and prominent nucleoli. Mitotic activity is low.
- The growth pattern is at least permeative. Variable amount of woven bone is present.
- Multinucleated osteoclast-like giant cells may be present.

Immunohistochemistry Technique

Clear cells of tumor are strongly positive for S-100 protein.

Differential Diagnosis

Clear cell chondrosarcoma should be differentiated from chondroblastic osteosarcoma and osteoblastoma.

Prognosis and Treatment

En bloc resection of clear cell chondrosarcoma tumor is curable. Incomplete curettage of tumor may result in metastasis and mortality in 15% of cases.

OSTEOGENIC TUMORS

Osteogenic tumors according to revised 2020 WHO classification of bone tumors are given in Table 27.24. Osteoid osteoma and osteoblastoma constitute 13% bone forming tumors. Facial bones are the most common site for osteoma. Osteoid osteoma originates in cortex of long bone; and is characterized by nidus of immature bone surrounded by sclerotic bone. Vertebrae and cortex of long bones are most common site for osteoblastoma. Osteosarcoma is a malignant osseous neoplasm and the most common primary cancer of childhood. Selected osteosarcoma variants, their age and salient pathologic features are given in Table 27.25.

OSTEOMA

Osteoma is a benign bone forming tumor and composed of cortical bone and less frequently cancellous bone. It is usually a solitary tumor except in Gardner's syndrome. Gardner's syndrome occurs due to APC gene mutation, and is characterized by formation of multiple osteomas, colonic polyps and soft tissue tumors.

Incidence and Locations

True incidence of osteoma is not known. Most common site is craniofacial skeleton around paranasal sinuses. Rare sites of tumor are spine and long bones.

Age and Sex Predilection

Osteoma is diagnosed in the middle-aged persons, which has slight male predilection.

Table 27.24 Osteogenic tumors according to revised 2020 WHO classification of bone tumors

Osteogenic Tumors	
Benign tumors	
▪ Osteoma ▪ Osteoid osteoma	
Intermediate tumors (locally aggressive)	
Osteoblastoma	
Malignant tumors	
▪ Low-grade osteosarcoma ▪ Conventional osteosarcoma (chondroblastic osteosarcoma, fibroblastic osteosarcoma) ▪ Telangiectatic osteosarcoma	▪ Small cell osteosarcoma ▪ Secondary osteosarcoma ▪ Parosteal osteosarcoma ▪ Periosteal osteosarcoma ▪ High-grade surface osteosarcoma

Table 27.25 Selected osteosarcoma variants, their age and salient pathologic features

Histologic Variant	Location	Age Group	Pathologic Features
Conventional osteoblastic osteosarcoma	Metaphysis of long bones around knee joint (distal end of femur or proximal end of tibia) are commonly affected in 65% of cases	10–20 years	Formation of lace-like pattern bone formed by the neoplastic cells is a diagnostic feature of conventional osteoblastic osteosarcoma
Low-grade osteosarcoma	Metaphysis of long bones (distal femur, proximal tibia) within medullary cavity	Third decade	Tumor is composed of spindle-shaped neoplastic cells forming variable amounts of osteoid formation. Neoplastic cells are arranged in interlacing bundles showing minimal atypia and a few mitoses
Telangiectatic osteosarcoma	Metaphysis (distal femur, proximal tibia) within medullary cavity	10–20 years	Blood filled spaces with highly malignant osteoid element
Parosteal osteosarcoma	Cortex outside periosteum (distal femur)	10–20 years	Abundant cartilage matrix with variable amount of malignant osteoid element
Periosteal osteosarcoma	Cortex inside periosteum (distal femur and upper tibia)	10–20 years	Osteoid bone formed by neoplastic cells composed of abundant cartilaginous matrix formed by neoplastic cells. Tumor cells show cytologic atypia

Clinical Features

Patient develops a sessile periosteal or endosteal growth in the skull and facial bones. Patient is usually asymptomatic and detected as incidental finding. Symptoms depend on location of tumor.

Radiologic Findings

Radiograph shows surface based, homogenous and well demarcated radiodense tumor.

Surgical Pathology: Osteoma

Gross Morphology

Osteoma tumor is hard, tan-white and mimicking normal bone.

Light Microscopy

- Osteoma tumor is composed of admixture of lamellar and woven bone mimicking compact cortical or trabecular bone. Bony trabeculae are broad, mature and irregular.
- Intertrabecular space is filled with fibrous tissue, which sometimes become vascularized. These intertrabecular spaces may contain foci of hematopoietic tissue (Fig. 27.18).

Prognosis and Treatment

Patient does not require any treatment unless becomes symptomatic. Excision of symptomatic tumor is curative. Large size osteoma in long bone may require biopsy to exclude a low-grade osteosarcoma.

OSTEOID OSTEOMA

Osteoid osteoma is a benign bone forming painful tumor and is characterized by a central osseous tissue; nidus measuring <2 cm surrounded by reactive sclerotic bone. It accounts for 11% of benign bone tumors.

Osteoid osteoma originates from the cortex of diaphysis of femur, humerus, radius and fibula. Proximal end of tibia is the most common sites. Other sites are phalanges, talus and calcaneum.

Age and Sex Predilection

Children and young adults during 5–25 years of age are affected with male predominance.

Clinical Features

Patient presents with osteoid osteoma tumor mass <2 cm size and severe localized nocturnal pain due to synthesis of prostaglandins by the tumor. Pain is relieved by administration of nonsteroidal anti-inflammatory

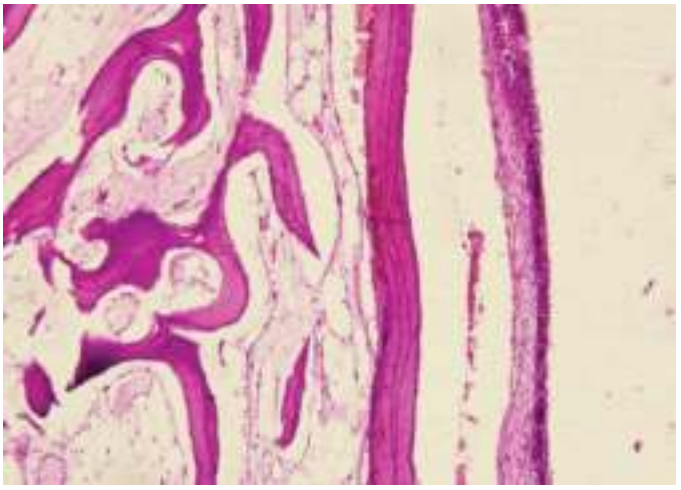


Fig. 27.18: Osteoma. Tumor is composed of admixture of lamellar and woven bone mimicking compact cortical or trabecular bone. Bony trabeculae are broad, mature and irregular. Intertrabecular space is filled with fibrous tissue, which sometimes become vascularized. These intertrabecular spaces may contain foci of hematopoietic tissue (400X).

aspirin drug. Tumor located close to joints may cause symptoms mimicking arthritis. Involvement of the vertebrae by the tumor can result in painful scoliosis.

Radiologic Findings

Radiograph shows well-demarcated radiolucent tumor with central mineralization surrounded by dense sclerosis.

Surgical Pathology: Osteoid Osteoma

Gross Morphology

- Osteoid osteoma is solitary tumor measuring <2 cm in diameter.
- The nidus of tumor is well demarcated, dark red, gritty and surrounded by dense sclerotic tissue (Fig. 27.19).

Light Microscopy

- In early stage, the nidus of osteoid osteoma is composed of inter-anastomosing woven bone with prominent osteoblastic rimming with intervening fibrovascular stroma.
- In later stage, there is presence of tangled islands and trabeculae of partially mineralized osteoid within the fibrous stroma; and densely packed irregular bony trabeculae.

Differential Diagnosis

Osteoid osteoma should be differentiated from osteoblastoma.

Immunohistochemistry Technique

Immunohistochemical stains demonstrate immunomarkers for nerve fibers within the tumor and surrounding reactive zone.

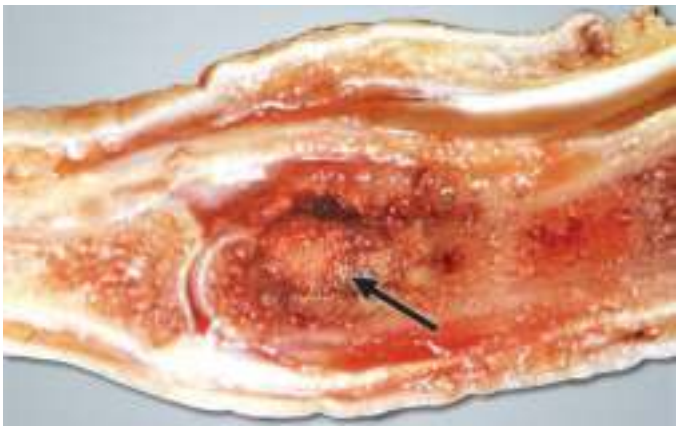


Fig. 27.19: Osteoid osteoma in the proximal phalanx of finger. The nidus of tumor is well demarcated, dark red, gritty and surrounded by dense sclerotic tissue (arrow). The treatment of choice is bone curettage. Proximal end of tibia is the most common sites.

Prognosis and Treatment

Patient is treated with aspirin and related analgesic drugs, surgical resection and percutaneous ablation. Local recurrence is seen in 5% of cases.

OSTEOBLASTOMA

Osteoblastoma is a benign bone forming tumor. Histologic features of osteoblastoma and osteoid osteoma are similar. Osteoblastoma measures >2 cm and osteoid osteoma <2 cm in diameter. Osteoblastoma lacks surrounding sclerotic bone, but osteoid osteoma has surrounding sclerotic bone. Comparison of osteoid osteoma and osteoblastoma is given in Table 27.26.

Table 27.26 Comparison of osteoid osteoma and osteoblastoma

Features	Osteoid Osteoma	Osteoblastoma
Age group	Children and young adults <25 years	Second to fourth decades of life
Sex distribution ratio	2:1	1:1
Size of tumor	<2 cm	>2 cm
Locations	Cortex of diaphysis of femur, humerus, radius and fibula. Proximal end of tibia is the most common site. Other sites are phalanges, talus and calcaneum	Metaphysis or diaphysis of femur, tibia, vertebrae, small bones of foot and ankle; and iliac bones
Pain in tumor	Severe nocturnal pain due to synthesis of prostaglandins and relieved by nonsteroidal anti-inflammatory aspirin and related analgesic drugs	Dull pain with minimal response to nonsteroidal anti-inflammatory aspirin and related analgesic drugs
Conventional radiograph	Well-demarcated radiolucent tumor with central mineralization surrounded by dense sclerosis	Well defined, predominantly osteolytic lesion surrounded by rim of sclerotic lesion
Gross morphology	Solitary round to oval tumor <2 cm with nidus dark red, gritty and surrounded by dense sclerotic tissue	Solitary round to oval tumor >2 cm with nidus dark red, gritty and surrounded by dense sclerotic tissue
Light microscopy (histological features similar)	Randomly interconnecting trabeculae of woven bone rimmed by osteoblasts with intervening fibrovascular stroma	Randomly interconnecting trabeculae of woven bone rimmed by osteoblasts with intervening fibrovascular stroma
Treatment and prognosis	Treated with aspirin, resection and percutaneous ablation; and local recurrence in 5% of cases	Treated by curettage or *en bloc* resection and local recurrence in 10–20% of cases

Incidence and Locations

Osteoblastoma accounts for 1% of primary bone tumors, which originates from metaphysis or diaphysis of femur and tibia. Other sites of tumor are vertebrae, small bones of hands and feet; and iliac bones.

Age and Sex Predilection

Osteoblastoma tumor occurs during second to fourth decades of life with male predominance.

Clinical Features

Patient with osteoblastoma presents with mass and dull pain in the femur or tibia of other bones. There is minimal response to nonsteroidal anti-inflammatory aspirin or related analgesic drugs to relieve pain.

Radiologic Findings

Radiograph shows well defined, predominantly osteolytic lesion surrounded by rim of sclerotic lesion of >2 cm in diameter.

Surgical Pathology: Osteoblastoma

Gross Morphology

Osteoblastoma is solitary round to oval tumor >2 cm with nidus dark red, gritty and surrounded by dense sclerotic tissue.

Light Microscopy

- Osteoblastoma is identical to giant osteoid osteoma. The nidus of tumor is composed of irregular reactive trabeculae of woven bone, lined by prominent osteoblastic rimming and osteoclasts separated by richly vascularized fibrovascular stroma.
- Tumor lacks mitosis and atypical cells (Fig. 27.20).

Differential Diagnosis

Osteoblastoma should be differentiated from osteoid osteoma, aneurysmal bone cysts, osteosarcoma and fibro-osseous lesions.

Prognosis and Treatment

Patient with osteoblastoma is treated by curettage and *en bloc* resection of tumor. Local recurrence after curettage of tumor occurs in 10–20% of cases.

CONVENTIONAL INTRAMEDULLARY OSTEOSARCOMA

Conventional intramedullary osteosarcoma is the most common malignant bone forming tumor; in which neoplastic cells form variable amount of osteoid, collagen and mineralized bone matrix. Histologic variants of osteosarcoma and their descriptive terms used in their diagnosis are given in Table 27.27.

Incidence and Locations

Conventional intramedullary osteosarcoma accounts for 20% of primary nonhematopoietic malignant bone tumors.

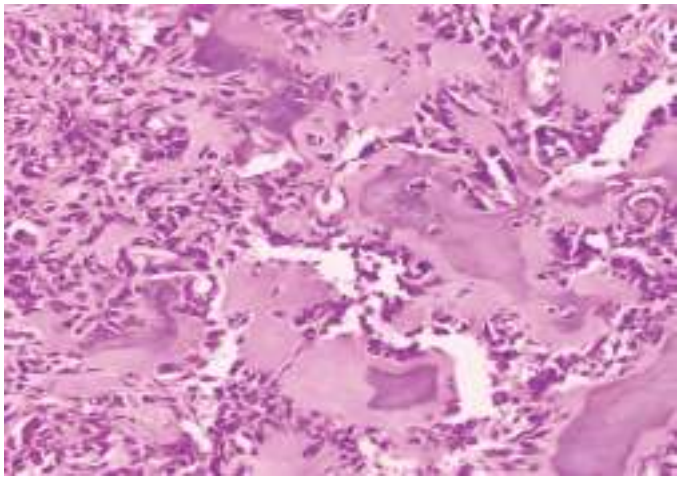


Fig. 27.20: Osteoblastoma. It is identical to giant osteoid osteoma. The nidus of tumor is composed of irregular reactive trabeculae of woven bone, lined by prominent osteoblastic rimming and osteoclasts separated by richly vascularized fibrovascular stroma. Tumor lacks mitosis and atypical cells (400X).

Table 27.27 Histologic variants of osteosarcoma and their descriptive terms used in their diagnosis

Intramedullary Osteosarcoma

- Conventional osteosarcoma
  - Osteoblastic osteosarcoma
  - Chondroblastic osteosarcoma
  - Fibroblastic osteosarcoma
  - Malignant fibrous histiocytoma-like osteosarcoma
  - Giant cell-rich osteosarcoma
  - Osteoblastoma-like osteosarcoma
  - Small cell osteosarcoma
  - Epithelioid osteosarcoma
- Telangiectatic osteosarcoma
- Well differentiated (low-grade intraosseous) osteosarcoma

Surface Osteosarcoma

- Parosteal (juxtacortical) osteosarcoma
- Periosteal osteosarcoma
- High-grade (*de novo* and dedifferentiated) osteosarcoma

Intracortical Osteosarcoma

Osteosarcoma arising within cortex

Extraskeletal Osteosarcoma

Osteosarcoma arising in extraskeletal region

Secondary Osteosarcoma

- Paget's disease of bone
- Post-radiation
- Bone infarct
- Fibrous dysplasia
- Metallic implant
- Chronic osteomyelitis

Osteosarcoma Associated with Specific Clinical Syndromes

- Hereditary retinoblastoma
- Li-Fraumeni syndrome
- Rothmund-Thomson syndrome
- Bloom syndrome (also known as Bloom-Torre-Machacek syndrome)
- Werner syndrome
- Diamond-Blackfan anemia
- Neurofibromatosis type 1
- Familial Paget's disease of bone
- Mazabraud syndrome
- McCune-Albright syndrome

- Long bones around knee joint (distal end of femur or proximal end of tibia) are commonly affected conventional intramedullary osteosarcoma in 65% of cases.
- However, it may originate from any bone, e.g. hip bone (15%), proximal humerus (10%) and mandible (8%). Rarely, it may occur in the upper end of femur.
- Tumor may extend into epiphysis, joint space and soft tissue. Unusual sites are bones distal to the wrists and joints. Locations of conventional intramedullary osteosarcoma and frequency are shown in Fig. 27.21 and Table 27.28.

Age and Sex Predilection

Conventional intramedullary osteosarcoma has bimodal age distribution, which affects below the age of 25 years (60%) and older than 40 years of age (10–30%). Peak incidence of tumor is seen during 10–20 years of age. Males and females are equally affected.

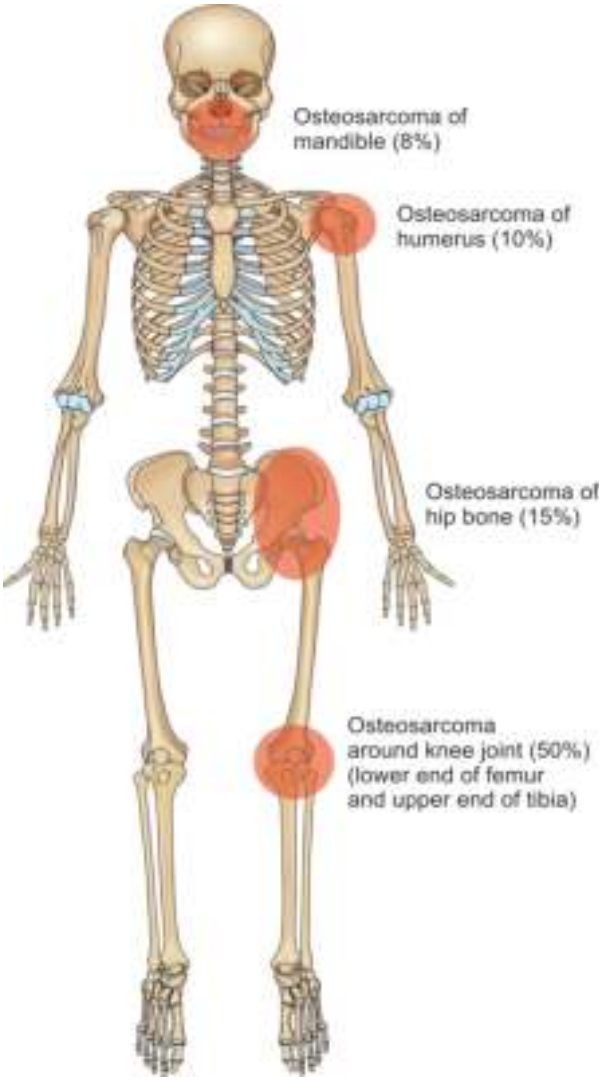


Fig. 27.21: Locations of conventional intramedullary osteosarcoma and frequency.

Table 27.28 Locations of conventional intramedullary osteosarcoma and frequency

Location of Conventional Osteosarcoma	Frequency
Around knee joint (distal end of femur or proximal end of tibia)	65%
Hip bones	15%
Proximal humerus	10%
Mandible	8%
Upper end of femur	Occasional case

Risk Factors

Risk factors for conventional intramedullary osteosarcoma are categorized in high risk, moderate risk and low risk precursor lesions (Table 27.29). Genetic or inherited disorders associated with increased risk for osteosarcoma are given in Table 27.30. Molecular genetic alterations in various histologic subtypes of conventional intramedullary osteosarcoma are given in Table 27.31.

Clinical Features

Initially patient with conventional intramedullary osteosarcoma presents with localized intermittent pain in deep-seated region with or without a mass.

Table 27.29 Risk factors for development of osteosarcoma

Categories of Risk Factors of Osteosarcoma	Examples of Precursor Lesions
High risk precursor lesions	▪ Familial retinoblastoma (RB gene mutation) ▪ Ollier disease (enchondromatosis) ▪ Maffucci syndrome (enchondromatosis associated with hemangiomas) ▪ Rothmund-Thomson syndrome ▪ Occupational exposure to radium used in watch-dial
Moderate risk precursor lesions	▪ Multiple osteochondromas ▪ Paget's disease of bone (polyostotic) results in parosteal osteosarcoma ▪ Previous exposure to radioisotope (phosphorus)
Low risk precursor lesions	▪ Fibrous dysplasia of bone ▪ Chronic osteomyelitis ▪ Bone infarct ▪ Giant cell tumor of bone ▪ Li-Fraumeni syndrome (germline TP53 gene mutation) ▪ Diamond-Blackfan anemia ▪ Werner syndrome ▪ Osteoblastoma ▪ Chondroblastoma ▪ Metallic and polyethylene implants ▪ Osteogenesis imperfecta

Table 27.30 Genetic or inherited disorders associated with increased risk for osteosarcoma

Genetic or Inherited Disorder	Inheritance Pattern	Gene Involved, Chromosomal Site and Molecule Malfunction	Other Associated Tumors
Hereditary retinoblastoma	Autosomal dominant	RB-1 cell cycle regulator gene on chromosome 13q14	Retinoblastoma, soft tissue sarcoma
Li-Fraumeni syndrome	Autosomal dominant	p53 (DNA repair protein) on chromosome 17q13.1	Breast carcinoma, adrenocortical carcinoma, bronchoalveolar carcinoma, soft tissue sarcoma including rhabdomyosarcoma, nervous system tumors, leukemia, lymphoma and myelodysplastic syndrome
Diamond-Blackfan anemia	Autosomal dominant	Ribosomal protein genes such as RPS19, RPL5 and others on multiple chromosomes	Leukemia, lymphoma, myelodysplastic syndrome
Rothmund-Thomson syndrome	Autosomal recessive	DNA helicase and REQL4 on chromosome 8q24.3	Leukemia, lymphoma, myelodysplastic syndrome, carcinoma
Bloom syndrome (also known as Bloom-Torre-Machacek syndrome)	Autosomal recessive	DNA helicase on chromosome 15q25.1	No other associated tumors
Werner syndrome	Autosomal recessive	DNA helicase on chromosome 8p12	Soft tissue sarcoma
Neurofibromatosis type 1	Autosomal dominant (50%) and sporadic (50%)	NF1 (neurofibromatosis 1) on chromosome 17q11	Neurofibromas, gastrointestinal stromal tumors, CNS tumors, leukemia, lymphoma, myelodysplastic syndrome, carcinoma
Familial Paget's disease of bone	Autosomal dominant	Multiple genes on multiple chromosomes 18q21, 5q31, 5q35 and others	Chondrosarcoma
Mazabraud syndrome	Unknown (sporadic)	GNAS1 (guanine nucleotide-binding protein; stimulating polypeptide 1) on chromosome 20q13	Polyostotic fibrous dysplasia, intramuscular myxomas
McCune-Albright syndrome	Unknown (sporadic)	GNAS1 (guanine nucleotide-binding protein; stimulating polypeptide 1) on chromosome 20q13	Polyostotic fibrous dysplasia

Table 27.31 Molecular genetic alterations in various histologic subtypes of conventional intramedullary osteosarcoma

Chromosomal Alterations	Chromosomal Aberrations	Comments
Numerical and structural chromosomal aberrations	1p36, 1q21.22, 6p12.21, 8q21.24, 12q11.14, 7p11.13 and 10q12.13	Recurrent amplification and DNA copy number gains at various chromosomal regions
Deletion and heterozygosity	3q13 involving LSAMP gene	Appears to correlate with progression and poor survival
Amplification of genes	▪ Amplification of RUNX2 gene mapping on 6p12.21 and other potential E2F3 and CDC5 genes ▪ VEGFA gene also maps on 6p12.21	▪ Promote terminal osteoblastic differentiation in conventional osteosarcoma associated with poor response to therapy ▪ Amplification of VEGFA gene promotes angiogenesis
Amplification and gain-of-function	Myc oncogene maps on 8q21.24	Amplification of Myc oncogene is demonstrated in 45–55% of cases of conventional osteosarcoma
Increased baseline activity of gene encoding protein synthesizing growth factor	PDGFB overexpression	Increases risk for osteosarcoma
Increased baseline activity of gene encoding protein synthesizing growth factor	FGF3 and HST1	Increases risk for osteosarcoma

Contd...

Table 27.31 Molecular genetic alterations in various histologic subtypes of conventional intramedullary osteosarcoma (*Contd...*)

Chromosomal Alterations	Chromosomal Aberrations	Comments
Chromothripsis	Small group of chromosomes	Chromothripsis has been proposed to involve shattering of small groups chromosome and their random assembly by non-homozygous end joining as a single event involved in *de novo* osteosarcoma
RB and TP53 gene mutations	RB gene (13q14), TP53 gene (Li-Fraumeni syndrome)	RB gene and TP53 gene increase risk for osteosarcoma
Amplification of cyclin-dependent kinase (CDK) gene	CDK4 (12q13.14), CCNE1 and CDKN2A	Loss of CDKN2A is associated with reduced survival in these cases
Exposure to occupational radiation (radium used in watch dial) induced osteosarcoma	Loss of 1p	Radiation-induced osteosarcoma in patient with bilateral retinoblastoma increases risk for osteosarcoma
Normal INK4a gene encoding $p14^{ARF}$, $p16^{NK4\alpha}$ function to arrest cell cycle	Inactivating of INK4α gene encoding $p14^{ARF}$, $p16^{NK4\alpha}$	INK4a gene mutations demonstrated in osteosarcoma

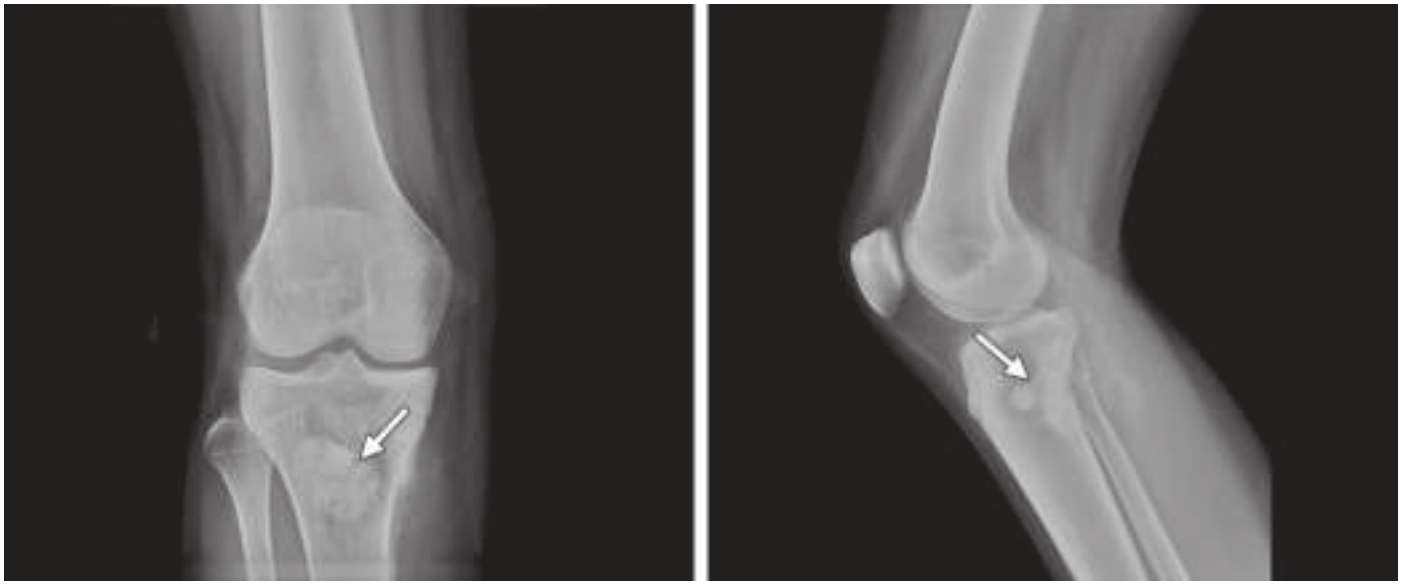


Fig. 27.22: Osteosarcoma of upper tibia in 16 years patient. Radiograph demonstrates a large irregularly marginated solid lesion in upper tibia involving epiphysis, upper growth plate, metaphysis and adjoining upper diaphysis. Tumor is extending up to subarticular region of upper tibia with erosion of articular surface in intercondylar eminence. Periosteal reaction is noted along posterior cortex with erosion and destruction of posterior cortex with associated soft tissue component posterior to upper tibia. Findings are suggestive of neoplastic etiology-osteosarcoma shown by arrow. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

Progressive enlargement of tumor may restrict range of movements, joint effusions and pathologic fracture. Pulmonary metastasis is associated with loss of weight and cachexia in advanced stage.

Radiologic Findings

- Radiologic appearance of conventional intramedullary osteosarcoma is extremely variable, i.e. large destructive poorly defined tumor or mixed osteoblastic and osteolytic mass that breaks the cortex and invades the soft tissue resulting in formation of mass in the soft tissue compartment.
- '**Codman triangle**' is a triangular shadow between the cortex and raised ends of periosteum demonstrated on radiograph. It is formed due to lifting of the periosteum away from the bone by the tumor. Bony spicules formed by the tumor cells give '**sunburst (sun ray) appearance**' (Fig. 27.22).
- Carcinomas of prostate and breast metastasizing to bones may show osteoblastic reaction. These tumors should be taken into consideration in differential diagnosis on radiological examination. Immunohistochemistry plays significant role in establishing metastatic bone disease.

Surgical Pathology: Conventional Intramedullary Osteosarcoma

Gross Morphology

- Gross morphology of conventional intramedullary osteosarcoma is variable depending on the presence of bone and other components.
- Cut surface of tumor shows variable appearance due to presence of osteoid, mineralized bone matrix, blood vessels in variable proportion; and presence of hemorrhage and necrosis. Gross morphology of conventional intramedullary osteoblastic osteosarcoma is shown in Fig. 27.23A and B.

Light Microscopy

Conventional intramedullary osteosarcoma is classified according to specific clinical features. However, there is no relationship between histologic variants (subtypes), treatment and prognosis.

- **Conventional intramedullary osteoblastic osteosarcoma:** Tumor is composed of malignant pleomorphic polyhedral and spindle-shaped neoplastic cells with hyperchromatic nuclei, numerous mitoses; and formation of 'lace-like' bone formation by neoplastic cells.
  - Formation of lace-like pattern of bone formed by the neoplastic cells is a diagnostic feature of conventional osteoblastic osteosarcoma.
  - The neoplastic cells also form island of reactive new woven bone (pinkish lace-like osteoid and calcified bone spicules) in response to the infiltration and destruction of the normal bone by the neoplastic cells.
  - Approximately in 60% of cases, there may be considerable histologic variation within a single tumor at primary site.
  - Metastasis may not exactly resemble the primary site tumor on histologic examination. Histologic features of conventional osteoblastic osteosarcoma are shown in Fig. 27.24.
- **Conventional intramedullary chondroblastic osteosarcoma:** Tumor is composed of pleomorphic polyhedral to spindle-shaped cells with hyperchromatic nuclei and numerous mitosis. Formation of hyaline chondroid islands by neoplastic cells is diagnostic feature. The lacunae contain neoplastic cells with severe cytological atypia (Fig. 27.25).
- **Conventional intramedullary fibroblastic osteosarcoma:** Tumor is composed of spindled neoplastic cells associated with extracellular collagen fibers arranged in 'storiform pattern'.
- **Conventional intramedullary giant cell-rich osteosarcoma:** Tumor is composed of neoplastic cells admixed with osteoclast type of giant cells.
- **Conventional intramedullary small cell-rich osteosarcoma:** Tumor is composed of small round cells that produce variable amount of osteoid.
- **Conventional intramedullary clear cell osteosarcoma:** Tumor is composed of neoplastic cells with sharply defined cell borders and clear or ground glass appearance cytoplasm containing vacuoles and central nuclei. There is presence of small trabeculae of reactive bone.
- **Conventional intramedullary chondroblastoma-like osteosarcoma:** Tumor is composed of neoplastic cells growing in an infiltrative pattern.
- **Conventional intramedullary malignant fibrohistiocytoma-like osteosarcoma:** Tumor is composed of lace-like osteoid deposits, hemangiopericytoma like blood vessels, formation of rosette-like structures and epithelioid neoplastic cells.

Electron Microscopy

Presence of mesenchymal neoplastic cells containing dilated rough endoplasmic reticulum, prominent Golgi apparatus, calcium hydroxyapatite crystals admixed with matrix collagen fibers in conventional osteoblastic osteosarcoma differentiate Ewing sarcoma/primitive neuroectodermal tumor (PNET), lymphoma and melanoma.

Immunohistochemistry Technique

Conventional intramedullary osteosarcoma, whether primary or secondary, has broad immunoprofile, but lacks specificity shown as below:

Markers	Expression
Osteocalcin	Positive
Osteonectin	Positive (neoplastic cells and neoplastic matrix)
S-100 protein	Positive
Actin	Positive
Smooth muscle actin	Positive
NSE (neuron-specific enolase)	Positive
Factor VIII	Positive
CD31	Positive
CD45	Positive
CK (cytokeratin)	Positive (diagnostic pitfall)
EMA (epithelial membrane antigen)	Positive (diagnostic pitfall)

Differential Diagnosis

Conventional intramedullary osteosarcoma should be differentiated from variety of benign and malignant tumors. In young patients, malignant bone tumors show prominent cartilaginous differentiation is almost conventional intramedullary chondroblastic osteosarcoma rather than conventional chondrosarcoma, which occurs in elderly patients. Conventional osteoblastoma like osteosarcoma can be confused with osteoblastoma and fibrous dysplasia. Aneurysmal bone cyst needs to be differentiated from telangiectatic osteosarcoma. Small cell osteosarcoma can mimic Ewing sarcoma and other round cell tumors. Stress fracture with callus formation and myositis ossificans should be differentiated from osteosarcoma.

Mode of Spread

Conventional intramedullary osteosarcoma invades surrounding soft tissue and metastasizes via

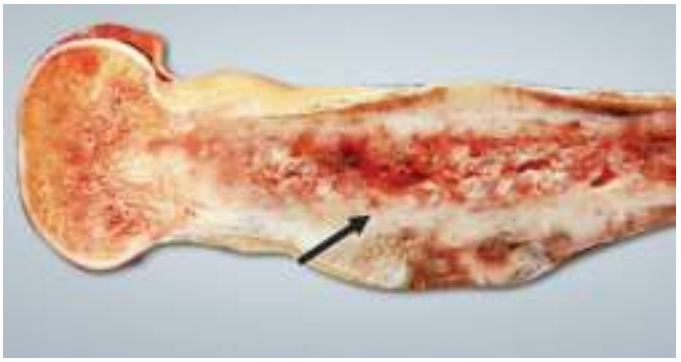


Fig. 27.23A: Osteosarcoma. Tumor has involved the medullary cavity of lower end of femur and broken through the cortical bone and caused elevation of the periosteum shown by arrow.

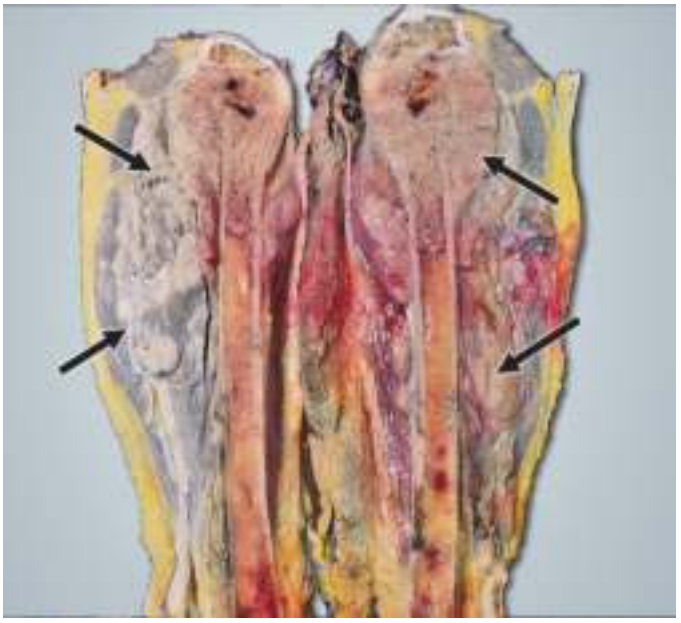


Fig. 27.23B: Conventional intramedullary osteosarcoma. Cut section of the specimen shows intramedullary mass arising from the metaphyseal region of tibia, which is permeating with in medullary region and surrounding soft tissues shown by arrows. (*Courtesy*: Dr. Tushar Kalonia.)

hematogenous route to the lungs (most common), liver, brain and other sites.

- **Local invasion:** Tumor spreads extensively within the medullary cavity bone marrow, evidentially eroding through the cortical plate and invading the surrounding tissue. Tumor usually does not cross the epiphyseal plate and thus spares involvement of joint cavity. Involvement of joint may occur due to either extension of tumor cells along the tendinoligamentous structures or through the attachment site within the joint capsule.
- **Hematogenous route:** Initially, the tumor metastasizes via hematogenous route to lungs in 20% of cases. Later, it may spread to liver, brain, other sites including involvement of other bones.

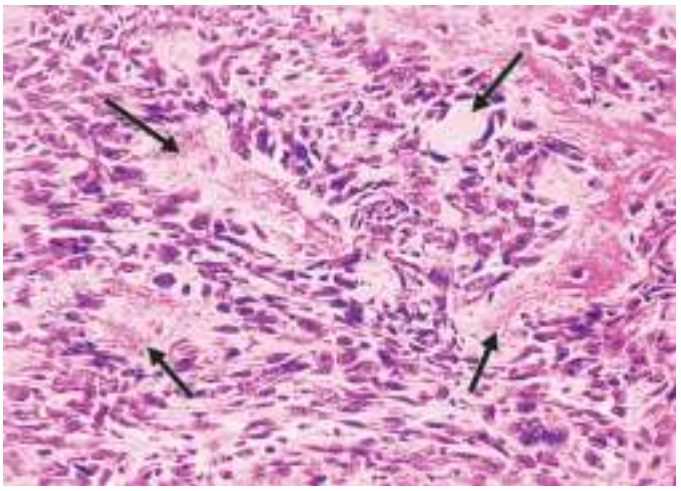


Fig. 27.24: Conventional intramedullary osteoblastic osteosarcoma. Tumor is composed of malignant pleomorphic neoplastic cells with hyperchromatic nuclei, numerous mitoses; and formation of 'lace-like' bone formation by neoplastic cells. Formation of lace-like pattern bone formed by the neoplastic cells is a diagnostic feature of conventional osteoblastic osteosarcoma. The neoplastic cells also form island of reactive new woven bone (pinkish lace-like osteoid and calcified bone spicules shown by arrows) in response to the infiltration and destruction of the normal bone by the neoplastic cells (400X).

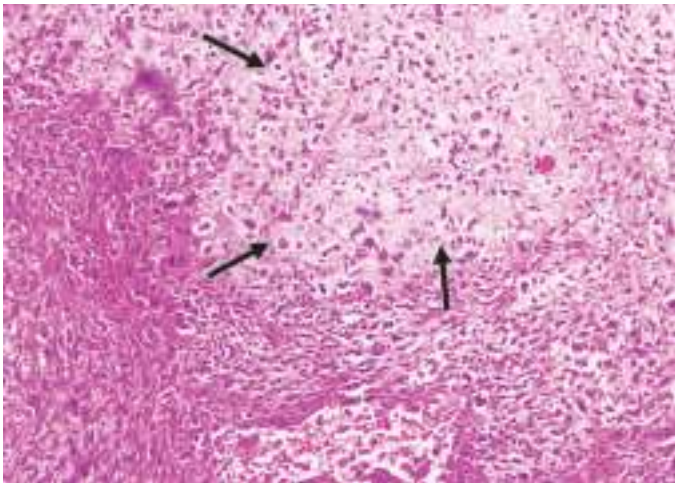


Fig. 27.25: Conventional intramedullary chondroblastic osteosarcoma. Tumor is composed of pleomorphic polyhedral to spindle-shaped cells with hyperchromatic nuclei and numerous mitosis. Formation of hyaline chondroid islands by neoplastic cells is diagnostic feature. The lacunae of neoplastic cells with severe cytological atypia (400X).

Prognosis and Treatment

Conventional intramedullary osteosarcoma is treated by combination of surgery and chemotherapy. Prognosis depends on histologic variants, grade of tumor, localized tumor and metastasis. Survival rate for localized tumor is higher than in tumor metastasizing to distant organs.

- Preoperative chemotherapy is administered in patient with conventional intramedullary high-grade osteosarcoma. Chemotherapy has improved five-year

survival rate in these patients. Accurate assessment of the chemotherapeutic response is critical. A good prognostic response is defined by the presence of >90% necrosis of neoplastic cells within the tumor.

- Combined chemotherapy and surgery are curable in children and adolescents in 80% of cases. Osteosarcoma affecting elderly person has less favorable response to chemotherapy. Prognosis in histologic variants of osteosarcoma are given in Table 27.32.

LOW-GRADE CENTRAL OSTEOSARCOMA

Low-grade central osteosarcoma is a malignant bone forming tumor, which arises within the medullary cavity of bone. It is also known as low-grade intramedullary osteosarcoma, which accounts for 1–2% of osteosarcomas. Most common sites are distal femur and proximal tibia in 80% of cases. However, any bone may be affected. Other sites are flat bones and small bone of hands and feet.

Age and Sex Predilection

Peak of low-grade central osteosarcoma incidence is in the third decade of life with slight male predominance.

Clinical Features

Patient presents with localized pain with or without swelling over months to years. Low-grade central osteosarcoma has indolent course when compared with conventional intramedullary osteosarcoma.

Radiologic Findings

Radiograph shows a large intramedullary poorly demarcated osteolytic mass, coarsely trabeculated with aggressive features in the metaphyseal or metadiaphyseal regions most often extending to the end of affected bone. CT scan and MRI are helpful in delineating the extent of tumor and identification of cortical abnormalities, that may not be evident on conventional radiograph.

Surgical Pathology: Low-grade Central Osteosarcoma

Gross Morphology

- Tumor shows a gritty gray medullary mass that may contain fleshy and fibrous areas. Cortical destruction may be demonstrated in some cases.
- Tumor may extend along the length of long bone with poor demarcation between the tumor and uninvolved bone.

Light Microscopy

- Tumor is composed of spindle-shaped neoplastic cells forming variable amounts of osteoid formation. Neoplastic cells are arranged in interlacing bundles showing minimal cytologic atypia and a few atypical mitoses.
- Some tumors contain irregular anastomosing branching and curved trabeculae mimicking woven bone in fibrous dysplasia. Tumor permeates the bone and extends into soft tissue. These histologic features are absent in fibrous dysplasia.

Immunohistochemistry Technique

- Tumor demonstrates one or more ring chromosomes showing amplification in the chromosomal 12q13.15 region.
- Potential target genes in this region include MDM2 and CDK4 showing amplification in majority of cases. Immunohistochemical analysis of MDM2 and CDK4 provides a useful diagnostic tool in low-grade central osteosarcoma.

Markers	Expression
MDM2	Positive
CDK4	Positive

Table 27.32 Prognosis in histologic variants of osteosarcoma

Features	Clinical Prognostic Significance
Osteosarcoma of jaw	Good prognosis (five-year survival in 80%)
Low-grade central osteosarcoma	Good prognosis
Osteosarcoma below knee joint	Better prognosis than located in proximal extremity
Parosteal osteosarcoma	Better prognosis than conventional osteosarcoma
Periosteal osteosarcoma	Better prognosis than conventional osteosarcoma
Osteosarcoma of vertebrae and skull	Poor prognosis
Multifocal osteosarcoma	Poor prognosis
Telangiectatic osteosarcoma	Poor prognosis
Osteosarcoma arising in Paget's disease of bone	Poor prognosis
High-grade surface osteosarcoma	Poor prognosis
Small cell osteosarcoma	Poor prognosis

Prognosis and Treatment

Patient with low-grade central osteosarcoma is treated with wide surgical excision of tumor. Prognosis is excellent in 90% of cases with five-year survival. Tumor may transform into high-grade osteosarcoma in 10–35% of cases. Such patients are treated with administration of chemotherapy.

TELANGIECTATIC OSTEOSARCOMA

Telangiectatic osteosarcoma is a high-grade malignant tumor and variant of central intramedullary osteosarcoma. It accounts for <4% of all osteosarcomas. Tumor is characterized by formation of large cystic spaces filled with blood. Cystic spaces show septations. Most common sites of tumor are metaphysis of distal femur (40%), proximal tibia (15%), proximal humerus (10%) and proximal femur (8%). Telangiectatic osteosarcoma is more prone to pathologic fracture.

Age and Sex Predilection

Telangiectatic osteosarcoma occurs in the second decade of life with male predominance.

Clinical Features

Patient with telangiectatic osteosarcoma presents with localized pain and enlarging. Massive bone destruction by the tumor may result in fracture in 25% of cases.

Radiologic Findings

- Radiograph shows osteolytic lesion with evidence of extensive destruction of bone and extension into the soft tissues. Most tumors are located in the metaphysis and extending into the soft tissue.
- Tumor most often expands the cortex and/or disrupts the cortex. Periosteal reaction forming 'Codman triangle' and 'onion-skin appearance' are frequent in these tumors.

Surgical Pathology: Telangiectatic Osteosarcoma

Gross Morphology

- Tumor shows a hemorrhagic multicystic lesion filled with blood clot, described a 'bag of blood'. Fleshy or sclerotic areas are not demonstrated.
- Extensive cortical erosion or destruction associated with involvement of soft tissue may be demonstrated in these cases.

Light Microscopy

- Tumor is composed of blood-filled or empty spaces simulating aneurysmal bone cyst. Cystic spaces are separated by septa of variable thickness. These cystic spaces are populated with pleomorphic neoplastic cells containing hyperchromatic nuclei. Some neoplastic cells float in the hemorrhagic areas. Atypical mitoses are demonstrated.
- Osteoclastic type of giant cells is demonstrated in the septa and edges of tumor.
- Osteoid formation by neoplastic cells is most often focal and confluent. Neoplastic cells permeating in pre-existing bone trabeculae are most often demonstrated.

Molecular Genetic Alterations

Telangiectatic osteosarcomas are genetically less complex than conventional intramedullary osteosarcoma. Trisomy and chromosomal aberrations and regional gains at 1q21–23.2, 1q25.2–31.1 and 7q21.13–21.2 are demonstrated by metaphase CGH and array CGS.

Prognosis and Treatment

Telangiectatic osteosarcoma is an aggressive tumor, but has sensitivity to chemotherapy and radiotherapy. The overall survival rate is similar to other histologic subtypes of osteosarcoma.

SMALL CELL OSTEOSARCOMA

Small cell osteosarcoma is high-grade bone forming tumor with variable degrees of osteoid element formed by neoplastic cells. It accounts for 2% of all osteosarcomas. Most common sites are **metaphysis of long bones**. It may affect 5–80 years of age group. Peak incidence of small cell osteosarcoma is during second decade of life with slight female predominance.

Clinical Features

Patient presents with localized pain with or without a mass. Patient may develop pathologic fracture.

Radiologic Findings

Radiograph shows destruction of cortex with osteolytic and radiodense components. Mineralized tissue in the tumor invading soft tissue strongly suggests small cell osteosarcoma.

Surgical Pathology: Small Cell Osteosarcoma

Gross Morphology

- Morphologic features of small cell osteosarcoma are similar to conventional intramedullary osteosarcoma.
- Tumor is firm gritty white, but not homogenous due to presence of neoplastic cells admixed with osteoid element, blood vessels and necrosis.

Light Microscopy

- Tumor is composed of small blue cells with scanty cytoplasm, round to oval nuclei with fine to chromatin and mitoses in the range of 3–5/high power fields.
- Lace-like osteoid production by neoplastic cells is always present and should be differentiated from fibrin deposits present in Ewing sarcoma.

Immunohistochemistry Technique

- Small cell osteosarcoma shows membrane positivity for **CD99** and osteoid element. Therefore, some authors consider this tumor as a variant of Ewing sarcoma with diverse differentiation showing positivity for CD99 and lacking osteoid element.
- Non-Hodgkin's lymphoma shows positivity for leukocyte common antigen (LCA), but lacks osteoid element.

Prognosis and Treatment

Small cell osteosarcoma has relatively poor prognosis than conventional intramedullary osteosarcoma. Patient is treated by surgery and chemotherapy.

PAROSTEAL OSTEOSARCOMA

Parosteal osteosarcoma is a low-grade malignant bone forming tumor arising from the **surface of long bones**. Osteoid element is formed by the neoplastic cells.

Incidence and Locations

Parosteal osteosarcoma accounts for 4% of all osteosarcomas. Most common site is the **surface of distal femur** in 70% of cases. Other important sites are proximal ends of tibia and humerus. The tumor wraps around the involved long bone. Flat bones are not involved.

Age and Sex Predilection

Parosteal osteosarcoma occurs in young adults during third decade of life with slight female predominance.

Clinical Features

Patient presents with painless swelling around knee joint or humerus, and inability to flex the joint. Sometimes, patient presents with painful swelling.

Radiologic Findings

Radiograph shows presence of heavily mineralized mass attached to the cortex with broad base. The tumor has a tendency to wrap around the involved bone. CT scan and MRI are useful in analyzing the extent of medullary involvement. Outer portion of tumor is less vascularized.

Surgical Pathology: Parosteal Osteosarcoma

Gross Morphology

- Tumor is hard lobulated mass affixed to the involved bone. In occasional case, presence of nodular cartilage with incomplete cap wrapping the tumor may simulate osteochondroma.
- Periphery of the tumor may be soft in consistency due to invasion of the tumor into surrounding soft tissue.
- Presence of fleshy areas in tumor suggests progression of the tumor to high-grade osteosarcoma. Focal invasion of the cortex and medullary cavity may be present.

Light Microscopy

- Tumor is composed of spindle cells with minimal cytologic atypia arranged in parallel pattern. Neoplastic cells form bony trabeculae. Hypercellular tumor composed of spindle cells show moderate cytologic atypia. Tumor may be hypocellular in <20% of cases.
- Cartilaginous differentiation in the tumor is demonstrated in 50% of cases. Tumor composed of high-grade spindle cells indicates progression of parosteal osteosarcoma to high-grade osteosarcoma.

Immunohistochemistry Technique

Immunohistochemical analysis of MDM2 (chromosome 12q region) and CDK4 provides a useful diagnostic tool in parosteal osteosarcoma.

Markers	Expression
▪ MDM2	▪ Positive
▪ CDK4	▪ Positive

Molecular Genetic Alterations

Parosteal osteosarcoma demonstrates one or more ring chromosomes showing amplification of genes in the chromosomal 12q13.15 region. Potential target genes in this region include MDM2 and CDK4 showing amplification in >85% of cases.

Prognosis and Treatment

Parosteal osteosarcoma is treated by wide local resection of tumor and prosthetic replacement. Prognosis is excellent with five-year survival in 90% of cases. Recurrence occurs in incompletely excised tumor and tumor undergoing dedifferentiation and progressing to high-grade osteosarcoma.

PERIOSTEAL OSTEOSARCOMA

Periosteal osteosarcoma is an intermediate grade malignant tumor, in which neoplastic cells form both cartilage and osteoid element. Tumor originates from the region between cortex and overlying periosteum on the surface of long bones, i.e. lower femur, upper tibia, fibula, humerus, ulna, and ilium.

Incidence and Locations

Tumor accounts for <2% of all osteosarcomas, which originates from diaphyseal or meta-diaphyseal region of distal femur and upper tibia in 80% of cases. Other sites are humerus, fibula, ulna and pelvis. Tumor may completely wrap around circumference of the long bones. Uncommon sites are clavicle, ribs, jaw and cranium.

Age and Sex Predilection

Periosteal osteosarcoma most often occurs during second to third decades of life with male predominance. Tumor may also affect persons >50 years of age.

Clinical Features

Patient presents with lump and/or associated pain over <6 moths to one-year duration.

Radiologic Findings

- Radiograph shows a soft tissue mass extending from cortex. Cortical thickening and extrinsic cortical scalloping are demonstrated in >80% of cases. Periosteal reaction caused by tumor perpendicular to the long axis of bone gives 'sun-burst' appearance or 'hair-on-end' pattern in 95% of cases.
- Both CT scan and MRI are useful to analyze tumor margins, medullary extension and relation of tumor to major blood vessels and peripheral nerves.

Surgical Pathology: Periosteal Osteosarcoma

Gross Morphology

- Tumor shows glistening gray cartilaginous lobulated mass over bony surface. Base of the tumor may be heavily ossified.
- Spiculed calcification perpendicular to the long axis of bone is demonstrated. Tumor may erode the cortex but not extend into the medullary cavity.

Light Microscopy

- Tumor is composed of abundant cartilaginous matrix formed by neoplastic cells. Tumor cells show cytologic atypia.
- Osteoid element or myxoid matrix or fibroblastic element may be demonstrated in the tumor.

Molecular Genetic Alterations

Point mutation of TP53 gene is demonstrated in 40% of cases. Chromosomal alterations are constant in periosteal osteosarcoma, i.e. gain of 8q, loss of 6q and amplification of 8q11.24 and 12q11.15.

Prognosis and Treatment

Patient with periosteal osteosarcoma is treated by surgical excision of tumor or limb salvage. Patient is not benefited by chemotherapy. Patient has good prognosis with five-year survival in >80% of cases. Metastasis may be demonstrated in >80% of cases.

HIGH-GRADE SURFACE OSTEOSARCOMA

High-grade surface osteosarcoma originates from cortical surface of long bones. Lace-like bone is formed by neoplastic cells. It accounts for <1% of all osteosarcomas. Most common sites of tumor are femur (50%), tibia (20%) and humerus (10%). It most often occurs in the second decade of life with male predominance.

Clinical Features

Patient presents with lump and pain in involved long bone over short duration.

Radiologic Findings

Radiologic findings of high-grade surface osteosarcoma and periosteal osteosarcoma are similar except the presence of mineralization of tumor. Radiograph shows a surface radiolucent tumor with spiculed calcification perpendicular to the long axis of bone. New bone formation is common in the peripheral portion of tumor.

Surgical Pathology: High-grade Surface Osteosarcoma

Gross Morphology

- Tumor is situated on the cortical surface of bone, and often eroding the cortex.
- Tumor is large lobulated and soft to firm in consistency depending on the presence of predominant osteoblastic, chondroblastic or fibroblastic component.
- Tumor extends into the surrounding soft tissue and forms a well circumscribed lobulated mass. Rarely, it may extend into medullary cavity.

Light Microscopy

- High-grade surface osteosarcoma and conventional central osteosarcoma have identical histologic features.
- Tumor is composed of polyhedral to spindle-shaped neoplastic cells containing hyperchromatic nuclei and numerous atypical mitoses.
- Formation of lace-like bone formation by neoplastic cells is diagnostic feature.
- Degree of cytologic atypia is greater in high-grade surface osteosarcoma than conventional central osteosarcoma.

Molecular Genetic Alterations

Amplification of the TSPAN31 (formerly known as SAS) gene located on 12q13.14 has been demonstrated in a case with high-grade surface osteosarcoma.

Mode of Spread

High-grade surface osteosarcoma spreads by local invasion into soft tissue compartment and via hematogenous route to lungs and other sites.

Prognosis and Treatment

- Prognosis is identical in high-grade surface osteosarcoma and conventional central osteosarcoma. Patient with unresectable tumor is treated by radiotherapy.
- Multi-agent chemotherapy has a dramatic impact on outcome of the disease. Five-year survival is demonstrated in >80% of cases.

EWING SARCOMA/PRIMITIVE NEUROECTODERMAL TUMOR

EWING SARCOMA

Ewing sarcoma and primitive neuroectodermal tumor (PNET) are related entities. Ewing sarcoma/PNET is composed of small cells with uniform round hyperchromatic nuclei.

- Distinguishing feature of Ewing sarcoma/PNET is degree of neural differentiation demonstrated by light microscopy (Homer-Wright rosettes), electron microscopy and immunohistochemistry. Approximately 20% cases of Ewing sarcoma show features of neural differentiation.
- Ewing sarcoma is currently used to describe a tumor composed of small, round cells lacking extracellular matrix, containing variable amounts of glycogen, and scant evidence of neuroectodermal derivation histologically, ultrastructurally and immunohistochemically.
- Ewing sarcoma tends to originate in skeletal sites populated by hematopoietic marrow of long bones in meta-diaphyseal regions.
- Ewing sarcoma is characterized by recurrent balanced chromosomal translocations in almost all cases. Reciprocal chromosomal translocation between chromosomes 11 and 22 results in fusion of the amino terminus of EWSR1 gene to the FLT1 gene (EWSR-FLT1 fusion gene), which encodes a nuclear transcription factor resulting in cellular proliferation.

Diagnostic Features

Generally, two or more major features of neural differentiation are essential for diagnosis of primitive neuroectodermal tumor (PNET). Approximately 20% cases of Ewing sarcoma show features of neural differentiation. Diagnostic criteria of Ewing sarcoma/primitive neuroectodermal tumor (PNET) are given in Table 27.33.

Incidence and Locations

Ewing sarcoma accounts 6–8% of primary most malignant tumors. It is the second most common bone sarcoma in children after osteosarcoma. Tumor originates from primitive bone marrow elements or immature mesenchymal cells of bones. Most common sites are meta-diaphyseal regions of femur, humerus; pelvis and ribs. Rare sites skull, scapula and short tubular bones of hands and feet.

Age and Sex Predilection

Ewing sarcoma most often occurs during second decade of life. Nearly 80% of patients are below the age of 20 years. Only 20% of patients are below the age of 8 years or older than 20 years.

Clinical Features

Patient presents with severe pain may be associated with a swelling in the bone. Systemic systems are intermittent low-grade fever, weight loss and early fatigued. Pathologic fracture of bone may be demonstrated in 10% of cases. Physical examination reveals localized tenderness associated with fullness or even mass in the bone. Clinically Ewing sarcoma simulates osteomyelitis. Laboratory findings are nonspecific, which include leukocytosis, increased erythrocyte sedimentation rate and anemia.

Ancillary Laboratory Tests

Ancillary laboratory tests performed in Ewing sarcoma are conventional cytogenetic analysis, FISH (fluorescence *in situ* hybridization) and PCR (polymerase chain reaction) are given in Table 27.34.

Radiologic Findings

- **Conventional radiograph:** Ewing sarcoma has no definite radiologic appearance. Tumor is most often radiolucent and ill-defined in the metaphysis or diaphysis of long bones giving 'moth-eaten' appearance. Degree of periosteal reaction may be disproportionate to the amount of bone destruction giving 'onion skin' appearance on radiograph. The outer cortex of bone may be identified from outside, even when the mass originates from soft tissue.
- **CT scan:** It may demonstrate cortical destruction by Ewing sarcoma.

Table 27.33 Diagnostic criteria of Ewing sarcoma/primitive neuroectodermal tumor (PNET)

Diagnostic Criteria (≥2 Major Features of Neural Differentiation)	Comments
Light microscopy	Neural differentiation (Homer-Wright rosettes)
Electron microscopy	Neural differentiation
Immunohistochemistry	Expression of neural differentiation markers, i.e. NSE (neuron-specific enolase), synaptophysin and chromogranin
MIC2 expression	Present
Vimentin expression	Present

Table 27.34 Ancillary laboratory tests performed in Ewing sarcoma

Ewing Sarcoma/PNET: Ancillary Laboratory Tests
Conventional cytogenetic analysis: It is performed to demonstrate reciprocal chromosomal translocation between chromosomes 11 and 22 (85%), chromosomes 21 and 22 (10–15%), chromosomes 7 and 22, chromosomes 17 and 22, chromosomes 2 and 22.
FISH (fluorescence *in situ* hybridization): FISH is performed to diagnose Ewing sarcoma.
PCR (polymerase chain reaction): PCR is performed to analyze **EWSR1/FLT1** and **EWSR1/ERG fusion genes**.

- **MRI scan:** It demonstrates extraosseous soft mass (Fig. 27.26).
- **Radionuclide scanning:** It demonstrates intramedullary spread better than on conventional radiograph.

Surgical Pathology: Ewing Sarcoma

Gross Morphology

- Ewing sarcoma is grayish yellow with soft consistency. Necrotic yellowish and semi-fluid material obtained from intramedullary or subperiosteal lesion on open biopsy erroneously interpreted as pus by the surgeons.
- Gross morphology demonstrates permeative destruction prior to chemotherapy.
- In majority of cases, overlying soft tissue tumor mass is connected to the intraosseous tumor on light microscopy; even though its gross continuity is not always obvious. Gross morphology of Ewing sarcoma is shown in Fig. 27.27.

Light Microscopy

- On histologic examination, Ewing sarcoma gives monotonous appearance on low magnification.
- Tumor is composed of uniform monotonous small round neoplastic cells with poorly defined cell borders. Neoplastic cells contain round dark nuclei, fine chromatin and nucleoli; and surrounded by scant clear to amphophilic cytoplasm.
- A few tumors are composed of large neoplastic cells containing prominent nuclei and increased mitotic activity. Scant mitotic activity is demonstrated. There is no extracellular matrix in the tumor (Fig. 27.28A).
- Aggregates of neoplastic cells may be arranged along delicate fibrous septae or around blood vessels forming 'Homer-Wright rosettes'.
- 'Necrosis' is a regular feature of Ewing sarcoma even in the absence of prior chemotherapy administered to the patient.

Histochemistry

'Neoplastic' cells of Ewing sarcoma contain glycogen in the cytoplasm demonstrated by PAS/diastase sensitive stain.

Differential Diagnosis

Ewing sarcoma has morphologic resemblance to various lesions. It should be differentiated from non-Hodgkin lymphoma, leukemia, osteomyelitis, metastatic neuroblastoma, small cell osteosarcoma, mesenchymal chondrosarcoma and embryonal sarcoma; by histologic examination, histochemistry, immunohistochemistry, conventional cytogenetic analysis, fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR) techniques for analysis of fusion protein.

Immunohistochemistry Technique

Ewing sarcoma shows positivity for panel of markers, which include MIC2, CD99, FLT1, vimentin, cytokeratins and neural differentiation markers, e.g. NSE (neuron-specific enolase), synaptophysin and neuron triplet filament protein. Ewing sarcoma shows positivity for CD99 and FLI-1 immunohistochemical markers (Fig. 27.28B and C).

Markers	Expression
MIC2 (specific marker)	Positive
CD99 (O-13, HBA-74)	Positive (membranous staining)
FLI-1 (sensitive but not specific)	Positive
Vimentin	Positive
CK (cytokeratins low molecular weight)	Positive
NSE (neuron-specific enolase), neural differentiation marker	Positive
Synaptophysin (neural differentiation marker)	Positive
Neuron triplet filament protein (neural differentiation marker)	Positive

Molecular Genetic Alterations

Specific molecular genetic aberrations in Ewing sarcoma/PNET and Ewing sarcoma-like tumors without ETS involvement are given in Table 27.35.

- About 85% of Ewing sarcomas harbor a somatic reciprocal chromosomal translocation between chromosomes 11 and 22 resulting in fusion of another amino terminus of the EWRS1 gene with FLT1 gene, leads to generation of EWRS1-FLT1 fusion protein that functions as an aberrant transcription factor required for cellular proliferation.
- In 10–15% of Ewing sarcomas harbor a somatic reciprocal chromosomal translocation between chromosomes 21 and 22. It leads to fusion of EWRS1 with members of ETS family resulting in generation of oncoprotein that function as an aberrant transcription factor required for unrestricted cellular proliferation.
- The remaining Ewing sarcomas show harbor a somatic reciprocal chromosomal translocation between chromosomes 7 and 22, chromosomes 17 and 22, chromosomes 2 and 22 and inversion of chromosome 22. The resulting chimeric proteins function as abnormal transcription factors required for unrestricted cellular proliferation.

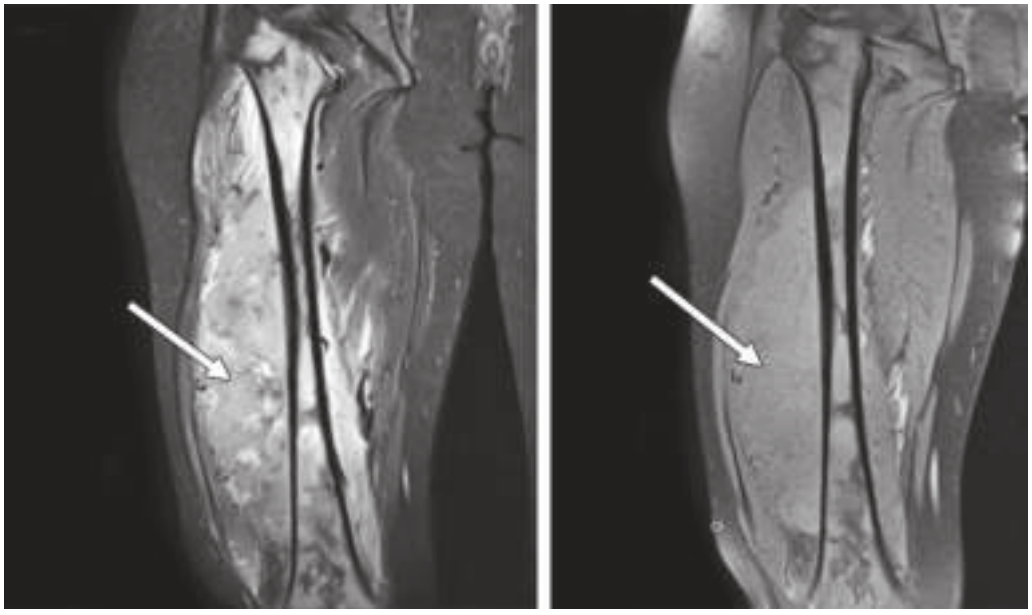


Fig. 27.26: Ewing sarcoma in the diaphysis of femur in a child. Post-contrast enhancement on magnetic resonance imaging demonstrates a heterogenous large mass lesion around the femoral diaphysis with few areas of calcification. The tumor is predominantly involving anterior and anterolateral compartment of thigh and smoothly elevating the vastus muscles. Possibility of round cell tumor likely Ewing sarcoma needs consideration. Other differential diagnosis includes lymphoproliferative disorder shown by arrows. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

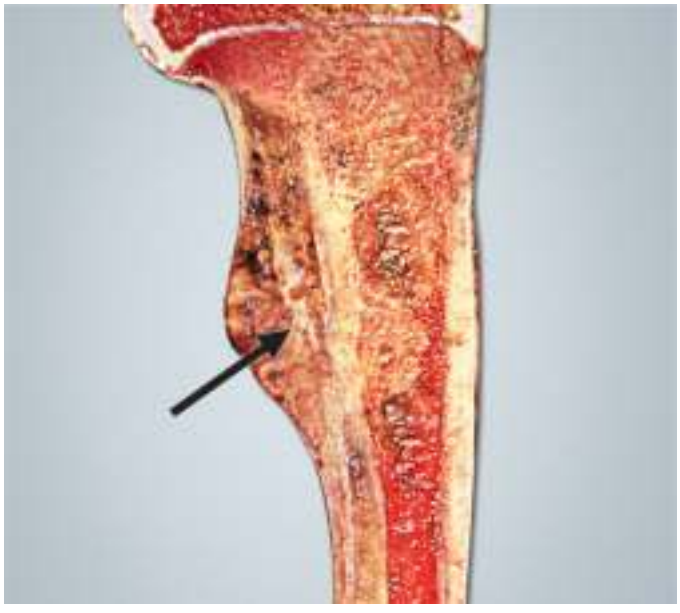


Fig. 27.27: Ewing sarcoma. A large tumor in the upper diaphysis of tibia has eroded the cortical bone and extended and duplicated beneath the periosteum having an onion-skin configuration. Tumor is grayish yellow necrotic with soft consistency (arrow).

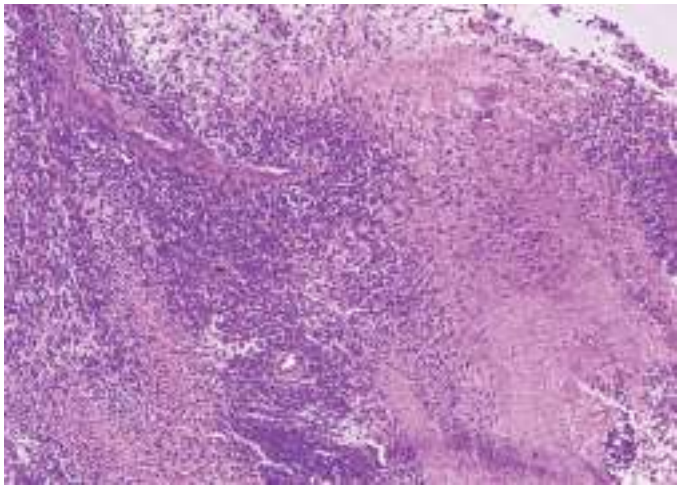


Fig. 27.28A: Ewing sarcoma. Tumor is composed of uniform monotonous small round neoplastic cells with poorly defined cell borders. Neoplastic cells contain round dark nuclei, fine chromatin and nucleoli; and surrounded by scant clear to amphophilic cytoplasm. Scant mitotic activity is demonstrated. There is no extracellular matrix in the tumor. Tumor cells forming Homer-Wright rosettes are also demonstrated in some cases. Cytoplasm of tumor cells is rich in glycogen demonstrated by PAS staining (100X).

Prognosis and Treatment

- Patients who present with metastatic disease have survival rates of about 20%. Ewing sarcoma follows an extremely adverse clinical course with early metastasis to lungs, liver, brain and other bones (multifocal lesions). Overall survival rate depends on anatomic location, size of tumor, and stage. Large size tumor arising in pelvis and spine, pathologic fracture and distant metastasis are poor prognostic factors and associated with poor prognosis.
- It has been reported that patients with Ewing sarcoma have most common type of fusion transcript (EWS/

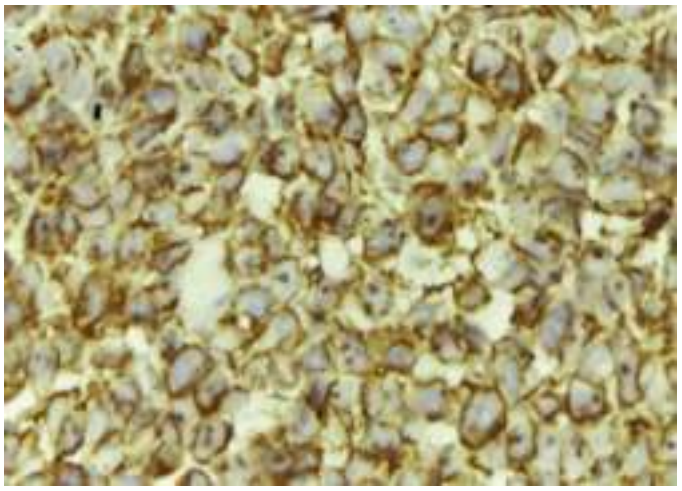


Fig. 27.28B: CD99 positivity in Ewing sarcoma. Certain types of soft tissue tumor are positive for CD99. CD99 is a membranous protein that is expressed in most cases of Ewing sarcoma (EWS), synovial sarcoma (SS) and low-grade fibromyxoid sarcoma.

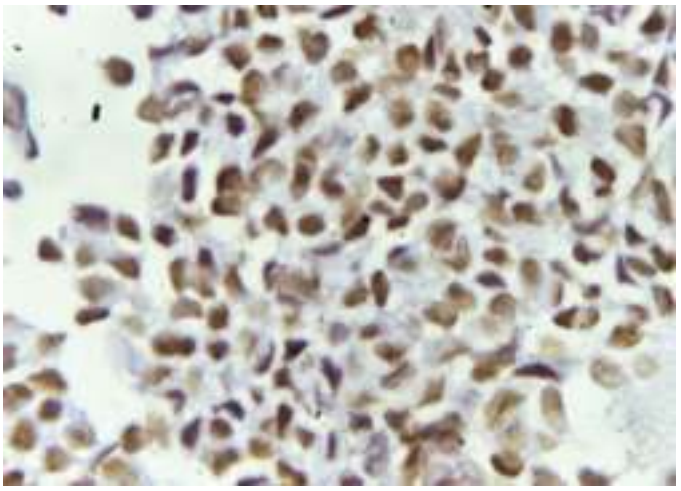


Fig. 27.28C: FLI-1 positivity in Ewing sarcoma. About 90% of Ewing sarcoma/primitive neuroectodermal tumors (PNET) have t(11;22) (q24;q12), which results in fusion of EWS to FLI-1.

Table 27.35 Specific genetic aberrations in Ewing sarcoma/PNET and Ewing sarcoma-like tumors without ETS involvement

Neoplasms	Chromosomal Translocation	Involved Genes and Fusion Protein	Frequency
Ewing sarcoma/ PNET	▪ t(11;22) (q24;q12) ▪ t(21;22) (q22;q12) ▪ t(7;22) (q22;q12) ▪ t(17;22) (q12;q12) ▪ t(2;22) (q33;q12) ▪ t(16;21) (p11;q22) ▪ t(2;16) (q35;p11)	▪ EWSR1-FLT1 ▪ EWSR1-ERG ▪ EWSR1-ETV1 ▪ EWSR1-ETV4 ▪ EWSR1-FEV ▪ FUS-ERG ▪ FUS-FEV	▪ 85–90% ▪ 10–15% ▪ <1% ▪ <1% ▪ <1% ▪ Rare ▪ Rare
Ewing sarcoma-like tumors without ETS involvement	▪ t(6;22) (p21;q12) ▪ Inv(22) (p12) ▪ t(4;22) (q31;q12) ▪ t(2;22) (q31;12) ▪ t(1;22) (q36.1;q12) ▪ Complex ring chromosome with amplification of the translocated segments ▪ t(4;19) (q35;q13) ▪ Inversion involving Xp11.4;Xp11.22	▪ EWSR1-POU5F1 ▪ EWSR1-ZSG (ZNF278) ▪ EWSR1-SMARCCA ▪ EWSR1-SP3 ▪ EWSR1-ZNF278 ▪ EWSR1-NFATc2 ▪ CIC-DUX4 ▪ BCOR-CCNB3	▪ Rare ▪ Rare ▪ Rare ▪ Rare ▪ Rare ▪ Rare ▪ Rare ▪ Rare

Ewing sarcoma/PNET and Ewing sarcoma-like tumors without ETS involvement are treated in a similar fashion.

FLI1) associated with better prognosis than those with EWS/ERG and other less common fusion transcripts. Recent studies revealed a strong positive correlation between telomerase activity and negative outcome in patients with nonmetastatic Ewing sarcoma tumors.

- Patient responds well to surgical excision of tumor, chemotherapy and radiotherapy. Five-year survival rate is demonstrated in 65–75% of cases. Patient receiving chemotherapy and local radiation has increased risk for osteosarcoma.

ADAMANTINOMA

Adamantinoma is a low-grade epithelial neoplasm involving predominantly tibia with or without involvement of fibula. Characteristics of classic and differentiated types of adamantinoma are given in Table 27.36.

- **Incidence and Locations:** Adamantinoma accounts for <0.5% of all primary malignant bone tumors. Most common sites are tibia (85%), and both tibia and fibula (10%). Less common sites are ulna, femur,

Table 27.36 Characteristics of classic and differentiated types of adamantinoma

Feature	Classic Adamantinoma	Differentiated Adamantinoma
Age distribution	Most often after 20 years	Most often below 20 years
Location	▪ Tibia ▪ Fibula	▪ Tibia ▪ Fibula
Radiologic findings	Lesion expands beyond periosteum	Intracortical lesion
Histology	Neoplastic cells are forming basaloid, spindled, tubular, or squamous pattern	Predominance of osteofibrous dysplasia pattern
Immunohistochemistry	Neoplastic cells show positivity for cytokeratin	Scattered neoplastic cells show positivity for cytokeratin

Local recurrence occurs 90% of cases in adamantinoma and lung metastasis in some cases.

humerus, radius, ribs, ischium, tarsal, metatarsal, and capitate.

- **Age and sex predilection:** Tumor most often occurs during 20–35 years of age with slight male predominance.
- **Clinical features:** Patient presents with slow-growing swelling with or without pain in the involved bone. Pathologic fracture occurs in some cases.
- **Radiologic findings:** Imaging techniques play important role in diagnosis, assessment of extent and invasiveness of adamantinoma.
  - Radiograph shows osteolytic, eccentric and expansile lesion in the medullary region of bone giving 'soap bubble appearance'. Sclerotic margin of lesion is indicative of slow growth of tumor. Longitudinal oriented average length of tumor is 11 cm. The expansile element of tumor tends to involve the anterior tibial surface, and the tumor may produce a bowing deformity of the tibia.
  - CT scan and MRI are useful in assessing extent and invasiveness of tumor. Extracortical extension of adamantinoma into the soft tissue is demonstrated in 15% of cases.

Surgical Pathology: Adamantinoma of Bone

Gross Morphology

Tumor is well-demarcated with peripheral lobulated contours. Eccentric tumor involves both cortex and medulla.

Light Microscopy

- **Squamous cell variant of adamantinoma:** Tumor resembles a well-differentiated squamous cell carcinoma and may exhibit keratinization or cell separation may result in formation of the stellate reticulum pattern. Tumor is composed of nests of epithelial cells within fibrous or fibro-osseous (woven or lamellar bone) stroma.
  - The epithelial cells are arranged in tubular, basaloid, squamous with stellate reticulum or spindle cell patterns.
  - Epithelial cells consist of peripheral tall columnar basal cells showing cytoplasmic vacuolation with reverse polarization of nuclei. Squamous metaplasia may be demonstrated.
- **Tubular variant of adamantinoma:** The most frequent is the tubular variant of adamantinoma in which narrow cords of epithelial cells form branching and anastomosing tubular structures. A central gland-like space produces tubular appearance due to cellular cohesion rather than true gland formation.
- **Basaloid variant of adamantinoma:** Tumor resembles basal cell carcinoma with palisading of its peripheral layer.
- **Spindle cell variant of adamantinoma:** Tumor is composed of spindle-shaped neoplastic epithelial cells, which may produce patterns resembling those of mesenchymal neoplasms such as storiform, fascicular and herring bone.
- **Ewing-like adamantinoma:** Tumor is composed of anastomosing cords of small round cells set in myxoid stroma.

Differential Diagnosis

Adamantinoma should be differentiated from oral-facial-digital syndrome, dedifferentiated adamantinoma, synovial sarcoma, epithelial hemangioendothelioma, and metastatic carcinoma to bone.

Immunohistochemistry Technique

Epithelial cells of tumor show positivity for pan cytokeratins. These cells exhibit a more specific subset profile, i.e. CK14 and CK19; CK5 and CK17.

Markers	Expression
▪ CK14 and CK19 ▪ CK5 and CK17	▪ Positive ▪ Positive

- **Prognosis and treatment:** Patient is treated by wide, local, limb-sparing resection of tumor. Radiotherapy and chemotherapy are ineffective. Local recurrence is reported in 90% of cases. It may metastasize to lung. Patient may have fatal outcome after surgical excision.

OSTEOCLASTIC GIANT CELL-RICH BONE TUMORS AND CYSTIC LESION

Osteoclastic giant cell-rich tumors according to recent 2020 WHO classification of bone tumors are given in Table 27.37.

Table 27.37 Osteoclastic giant cell-rich tumors according to recent 2020 WHO classification of bone tumors

Osteoclastic Giant Cell-rich Tumors
▪ Benign tumors • Giant cell tumor of the small bones
▪ Intermediate tumors (locally aggressive, rarely metastasizing) • Giant cell tumor of bone
▪ Giant cell tumor, malignant

GIANT CELL TUMOR OF BONE (OSTEOCLASTOMA)

Giant cell tumor of bone is potentially low-grade locally aggressive neoplasm, which is derived from monocyte-macrophage lineage. Tumor is composed of sheets of ovoid or spindled mononuclear cells interspersed with numerous osteoclast-like multinucleated giant cells.

Incidence and Locations

Giant cell tumor of bone accounts for 5% of all bone tumors, which originates from epiphysis or metaphysis of distal femur, proximal tibia and distal radius bones. Less common sites are sacrum and vertebral bodies. Tumor expands into the metaphysis of long bones resulting in erosion of the bone cortex. Tumor is confined proximally by growth plate, hence limited to metaphysis of the bone. As the tumor expands, it produces a bright rim of overlying reactive bone formation.

Age and Sex Predilection

Tumor occurs in mature skeleton mainly young adults during third to fourth decades of life with slight female predominance.

Clinical Features

Patient with giant cell tumor of bone presents with pain in the involved bone as a result of expansion of tumor near a joint. The weakened bone may undergo pathologic fracture. Clinical examination reveals tender swelling in the involved bone.

Radiologic Findings

- Radiograph shows osteolytic eccentric lesion with either well-defined without sclerotic margins or poorly differentiated margins giving '**soap bubble**' appearance.
- Aggressive giant cell tumor of bone may show cortical destruction and occasionally periosteal reaction.
- Soft tissue extension or recurrence of tumor shows marginal calcification giving 'egg-shell' appearance (Fig. 27.29).



Fig. 27.29: Giant cell tumor of femur in 56-year-old patient. Radiograph demonstrates a well-defined osteolytic lesion with narrow zone of transition with no sclerotic margins in lateral portion of lower femoral metaphysis extending into lateral femoral condyle. No periosteal reaction is noted. Findings are suggestive of giant cell tumor of femur shown by arrow. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

Surgical Pathology: Giant Cell Tumor of Bone

Gross Morphology

- Giant cell tumor of bone is large red brown, fleshy well-demarcated mass. Cut surface shows yellow and/or cystic areas.
- Areas of hemorrhage and necrosis are common. Hemosiderin deposition and reactive bone formation are demonstrated in the tumor. Gross morphology of giant cell tumor is shown in Fig. 27.30.

Light Microscopy

- Tumor is composed of ovoid mononuclear cells with indistinct cellular outlines growing in the form syncytium; interspersed with numerous osteoclast-like multinucleated giant cells containing >100 nuclei scattered among mononuclear cells. Mitotic figures are common in mononuclear cells.
- Morphologic features of nuclei of stromal mononuclear cells and osteoclast-like multinucleated giant cells are identical. There may be presence of aggregates of foam cells, focal osteoid or bone production, aneurysmal bone cyst like changes, storiform areas and necrosis (Fig. 27.31).

Differential Diagnosis

Giant cell tumor of bone should be differentiated from other bone tumors and tumor-like lesions showing multinucleated giant cells, which include aneurysmal bone cyst, chondroblastoma, benign fibrous histiocytoma, chondromyxoid fibroma, osteoblastoma, metaphyseal fibrous defect (nonossifying fibroma), brown tumor of hyperparathyroidism, malignant fibrous histiocytoma, fibrosarcoma of bone and giant cell variant of osteosarcoma. Bone tumors and tumor-like lesions show multinucleated giant cells are given in Table 27.38.

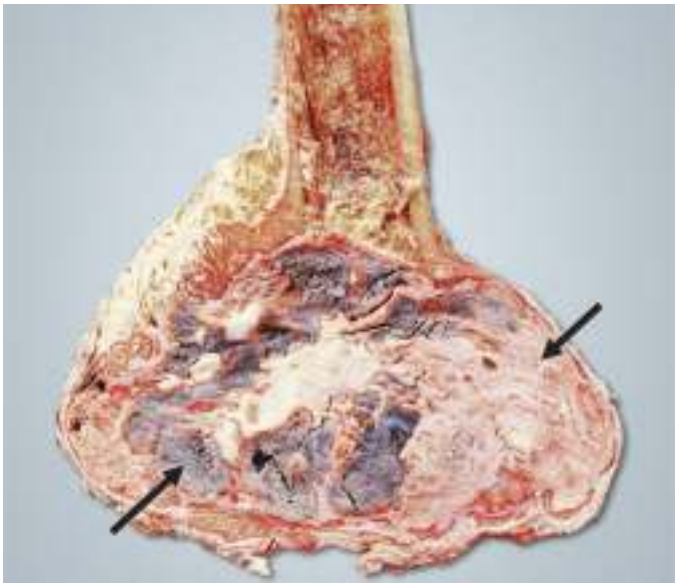


Fig. 27.30: Giant cell tumor of bone. Tumor of bone is large red brown, fleshy well-demarcated mass. Cut surface shows yellow and/or cystic areas. Areas of hemorrhage and necrosis are common. Hemosiderin deposition and reactive bone formation are demonstrated in the tumor (arrows). (*Courtesy:* Dr. Sujata Kanetkar, Professor and Head, Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra).

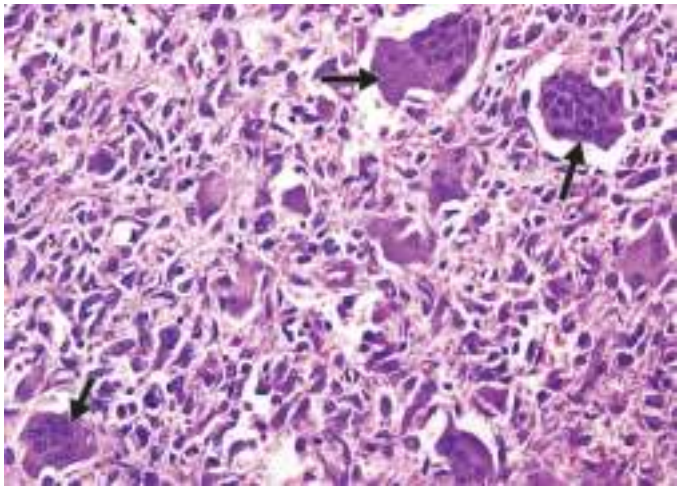


Fig. 27.31: Giant cell tumor of bone. Tumor is composed of ovoid mononuclear cells with indistinct cellular outlines growing in the form syncytium; interspersed with numerous osteoclast-like multinucleated giant cells containing >100 nuclei scattered among mononuclear cells. Mitotic figures are common in mononuclear cells. Morphological features of nuclei of stromal mononuclear cells and osteoclast-like multinucleated giant cells are identical. There may be presence of aggregates of foam cells, focal osteoid or bone production, aneurysmal bone cyst like changes, storiform areas and necrosis (400X).

Prognosis and Treatment

- Giant cell tumor of bone (osteoclastoma) is treated by surgical excision or curettage. Radiotherapy is advised inaccessible lesions. Recurrence most often occurs within two years of surgical intervention.

Table 27.38 Bone tumors and tumor-like lesions show multinucleated giant cells

Benign Bone Tumors and Tumor-like Lesions
Giant cell tumor of bone (osteoclastoma)
Aneurysmal bone cyst
Chondroblastoma
Benign fibrous histiocytoma
Chondromyxoid fibroma
Osteoblastoma
Metaphyseal fibrous defect (nonossifying fibroma)
Brown tumor of hyperparathyroidism
Malignant Bone Tumors
Malignant fibrous histiocytoma
Fibrosarcoma of bone
Giant cell variant of osteosarcoma

- Histologic examination does not predict the aggressiveness of the neoplasm. Lung metastasis is demonstrated in 2% of cases. Histologic features of giant cell tumor of bone are identical at primary site and metastatic site.
- Malignant transformation of giant cell tumor of bone may occur in 2% of cases. Prognosis is similar in malignant change in giant cell bone tumor and high-grade spindle cell sarcoma.

ANEURYSMAL BONE CYST

Aneurysmal bone cyst (ABC) is a benign locally destructive non-neoplastic multiloculated blood filled cystic lesion. Aneurysmal bone cyst originates from metaphysis of long bone (femur, tibia), vertebral column and flat bones. ABC may arise in *de novo* (primary) or secondary in previously existing lesions. Aneurysmal bone cyst accounts for 2% of bone tumors. Precursor lesions for secondary aneurysmal cyst formation are given in Table 27.39.

Age and Sex Predilection

Aneurysmal bone cyst most often occurs during 5–20 years of age with equal sex distribution.

Clinical Features

Patient presents with painful swelling in the involved bone. Aneurysmal bone cyst in vertebral column may cause spinal cord compression.

Radiologic Findings

- Radiograph shows eccentric osteolytic lesion in the metaphyseal region of femur or tibia, balloon distension with cortical destruction exhibiting 'soap bubble appearance'. Periosteal eggshell appearance of bone is demonstrated.

Table 27.39 Precursor lesions for secondary aneurysmal cyst formation

Giant cell tumor of bone
Giant cell reparative granuloma
Osteoblastoma
Osteosarcoma
Chondroblastoma
Chondromyxoid fibroma
Chondrosarcoma
Nonossifying fibroma
Fibrous dysplasia
Eosinophilic granuloma
Hemangioma
Myositis ossificans
Malignant fibrous histiocytoma
Metastatic carcinoma
Hamartoma (rich vascular network)

- CT scan and MRI demonstrate multiloculated lesion in the bone often filled with fluid levels. Vertebral collapse is demonstrated in case of lesion in the vertebral column.

Surgical Pathology: Aneurysmal Bone Cyst

Gross Morphology

- Curettage fragments of aneurysmal bone cyst are red brown and soft in consistency.
- Resected specimen shows well demarcated lesion with blood filled cystic spaces of variable size and spongy in consistency. Well-defined margins with peripheral shell of bone are demonstrated. Gross morphology of aneurysmal bone cyst is shown in Fig. 27.32.

Light Microscopy

- Aneurysmal bone cyst is composed of variable sized blood spaces lined by thin membranous septae of loose fibrous tissue admixed with numerous osteoclast-like giant cells.
- Reactive bone trabeculae are lined by osteoblasts. Immature osteoid is arranged in a linear manner along septae. Irregular immature chondroid areas and calcification are demonstrated. There is no evidence of cytologic pleomorphism and cellular atypia.
- Solid areas with xanthogranulomatous appearance admixed with reactive bone trabeculae are also demonstrated (Fig. 27.33).

Differential Diagnosis

Aneurysmal bone cyst should be differentiated from telangiectatic osteosarcoma, giant cell tumor of bone, chondroblastoma, osteoblastoma, simple bone cyst and giant cell reparative granuloma.



Fig. 27.32: Aneurysmal bone cyst. Surgically resected specimen shows well-demarcated lesion with blood filled cystic spaces of variable size and spongy in consistency. Well-defined margins with peripheral shell of bone in aneurysmal bone cyst are demonstrated (arrows).

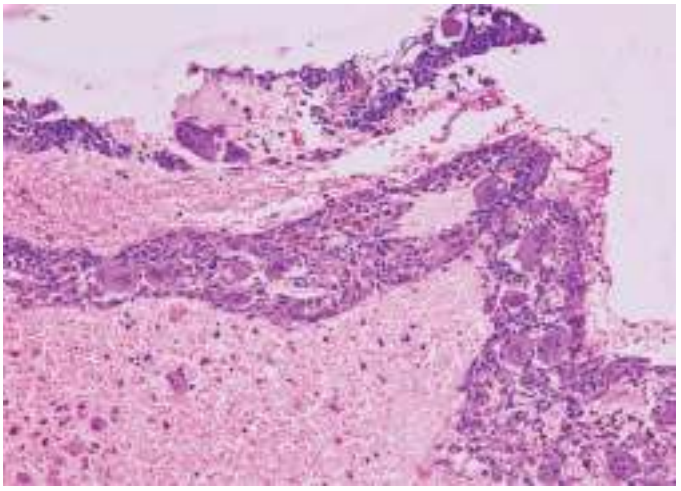


Fig. 27.33: Aneurysmal bone cyst. It is composed of variable sized blood spaces lined by thin membranous septae of loose fibrous tissue admixed with nemerous osteoclast-like giant cells. Reactive bone trabeculae are lined by osteoblasts. Immature osteoid is arranged in a linear manner along septae. Irregular immature chondroid areas and calcification are demonstrated. There is no evidence of cytologic pleomorphism and atypia. Solid areas with xanthogranulomatous appearance admixed with reactive bone trabeculae are also demonstrated (400X).

Prognosis and Treatment

Aneurysmal bone cyst is treated curettage or surgical resection. Bone grafting with preoperative embolization and or cryotherapy. Radiotherapy is advised for unresectable lesions. Prognosis is good in 90% of post-operative cases. Recurrence may occur after surgery in 20–70% of cases. Spontaneous malignant transformation is rare especially after post-radiation.

Table 27.40 Notochordal tumors classification according to recent 2020 World Health Organization classification of bone tumors

Notochordal Tumor: Histologic Variant	Behavior	Additional Comments
Conventional chordoma	Low malignant tumor	▪ Arising from sacrococcygeal region (35–50%), spheno-occipital region in the base of skull (30–40%), and vertebrae of mobile spine (20–30%) ▪ Slight risk for malignant transformation
Chondroid chordoma (conventional chordoma with chondroid component)	Malignant tumor	▪ Sporadic or familial tumor rising from base of skull ▪ Histologically closely resembles chondrosarcoma ▪ Immunohistochemical epithelial marker positive. Brachyury is a new marker for chondroid chordoma
Poorly differentiated chordoma	Malignant tumor	▪ Arising from base of skull in children and adolescents ▪ Highly cellular tumor with poor prognosis
Dedifferentiated chordoma	Malignant tumor	Some cases are associated with postradiotherapy

NOTOCHORDAL TUMORS

Notochordal tumors are classified according to old 2013 World Health Organization into four histologic variants: (a) conventional chordoma, (b) chondroid chordoma (conventional chordoma with chondroid component), (c) poorly differentiated chordoma, and (d) dedifferentiated chordoma. Notochordal tumors classification according to recent 2020 World Health Organization classification of bone tumors is given in Table 27.40.

CONVENTIONAL CHORDOMA

Notochord is a midline ectoderm derived structure in human beings. Degeneration of notochord begins during the development and completes by the second decade of life. Notochord remnants in adults are represented by nucleus pulposus in the intervertebral discs. Chordoma represents a low malignant mesenchymal neoplasm with distinct clinical and pathologic features and histologic similarities to notochord.

Incidence and Locations

Conventional chordoma accounts for 4% of malignant bone tumors. Various sites of conventional chordoma are sacrococcygeal region (35–50%), spheno-occipital region in the base of skull (30–40%), and vertebrae of mobile spine (20–30%).

- Nasopharyngeal chordoma arises from ectopic residues of chorda dorsalis.
- Extraosseous growth develops in the soft tissue around sacrum completely independent of origin from bone. But it is difficult to be sure that chordoma in either situation does not have a long filament osseous attachment. Locations and frequency of conventional chordoma in various age groups are given in Table 27.41.

Table 27.41 Locations and frequency of conventional chordoma in various age groups

Locations of Conventional Chordoma	Frequency	Age Group
Sacrococcygeal chordoma	30–50%	40–60 years
Spheno-occipital region in base of skull	30–40%	40–60 years
Vertebrae of mobile spine	20–30%	20–40 years

Age and Sex Predilection

Conventional chordoma occurs in infants and older persons. It most often affects 40–60 years of age group in sacrococcygeal and spheno-occipital region. It occurs in vertebrae of mobile spine in 20–40 years of age group.

Clinical Features

Patient with sacrococcygeal chordoma presents with low pain in the back region.

- Cranial chordoma presents with headache, visual disturbances (bilateral hemianopia) and symptoms related to multiple cranial palsies.
- Chordoma located in nasal cavity, paranasal sinuses and nasopharyngeal region presents respiratory difficulty.

Radiologic Findings

Radiograph shows osteolytic lesion. Rarely osteoblastic lesion is demonstrated.

Surgical Pathology: Conventional Chordoma

Gross Morphology

- Conventional chordoma has a characteristic lobulated structure. The lobules are separated by thin whitish septae.
- Cut surface of tumor is shiny, translucent and blue gray. Gross resection of tumor shows soft tissue extension with pushing border (Fig. 27.34).

Light Microscopy

- At low magnification, conventional chordoma demonstrates lobular growth pattern separated by thin collagenous septae. Majority of tumor volume is composed of abundant basophilic myxoid stroma.
- Tumor is composed of round to ovoid or polygonal neoplastic cells arranged in strands, cords and clusters. Tumor cells contain pale eosinophilic cytoplasm and central round nuclei.
- Numerous tumor cells contain one or several intracytoplasmic vacuoles creating bubbly appearance and small and central nuclei known as 'physaliphorous neoplastic cells'.
- Occasional tumor is composed of pleomorphic neoplastic cells with hyperchromatic nuclei, but retaining nuclear cytoplasmic ratio, which does not predict a worse prognosis.
- Areas of necrosis are demonstrated in 35% of cases. Mitotic activity is rare (Fig. 27.35).

Differential Diagnosis

Conventional chordoma should be differentiated from metastatic adenocarcinoma, giant notochordal rest, chondrosarcoma and extraskeletal myxoid chondrosarcoma.

Immunohistochemistry Technique

Conventional chordoma is positive for CK8, CK18, CK19, epithelial membrane antigen (EMA) and S-100 protein.

Markers	Expression
▪ CK8	▪ Positive
▪ CK18	▪ Positive
▪ CK19	▪ Positive
▪ EMA	▪ Positive
▪ S-100 protein	▪ Positive

Brachyury is a new marker for chondroid chordoma.

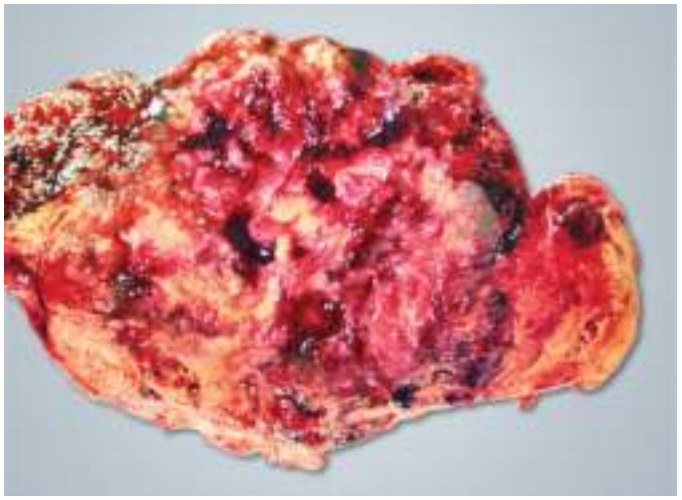


Fig. 27.34: Conventional chordoma. Tumor has a characteristic lobulated structure. The lobules are separated by thin whitish septae. Cut surface of tumor is shiny, translucent and blue gray. Gross resection of tumor shows soft tissue extension with pushing border.

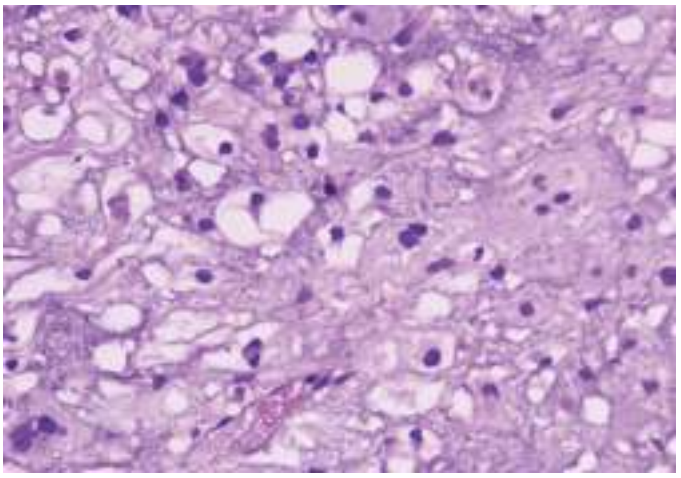


Fig. 27.35: Conventional chordoma. Tumor is composed of polygonal large cells with central round nuclei and abundant clear to eosinophilic cytoplasm that have a bubbly/vacuolated appearance (physaliphorous cells) and abundant vacuolated cytoplasm in myxoid stroma (400X).

Prognosis and Treatment

Conventional chordoma is treated by complete surgical resection with wide tumor-free margins. Cranial chordoma is treated by adjuvant radiation therapy followed by surgery. Metastases are demonstrated in ≤30% of cases. Morbidity and mortality occur due to destructive recurrence of conventional chordoma.

FIBROGENIC BONE TUMORS

Fibrogenic bone tumors are uncommon, which include desmoplastic fibroma and fibrosarcoma. Fibrogenic tumors classification according to recent 2020 WHO classification of bone tumors is given in Table 27.42.

DESMOPLASTIC FIBROMA OF BONE

Desmoplastic fibroma of bone is extremely rare benign locally aggressive intraosseous tumor due to proliferation of fibroblasts. This lesion is equivalent to soft tissue fibromatosis, which does not metastasize. But it may recur following incomplete surgical excision.

Incidence and Locations

Most common sites of tumor are distal femur, proximal tibia and pelvis. The other site is mandible.

Table 27.42 Fibrogenic tumors classification according to recent 2020 WHO classification of bone tumors

Fibrogenic Tumors
▪ Intermediate tumor (locally aggressive) • Desmoplastic fibroma of bone
▪ Malignant tumors • Fibrosarcoma of bone

Age and Sex Predilection

Tumor most often affects children and adolescents during 15–25 years of age. However, it may affect any age group between 20 months and 60 years of age.

Clinical Features

Patient presents with pain in the involved bone over years.

Radiologic Findings

Radiograph shows osteolytic lesion with multicystic pattern and destruction of cortex.

Surgical Pathology: Desmoplastic Fibroma of Bone	
Gross Morphology	
Desmoplastic fibroma of bone is gray and shining lesion measuring 3–10 cm in size.	
Light Microscopy	
▪ Histology of desmoplastic fibroma of bone is identical to soft tissue fibromatosis. ▪ Tumor is composed of spindle-shaped fibroblasts in a collagenized extracellular matrix. ▪ Spindle-shaped fibroblasts have bland oval or elongated nuclei usually without nucleoli. ▪ Proliferation of fibroblasts exhibits infiltrative growth pattern often entrapping native bone trabeculae.	
Differential Diagnosis	
Desmoplastic fibroma of bone should be differentiated from low-grade fibrosarcoma, fibrous dysplasia and nonossifying fibroma.	
Immunohistochemistry Technique	
Desmoplastic fibroma of bone shows positivity for vimentin.	
Marker	**Expression**
Vimentin	Positive

Prognosis and Treatment

Desmoplastic fibroma of bone is treated by surgical resection with tumor-free margin. Curettage of tumor may recur in 50% of cases.

FIBROSARCOMA OF BONE

Fibrosarcoma of bone is a rare spindle cell neoplasm of low to intermediate grade malignant tumor.

Incidence and Locations

Most common sites of fibrosarcoma of bone are distal femur and proximal tibia.

Age and Sex Predilection

Fibrosarcoma of bone most often affects adults. Peak incidence is seen in the fifth decade of life. It has also been reported in infants.

Clinical Features

Patient presents with pain in involved bone, which may produce pathologic fracture.

Radiologic Findings

Radiograph shows a poorly defined osteolytic lesion (permeative and destructive).

Surgical Pathology: Fibrosarcoma of Bone	
Gross Morphology	
Tumor is white soft tissue mass with firm consistency. It demonstrates replacement of cancellous and cortical bone.	
Light Microscopy	
▪ Tumor is composed of spindle cell arranged in a fascicular or 'herringbone' pattern. It also contains varying amount of collagen. ▪ Low-grade fibrosarcoma of bone is composed of uniform spindle cells with mild to moderate atypia. ▪ High-grade fibrosarcoma of bone is a highly cellular neoplasm composed of spindle cells with cytologic atypia and numerous mitotic figures (Fig. 27.36).	
Differential Diagnosis	
Fibrosarcoma of bone should be differentiated from desmoplastic fibroma of bone and malignant fibrous histiocytoma.	
Immunohistochemistry Technique	
Tumor shows positivity for vimentin. A few neoplastic cells may be weakly positive for smooth muscle actin.	
Markers	**Expression**
▪ Vimentin ▪ Smooth muscle actin	▪ Positive ▪ Positive (few neoplastic cells)

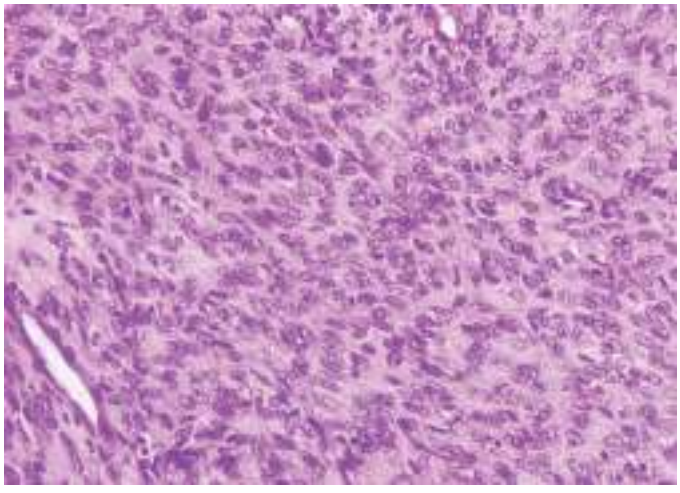


Fig. 27.36. Fibrosarcoma of bone. Tumor is composed of spindle cell with cytologic atypia and numerous mitotic figures arranged in a fascicular or 'herringbone' pattern. It also contains varying amount of collagen (400X).

Prognosis and Treatment

Fibrosarcoma of bone is treated by surgical ablation with disease-free margins. Low-grade fibrosarcoma of bone has a 10-year survival rate in 80% of cases. High-grade fibrosarcoma of bone has a lower survival rate.

FIBROHISTIOCYTIC TUMORS OF BONE

Fibrohistiocytic tumors of the bone include nonossifying fibroma (metaphyseal fibrous defect) of long bones, benign fibrous histiocytoma of flat bones and vertebrae, and malignant fibrous histiocytoma. The term benign fibrous histiocytoma of bone is only applied, when lesion affects flat bones and vertebrae. Tumor is composed of spindle cells arranged in storiform pattern, foam cells and multinucleated giant cells.

Fibrohistiocytic tumors classification according to revised 2020 WHO classification of bone tumors is given in Table 27.43.

BENIGN FIBROUS HISTIOCYTOMA/NONOSSIFYING FIBROMA (METAPHYSEAL FIBROUS DEFECT)

Benign fibrous histiocytoma/nonossifying fibroma (metaphyseal fibrous defect) of bone is a spindle cell neoplasm arising from the metaphyseal region of long bones (distal femur or proximal tibia, fibula, radius).

- Tumor occurs during skeletal growth due to exaggerated subperiosteal osteoclastic resorption of bone while modeling of the bone in the metaphyseal region.
- Osteoclastic resorption of bone most often begins at the site of tendon insertion. It never undergoes malignant transformation.

Incidence and Locations

Most common sites of benign fibrous histiocytoma/nonossifying fibroma of bone are distal femur or proximal tibia in 80% of cases. It may also occur in both ends of fibula and distal radius.

Age and Sex Predilection

Benign fibrous histiocytoma/nonossifying fibroma of bone affects equally male and female children during skeletal growth.

Table 27.43 Fibrohistiocytic tumors classification according to revised 2020 WHO classification of bone tumors

Fibrohistiocytic Tumors
▪ Benign tumors • Benign fibrous histiocytoma/nonossifying fibroma
▪ Malignant tumors • Malignant fibrous histiocytoma



Fig. 27.37: Benign fibrous histocytoma/nonossifying fibroma (metaphyseal fibrous defect) in right forearm radius bone in radiograph. Expansile osteolytic lesion is observed in lower radial diaphysis with a narrow zone of transition and mild sclerotic rim. There is marked thinning of overlying radial cortex. Chondroid matrix within lesion is absent. There is no break in the underlying cortex, and absence of periosteal reaction. Advised surgical biopsy and histological correlation are shown by arrow. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

Clinical Features

Patient is usually asymptomatic. Large size lesion may cause pain and pathologic fracture. Jaffe-Campanacci syndrome is characterized by formation of non-ossifying fibromas in multiple bones and *café au lait* spots on skin.

Radiologic Findings

Radiograph shows a well-defined eccentric osteolytic lesion in the metaphyseal region of a long bone. Margins of lesion are scalloped and surrounded by a sclerotic rim. Small lesion measuring 1–2 cm is regarded as fibrous cortical defect. Large lesion measuring 7 cm may undergo pathologic fracture due to cortical expansion (Fig. 27.37).

Surgical Pathology: Benign Fibrous Histiocytoma/ Nonossifying Fibroma (Metaphyseal Fibrous Defect)
Gross Morphology
Curettages of benign fibrous histiocytoma/nonossifying fibroma are friable, yellow brown and soft in consistency with an intact shell of overlying bone.
Light Microscopy
▪ Tumor is composed of spindle cell stroma with scattered a few to many multinucleated giant cells, hemosiderin pigment-laden macrophages and foam cells.

- Spindle cells of tumor are arranged in a whorled or storiform pattern. In occasional lesion, spindle cells have plump nuclei and typical mitotic figures. Reactive bone formation is most often demonstrated in older lesions (Fig. 27.38).

Differential Diagnosis

Benign fibrous histiocytoma/nonossifying fibroma should be demonstrated from giant cell tumor of bone, malignant fibrous histiocytoma and fibrous dysplasia.

Prognosis and Treatment

Benign fibrous histiocytoma/nonossifying fibroma can be left untreated. Large lesions are treated by curettage.

MALIGNANT FIBROUS HISTIOCYTOMA OF BONE

Malignant fibrous histiocytoma of bone is a high-grade neoplasm. Tumor is composed of fibrogenic spindle cells and histiocytic cells arranged in storiform pattern. It may also arise in pre-existing osseous lesions, Paget's disease of bone and cartilaginous neoplasms.

Incidence and Locations

Most common sites of tumor are femur in 33% of cases followed by tibia and humerus.

Age and Sex Predilection

Malignant fibrous histiocytoma of bone most often affects adults >40 years of age, which may also affect children and elderly persons of >75 years.

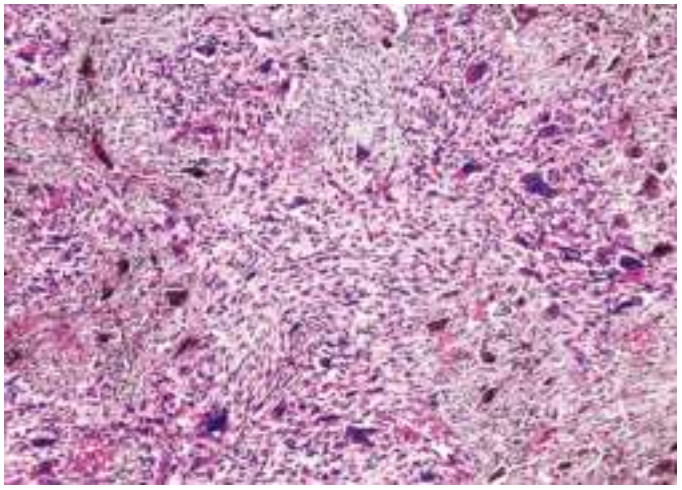


Fig. 27.38: Benign fibrous histiocytoma/nonossifying fibroma. Tumor is composed of spindle cell stroma with scattered a few to many multinucleated giant cells, hemosiderin pigment laden macrophages and foam cells. Spindle cells of lesion are arranged in a whorled or storiform pattern. In occasional lesion, spindle cells have plump nuclei and typical mitotic figures. Reactive bone formation is most often demonstrated in older lesions (400X).

Clinical Features

Patient presents with pain and swelling in tibia or humerus. Sometimes, pathologic fracture may occur in the involved bone.

Radiologic Findings

Radiograph shows poorly circumscribed osteolytic lesion with a permeative pattern of bone destruction. Cortex is most often destroyed.

Surgical Pathology: Malignant Fibrous Histiocytoma of Bone

Gross Morphology

Tumor is gray tan fleshy tumor. Cut surface shows larger areas of necrosis.

Light Microscopy

- Tumor is composed of spindle cells, round histiocyte-like cells or combination of both types of cells in varying proportions.
- Spindle cells contain oval nuclei with mild to moderate pleomorphisms. Spindle cells are arranged in a storiform pattern.
- Some malignant fibrous histiocytomas show histiocyte-like differentiation. Histiocyte-like cells contain large or lobulated nuclei and prominent nucleoli. Hemosiderin and lipid can be demonstrated in histiocyte-like cells. Multinucleated giant cells are most often present in the tumor (Fig. 27.39).

Differential Diagnosis

Malignant fibrous histiocytoma of bone should be differentiated from metastatic carcinoma especially renal cell carcinoma showing sarcomatoid growth pattern and osteosarcoma showing little osteoid production.

Immunohistochemistry Technique

Tumor cells show strong positivity for vimentin. Occasional tumor demonstrates positivity for α-actin, calponin, caldesmon, smooth muscle myosin heavy chain, cytokeratins and epithelial membrane antigen (EMA).

Markers	Expression
▪ Vimentin	▪ Positive (strong staining)
▪ α-Actin	▪ Positive/negative
▪ Calponin	▪ Positive/negative
▪ Caldesmon	▪ Positive/negative
▪ Smooth muscle myosin heavy chain (SMMHC)	▪ Positive/negative
▪ Cytokeratins	▪ Positive/negative
▪ Epithelial membrane antigen	▪ Positive/negative

Prognosis and Treatment

Malignant fibrous histiocytoma of bone is treated by complete surgical ablation with tumor-free margins and chemotherapy. Five-year survival rate is demonstrated in 35–50% of cases.

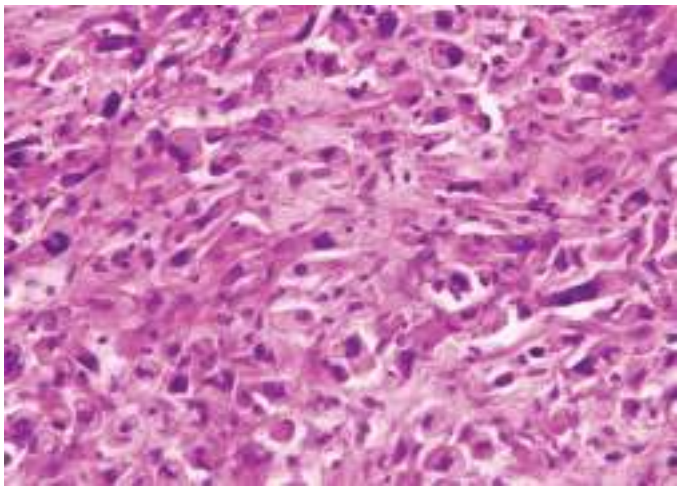


Fig. 27.39: Malignant fibrous histiocytoma of bone. Tumor is composed of spindle cells, round histiocyte-like cells or combination of both types of cells in varying proportions. Spindle cells contain oval nuclei with mild to moderate pleomorphisms. Spindle cells are arranged in a storiform pattern (400X).

HEMATOPOIETIC NEOPLASMS OF BONE

Lymphocytes and plasma cells reside in the company with hematopoietic elements of bone marrow. Therefore, malignant proliferation of lymphocytes and plasma cells occur in the center of the bone marrow and destroy bone marrow secondarily.

- Examples of hematopoietic neoplasms are plasma cell myeloma (multiple myeloma), solitary plasmacytoma of bone, non-Hodgkin's lymphoma and Hodgkin's disease. Solitary hematopoietic neoplasms confined to the bone marrow respond well to chemotherapy and radiotherapy and have excellent prognosis.
- Hematopoietic neoplasms can disseminate rapidly resulting in poor prognosis. These tumors most often occur in elderly persons, may also affect children. Hematopoietic neoplasms according to recent 2020 WHO classification of bone tumors are given in Table 27.44.

MULTIPLE MYELOMA

Multiple myeloma is monoclonal proliferation of plasma cells involving multiple sites in the skeleton.

Table 27.44 Hematopoietic neoplasms according to recent 2020 WHO classification of bone tumors

Hematopoietic Neoplasms
Malignant tumors
Plasma cell myeloma
Solitary plasmacytoma of bone
Primary non-Hodgkin's lymphoma of bone

Malignant plasma cells synthesize excess of monoclonal immunoglobulin that can be detected in the serum and urine. Monoclonal immunoglobulin, known as 'M-protein', is usually an IgG. Light chains released from the breakdown of monoclonal immunoglobulin is excreted in the urine, known as 'Bence Jones proteins' identified by urinary immunoelectrophoresis. Solitary plasmacytoma of bone is caused by neoplastic proliferation of plasma cells involving a solitary bone.

- Diagnosis of multiple myeloma rests on meeting specific diagnostic criteria including presence of ≥10% plasma cells on the bone marrow, increased M-protein in serum and urine, and evidence of end-organ damage (hypercalcemia, renal insufficiency, anemia or bone lesions).
- Majority of patients are diagnosed by clinical history, imaging of skeletal survey, hematologic findings (peripheral blood smear examination, erythrocyte sedimentation rate, bone marrow aspiration and trephine biopsy examination), serum calcium, blood urea, serum creatinine, and immunoelectrophoresis and immunofixation of serum and urine.

Incidence and Locations

Vertebral column is the most common site especially in lumbar region. The other bones affected in the descending frequency are skull, ribs, sternum, pelvis, femur, clavicle and scapula.

Age and Sex Predilection

Most cases of multiple myeloma occur during 50–80 years of age. Less than 2% of cases affect <40 years of age. Osteoclastic activating factor synthesized by monoclonal plasma cells cause osteolytic lesions in the skeleton. The lesion begins in the medullary cavity, erodes cancellous bone and progressively destroy the cortex of bone. Skeletal lesions are associated with bone pain and pathological fracture.

Clinical Features

Patient presents with bone pain (85%) and pathologic fracture of vertebrae and rib. Spine involvement causes compression of the spinal cord. Other presenting symptoms are recurrent bacterial infections, epistaxis, fever, anemia and weakness. Systemic complications due to M protein (myeloma protein) are renal insufficiency, hypercalcemia and amyloid deposition in the late stage disease.

Numerous other systems may be involved and known as **POEM syndrome** characterized by polyneuropathy, organomegaly, endocrinopathy and M protein. Myeloma kidney is caused by deposition of waxy casts of precipitated nephrotoxic monoclonal light chains

and blocking in the renal tubules. λ Light chains are more nephrotoxic then κ chains.

Radiologic Findings

Early involvement of bone in multiple myeloma shows only changes of osteoporosis. Well-developed skeletal lesions are usually multiple, discrete, punched out and radiolucent demonstrated on radiograph. Rarely, the skeletal lesion may be osteoblastic on skull radiograph, it shows multiple punched out osteolytic lesions as shown in Fig. 27.40.

Surgical Pathology: Multiple Myeloma

Gross Morphology

- Early skeletal lesion in multiple myeloma may show involvement of red bone marrow without destruction of cancellous bone.
- In advanced skeletal lesion, bone marrow is replaced by hemorrhagic friable soft tissue resembling 'currant jelly'. However, tissue fragments may show 'fish flesh' appearance. Humerus and femur involved by multiple myeloma is shown in Fig. 27.41.

Light Microscopy

- Trephine bone marrow biopsy fragments in multiple myeloma show replacement of bone marrow by diffuse sheets of plasma cells.
  - Plasma cells contain abundant blues to pink cytoplasm and eccentrically placed nuclei with clumped chromatin giving 'clock faced' appearance.
  - Sometime, plasma cells are poorly differentiated with large nuclei and prominent nucleoli (Fig. 27.42).
- Amyloid deposits (AL-amyloid) may be demonstrated in bone marrow and viscera in 10–15% of cases. Amyloid deposits in various sites indicate progression of the disease.

Differential Diagnosis

Multiple myeloma should be differentiated from malignant lymphoma, metastatic carcinoma and chronic osteomyelitis. Comparison of multiple myeloma and chronic osteomyelitis is given in Table 27.45.

Immunohistochemistry Technique

Neoplastic plasma cells consistently stain with specific marker CD138. These cells are also positive for κ or λ light chains.

Markers	Expression
▪ CD138	▪ Positive
▪ κ or λ light chains	▪ Positive

Prognosis and Treatment

Patient of multiple myeloma is treated with administration of chemotherapeutic agent melphalan and prednisone, and bone marrow transplantation.

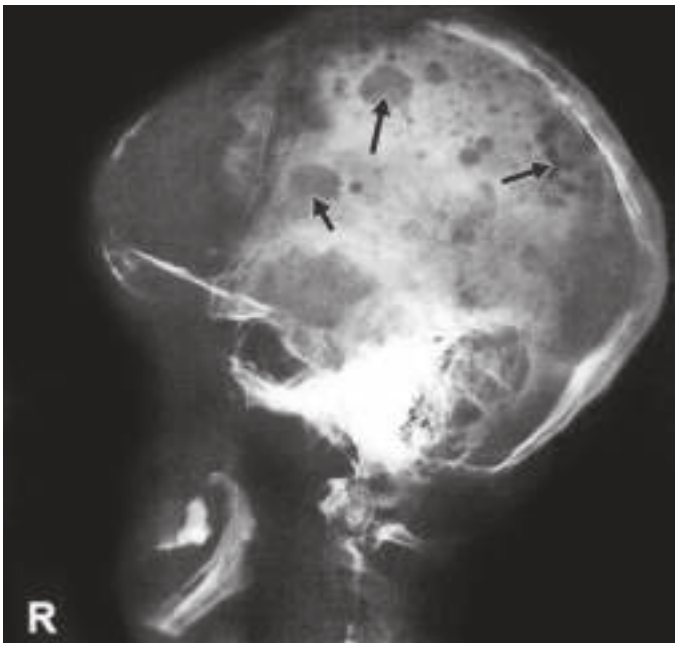


Fig. 27.40: Skull radiograph in multiple myeloma. Skull radiograph shows multiple punched out osteolytic lesions (arrows).



Fig. 27.41: Humerus and femur involved in multiple myeloma. Bone marrow cavity of humerus and femur bones is replaced by soft, gelatinous 'fish flesh hemorrhagic tissue. Patient presents with bone pain, lytic bone lesions (thoracic vertebrae most common, also ribs, skull, pelvis, femur); spinal cord compression and peripheral neuropathy are less common presenting symptoms, renal failure and recurrent infections shown by arrows.

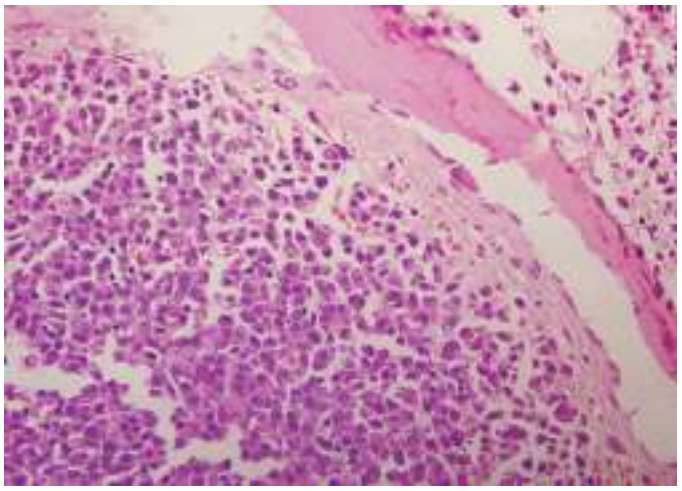


Fig. 27.42: Multiple myeloma. Trephine bone marrow biopsy fragments in multiple myeloma show replacement of bone marrow by diffuse sheets of plasma cells. Plasma cells contain abundant blues to pink cytoplasm and eccentrically placed nuclei with clumped chromatin giving 'clock faced appearance'. Sometime, plasma cells are poorly differentiated with large nuclei and prominent nucleoli (400X).

Radiotherapy is indicated in specific clinical situations. Median survival of multiple myeloma patients is three years. About 15% of solitary plasmacytoma cases experience progression to multiple myeloma after a median duration of three years.

PRIMARY BONE MARROW LYMPHOMA (PBML)

Lymphomas (non-Hodgkin's lymphoma and Hodgkin's disease) primarily involve lymph nodes, which may disseminate to other visceral organs and skeleton during course of disease. About 66% of lymphomas originate in other tissues (e.g. gastrointestinal tract, lung and bones) other than lymph nodes. About 5% of extra-nodal lymphomas are primary lymphomas of bone. Diagnostic criteria of primary bone marrow lymphoma include isolated bone marrow infiltration with no evidence of nodal or extranodal involvement, including bone and exclusion of leukemias/lymphomas that are considered to primarily involve bone marrow.

Incidence and Locations

Primary lymphoma of bone accounts for 7% of all malignant bone tumors. Most common sites are diametaphysis of femur and pelvis. It has aggressive pattern of osteolytic lesion associated with soft tissue mass. Other sites of bone tuomr are spine and other long bones. Involvement of solitary bone is demonstrated in 75% and multiple in 25% of cases.

Age and Sex Predilection

Primary lymphoma of bone most affects patients >40 years of age in 50% of cases. However, it may affect any age group. Precursor B cell lymphoblastic lymphoma affects children.

Clinical Features

Patient presents with symptoms depending on bone involved.

Radiologic Findings

Radiograph shows poorly defined lesion with permeative pattern of bone destruction. Primary lymphoma of bone marrow easily penetrates the cortex and invades soft tissue in 50% of cases. Some lesions show intraosseous reactive bone formation that can cause focal radiodensity. Pathologic fracture is demonstrated in 25% of cases.

Surgical Pathology: Primary Bone Marrow Lymphoma

Gross Morphology

Tumor is fleshy hemorrhagic due to bone destruction by primary bone marrow lymphoma.

Light Microscopy

Almost all primary bone marrow lymphomas are diffuse large B cell lymphoma (DLBCL). Others are immunoblastic lymphoma, anaplastic large T cell lymphoma, lymphoblastic lymphoma and Hodgkin's disease. Neoplastic cells diffusely infiltrate the bone marrow with intact bony trabeculae.

- **Diffuse large B cell lymphoma (DLBCL):** Majority of neoplastic cells display population of pleomorphic centroblasts with scant cytoplasm, hyperchromatic nuclei with finely clumped chromatic and two to three nucleoli. In occasional case tumor is composed of immunoblasts with round nuclei and abundant amphophilic cytoplasm.
- **Anaplastic large T cell lymphoma:** Tumor is composed of pleomorphic nuclei especially horseshoe nuclei.
- **Lymphoblastic lymphoma in children:** Tumor is composed of sheets of neoplastic cells with containing round nuclei with a fine stippled chromatin infiltration in the bone marrow.
- **Hodgkin's disease:** Tumor is composed of Reed-Sternberg cells admixed with population of inflammatory cells in mixed cellularity variant of Hodgkin's disease.

Differential Diagnosis

Primary bone marrow lymphoma should be differentiated from Ewing sarcoma, metastatic carcinoma, multiple myeloma and sarcomas with extensive fibrosis.

Immunohistochemistry Technique

Diffuse large B cell lymphoma (DLBCL) shows positivity for CD45, pan B cell markers (CD19, CD20, CD22, CD79a, PAX5). CD20 reactivity is lost in DLBCL treated by rituximab (anti-CD20 antibody), but reactivity is intact for CD19, CD22 and CD79a. There is variable expression of CD10, CD15, BCL-6, BCL-2, sIg and Ki-67 in diffuse large B cell lymphoma.

Markers	Expression
▪ CD45	▪ Positive
▪ Pan B cell markers (CD19, CD20, CD22, CD79a, PAX5)	▪ Positive
▪ CD10	▪ Positive (variable)
▪ CD15	▪ Positive (variable)
▪ BCL-6	▪ Positive (variable)
▪ BCL-2	▪ Positive (variable)
▪ sIg	▪ Positive (40–50%)
▪ Ki-67	▪ Positive (40–50%)

Prognosis and Treatment

Primary bone marrow lymphomas are treated with radiation and chemotherapy. Five-year survival rate is demonstrated in 50–90% of cases. Patients above 60 years of age have poor prognosis.

BONE TUMORS OF UNDEFINED NEOPLASTIC NATURE

A group of lesions that affect bone undergo into malignant neoplasms and have distinct histologic appearance and cytogenetic or molecular alterations, but the clinical behavior is suggestive of a non-neoplastic process. Tumors of undefined neoplastic nature according to recent 2020 WHO classification of bone tumors are given in Table 27.46.

SIMPLE BONE CYST

Simple bone cyst is a central intramedullary unilocular cystic cavity containing serous or serosanguineous fluid lined by a thin fibrovascular connective tissue membrane. It occurs in the metaphyseal region of immature skeleton of proximal femur and humerus, which may also occur in jaw.

Incidence and Locations

Simple bone cyst occurs in the metaphyseal region of immature skeleton of proximal femur and humerus. Its pathogenesis is unknown. But it is considered to be reactive or developmental lesion, probably be related to disturbance in the growth at the epiphyseal plate. It may also occur in jaw.

Age and Sex Predilection

Simple bone cyst occurs during first and second decades of life with male predominance.

Clinical Features

Simple bone cyst is detected as incidental finding on radiograph in majority of cases. Some patients may present with painful swelling and pathologic fracture.

Radiologic Findings

Simple bone cyst is a central intramedullary metaphyseal lesion. It is well circumscribed lucent lesion with

Table 27.45 Comparison of multiple myeloma and chronic osteomyelitis

Parameters	Multiple Myeloma	Chronic Osteomyelitis
Population of cells on histology	Plasma cells with abundant blue to pink cytoplasm with eccentrically placed nuclei with clumped chromatin giving 'clock faced appearance'	Plasma cells admixed with lymphocytes and neutrophils
Granulation tissue	Absent	Present
Immunohistochemistry	CD138 positive (monoclonal plasma cells)	CD138 positive (polyclonal plasma cells)

Table 27.46 Tumors of undefined neoplastic nature according to recent 2020 WHO classification of bone tumors

Tumors of Undefined Neoplastic Nature
Benign tumors
▪ Simple bone cyst ▪ Fibrous dysplasia ▪ Osteofibrous dysplasia ▪ Chondromesenchymal hamartoma ▪ Rosai-Dorfman disease
Intermediate tumors (locally aggressive)
▪ Aneurysmal bone cyst ▪ Langerhans cell histiocytosis (monostotic, polyostotic) (Letterer-Siwe disease, Hand-Schüller-Christian disease and eosinophilic granuloma) ▪ Erdheim-Chester disease

fine trabeculation with scalloped borders and cortical thinning; but without disruption, unless bone fracture has taken place. In some instances, a bony fragment within the cyst lumen known as 'fallen fragment' is demonstrated on radiograph.

Surgical Pathology: Simple Bone Cyst

Gross Morphology

- Thin translucent fibrous membrane <1 mm thick is demonstrated in minimal curettage material obtained in surgical specimen.
- Cyst is communicating directly with the medullary cavity of shaft of long bone.
- Intact simple bone cyst contains clear to yellowish fluid with or without internal septations.
- Presence of hemorrhagic fluid in the cyst may reflect prior bone fracture.

Light Microscopy

- Simple bone cyst is lined by a thin fibrovascular connective tissue membrane.
- There may be presence of scattered osteoclasts admixed with inflammatory cells and hemosiderin deposits in a case prior to bone fracture.
- Reactive bone formation is most often demonstrated in simple bone cyst following bone fracture. Cementum-like material is also demonstrated.

Differential Diagnosis

Simple bone cyst should be differentiated from aneurysmal bone cyst and intraosseous ganglion.

Prognosis and Treatment

Simple bone cyst is treated by curettage and bone grafting. It can be repeated in the rare event of recurrence of the lesion.

FIBROUS DYSPLASIA OF BONE

Fibrous dysplasia of bone is non-inherited dysplastic developmental disorder characterized by fibrous stroma with trabeculae of immature woven bone reflecting defective bone maturation due to post-zygotic GNAS1 gene mutation; which encodes G protein-coupled receptor activating adenyl cyclase resulting in excessive production of adenosine monophosphate that derives unrestricted cellular proliferation. Transformation of fibrous dysplasia of bone to osteosarcoma is rare.

Incidence and Locations

Fibrous dysplasia of bone accounts for 1% of bone tumors. Most common sites are neck of femur, tibia, humerus, craniofacial bones (maxilla, mandible) and ribs. It may involve single bone (monostotic) or multiple bones (polyostotic). Spine is rarely involved. Features of fibrous dysplasia of bone are given in Table 27.47.

- **Monostotic fibrous dysplasia:** It involves single bone and accounts for 70–75% of cases affecting older children and adolescents.
- **Polyostotic fibrous dysplasia:** It involves multiple bones and accounts for 25–30% of cases affecting children. Craniofacial involvement is demonstrated in 50% of cases.
  - Polyostotic fibrous dysplasia of bone is associated with McCune-Albright syndrome in 3–5% of cases affecting children; in the patient presents with precocious puberty and abnormal skin pigmentation (*café au lait* spots on skin).
  - Mazabraud syndrome is characterized by polyostotic fibrous dysplasia, skin lesions and hyperplasia of endocrinal glands.
  - Monomelic polyostotic fibrous dysplasia of bone involves unilateral one extremity and homolateral pelvis or scapula. On the other hand, polymelic polyostotic fibrous dysplasia involves widespread skeleton.

Age and Sex Predilection

Fibrous dysplasia of bone most often affects infants, children and adolescents <30 years of age. Monostotic

Table 27.47 Features of fibrous dysplasia of bone

Fibrous Dysplasia: Variants and Frequency	Age Group Distribution	Distribution of Lesions and Clinical Features
Monostotic fibrous dysplasia (70–75%)	Older children, adolescents	Single focus in femur, humerus, craniofacial bones, rib
Polyostotic fibrous dysplasia (25–30%)	Young children	▪ Multiple foci in several bones, i.e. femur, humerus, craniofacial bones and ribs ▪ Monomelic polyostotic fibrous dysplasia involves unilateral one extremity and homolateral pelvis or scapula ▪ Polymelic polyostotic fibrous dysplasia involves widespread skeleton
McCune-Albright syndrome	Young children	Polymelic polyostotic fibrous dysplasia with endocrinal abnormalities, i.e. precocious puberty in females, acromegaly, Cushing syndrome, hyperthyroidism, hyperparathyroidism
Mazabraud syndrome	Young persons	Polymelic polyostotic fibrous dysplasia associated with soft tissue myxoma

fibrous dysplasia occurs during second to third decades of life. On the other hand, polyostotic fibrous dysplasia occurs during first decade of life. Fibrous dysplasia of rib occurs during fourth to fifth decades of life.

Clinical Features

- **Monostotic fibrous dysplasia:** Patient is most often asymptomatic. Fibrous dyspasia of bone may be an incidental finding. Some patients present with deformity of craniofacial bones and long bone fracture.
- **Polyostotic fibrous dysplasia:** Patient with McCune-Albright syndrome may present with bone deformity, bone fracture and symptoms related to hyperplasia of endocrinal glands such as sexual precocity, acromegaly, hyperthyroidism, hyperparathyroidism, Cushing syndrome and hyperprolactinemia.

Radiologic Findings

Radiograph shows well circumscribed lesion with sclerotic rim of variable density giving 'ground glass appearance'. Lesion in femur, tibia and humerus is located in the metaphyseal region involving medullary cavity. Shepherd's crook deformity occurs in the femur, characterized by a coxa-vara angulation of the proximal femur. Lesion produces expansion in small bones (Fig. 27.43).

Surgical Pathology: Fibrous Dysplasia of Bone

Gross Morphology

- Fibrous dysplasia of long bones is well-defined showing thin cortex and expanded contours.
- Curettage shows tan gray fibrous tissue with gritty consistency.
- Areas of hemorrhage with or without cysts are demonstrated. Gross morphology of fibrous dysplasia is shown in Fig. 27.44.

Light Microscopy

- Fibrous dysplasia of bone is composed of irregular trabeculae of immature woven bone lacking osteoblastic rimming. The irregular trabeculae of immature woven bone are present in the stroma and giving 'storiform appearance'.
- There may be presence of foam cells, occasional stromal myxoid and/or cystic change. Sarcomatous transformation in fibrous dysplasia is rare (Fig. 27.45).

Differential Diagnosis

Fibrous dysplasia of bone should be differentiated from osteofibrous dysplasia, cemento-ossifying fibroma of gnathic bones (maxilla, mandible), desmoplastic fibroma, parosteal osteosarcoma, low-grade osteosarcoma and chondrosarcoma with prominent chondroid component.



Fig. 27.43: Fibrous dysplasia of fourth metacarpal bone. Radiograph of right hand demonstrates expansion of fourth metacarpal shaft due to an osteolytic lesion with ground glass density with cortical thinning with break in cortex on radial side. Other bones and joints appear normal. Soft tissue is normal. Findings are suggestive of fibrous dysplasia with pathological fracture shown by arrow. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

Prognosis and Treatment

Conservative treatment is recommended in monostotic fibrous dysplasia; because the lesion stabilizes after puberty.

- Prognosis is excellent in fibrous dysplasia involving single bone.
- In polyostotic fibrous dysplasia, prognosis depends on extent and severity of bone lesions and hyperplasia of endocrinal glands related symptoms

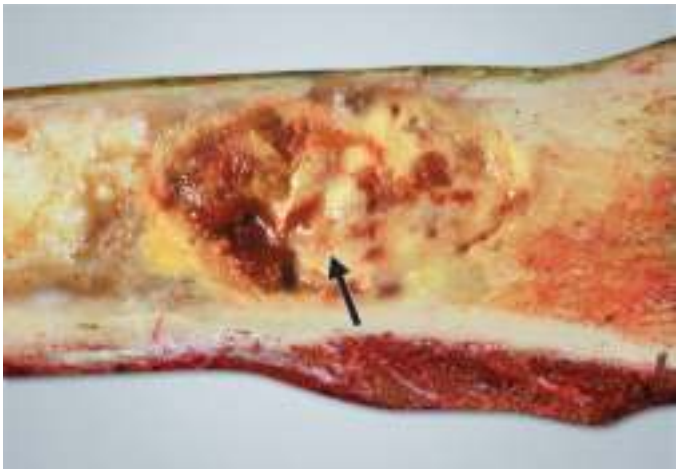


Fig. 27.44: Fibrous dysplasia of long bone. It is well-defined lesion showing thin cortex and expanded contours, tan gray fibrous tissue with gritty consistency. Areas of hemorrhage with or without cysts are demonstrated (arrow).

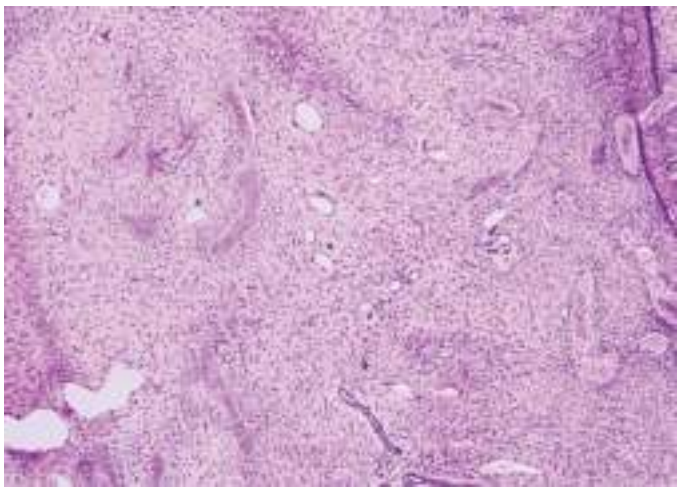


Fig. 27.45: Fibrous dysplasia. It is composed of irregular trabeculae of immature woven bone lacking osteoblastic rimming. The irregular trabeculae of immature woven bone are present in the stroma and giving 'storiform appearance'. There may be presence of foam cells, occasional stromal myxoid and/or cystic change (400X).

in McCune-Albright syndrome and Mazabraud syndrome.

OSTEOFIBROUS DYSPLASIA (OSSIFYING FIBROMA)

Osteofibrous dysplasia (also known as ossifying fibroma) is a benign intracortical lesion in the midshaft of tibia with or without involvement of fibula affecting children. Radius and ulna are rarely involved.

Incidence and Locations

Osteofibrous dysplasia most often occurs in the midshaft of tibia with or without involvement of fibula. Fibula alone is rarely involved. Rarely, radius and ulna may be involved.

Age and Sex Predilection

Osteofibrous dysplasia most often occurs in young children below the age of 15 years. Males and females are equally affected.

Clinical Features

Patient presents with painful swelling and deformity of tibia in the diaphyseal region.

Radiologic Findings

Radiograph shows several centimeters long intracortical radiolucent lesion in the diaphyseal region of tibia and sclerosis surrounding the lesion giving ground glass appearance. Involvement of medulla of bone results in narrowing of medullary cavity. Involved bone shows bowing. Lesion does not involve soft tissue.

Surgical Pathology: Osteofibrous Dysplasia

Gross Morphology

Osteofibrous dysplasia is confined to the cortex of tibia. It is gray white to yellow brown. Consistency is firm fibrous to gritty texture.

Light Microscopy

Osteofibrous dysplasia has zonal architecture, i.e. central and peripheral zones.

- **Central zone of osteofibrous dysplasia:** It is composed of loose fibrous tissue with myxoid appearance, arranged in storiform pattern admixed with immature woven bony trabeculae.
- **Peripheral zone of osteofibrous dysplasia:** It is composed of a mixture of mature lamellar bone and immature woven bone.
  - Mature lamellar bone merges with either the cortical bone or reactive sclerotic bone at the medullary aspect.
  - Bony trabeculae are rimmed by layer of osteoblasts throughout the lesion.

Differential Diagnosis

Osteofibrous dysplasia should be differentiated from fibrous dysplasia, osteofibrous dysplasia like differentiated lesion, classic adamantinoma with focal osteofibrous dysplasia like component.

Immunohistochemistry Technique

As osteofibrous dysplasia lacks epithelial cells, but shows positivity for cytokeratin AE1/AE3 in scattered cells within fibrous stroma. Cytokeratin profile is that of basal phenotype showing expression of CK14 and CK19. CK1 and CK5 are expressed in some lesions.

Markers	Expression
▪ CK14 (AE1/AE3)	▪ Positive
▪ CK19 (AE1/AE3)	▪ Positive
▪ CK1	▪ Positive (some cases)
▪ CK5	▪ Positive (some cases)

Prognosis and Treatment

Osteofibrous dysplasia regresses spontaneously after puberty in majority of cases. Lesion may recur after biopsy or surgical excision in 25% of cases. Severe bone deformity is treated by surgery.

LANGERHANS CELL HISTIOCYTOSIS

Langerhans cell histiocytosis (LCH) is a spectrum of clinical syndromes characterized by proliferation of antigen-presenting dendritic cells derived from CD34 positive hematopoietic stem cells of bone marrow, which express major histocompatibility complex class II molecules and bear C3 receptors and CD1A.

Table 27.48 Clinical forms of Langerhans cell histiocytosis (LCH)

Clinical Forms	Age Group	Organs Involved	Prognosis
Letterer-Siwe disease (generalized diffuse)	Infancy	Bone, bone marrow, skin, lymph nodes, liver, spleen, thymus, lung	Usual fatal
Hand-Schüller-Christian disease (generalized diffuse)	Childhood, young adults	▪ *Adults:* Bone (ribs, ramus of mandible, vertebral bodies and orbit), lung, gastrointestinal tract, skin ▪ *Children:* Head and femur	Chronic disabling disease
Eosinophilic granuloma of bone (localized diffuse)	Childhood, young adults	Bone	Variable stable to remission
Eosinophilic granuloma of lung (localized diffuse)	Young adults	Lung	Variable stable to remission

- Langerhans cell histiocytosis contains tennis racket-shaped cytoplasmic 'Birbeck granules' demonstrated by electron microscopy, which have poor phagocytic activity.
- Langerhans cell histiocytosis is positive for S-100 protein, CD1a, CD207 (langerin) and CD68.
- Langerhans cell histiocytosis is classified into three clinical forms according to the extent of involvement of organs, i.e. skeletal and extraskeletal regions (lymph node, skin, lung, gastrointestinal tract), e.g. Letterer-Siwe disease, Hand-Schüller-Christian disease and eosinophilic granuloma. There can be unifocal or severe form multifocal involvement of organs. Clinical forms of Langerhans cell histiocytosis (LCH) are given in Table 27.48.

Langerhans Cell Histiocytosis (LCH): Clinical Forms

Letterer-Siwe Disease

Letterer-Siwe disease is an acute disseminated Langerhans cell histiocytosis, which affects infants and children. It has a rapid aggressive clinical course with fatal outcome.

- **Clinical Features:** Patient presents with painless yellow brown, maculopapular skin lesions over face (especially around eyes), mouth, trunk, perineum and axilla and recurrent infection as a result of widespread proliferation of Langerhans cell histiocytosis. Physical examination reveals hepatosplenomegaly, lymphadenopathy and pulmonary involvement (honeycomb appearance, pleural effusion and spontaneous pneumothorax).
- **Light Microscopy:** Histologic examination of the lesion shows numerous histiocytes lacking lipids and foci of necrosis.

Hand-Schüller-Christian Disease

Hand-Schüller-Christian disease is an acute onset disease followed by chronic clinical course as a result of proliferation of Langerhans cell histiocytosis, which affects infants, adolescents and elderly persons. It most often manifests before the age of 5 years. It has better prognosis than Letterer-Siwe disease.

- **Clinical Features:** Patient presents with clinical triad of manifestations: (a) skull lesions (orbit, tooth-bearing area of mandible, mastoid bone), (b) diabetes insipidus due to involvement of sella turcica, and (c) exophthalmos due to involvement of orbit. There may also be involvement of femur, pelvis, rubs, shaft of humerus. Hepatosplenomegaly and lymphadenopathy are demonstrated in 25% of cases.
- **Light Microscopy:** Histologic examination of the lesion (skull, liver, spleen, other tissues) is composed of histiocytes admixed with inflammatory cells.

Eosinophilic Granuloma

Eosinophilic granuloma is a rare benign-like disorder characterized by clonal proliferation of Langerhans cell histiocytosis. It is the most common variant of Langerhans cell histiocytosis. It may be unifocal or multifocal involvement of organs. It mainly involves skull, mandible, vertebrae, pelvis and ribs in children. It involves long bones, lung and gastrointestinal tract in adults.

- **Clinical Features:** Patient presents with bone pain, pathological fracture of long bone and vertebral collapse. Pulmonary manifestations are cough, breathlessness and pneumothorax. Gastrointestinal lesion may simulate polyp, duodenal ulcer and gastric carcinoma. Gastrointestinal lesion usually heals without treatment.
- **Light Microscopy:** Histologic examination of the eosinophilic granuloma is composed of numerous mononuclear histiocytes admixed with eosinophils and lymphocytes (Fig. 27.46).

Radiologic Findings

- Radiologic findings of clinical variants of Langerhans cell histiocytosis are variable. Radiograph shows geographic osteolytic lesion in the medullary region of long bones.
- Skull radiograph shows most often well defined 1–2 cm size nonsclerotic round or oval lesion with beveled edge. In long bones, radiograph demonstrates lesion reaching 6 cm in size within cortical region. Soft tissue involvement is well documented.

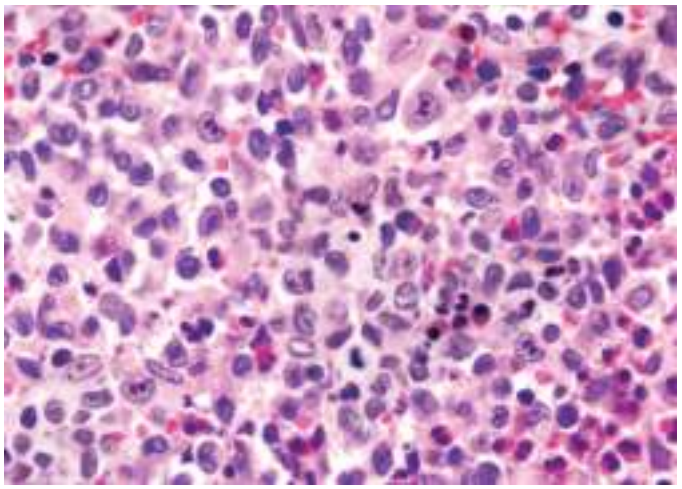


Fig. 27.46: Eosinophilic granuloma. It is composed of numerous mononuclear histiocytes admixed with eosinophils and lymphocytes (400X).

Surgical Pathology: Langerhans Cell Histiocytosis

Gross Morphology

- Gross morphology of curettage of Langerhans cell histiocytosis is gray yellow to tan or hemorrhagic depending on the proportion of these components.
- Small fragments are soft in consistency.
- Surgical excision of complete lesion is well circumscribed gray, yellow to red.
- Cortical destruction of long bone is most often demonstrated. Extension of the lesion into soft tissue is demonstrated.

Light Microscopy

- Langerhans cell histiocytosis lesion is composed of numerous histiocytes admixed with eosinophils, foamy macrophages, plasma cells, neutrophils, scattered osteoclast-like giant cells and scattered mitotic figures. Atypical cells and fibrous tissue in the background are absent.
- Histiocytes contain abundant eosinophilic to vacuolated cytoplasm and vesicular nuclei with well defined nuclear borders and prominent indentations giving 'coffee bean reniform' appearance. Binucleated and multinucleated forms are common (Fig. 27.47).

Electron Microscopy

Langerhans cell histiocytosis contains tennis racket-shaped cytoplasmic '**Birbeck granules**' demonstrated by electron microscopy.

Differential Diagnosis

Langerhans cell histiocytosis should be differentiated from osteomyelitis, granulation tissue, Hodgkin's disease, non-Hodgkin's lymphoma, Rosai-Dorfman disease and fibrohistiocytic tumor (nonossifying fibroma).

Immunohistochemistry Technique

Cells of Langerhans cell histiocytosis are positive for S-100 protein, CD1a, CD207 (langerin) and CD68. These cells are negative for CD45, AE1/AE3, CD15 and CD30.

Markers	Expression
S-100 protein	Positive
CD1a	Positive
CD207 (langerin)	Positive
CD68	Positive
CD45	Negative
AE1/AE3	Negative
CD15	Negative
CD30	Negative

Prognosis and Treatment

Prognosis of Langerhans cell histiocytosis depends on site and extent of lesion in the bones and other tissues. Localized lesion may regress spontaneously. Some lesions are treated by simple curettage or administration of steroids in the lesion. Multifocal systemic disease has a poor prognosis. Poor prognostic factors are hepatosplenomegaly, polyostotic disease, anemia, thrombocytopenia and affecting children of <3 years of age. Multiagent chemotherapy has been some success in treating these cases.

ERDHEIM-CHESTER DISEASE

Erdheim-Chester disease is a rare disseminated xanthogranulomatous and infiltrative disease of unknown etiology. It is characterized by involvement of bone and

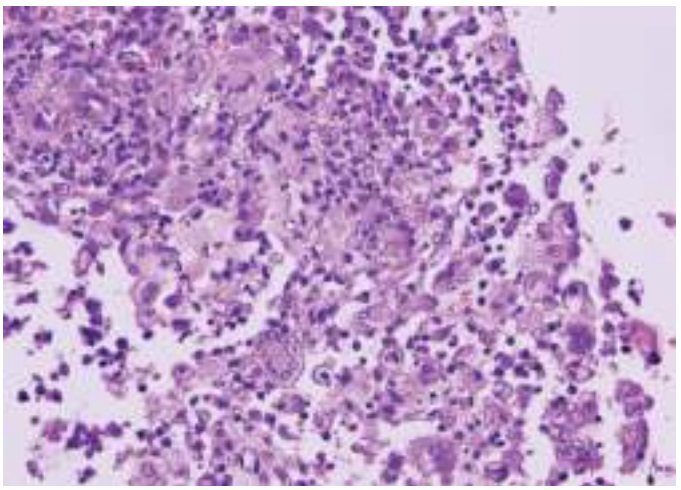


Fig. 27.47: Langerhans cell histiocytosis. It is composed of numerous Langerhans cell histiocytosis admixed with eosinophils, foamy macrophages, plasma cells, neutrophils, scattered osteoclast-like giant cells and scattered mitotic figures (400X).

systemic organs. Recent cytogenetic HUMARA (human androgen receptor-α)-PCR (polymerase chain reaction)-based studies suggest it a neoplastic disease.

Incidence and Locations

Erdheim-Chester disease involves skull, bilateral femur and tibia; heart, lung and retroperitoneum.

Age and Sex Predilection

Erdheim-Chester disease affects children to elderly persons between 7 and 84 years of age with slight male predominance. Peak incidence is observed during fifth to seventh decades of life.

Clinical Features

Involvement of orbit produces proptosis and exomphalos (birth defect of the abdominal wall). Involvement of pituitary gland manifests with diabetes insipidus. Cerebral and cerebellar involvement produces a multiple sclerosis-like syndrome. Involvement of myocardium and pericardium leads to cardiac failure. Pulmonary involvement produces interstitial lung disease. Bone marrow involvement occurs in bilateral distal femur, proximal tibia and fibula in the metaphyseal region.

Radiologic Findings

Radiograph shows bilateral symmetrical osteosclerotic lesions in the metaphyseal region of distal femur, proximal tibia and fibula. Epiphysis of bones is usually spared. Skull and ribs may demonstrate osteolytic lesions. In an occasional case, mixed sclerotic and osteolytic lesions may be demonstrated in distal femur, proximal tibia and fibula.

Surgical Pathology: Erdheim-Chester Disease

Gross Morphology

Biopsy fragments from lesion in Erdheim-Chester disease is composed of tan brown tissue with yellowish areas corresponding to xanthogranulomatous change.

Light Microscopy

- Osseous lesion of Erdheim-Chester disease is composed of numerous histiocytes admixed with multinucleated giant cells, Touton cells, lymphocytes and plasma cells lying in stroma.
- Thickening occurring in woven and lamellar bone may exhibit a pagetoid appearance.

Differential Diagnosis

Erdheim-Chester disease should be differentiated from chronic osteomyelitis, Langerhans cell histiocytosis and Rosai-Dorfman/sinus histiocytosis with massive lymphadenopathy.

Immunohistochemistry Technique

Osseous lesion of Erdheim-Chester disease is positive for CD68 and CD163 and negative for S-100 protein and CD1a.

Markers	Expression
▪ CD68	▪ Positive
▪ CD163	▪ Positive
▪ S-100 protein	▪ Negative
▪ CD1a	▪ Negative

Prognosis and Treatment

Prognosis of Erdheim-Chester disease depends on the extent of systemic involvement. Majority of patients have prolonged indolent course. Approximately 33% of cases have fatal outcome within 10 years from systemic effects. There is no definite treatment of the disease. Chemotherapy and corticoids are most often used to treat these cases.

CHONDROMESENCHYMAL HAMARTOMA

Chondromesenchymal hamartoma is a rare lesion in the rib cage of infants. It is composed of an admixture of spindle cells, cartilage and hemorrhagic cysts in variable proportion.

Incidence and Locations

Chondromesenchymal hamartoma most often occurs either in the medullary cavity or on the surface of rib cage. Lesion may be bilateral or multicentric.

Age and Sex Predilection

Chondromesenchymal hamartoma most often occurs in infants with male predominance, which rarely affects adults.

Clinical Features

Patient may be asymptomatic or having respiratory distress. Chondromesenchymal hamartoma may be detected incidentally as a palpable mass in the chest region.

Radiologic Findings

Radiograph and CT scan demonstrate an expansile mass with well-defined sclerotic margins, and areas of calcification and ossification. MRI shows solid and cystic components of the lesion.

Surgical Pathology: Chondromesenchymal Hamartoma

Gross Morphology

- Chondromesenchymal hamartoma can be of considerable size.
- Cut surface shows solid and cystic components with areas of hemorrhage. Cartilage is demonstrated in the solid component of the lesion.

Light Microscopy

- Solid component of chondromesenchymal hamartoma is composed of spindle cells with admixture of woven bone, cartilage and collagen in varying proportion. Enchondral ossification is frequently demonstrated.
- Cystic component of the lesion shows areas of hemorrhage. Its histologic features resemble aneurysmal bone cyst. Walls of septae of cystic component are composed of fibrous tissue, reactive bone and scattered osteoclast-like giant cells.

Differential Diagnosis

Chondromesenchymal hamartoma should be differentiated from aneurysmal bone cyst.

Molecular Genetic Alterations

Chondromesenchymal hamartoma arising in the nasal septum demonstrates chromosomal translocation t(12;17)(q24.1;q21).

Prognosis and Treatment

Chondromesenchymal hamartoma is treated by surgical excision. Asymptomatic patients can be managed by conservative treatment. Sporadic cases have fatal outcome due to respiratory insufficiency. Lesion may regress spontaneously.

ROSAI-DORFMAN DISEASE

Rosai-Dorfman disease is a rare disorder involving multiple organs and characterized by proliferation of histiocytes in the metaphyseal region of long bones and craniofacial bones.

Incidence and Locations

Most common sites of Rosai-Dorfman disease are long bones and craniofacial skeleton. Lesion is solitary in majority of cases. There may involvement of two or more separate bones in 20% of cases.

Age and Sex Predilection

Rosai-Dorfman disease affects 3–56 years of age group without sex predominance.

Clinical Features

Patient presents with pain in the localized area of involved bone.

Radiologic Findings

Radiograph shows well-defined osteolytic lesion and sometimes expansile separate mass. Cortical thickening and periosteal reaction are demonstrated in minority of cases.

Surgical Pathology: Rosai-Dorfman Disease

Gross Morphology

Osseous lesion of Rosai-Dorfman disease measures 1–7 cm in size. Osseous lesion measures <5 cm in diameter in majority of cases. Osseous lesion is well defined tan-gray and soft or gray gritty in consistency.

Light Microscopy

- Osseous lesion of Rosai-Dorfman disease is composed of numerous large size histiocytes admixed with plasma cells, lymphocytes, neutrophils, foamy macrophages and occasional eosinophils. Plasma cells most often contain Russell bodies.
- Large size histiocytes contain abundant eosinophilic cytoplasm and round or oval or kidney bean-shaped nuclei with fine or vesicular chromatin and prominent nucleoli. Microabscesses may be demonstrated in the lesion.

Immunohistochemistry Technique

Large size histiocytes show positivity for S-100 protein, CD8, CD163, and negative for CD1a.

Markers	Expression
S-100 protein	Positive
CD8	Positive
CD163	Positive
CD1a	Negative

Prognosis and Treatment

Rosai-Dorfman disease has a good prognosis. Approximately 40% of cases develop extraosseous foci of disease. Osseous lesions are treated by curettage.

ACHONDROPLASIA

Achondroplasia is an autosomal disorder characterized by short-limbed dwarfism and macrocephaly. Limb bones become short and thick. Anchondroplasia represents a failure of normal epiphyseal cartilage formation.

Pathogenesis

Achondroplasia occurs due to mutation of fibroblast growth factor receptor 3 (FGFR3) located at 4p16.3. FGFR3 gene mutation arrests chondrocyte proliferation, differentiation and development of epiphyseal plates.

Narrowing of epiphyseal plates, and bony sealing of the area between the epiphyseal plate and the metaphysis lead to failure of elongation of limb bones. A defective growth hormone receptor is responsible for rare cases of dwarfism (Laron dwarfism).

OSTEOGENESIS IMPERFECTA

Osteogenesis imperfecta is an autosomal dominant disorder and also known as brittle bone disease.

- **Physiologic state:** Normally, collagen genes (COLLA1 and COLLA2) encode α_1- and α_2-chains of type 1 procollagen fibers result in synthesis of collagen fibers, the major structural protein of bone.
- **Pathologic state:** Collagen genes (COLLA1 and COLLA2) lead to defective collagen synthesis in various organs such as skeleton (brittle bones), joints, ears, ligaments, teeth, sclerae, and skin.

Molecular genetic defects in four types of osteogenesis imperfecta are heterogeneous, but all adversely affect the synthesis of type 1 collagen fibers.

Clinical Features

Due to defective synthesis of collagen fibres in various organs (skeleton, joints, ligaments, sclerae, ear, teeth and skin, patients of osteogenesis imperfecta develop brittle bone disease, blue sclera, hearing loss and fractures during infancy and childhood.

OSTEOPETROSIS

Osteopetrosis is inherited disorder also known as marble disease or Albers-Schönberg disease, which occurs in two major clinical forms: an autosomal recessive variant of osteopetrosis that is usually fatal in infancy and a less severe autosomal dominant variant.

Age Group

Osteopetrosis affects infants and children, which occurs due to mutations in genes that govern osteoclast formation or function.

Pathology

Osteopetrosis results in increased bone density (thickened cortex), and lack of funnelization (funnel-like appearance) of the metaphysis and retention of the primary spongiosum with its cartilage cores. Decreased bone marrow space results in anemia.

- Due to narrowing of foramina results in blindness, deafness, due to pressure exerted on cranial nerves.
- Patient develops 'multiple fractures' in spite of increased bone density.
- Bone marrow transplant reverses many skeletal abnormalities. Human interferon gamma (IFN-γ) is administered in these patients.

Radiologic Findings

Long bones are shortened with loss of metaphyseal flare (Erlenmeyer, flask deformity, uniform opacity of pelvis and peripheral bones) alternating with normal bone cause a stippled appearance. It may cause spinal spondylolithiasis.

Surgical Pathology: Osteopetrosis

Gross Morphology

- Bones are solid and heavy without medullary canal.
- Long ends of bones are bulbous, small neural foramina, which compress the nerves.

Light Microscopy

- Osteopetrosis is primarily woven bone which does not undergo remodeling.
- Central core of the cartilage with dense and irregular bone trabeculae are formed.
- There is often presence of abundant osteoclasts.
- Reduced marrow space is present.
- Histology of osteopetrosis is seen in Fig. 27.48.
- Masson's trichrome stained section of osteopetrosis is shown in Fig. 27.49.

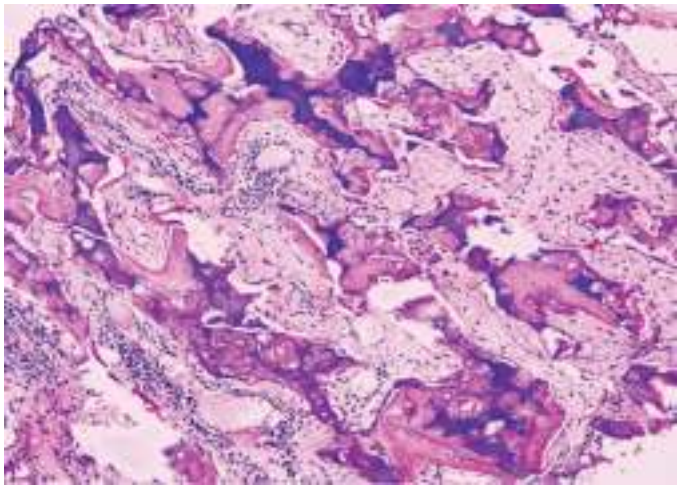


Fig. 27.48: Osteopetrosis. It shows increased bone density (thickened cortex) and decreased bone marrow space (100X).

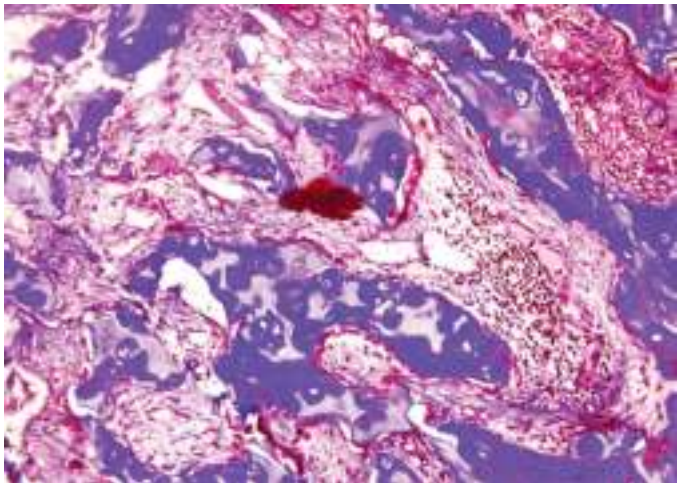


Fig. 27.49: Osteopetrosis. Masson's trichrome stain is demonstrated in osteopetrosis.

OSTEONECROSIS (AVASCULAR NECROSIS)

Osteonecrosis refers to the death of bone marrow in the absence of infection. Growing bones of children and adolescents are often affected.

Etiopathogenesis

Osteonecrosis is caused by trauma, thromboemboli, sickle cell anemia, decompression syndrome or the bends, in adults, which lead to interruption of blood supply to bone marrow. Causes of osteonecrosis are given in Table 27.49.

Pathologic Entities

- **Legg-Calvé-Perthes disease:** It refers to osteonecrosis in the femoral head in children. Collapse of the femoral head may lead to joint incongruity and severe osteoarthritis (Fig. 27.50).
- **Osgood-Schlatter disease:** It refers to osteonecrosis of tibial tubercle.
- **Köhler bone disease:** It refers to osteonecrosis of **navicular bone**.

Table 27.49 Causes of osteonecrosis

Mechanical disruption of blood vessels
Blood vessel injury
Vasculitis
Bone fractures
Thromboembolism
Sickle cell disease
Nitrogen bubbles in decompression
Gaucher disease
Corticosteroid therapy
Radiation therapy
Legg-Calvé-Perthes disease
Blount disease
Systemic lupus erythematosus
Rheumatoid arthritis
Chronic pancreatitis
Tumors
Epiphyseal disorders

SECONDARY BONE TUMORS (METASTATIC BONE DISEASE)

Secondary bone tumors (metastatic bone disease) in various age groups are given in Table 27.50.

METASTATIC BONE DISEASE

Secondary bone involvement by a malignant tumor originating from another site is known as metastatic bone disease. After lung and liver, skeleton is the most common site of metastatic bone disease.



Fig. 27.50: Avascular necrosis of bilateral femoral heads. Radiograph and post-contrast magnetic resonance imaging demonstrate flattening and irregularity of articular surfaces of bilateral femoral heads. Areas altered marrow signal intensity are seen in bilateral femoral heads. There is involvement of almost entire right femoral head and around 40–50% of left femoral head. Few tiny degenerative changes and cystic areas are seen in right acetabulum. Left acetabulum reveals normal marrow signal. Findings are suggestive of avascular necrosis of bilateral femoral heads shown by arrows. (*Courtesy:* Dr. Vigyat and Dr. Anubhav, Dr. DY Patil Medical College, Pune, Maharashtra.)

Table 27.50 Secondary bone tumors (metastatic bone disease) in various age groups

Secondary Bone Tumors (Metastatic Bone Disease)	
Adult males	
▪ Prostatic carcinoma	▪ Lung carcinoma
Adult females	
▪ Breast carcinoma	▪ Endometrial carcinoma
▪ Thyroid carcinoma	▪ Cervical carcinoma
▪ Renal cell carcinoma	
Children (males and females)	
▪ Rhabdomyosarcoma	▪ Clear cell sarcoma of kidney
▪ Neuroblastoma	

- During course of primary carcinomas originating from breast, prostate, thyroid, lung, gastrointestinal tract, kidney, urinary bladder and melanoma; the adult patients have metastatic bone disease during fourth to seventh decades of life.
- In children, neuroblastoma, renal clear cell sarcoma, osteosarcoma, Ewing sarcoma/primitive neuroectodermal tumor (PNET), and rhabdomyosarcoma metastasize to the bones.
- Basal cell carcinoma, glioma and soft tissue sarcomas (except rhabdomyosarcoma) rarely metastasize to the bones.
- Immunohistochemistry technique is used to diagnose primary carcinoma involving bone.
- Combination of surgical procedures and radiation or other therapies is useful in the management of metastatic bone disease. Metastatic deposits in hematopoietic bone marrow in descending order are given in Table 27.51.

Bones Involvement: Mechanism

Primary malignancies invade bone by direct extension and lymphatic and hematogenous route including Batson paravertebral venous plexus. Skip metastasis or bone-to-bone metastasis has been reported.

- Batson paravertebral venous plexus has communication with vertebral venous plexus and vena cava.
- Vertebral venous plexus is extensive and valveless and having constant communication with breast, thyroid, lung and prostate gland.
- In addition, vertebral venous plexus has communication with dural sinus and vasa vasorum of humerus and femur. There are numerous inter-communications between vascular and lymphatic channels.

Table 27.51 Metastatic deposits in hematopoietic bone marrow in descending order

Metastatic Deposits in Bone Marrow
Vertebral column (most common site)
Pelvis
Ribs
Skull
Humerus proximal end
Femur proximal end

Skeletal metastases are uncommon below knee or elbow joints. Tumors rarely metastasizing to bones include basal cell carcinoma, gliomas and soft tissue sarcomas except embryonal rhabdomyosarcoma.

Solitary and Multifocal Bone Disease

Solitary and multifocal bone metastatic disease is given in Table 27.52.

- **Solitary bone metastatic disease:** It is an independent good prognostic factor. Patients with solitary metastatic bone disease live longer than with multifocal metastatic bone disease. Examples of solitary metastatic bone disease are lung carcinoma, renal cell carcinoma and well-differentiated thyroid carcinoma.
- **Multifocal bone metastatic disease:** Prognosis is poor in patient presenting with involvement of multiple bones by a malignant tumor. Examples of multifocal metastatic bone disease are mammary ductal carcinoma, gastric carcinoma, colorectal carcinoma and neuroblastoma.

Incidence and Locations

Metastatic bone disease is more common than primary malignant bone tumors. Metastases predominantly occur in the red bone marrow of vertebrae, cranium, ribs, sternum, sacrum, pelvic girdle, proximal femur and proximal humerus.

- Approximately 90% of patients have metastatic bone disease due to carcinoma originating from breast, lung, thyroid, prostate, stomach, colorectal region and malignant melanoma.
- Metastasis tends to involve the metaphysis of long bones.

Table 27.52 Solitary and multifocal bone metastatic disease

Bone Metastatic Disease	Example
Solitary metastatic bone disease	▪ Lung carcinoma ▪ Renal cell carcinoma ▪ Well differentiated thyroid carcinoma
Multifocal metastatic bone disease	▪ Mammary adenocarcinoma ▪ Gastric carcinoma ▪ Colorectal carcinoma ▪ Neuroblastoma

- Bone metastases are uncommon below knee or elbow joints. Metastases are rare in small bones of hands and feet.

Age and Sex Predilection

Metastatic bone disease most often occurs in adults 40–60 years of age; and children. Metastatic bone disease in adults and children is given in Table 27.53.

Clinical Features

Symptoms of metastatic bone disease are nonspecific. Patient presents with bone pain with or without swelling; and pathologic fracture. Metastases in vertebrae produce neurological symptoms.

- Hypercalcemia occurs due to osteolytic lesion produced by metastasis.
- Metastases involving skull base may cause Collet-Sicard syndrome characterized by combined and unilateral palsy of cranial nerves IX, X, XI and XII.

Table 27.53 Metastatic bone disease in adults and children

Metastases Involving Bone in Adults
Prostatic carcinoma
Mammary ductal carcinoma
Well-differentiated thyroid carcinoma
Renal cell carcinoma
Transitional cell carcinoma of urinary bladder
Lung adenocarcinoma
Serous cystadenocarcinoma of ovary
Gynecologic endometrioid carcinoma
Gastric carcinoma
Colorectal carcinoma
Hepatocellular carcinoma
Pancreatic carcinoma
Small cell lung carcinoma (neuroendocrine tumor)
Giant malignant pheochromocytoma (neuroendocrine tumor)
Paraganglioma (neuroendocrine tumor)
Malignant melanoma (melanocytic tumor)
Angiosarcoma
Rhabdomyosarcoma
Leiomyosarcoma
Liposarcoma
Malignant peripheral nerve sheath tumor
Alveolar soft part sarcoma
Synovial sarcoma
Undifferentiated pleomorphic sarcoma
Metastases Involving Bone in Children
Rhabdomyosarcoma
Osteosarcoma
Renal clear cell sarcoma

Radiologic Findings

Metastatic bone disease produces either osteolytic (destructive) or osteoblastic lesions. Less frequently, some tumors metastasizing to bone may produce osteolytic/osteoblastic mixed patterns.

- Neoplastic cells synthesize transforming growth factor β (TGF-β), fibroblastic growth factor (FGF), bone morphogenic proteins, prostaglandins, interleukin 1 (IL-1) and many other proteins, which modulate bone changes. Activity of the osteoblasts may also produce structural changes.
- Initial evaluation of metastatic bone disease should begin with conventional radiograph. Metastatic bone lesion in vertebrae may be detected by radiograph in 25% of cases; and CT scan and MRI in almost all cases. Radioisotope scintigram is done to detect multiple bone metastatic disease from carcinomas in almost all cases.

Osteolytic Lesions in Metastatic Bone Disease

Renal cell carcinoma, well-differentiated thyroid carcinoma, gastric adenocarcinoma, colorectal carcinoma, melanoma, non-Hodgkin's lymphoma and neuroblastoma metastasizing to the bone produce osteolytic and destructive lesions resulting in hypercalcemia and increased excretion of calcium in urine. Increased excretion of hydroxyproline containing peptide in urine indicates bone matrix destruction.

Osteoblastic Lesions in Metastatic Bone Disease

Prostatic adenocarcinoma, mammary ductal carcinoma and Hodgkin's disease metastasizing to the bone produce osteoblastic lesion.

- Prostatic adenocarcinoma metastasizing to the bone most often presents with osteoblastic lesion associated with periosteal reaction simulating primary bone sarcoma.
- Metastatic bone disease involving metaphysis can simulate primary bone sarcoma.
- Pathologic fracture in metastatic bone disease produces florid ossification demonstrated on radiograph may simulate osteosarcoma.
- Osteoblastic lesion in metastatic bone disease is associated with increased serum alkaline phosphatase and decreased serum calcium level.

Table 27.54 Osteolytic or osteoblastic lesion on radiograph in metastatic bone disease

Findings on Radiograph (Tumors Metastasizing to Bone)	Comments
Osteolytic and destructive lesion on radiograph	
▪ Renal cell carcinoma ▪ Well differentiated thyroid carcinoma ▪ Gastric adenocarcinoma ▪ Colorectal carcinoma ▪ Malignant melanoma ▪ Non-Hodgkin's lymphoma ▪ Neuroblastoma	▪ Increased serum calcium ▪ Increased excretion of calcium in urine ▪ Increased excretion of hydroxyproline containing peptide in urine indicates bone matrix destruction
Osteoblastic lesions on radiograph	
▪ Prostatic adenocarcinoma ▪ Mammary ductal carcinoma ▪ Hodgkin's disease	▪ Increased serum alkaline phosphatase ▪ Decreased serum calcium
Either osteolytic or osteoblastic lesion on radiograph	
▪ Small cell lung carcinoma ▪ Mammary ductal carcinoma	▪ Serum and urinary findings depend on type of lesion

Osteolytic/Osteoblastic Lesions in Metastatic Bone Disease

Small cell lung carcinoma and mammary ductal carcinoma metastasizing to the bone may produce either osteolytic or osteoblastic lesion on radiograph. Osteolytic or osteoblastic lesion on radiograph in metastatic bone disease is given in Table 27.54.

Surgical Pathology: Metastatic Bone Disease

Gross Morphology

- Gross morphologic features of metastatic bone disease are usually nonspecific simulating primary bone sarcomas.
- Metastatic bone disease lesions are osteolytic with soft in consistency and areas of hemorrhage.
- Desmoplastic reaction of stroma in metastatic bone disease produces grayish white lesion with firm in consistency.
- Prostatic adenocarcinoma metastasizing to the bone produces dense hard osteoblastic lesion.
- Periosteal new bone formation is infrequent.
- Extraosseous extension of metastatic bone disease may be demonstrated.

Light Microscopy

- Well-differentiated carcinoma from breast, prostate, kidney and thyroid gland metastasizing to bone resemble the histology of primary tumor.
- Undifferentiated or poorly differentiated tumors metastasizing to bone poses diagnostic difficulty on histologic examination.
- Sarcomatoid carcinomas of kidney, thyroid gland and lung lack apparent epithelial differentiation and therefore simulate spindle cell sarcoma of bone.
- Prostatic adenocarcinoma, mammary ductal carcinoma and neuroendocrine tumors produce reactive bone formation simulating osteosarcoma.
- Presence of numerous osteoclastic multinucleated giant cells in metastatic bone disease can be confused with giant cell tumor of bone.

Immunohistochemistry Technique

Immunohistochemistry technique plays an important role to differentiate metastatic bone disease and primary bone sarcomas.

- Immunostains for cytokeratins (AE1/AE3, CAM 5.2, CK7 and CK20) and epithelial membrane antigen (EMA) are most often positive in epithelial and negative in mesenchymal malignancies.
- In some cases, immune markers may be helpful to diagnose metastatic bone disease.
- Some examples are TTF-1 (thyroid transcription factor 1) for well differentiated thyroid carcinoma and lung adenocarcinoma; PSA (prostate-specific antigen) for prostatic adenocarcinoma, S-100 protein, MHB-45 and melan-A (A103) and MART-1 (melanoma-associated antigen recognized by T cells) for malignant melanoma.
- Other examples of immunohistochemical markers include NSE (neuron-specific enolase), synaptophysin and chromogranin A for neuroendocrine tumors; ER/PR, GCDFP (gross cystic disease fluid protein), mammaglobin and GATA3 for mammary ductal carcinoma, CDX2 and villin for gastric adenocarcinoma and colorectal carcinoma.
- Applications of immunohistochemistry technique in analysis of cytokeratins are given in Table 27.55. CK7 and CK20 immunohistochemistry technique expression profiles are given in Table 27.56. Role of immunohistochemistry technique in breast carcinoma is given in Table 27.57.

Table 27.55 Applications of immunohistochemistry technique in analysis of cytokeratins

Monoclonal Antibody Used	Analysis of Various Types of Cytokeratins
AE1 antibody	All acidic cytokeratins except CK9, CK12, CK17, CK18
AE3 antibody	All basic cytokeratins
AE1/AE3 antibody (pan-CK)	▪ Broad spectrum pan-cytokeratins antibody to identify both LMWCK and HMWCK ▪ AE1/AE3 antibody (pan-CK) is the first line marker, which detects both LMWCK and HMWCK present in squamous cell carcinoma, colon adenocarcinoma, prostatic carcinoma, hepatocellular carcinoma and renal cell carcinoma, adrenocortical neoplasms ▪ AE1/AE3 antibody reactivity is variable in neuroendocrine tumors. Therefore, precautions should be taken in interpretation of results
OSCAR, PANK (MNF-116) antibody	These are also broad spectrum cytokeratins similar to AE1/AE3 antibody used to identify both LMWCK and HMWCK
CAM 5.2 (34bH11) antibody	▪ CAM 5.2 (34βH11) antibody is a good complement to AE1/AE3 antibody (pan-CK). It detects those carcinomas missed by AE1/AE3 antibody (pan-CK) ▪ CAM 5.2 (34βH11) antibody demonstrates LMWCK (CK8, CK18) expressed by simple/non-squamous keratins in glandular epithelia (e.g. colon adenocarcinoma, prostatic adenocarcinoma); and visceral parenchyma (e.g. hepatocellular carcinoma, renal cell carcinoma)
CK903 (K903;34bE12) antibody and CK5/CK6 antibody	CK903 and CK5/CK6 antibodies demonstrate HMWCK (CK1, CK5, CK10, CK14) without LMWCK (CK1, CK5, CK10, CK14) expressed in keratinizing squamous epithelia and in majority of squamous cell carcinoma, however, some poorly differentiated non-keratinizing carcinomas show positivity for both HMWCK and LMWCK
Expression of cytokeratins (and EMA) is key diagnostic feature of carcinomas. Cytokeratin positivity may also be seen synovial sarcoma, epithelioid sarcoma, epithelioid angiosarcoma, non-seminomatous germ cell tumors (yolk sac tumor, embryonal carcinoma), trophoblastic tumors, mesothelioma, chordoma and thymoma, and aberrant expression of leiomyosarcoma, melanoma and peripheral nerve sheath tumor	
HMWCK (high molecular weight cytokeratin), LMWCK (low molecular weight cytokeratin)	

Table 27.56 CK7 and CK20 immunohistochemistry technique expression profiles

CK7 and CK20 Immunohistochemistry Technique	Location of Organs	Examples of Malignancy of Organs	
CK7+ and CK20–	Organs above the diaphragm	▪ Breast ▪ Lung and pleura ▪ Thyroid gland ▪ Salivary gland	▪ Kidney (renal papillary carcinoma) ▪ Female genital system (uterus and ovary)
CK7– and CK20+	Organs below the diaphragm	▪ Colon ▪ Rectum	▪ Merkel cell carcinoma
CK7+ and CK20+	Organs in peridiaphragmatic region	▪ Stomach ▪ Pancreas	▪ Biliary tree ▪ Urinary bladder
CK7– and CK20– (except colon)	Simple visceral epithelia	▪ Liver ▪ Kidney	▪ Prostate gland

Prognosis and Treatment

Prognosis of metastatic bone disease depends on the primary site of the tumor, histologic type and extent of disease. Aim of treatment of metastatic bone disease is to relieve pain and prevent pathologic fracture. Localized solitary metastatic deposit may be surgically resected. Radiotherapy and chemotherapy may be effective in controlling the symptoms. Specific therapies should be administered to the patients. ER/PR positive mammary ductal carcinoma is treated by tamoxifen. Herceptin is administered in HER2/neu positive mammary ductal carcinoma.

Table 27.57 Role of immunohistochemistry technique in breast carcinoma

Immunohistochemical Markers	Expression
Ductal carcinoma of breast in general	
▪ Estrogen/progesterone receptors (ER/PR) ▪ GCDFP-15 (gross cystic disease fluid protein 15) ▪ Mammoglobin ▪ HER2/neu growth factor receptor ▪ Ki-67 (proliferative marker) ▪ p53 ▪ CK7 ▪ GATA3	▪ Positive (nuclear staining in 50% of cases) ▪ Positive (cytoplasmic staining in 50% of cases) ▪ Positive (cytoplasmic staining in 50% of cases) ▪ Positive (membranous staining in 10–30% of cases) ▪ Positive ▪ Positive ▪ Positive (cytoplasmic staining) ▪ Positive
Lobular carcinoma of breast (*in situ* and invasive)	
▪ Loss of E-cadherin ▪ GCDFP-15 (gross cystic disease fluid protein 15)	▪ Positive ▪ Positive (cytoplasmic staining in 100% of cases)
Metaplastic carcinoma of breast	
▪ CK903 ▪ p63 ▪ CAM 5.2 (most reliable HMWCK marker) ▪ CK7 (most reliable HMWCK marker)	▪ Positive (cytoplasmic staining) ▪ Positive ▪ Positive (cytoplasmic staining) ▪ Positive (cytoplasmic staining)

DISORDERS OF JOINTS

CLASSIFICATION OF JOINTS

Joints are classified into three categories described as under:

- **Synarthroses joints:** Synarthroses joints are fibrous immovable joints, which do not allow any movement. Examples are coronal suture of skull and root of teeth in socket.
- **Amphiarthroses joints:** Amphiarthroses joints are cartilaginous or lightly movable joints. The bones are joined together with the help of cartilages, which permit limited movements. Examples are intervertebral joints and pubic symphysis.
- **Diarthroses synovial joints:** Diarthroses synovial joints contain synovial fluid in synovial cavity between the articulating surfaces of the two bones. Such an arrangement allows considerable free movements. Examples of synovial joints are given in Table 27.58.
  - Synovium is a glistening white membrane with villous projections. Synovium lines the inner surface of the joint capsule.
  - Factors affecting movement of diarthroses joints include structure or shape of the articulating bones, strength and tension of the joint ligaments and arrangement, and tension of the skeletal muscles around the joints (Fig. 27.51).

Table 27.58 Salient features of diarthroses synovial joints

Diarthroses Synovial Joints	Examples
Ball and socket joints	Shoulder joint (between humerus and pectoral girdle)
Hinge joint	Knee and elbow joints
Pivot joint	Atlas rotation around axis vertebrae
Gliding joint	▪ Joint between carpal bones of upper limb ▪ Joint between navicular and tarsus bone of foot
Saddle joint	Joint between trapezium and metacarpal of thumb
Condyloid joint	Joint between carpals and radius

Arthroscopy is a procedure that involves examination of the interior of joint especially knee to determine the nature and extent of injury.

ARTHRITIS OF PROBABLE AUTOIMMUNE ORIGIN

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a chronic multisystem disorder of unknown cause. Its hallmark is persistent

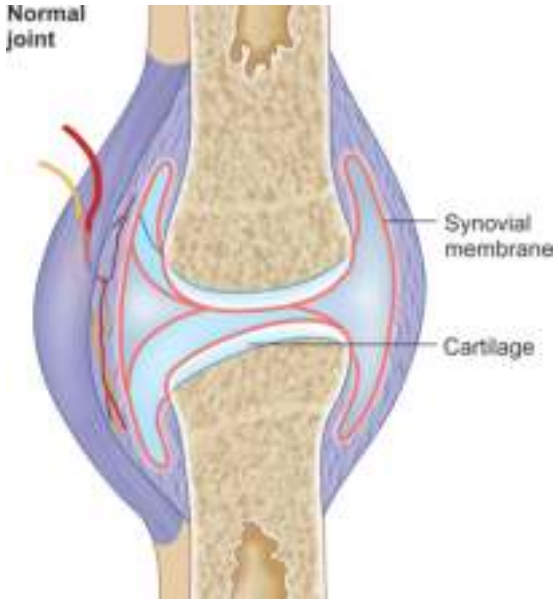


Fig. 27.51: Synovial joint is most common type of diarthrodial joints, which is covered by hyaline articular cartilage composed of collagen proteoglycans and water. This joint is enclosed in capsule. Joints are affected mostly by inflammatory and degenerative diseases.

inflammatory synovitis involving bilateral peripheral joints (proximal interphalangeal and metacarpophalangeal joints of the hands) in a symmetric distribution. Surrounding skeletal muscles, tendons, ligaments, blood vessels and knee joints may also be involved. Comparison of osteoarthritis and rheumatoid arthritis is given in Table 27.59.

Age Group

Rheumatoid arthritis is potentially crippling disease affecting more often women between 20 and 50 years of age. It occurs most often in HLA-DR4 positive individuals.

Hallmark of Rheumatoid Arthritis

Subcutaneous nodules and rheumatoid factor (autoantibodies principally IgM or IgG or IgA) are hallmarks of rheumatoid disease. These autoantibodies are directed against the Fc fragment of IgG.

- Rheumatoid factor (RF) is demonstrated in the serum in 80% of classic rheumatoid arthritis patients. Rheumatoid factor is useful in diagnosis.
- Significant titers of RF may also be demonstrated in related collagen vascular diseases, such as systemic lupus erythematosus (SLE), scleroderma, and dermatomyositis.

Pathologic Changes in Rheumatoid Arthritis

Pathologic changes in rheumatoid arthritis are shown in Fig. 27.52A and B.

Table 27.59 Comparison of osteoarthritis and rheumatoid arthritis

Feature	Osteoarthritis	Rheumatoid Arthritis
Joints involvement	▪ Weight bearing joints (hips, knees) ▪ Proximal interphalangeal (PIP) joints ▪ Distal interphalangeal (DIP) joints	Metacarpophalangeal (MCP) joints, proximal interphalangeal (PIP) joints, feet, wrists, ankles, elbow, knees
Morning stiffness of joints	<30 minutes	>1 hour
Clinical features	Pain occurs with movement of joints, feels better after rest	Stiffness and pain in joints after inactivity
Physical examination	Heberden and Bouchard lymph nodes	Rheumatoid nodules, radial deviation of wrist and ulnar deviation

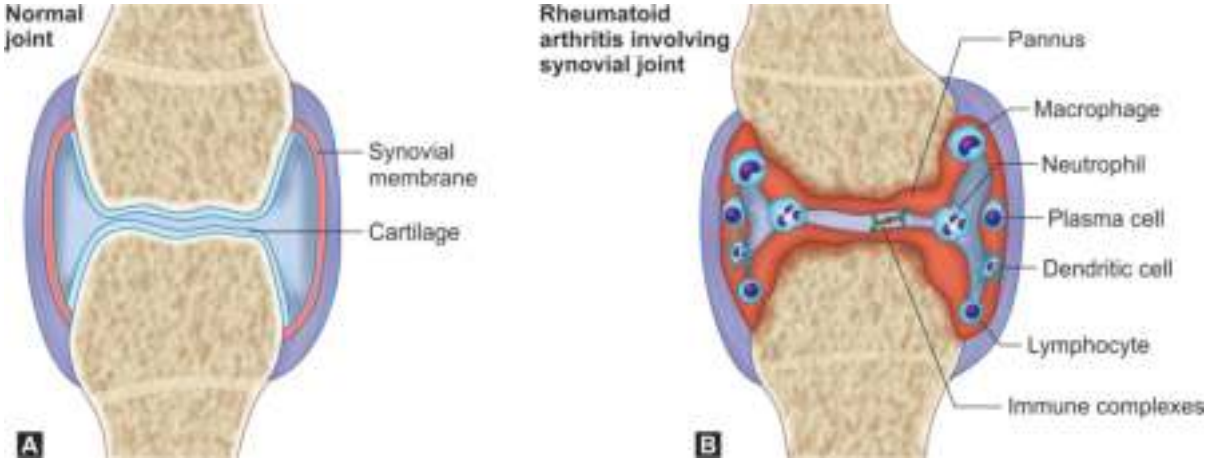


Fig. 27.52: Rheumatoid arthritis. (A) Normal synovial joint, (B) pathologic changes in rheumatoid arthritis.

- **Inflammatory stage:** Synovitis develops from congestion and edema of the synovial membrane and joint capsule. Initially, it shows acute inflammation followed by infiltration by lymphocytes and plasma cells. Synovial cells undergo hyperplasia and hypertrophy with formation of numerous villi and frond-like folds.
- **Formation of pannus (thickened layers of granulation tissue):** It covers and invades articular cartilage and subchondral bone, which eventually erodes and destroys the joint capsule and bone.
- **Fibrous ankylosis:** It occurs as a result of fibrous invasion of the pannus and scar formation that occludes the joint space. Bone atrophy and misalignment cause visible deformities and restrict movement, causing muscle atrophy, imbalance, and possibly partial dislocations.
- **Bony ankylosis:** Fibrous tissue calcifies, resulting in bony ankylosis (fixation of a joint) and total immobility. Pain associated with movement may restrict active joint use and resulting in fibrous or bony ankylosis, soft tissue contractures, and joint deformities. Subcutaneous nodules develop in approximately 33% of patients.

Clinical Features

- **Acute phase of disease:** Patient presents with fatigue, malaise, anorexia, weight loss, fever, and myalgia, bilateral symmetrical swelling and stiffness of joints especially in the morning or after inactivity.
- **Chronic phase of disease:** Patient develops flexion contracture of small joints of hands resembling curve of a swan-neck as a result of flexion of metacarpophalangeal joints and distal interphalangeal joints.
  - Boutonnière deformity occurs as a result of flexion of proximal interphalangeal joints with compensatory hyperextension of distal interphalangeal joints.
  - Patient develops ulnar deviation (drift) of fingers resulting from stretching of articular capsule and muscle imbalance. Minimal radial deviation of the wrist may occur. Rheumatoid nodules are formed in metacarpophalangeal joints and distal interphalangeal joints (Figs 27.53 and 27.54).

Extra-articular Manifestations

Extra-articular manifestations in rheumatoid arthritis include effusions (pleura, pericardium), vasculitis, and anemia of chronic disease, neurologic manifestations, lymphadenopathy and secondary reactive amyloidosis.

Association of Rheumatoid Arthritis

- **Sjögren syndrome:** Patient with Sjögren syndrome is associated with rheumatoid arthritis. Sjögren syndrome is a chronic inflammatory disease of the salivary (xerostomia) and lacrimal glands (keratoconjunctivitis sicca), which may be associated with a systemic collagen vascular disease. Malignant lymphoma is a frequent complication.

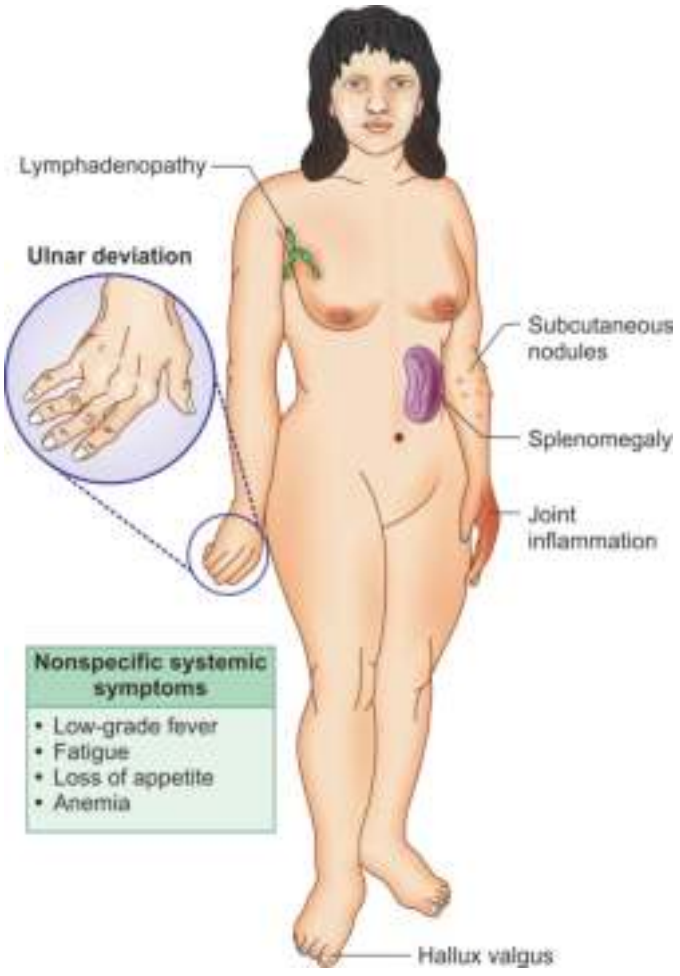


Fig. 27.53: Rheumatoid nodules over an interphalangeal joint of a patient with rheumatoid arthritis.



Fig. 27.54: Rheumatoid nodule (arrows).

- **Felty syndrome:** It is characterized by splenomegaly, neutropenia, and rheumatoid arthritis.
- **Still disease (juvenile rheumatoid arthritis):** Approximately 20% of children with juvenile arthritis (polyarticular) present with fever, rash, hepatosplenomegaly, lymphadenopathy, pleuritis, and anemia. Many children with juvenile arthritis develop ankylosing spondylitis, rheumatoid arthritis, psoriatic arthritis, and other connective tissue diseases.

Surgical Pathology: Rheumatoid Arthritis

Light Microscopy

- Histologic changes in rheumatoid arthritis occur due to destruction of the joint.
- Synovium undergoes hypertrophy and hyperplasia along with lymphocytes and plasma cells infiltration.
- Lymphoid follicles, acute fibrinous surface exudate, synovial giant cells may be seen.
- Destruction of cartilage and joint fusion (ankylosis) may occur due to formation of pannus.
- Pannus is composed of inflamed synovium and granulation tissue over the surface of overlying cartilage (Fig. 27.55).

ANKYLOSING SPONDYLITIS

Ankylosing spondylitis is probably autoimmune disorder with genetic component. It most often affects vertebral column and sacroiliac joints especially in young men. Bony fusion (ankylosis) causes rigidity and fixation of the spine (Fig. 27.56).

Etiopathogenesis

Ankylosing spondylitis occurs in various settings.

- **Genetic predisposition:** Almost all patients are positive for HLA-B27 antigen in 90% of cases.

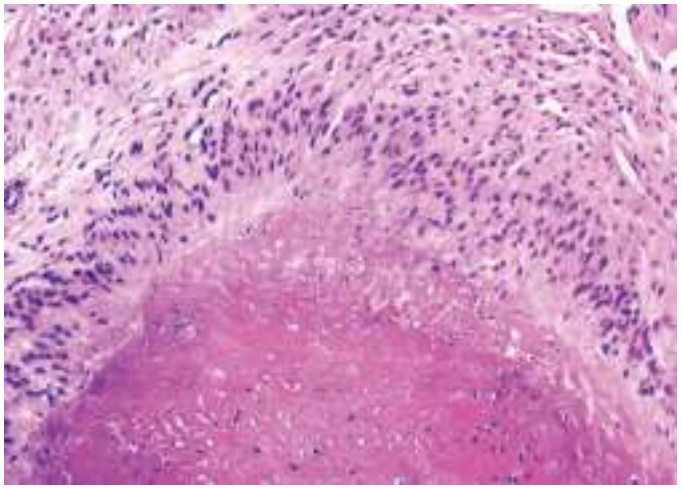


Fig. 27.55: Rheumatoid nodule. It is composed of lymphocytes, plasma cells, lymphoid follicles, acute fibrinous surface exudate, synovial giant cells may be seen (100X).

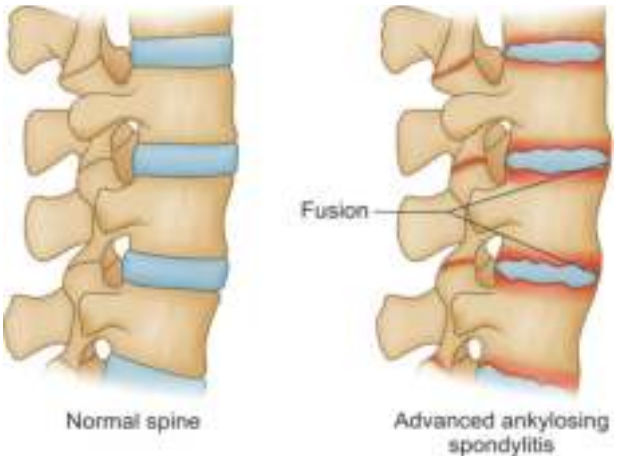


Fig. 27.56: Ankylosing spondylitis. It shows fusion of vertebrae.

- **Reiter syndrome:** It is triad that includes seronegative polyarthritis, conjunctivitis, and urethritis. It is often associated with venereal or bacillary dysentery in men. It is also associated with HLA-B27 antigen in up to 90% of patients.
- **Psoriatic arthritis:** It occurs in 10% of patients with psoriasis. Patient develops peripheral arthritis or ankylosing spondylitis complicating psoriasis.
- **Arthritis associated with inflammatory bowel disease:** Patient develops peripheral arthritis or ankylosing spondylitis complicating ulcerative colitis or Crohn's disease.

DEGENERATIVE DISEASE OF JOINTS

OSTEOARTHRITIS

Osteoarthritis is a slowly progressive destruction of the articular cartilage. Primary osteoarthritis is of unknown etiology and related to mechanical injury (wear-and-tear) and genetic predisposition. Secondary osteoarthritis occurs due to mechanical factors, ochronosis and inflammatory disorders.

Age Group

Osteoarthritis involves weight-bearing joints and interphalangeal joints especially in women more than 50 years of age.

Pathogenesis

Articular cartilage loses its elasticity resulting in fragmentation, which floats into synovial fluid. Floating cartilage erodes bone, which exhibits polished, ivory-like appearance (eburnation). It is accompanied by formation of new bone (osteocytes) subchondrally and at the margins of the affected joint.

Clinical Examination

Osteophytes (bony spurs) are formed at points of ligamental attachment to bone.

- Osteophytes formed at distal interphalangeal joints are known as Heberden nodes and Bouchard nodes at proximal interphalangeal joints.
- Osteophytes undergo fracturing and floating into synovial fluid known as joint mice.

Surgical Pathology: Osteoarthritis
Gross Morphology
▪ Gross examination reveals cartilaginous thinning, disruption of joints. ▪ There is complete loss of cartilage resulting in exposure of underlying bone exhibiting dense polished marble-like appearance.
Light Microscopy
▪ Histopathologic examination shows characteristic vertical clefts in the cartilage. ▪ There may be associated chronic inflammation of synovium and villus hyperplasia. ▪ Later findings should not be confused with rheumatoid arthritis.

ARTHRITIS OF METABOLIC ORIGIN

GOUTY ARTHRITIS

Gout is a metabolic disorder of disturbed purine metabolism, associated with elevated serum uric acid, which occurs primarily in men over 40 years of age.

- The deposition of monosodium urate crystals leads to formation of gouty tophi in metatarsophalangeal joint of the great toe (podagra), cartilage of helix and antihelix of the ear, and the Achilles tendon.
- Joint involvement is extremely painful in acute arthritis and bursitis.

Pathogenesis

When sodium urate crystals precipitate from supersaturated body fluids, which absorb fibronectin, complement, and other proteins on joint surfaces and initiate intense inflammatory reaction with opsonization of crystals by IgG.

- Neutrophils phagocytose urate crystals resulting in release of proteolytic lysosomal enzymes and oxygen-derived free radicals, which mediate tissue injury and promote an inflammatory response. It eventually leads to formation of nodular gouty tophi in the joints.
- Gouty tophus is an extracellular soft tissue deposit of urate crystals surrounded by fibrous connective tissue, foreign body giant cells and mononuclear cells.

Variants of Gout

- **Primary gout:** Primary gout is most common type of gout (85%) and is characterized by hyperuricemia without evident cause affecting middle-aged men.
- **Secondary gout:** Secondary gout is much less common and is characterized by hyperuricemia with evident cause, such as multiple myeloma, leukemia, myeloproliferative syndromes and chronic renal disease.
  - Patient with chronic renal disease has decreased urate excretion.
  - **Lesch-Nyhan syndrome** is an X-linked disorder due to deficiency of hypoxanthine-guanine phosphoribosyl transferase which produces hyperuricemia with severe neurological manifestations.

Clinical Features

Gouty arthritis is characterized by severe pain, redness and tenderness in joints often big toe. Severe attack of pain can occur suddenly often at night. During an acute attack, anti-inflammatory medication (NSAIDs) can help to reduce pain and shorten the duration of attack.

Patient presents with features due to acute gout arthritis and urate nephropathy described as under:

- **Acute gouty arthritis:** Acute gout usually involves first metatarsophalangeal joint. Clinical examination reveals redness, swelling, heat and extreme tenderness. In this characteristic location is known as podagra. A large meal or alcohol intake may increase hyperuricemia, often precipitates an exacerbation of the disorder.
- **Urate nephropathy:** Gout often leads to urate nephropathy characterized by interstitial deposition of urate crystals and obstruction of collecting tubules due to deposition of urate crystals and formation of urate and calcium stones.

Handling Fresh Specimen of Gouty Arthritis

In suspected cases of gout, fresh specimen should be handled carefully. Because sodium urate crystals are soluble in water component of 10% formalin preparation. Therefore preservation of sodium urate crystals is done by preparing touch preparation fixed in 100% alcohol.

Surgical Pathology: Gouty Arthritis
Light Microscopy
▪ Chronic accumulation of uric acid crystals leads to the formation of nodules (gouty tophi) in joint that contain granuloma-like aggregates of macrophages. ▪ Gouty tophus is composed of multiple irregular foci of amorphous pink stained material surrounded by foreign body giant cells reaction (Fig. 27.57). ▪ Gouty tophi may show secondary calcification and ossification. Gouty tophi may be demonstrated by De Galantha (silver impregnation) stain.

Laboratory Diagnosis

The diagnosis of gout is based on the finding of hyperuricemia along with urate crystals and neutrophils in synovial fluid or with biopsy evidence of tophaceous deposits. Urate crystals are needle-shaped and negatively birefringent under polarized light.

Autopsy Findings

During postmortem examination in suspected case of gout, one should expect gouty tophi, nephrolithiasis, white chalky paste-like material in synovium and joint deformity in long-standing gout.

CHONDROCALCINOSIS (PSEUDOGOUT)

Chondrocalcinosis (pseudogout) occurs due to deposition of calcium pyrophosphate dihydrate crystals in the synovial membranes (pseudogout), joint cartilage, ligaments, and tendon; which elicits an inflammatory reaction in cartilage.

- Under polarized light, these rhomboid-shaped crystals are positively birefringent.
- Pseudogout clinically resembles gout. It refers to self-limited attacks of acute arthritis lasting from 1 day to 4 weeks and involving one or two joints. It most often affects older than 85 years.

Clinical Features

Patient presents with acute onset of gout-like symptoms, manifesting as inflammation and swelling of knees, ankles, wrists, elbows, hips, or shoulders.

OCHRONOSIS OF JOINTS

Ochronosis is deposition of polymerized homogentisic acid, a black pigment, which is the product of incomplete degradation of tyrosine and phenylalanine occurring due to inborn defect of enzyme homogentisic acid oxidase.

Ochronosis may result in black discoloration of the tracheal rings, nephrolithiasis and vertebral ankylosis on postmortem examination.

Joints Involved

Homogentisic acid is deposited in articular cartilage of vertebral column and later hips, knees and shoulder joints.

Cartilage of nose and ears is also affected. Larger joints may be distended by noninflammatory effusions and hypertrophy of synovium.

Surgical Pathology: Ochronosis of Joints

Light Microscopy

Biopsy from tissue involved in ochronosis shows granular pigment in chondrocytes and matrix is diffusely stained. Synovium shows evidence of acute or chronic inflammation (Fig. 27.58).

INFECTIVE ARTHRITIS

Infective arthritis is characterized by purulent synovial fluid.

GONOCOCCAL ARTHRITIS

Gonococcal arthritis is caused by *Neisseria gonorrhoeae*. It is the most common form of bacterial arthritis (monoarticular). It most often involves knee joint. Other joints such as wrist and small joints of the hand may also be affected.

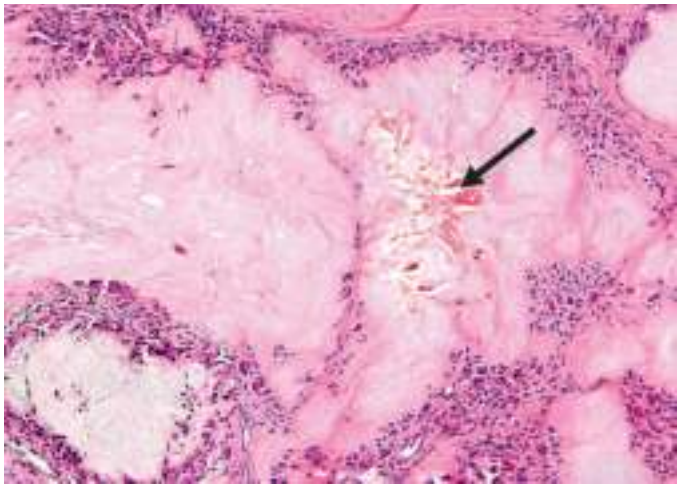


Fig. 27.57: Gouty tophus. It shows multiple irregular foci of amorphous pink stained material of surrounded foreign body giant cells reaction (arrow) (100X).

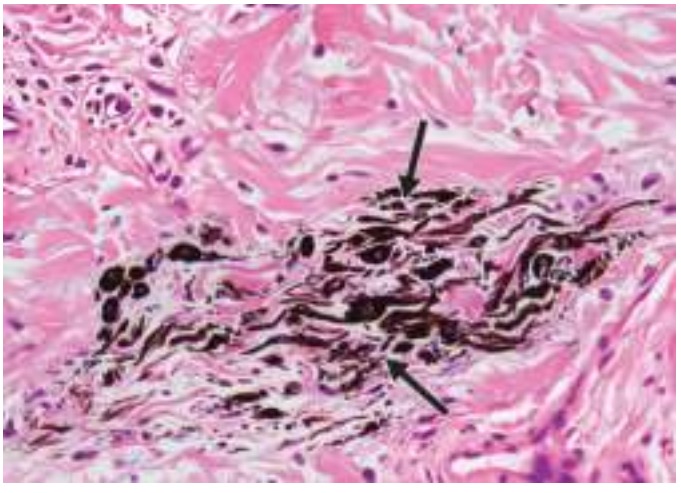


Fig. 27.58: Ochronosis. It occurs due to excessive deposition of homogentisic acid in connective tissues, cartilage and sclera. Histologic examination shows yellow-brown deposits in the cartilage (arrows).

LYME ARTHRITIS

Spirochete *Borrelia burgdorferi* transmitted by a tick causes Lyme disease. Lyme arthritis occurs when *Borrelia burgdorferi* enters joint tissue and induces inflammation. If Lyme arthritis left untreated permanent damage to the joint can occur.

Clinical Features

Patient presents with erythema chronicum migrans with central fading (bull's eye lesion), polyarticular arthritis involving larger joints (especially knee joints). It can also lead to myocardial, pericardial, or neurologic changes as late sequelae.

Laboratory Diagnosis

Lyme disease is diagnosed by demonstration of IgM serum antibodies to *Borrelia burgdorferi.*

Treatment

Response to early treatment with antibiotics is good.

TUBERCULAR ARTHRITIS

Tubercular arthritis is caused by the bacteria, *Mycobacterium tuberculosis*. Hip joint is most often involved in tuberculosis. There is fullness of groin and lower buttocks with loss of gluteal fold on affected side, flexion of hip or thigh and pain on pressure.

MISCELLANEOUS DISORDERS OF JOINTS

HYPERTROPHIC OSTEOARTHROPATHY

Hypertrophic osteoarthropathy is associated with systemic diseases such as chronic lung interstitial disease, congenital cyanotic heart disease, cirrhosis of liver, and inflammatory bowel disease.

- Patient develops clubbing of the fingers (most frequent), inflammation of distal end of the radius and ulna.
- Patient presents with painful swelling and tenderness of the peripheral joints.

GANGLION CYST

Ganglion cyst is small cystic and painless nodule arising in the tendon sheath or the joint capsule of wrist, which is thought to be caused by myxoid degeneration of connective tissue.

TIBIAL PSEUDOARTHROSIS

If a tibial fracture site does not heal, the condition is termed nonunion. Pseudoarthrosis occurs due to interposition of soft tissues at the fracture site, excessive motion, infection, and poor blood supply. Continued movement at the unhealed fracture may produce pseudoarthrosis. The joint-like material must be surgically excised for the fracture to heal properly.

Bone Specimen Handling in Surgical Pathology

Bone biopsies may be done to evaluate bone pain or tenderness, investigate an abnormality on radiographs, nature of bone tumor and find the cause of an unexplained inflammation. Magnetic resonance imaging is the gold standard for detailed evaluation of bone tumors and tumor-like lesions. Bone specimen requirements should be standardized and reported on every specimen. There are three main areas of grossing technology (i.e. tissue fixation, tissue sampling and embedding orientation for optimal microtomy), which can be beneficial to standardization of digital microscopic images of hematoxylin and eosin-stained tissue sections. Gross technology contributes to microslide images standardization.

- **Bone specimen collection and handling:** Patient's identity such as full name, age, sex, and identification number must be verified at the time of specimen collection. Specimen is labeled in the presence of the patient. All subsequent labeling of patient's blocks and slides must follow same patient-specific identifying process.
  - Recommended tissue fixative is 10% neutral buffered formalin for a minimum of six hours. Volume of the fixative to tissue ratio must be included in the collection/handling and submission procedures, i.e. 10% neural buffered formalin volume should be 15–20 times the volume of the tissue specimen.
  - Specimen must be placed in leak proof container properly labeled and transported to the laboratory immediately after collection. Containers should be rigid, impermeable, unbreakable, and non-reactive to fixative solutions.
  - All unfixed specimens should be placed in a sterile saline soaked sponge, transported to the pathology laboratory as soon as possible and refrigerated until placed into appropriate fixative. Written request for patient testing to include clinical history and all relevant investigations carried out.
- **Standard laboratory processing technique:** The use of surgical instruments should be avoided to prevent crushing or damaging the soft tissue attached to bone specimen. Adequate neutral buffered formalin fixative should be added to the specimen container as soon as possible. If insufficient tissue fixative is present when the specimen is received in the laboratory additional fixative should be added.
  - Pathologist determines correct orientation of bone specimen, immobilization, and appropriate cutting instrument. From grossing point of view, there are two types of bones: (a) compact bones (e.g. shaft of long bones, femoral head, and flat bones of skull), and (b) cancellous, trabecular, or spongy bones (e.g. vertebrae, ribs and epiphysis of long bones posing a challenge in the grossing room). Bone resections are performed for both benign and malignant tumors. Pathologist should review the results of prior biopsy along with the patient's clinical history and radiologic findings.

- Bone consists of osteocytes surrounded by a calcified matrix containing type 1 collagen fibers. In the calcified matrix, calcium is present in the form of hydroxyapatite crystals (i.e. calcium phosphate) deposited between the fibrous elements. Therefore, it is impossible to prepare in sections from 10% buffered neutral formalin-fixed paraffin-embedded tissue by standard microtomy without proper prior decalcification of the tissue containing bone, teeth, or calcifications.
- Decalcification technique removes minerals from the bone, so that good quality paraffin sections can be prepared that preserve all the essential microscopic elements. Decalcification is carried out by immersing the tissue in decalcification fluids (e.g. inorganic and organic acids, neutral fluid containing EDTA, and Bouin's fluid) after the surgical specimen has been thoroughly fixed and prior to routine processing to prepare paraffin block. Monitor the specimen frequently decalcification is detrimental to histologic quality.
- Scant bony fragments can be frozen fresh without decalcification and cut with cryostat. All heme bone core biopsies should be decalcified in ethylenediaminetetraacetic acid (EDTA), as the need for molecular genetic testing cannot always be predicted. Strong hydrochloric acid quickly decalcifies bone, but destroys nucleic acids. Formic acid slowly decalcifies bone, but also destroys nucleic acids. Chelating agent such as EDTA is the slowest, but also best at preserving nucleic acids.
- Cutting unform thin tissue sections 3–5 µm, with minimal artifacts is one of the most important steps in diagnostic pathology. Tissue sample should be submitted for light microscopy (histologic type, grade of bone tumor and adequacy of tumor margins), electron microscopy, cytogenetics, and flow cytometry.

CHAPTER

28

Soft Tissue Tumors and Skeletal Muscle Non-Neoplastic Disorders

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

SOFT TISSUE TUMORS

- Soft tissue tumors: overview
  - Clinical history
  - Histopathologic examination
  - Immunohistochemistry technique
- Adipocytic tumors
  - Lipoma
  - Hibernoma
  - Well-differentiated liposarcoma/atypical lipomatous tumor
  - Dedifferentiated liposarcoma
  - Myxoid liposarcoma
  - Pleomorphic liposarcoma
- Fibroblastic/myofibroblastic tumors and other lesions
  - Keloid
  - Fibromatosis colli
  - Nodular fasciitis
  - Superficial fibromatosis (Dupuytren's disease or contracture)
  - Infantile fibrosarcoma (congenital fibrosarcoma)
  - Dermatofibrosarcoma protuberans
  - Conventional adult fibrosarcoma and other histologic variants
  - Myxofibrosarcoma
- Fibrohistiocytic tumors
  - Benign fibrous histiocytoma
  - Tenosynovial giant cell tumor localized type
  - Juvenile xanthogranuloma
  - Xanthoma
  - Atypical fibroxanthoma
  - Plexiform fibrohistiocytic tumor
  - Angiomatoid fibrous histiocytoma
  - Soft tissue giant cell tumor with low malignant potential
  - Undifferentiated pleomorphic sarcoma
- Smooth muscle tumors
  - Soft tissue leiomyoma
  - Soft tissue leiomyosarcoma
- Skeletal muscle tumors
  - Rhabdomyoma
  - Embryonal rhabdomyosarcoma
  - Alveolar rhabdomyosarcoma
  - Pleomorphic rhabdomyosarcoma
  - Adult sclerosing rhabdomyosarcoma
- Gastrointestinal stromal tumors
  - Gastrointestinal stromal tumors (benign/low-grade and malignant)
- Vascular tumors of soft tissue
- Pericytic/perivascular tumors
  - Hemangiopericytoma, solitary fibrous tumor of soft tissue and related lesions
  - Myopericytoma family of tumors
  - Glomus tumor
  - Atypical and malignant glomus tumors
- Neural and perineural tumors and other lesions
  - Traumatic neuroma
  - Granular cell tumor
  - Neurofibroma, neurofibromatosis type 1 and malignant change in neurofibroma
  - Schwannoma and neurofibromatosis type 2
  - Psammomatous melanocytic schwannoma
  - Perineuroma (sclerosing, intraneural, soft tissue and reticular perineuromas)
  - Nerve sheath myxoma
  - Malignant peripheral nerve sheath tumor
- Miscellaneous soft tissue tumors of uncertain lineage
  - Myxoma (intramuscular myxoma and juxta-articular myxoma)
  - Synovial sarcoma
  - Epithelioid sarcoma
  - Alveolar soft part sarcoma
  - Clear cell sarcoma of soft tissue
- Chondro-osseous tumors and other lesions
  - Myositis ossificans
  - Fibro-osseous pseudotumor of digits
  - Extraskeletal osteosarcoma
  - Soft tissue chondroma
  - Extraskeletal myxoid chondrosarcoma
  - Extraskeletal mesenchymal chondrosarcoma

NON-NEOPLASTIC DISORDERS OF SKELETAL MUSCLE

- Skeletal muscle: structure
- Myopathies
  - Inherited myopathies
    - Muscular dystrophies (Duchenne, Becker, myotonic, facioscapulohumeral, limb–girdle, Emery-Dreifuss, and oculopharyngeal muscular dystrophies)
    - Congenital myopathy (nemaline, central core, centronuclear, myotubular, and hyaline body myopathies)
    - Mitochondrial myopathy
    - Metabolic myopathies
  - Acquired myopathies
    - Inflammatory myopathies
    - Endocrine myopathies
    - Toxic myopathies
    - Autoimmune myopathies
  - Miscellaneous non-neoplastic disorders of skeletal muscle
    - Skeletal muscle atrophy
    - Myasthenia gravis
    - Lambert-Eaton myasthenic syndrome
    - Myositis ossificans
    - Rhabdomyolysis
    - Skeletal muscle hypertrophy

SOFT TISSUE TUMORS

SOFT TISSUE TUMORS: OVERVIEW

Soft tissue tumors comprise a group of entities exhibiting mesenchymal differentiation, which arise in skin, subcutis or deep soft tissue and are located in the deep soft tissues in the subfascial regions of extremities, shoulder, pelvis, thorax and intracranial regions. Similar tumors can occur in the viscera. According to revised 2020 World Health Organization (WHO), soft tissue tumors are categorized into adipocytic, fibrohistiocytic/myofibroblastic, fibrohistiocytic, smooth muscle, skeletal muscle, vascular, pericytic/perivascular, neural/perineural, chondro-osseous, miscellaneous tumors of uncertain origin and metastatic soft tissue tumors. Revised 2020 WHO classification of soft tissue tumors is given in Table 28.1. Based on clinical behavior, soft tissue tumors can be benign, intermediate and malignant. Most common metastatic tumors to soft tissues are given in Table 28.2.

Table 28.1 Revised 2020 World Health Organization classification of soft tissue tumors

Adipocytic Tumors

- Benign tumors
  - Lipoma and lipomatosis
  - Lipoblastoma
  - Lipomatosis of nerve
  - Lipoblastoma/lipoblastomatosis
  - Angiolipoma
  - Myolipoma
  - Chondroid lipoma
  - Extrarenal angiomyolipoma
  - Extra-adrenal myelolipoma
  - Spindle cells/pleomorphic lipoma
  - Hibernoma
- Intermediate (locally aggressive and recurrence after surgical excision) tumors
  - Atypical lipomatous tumor/well-differentiated liposarcoma
- Malignant tumors
  - Well differentiated liposarcoma: lipoma-like, sclerosing, inflammatory
  - Dedifferentiated liposarcoma
  - Myxoid liposarcoma/round cell liposarcoma
  - Pleomorphic liposarcoma
  - Mixed type liposarcoma
  - Liposarcoma, not otherwise specified

Fibroblastic/Myofibroblastic Tumors

- Benign tumors
  - Nodular fasciitis
  - Proliferative fasciitis
  - Proliferative myositis
  - Myositis ossificans and fibro-osseous pseudotumor of digits
  - Fibro-osseous pseudotumor of digits
  - Ischemic fasciitis
  - Elastofibroma
  - Fibrous hamartoma of infancy
  - Myofibroma/myofibromatosis
  - Fibromatosis colli
  - Juvenile hyaline fibromatosis
  - Inclusion body fibromatosis
  - Fibroma of tendon sheath
  - Desmoplastic fibroblastoma
  - Mammary-type fibroblastoma
  - Calcifying aponeurotic fibroma
  - Angiomyoblastoma
  - Cellular angiofibroma
  - Nuchal-type fibroma
  - Calcifying fibrous tumor
  - Giant cell angiofibroma
  - EWSR1-SMAD3 positive fibroblastic tumor (merging)
- Intermediate (locally aggressive and recurrence after surgical excision) tumors
  - Superficial fibromatosis (palmar/plantar)
  - Desmoid-type fibromatosis
  - Lipofibromatosis
  - Giant cell fibroblastoma
- Intermediate (rarely metastasizing) tumors
  - Dermatofibrosarcoma protuberans
    - Fibrosarcomatous dermatofibrosarcoma protuberans
    - Pigmented dermatofibrosarcoma protuberans
  - Solitary fibrous tumor
    - Solitary fibrous malignant tumor
  - Inflammatory myofibroblastic tumor
  - Myxoinflammatory fibroblastic sarcoma
    - Atypical myxoinflammatory fibroblastic sarcoma
  - Infantile fibrosarcoma
- Malignant tumors
  - Adult fibrosarcoma
  - Myxofibrosarcoma
  - Low-grade fibromyxoid sarcoma/hyalinizing spindle cell tumor
  - Sclerosing epithelioid fibrosarcoma

Fibrohistiocytic Tumors

- Benign tumors
  - Tenosynovial giant cell tumor (giant cell tumor of tendon sheath)
    - Localized tenosynovial giant cell tumor
    - Diffuse tenosynovial giant cell tumor
    - Malignant tenosynovial giant cell tumor
  - Deep benign fibrous histiocytoma
  - Juvenile xanthogranuloma
  - Xanthogranuloma

Contd...

Contd...

Table 28.1 Revised 2020 World Health Organization classification of soft tissue tumors (*Contd...*)

- Intermediate (rarely metastasizing) tumors
  - Atypical fibroxanthoma
  - Plexiform fibrohistiocytic tumor
  - Angiomatoid (malignant) fibrous histiocytoma
  - Giant cell tumor of soft tissue
- Malignant tumors
  - Undifferentiated pleomorphic sarcoma
  - Undifferentiated pleomorphic sarcoma with giant cells
  - Undifferentiated pleomorphic sarcoma with prominent inflammation

Smooth Muscle Tumors

- Benign tumors
  - Leiomyoma
    - Genital leiomyoma
    - Pilar leiomyoma
    - Leiomyoma of deep soft tissue
    - Vascular leiomyoma (angioleiomyoma)
    - Leiomyoma of retroperitoneal region
- Malignant tumors
  - Leiomyosarcoma
    - Leiomyosarcoma of deep soft tissue
    - Cutaneous leiomyosarcoma
    - Vascular leiomyosarcoma
    - Leiomyosarcoma of peritoneum
    - Epstein-Barr-associated smooth muscle tumor

Skeletal Muscle Tumors

- Benign tumors
  - Adult rhabdomyoma
  - Fetal rhabdomyoma
  - Genital rhabdomyoma
- Malignant tumors
  - Embryonal rhabdomyosarcoma
    - Conventional embryonal rhabdomyosarcoma
    - Spindle cell rhabdomyosarcoma (pediatric group)
    - Botryoid rhabdomyosarcoma
    - Anaplastic rhabdomyosarcoma
  - Alveolar rhabdomyosarcoma including solid alveolar rhabdomyosarcoma
  - Pleomorphic rhabdomyosarcoma
  - Spindle cell/sclerosing rhabdomyosarcoma (adult)

Vascular Tumors

- Reactive vascular hyperplasia
  - Papillary endothelial hyperplasia
  - Pyogenic granuloma (lobular capillary hemangioma)
  - Bacillary angiomatosis
  - Glomeruloid hemangioma
- Benign tumors
  - Capillary hemangioma
  - Cavernous hemangioma
  - Hemangioma of subcutaneous/deep soft tissue
  - Angiomatosis
  - Epithelioid hemangioma (angiolymphoid hyperplasia with eosinophilia)
  - Intramuscular hemangioma
  - Spindle cell hemangioma
  - Lymphangioma
- Intermediate (borderline) malignant tumors
  - Kaposiform hemangioendothelioma
  - Dabska-type (papillary intralymphatic angioendothelioma) and retiform hemangioendothelioma
  - Kaposi sarcoma
- Malignant tumors
  - Angiosarcoma
  - Epithelioid hemangioendothelioma

Pericytic/Perivascular Tumors

- Benign tumors
  - Hemangiopericytoma and related tumors
    - Giant cell angiofibroma
    - Lipomatous hemangiopericytoma
  - Glomus tumor
    - Solid glomus tumor
    - Extra-tumoral perivascular sheathing by neoplastic glomus cells
    - Glomangiomyoma
    - Multiple glomus tumors
    - Glomus tumor with marked hemangiopericytic vessels
    - Symplastic glomus tumor
    - Glomus tumor with epithelioid cells
    - Glomus tumor with oncocytes
    - Glomus tumor with extensive myxoid change
    - Vascular invasion in otherwise benign glomus tumor
    - Glomangiomatosis
  - Myopericytic family of tumors
    - Infantile myofibromatosis
    - Solitary myofibroma in infants and children
    - Myopericytoma
    - Glomangiopericytoma
- Intermediate tumors
  - Glomus tumor of uncertain malignant potential
  - Atypical glomus tumor
- Malignant tumors
  - Malignant glomus tumor/glomangiosarcoma

Chondro-osseous Tumors

- Benign tumors/lesions
  - Soft tissue chondroma
  - Myositis ossificans
  - Fibro-osseous pseudotumor of digits
- Malignant tumors
  - Primary soft tissue osteosarcoma
  - Mesenchymal chondrosarcoma

Contd...

Table 28.1 Revised 2020 World Health Organization classification of soft tissue tumors (*Contd...*)

Gastrointestinal Stromal Tumors

- Benign tumors
  - Benign gastrointestinal stromal tumor
- Intermediate (locally aggressive) tumors
  - Gastrointestinal stromal tumor of uncertain malignant potential
- Malignant tumors
  - Malignant gastrointestinal stromal tumor

Neural and Perineural Tumors/Lesions

- Benign tumors
  - Neuromuscular hamartoma (benign Triton tumor)
  - Neuromas
    - Traumatic neuroma
    - Morton neuroma
  - Granular cell tumor
  - Schwannoma
    - Conventional schwannoma
    - Cellular schwannoma
    - Plexiform schwannoma
    - Melanotic schwannoma
    - Microcytic/reticular schwannoma
    - Other schwannomas
  - Neurofibroma
    - Circumscribed neurofibroma
    - Plexiform neurofibroma
    - Other neurofibromas
  - Perineuroma
    - Intraneural perineuroma
    - Soft tissue perineuroma
    - Sclerosing perineuroma
    - Reticular perineuroma
  - Hybrid peripheral nerve sheath tumor
- Intermediate tumors
  - Nerve sheath myxoma
  - Atypical granular tumor
- Malignant tumors
  - Malignant peripheral nerve sheath tumor (MPNST)
    - Low-grade malignant peripheral nerve sheath tumor
    - High-grade malignant peripheral nerve sheath tumor
    - Epithelioid peripheral nerve sheath tumor
    - MPNST ex-schwannoma
    - Malignant peripheral nerve sheath tumor
  - Synovial sarcoma
  - Malignant granular cell tumor
  - Malignant perineuroma
- Benign to malignant tumors
  - Paraganglioma of head and neck region
- Intermediate to malignant tumors
  - Ependymoma of soft tissue

Tumors of Uncertain Differentiation or Uncertain Lineage

- Benign tumors
  - Acral fibromyxoma
  - Intramuscular myxoma including cellular variant
  - Juxta-articular myxoma
  - Deep aggressive angiomyxoma
  - Ectopic hamartomatous thymoma
- Intermediate (locally aggressive) malignant tumors
  - Hemosiderotic fibrolipomatous tumor
- Intermediate (rarely metastasizing) malignant tumors
  - Atypical fibroxanthoma
  - Angiomatoid fibrous histiocytoma
  - Ossifying fibromyxoid tumor malignant
  - Mixed tumor, not otherwise specified
  - Mixed tumor, not otherwise specified malignant
  - Myoepithelioma
  - Myoepithelial carcinoma
  - Phosphaturic mesenchymal tumor usually benign
  - Phosphaturic mesenchymal tumor usually malignant
  - Pleomorphic hyalinizing angiectatic tumor
  - Hemosiderotic fibrolipomatous tumor
- Malignant tumors
  - Synovial sarcoma
    - Synovial sarcoma, spindle cell variant
    - Synovial sarcoma, biphasic
  - Epithelioid sarcoma
  - Alveolar soft part sarcoma
  - Clear cell sarcoma of soft tissue
  - Extraskeletal myxoid chondrosarcoma
  - Extraskeletal Ewing sarcoma
  - Desmoplastic small round cell tumor
  - Extrarenal rhabdoid tumor
  - Neoplasms with perivascular epithelioid cell differentiation (PEComa)
    - PEComa not otherwise specified, benign
    - PEComa not otherwise specified, malignant
  - Intimal sarcoma

Undifferentiated/Unclassified Sarcomas

- Undifferentiated spindle cell sarcoma
- Undifferentiated pleomorphic sarcoma
- Undifferentiated round cell sarcoma
- Undifferentiated epithelioid sarcoma
- Undifferentiated sarcoma, not otherwise specified

Metastatic Tumors of Soft Tissue

- Metastases in adult males
  - Lung carcinoma
  - Malignant melanoma
  - Renal cell carcinoma
  - Colon carcinoma
- Metastases in adult females
  - Lung carcinoma
  - Malignant melanoma
  - Renal cell carcinoma
  - Colon carcinoma
  - Breast carcinoma

Trunk, thigh, muscles, iliopsoas, paraspinal muscles are sites of metastases.

Contd...

Table 28.2 Most common metastatic tumors to soft tissues

Age Group and Gender	Most Common Metastatic Tumors to Soft Tissues	Most Common Soft Tissue Sites
Adult males	▪ Lung carcinoma ▪ Melanoma ▪ Renal cell carcinoma ▪ Colorectal carcinoma	▪ Trunk muscle ▪ Thigh muscle ▪ Iliopsoas muscle ▪ Paraspinal muscles
Adult females	▪ Melanoma ▪ Breast carcinoma ▪ Lung carcinoma ▪ Renal cell carcinoma	▪ Trunk muscle ▪ Thigh muscle ▪ Iliopsoas muscle ▪ Paraspinal muscles
Children (males and females)	No data available	No data available

- **Benign soft tissue tumors:** These tumors are cured by surgical excision. More superficial tumor <5 cm size can be excised.
- **Intermediate soft tissue tumors:** These tumors are locally aggressive. These can recur after surgical excision and rarely metastasize.
- **Malignant soft tissue tumors:** These tumors most often metastasize. Deep seated soft tissue tumors tend to be malignant than located in the superficial region. Large size soft tissue tumors tend to be malignant. Rapidly growing soft tissue tumors with infiltrative margins are malignant in nature. Deep seated tumors most often grow to a large size. These tumors are diagnosed for optimal management by clinical history, physical examination, radiologic examination, fine needle aspiration cytology, histologic examination (minimum of three biopsy cores), immunohistochemistry, electron microscopy, cytogenetic study and molecular analysis. Common diagnostic genetic chromosomal abnormalities in selected soft tissue tumors are given in Table 28.3.

Table 28.3 Common diagnostic genetic chromosomal abnormalities in selected soft tissue tumors

Histologic Type	Chromosomal Abnormalities	Fusion Gene or other Genetic Alteration
Adipocytic tumors		
Well-differentiated liposarcoma	12q14-15 amplification	MDM2/CDK4 amplification
Dedifferentiated liposarcoma	12q14-15 amplification	MDM2/CDK4 amplification
Myxoid/round cell liposarcoma	▪ t(12;16)(13;11) ▪ t(12;22)(13;12)	▪ FUS-DD1T3 ▪ EWSR1-DD1T3
Pleomorphic liposarcoma	Complex changes	Complex changes
Fibroblastic/histiocytic tumors		
Nodular fasciitis	t(17;22)	UPP6-MYH9
Tenosynovial giant cell tumor (giant cell tumor of tendon sheath)	t(1;2) (q13;q37)	COL6A3-CSF1
Solitary fibrous tumor	12q13	NAB2-STAT6
Inflammatory myofibroblastic tumor	Alterations of chromosome 2	Multiple translocations involving ALK
Dermatofibrosarcoma protuberans	t(17;22)	COL1A1-PDGFB
Infantile fibrosarcoma	t(12;15)	ETV6-NTRK3
Low-grade fibromyxoid sarcoma	▪ t(7;16) (q33;p11) ▪ t(11;16)(q33;p11)	▪ FUS-CREB3L2 (most common) ▪ FUS-CREB3L1 (exceptional)
Myxoinflammatory fibroblastic sarcoma	▪ t(1;10) (p22;q24) ▪ t(2;6) (p31;q21.3)	TGFBR3-MGEA5
Skeletal muscle tumors		
Alveolar rhabdomyosarcoma	t(X;17)	ASPSCR1-TFE3
Spindle cell rhabdomyosarcoma	t(6;8)	SRF-NCOA2

Contd...

Table 28.3 Common diagnostic genetic chromosomal abnormalities in selected soft tissue tumors (*Contd...*)

Histologic Type	Chromosomal Abnormalities	Fusion Gene or other Genetic Alteration
Vascular tumors		
Epithelioid hemangioendothelioma	t(1;3) or t(X;11)	WWTR1-CAMTA1 or YAPT1-TFE3
Gastrointestinal stromal tumors		
Clear cell sarcoma (GIT)	t(2;22)(q33;q12)	EWSR1-CREB1
Chondro-osseous tumors		
Extraskeletal mesenchymal chondrosarcoma	t(8;8)	HEY1-NCOA2
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;12)	EWS-NR4A3 fusion transcript
Soft tissue tumors of uncertain differentiation		
Myoepithelioma of soft tissue	Chromosome 22 translocations	Multiple chromosomal translocations involving EWSR1
Ossifying fibromyxoid tumor	t(6;12)	EP400-PHF1
Alveolar soft part sarcoma	t(X;17)	ASPSCR1-TFE3
Clear cell sarcoma of soft tissue	▪ t(12;22)(q13;q12) ▪ t(2;22)(q34;q12)	▪ EWSR1-ATF1 fusion transcript ▪ EWSR1-CREB1
Ewing's sarcoma/PNET	▪ t(11;22)(q24;q12) ▪ t(21;22)(q22;q12) ▪ t(7;22)(q22;q12) ▪ t(17;22)(q12;q12) ▪ t(2;22)(q33;q12) ▪ t(16;21)(p11;q22) ▪ t(2;16)(q35;p11)	▪ EWSR1-Flt-1 ▪ EWSR1-ERG ▪ EWSR1-ETV1 ▪ EWSR1-ETV4 ▪ EWSR1-FEV ▪ FUS-ERG ▪ FUS-FEV
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	EWSR1-WT1 fusion transcript
Epithelioid sarcoma	Deletion 22q	Inactivation of SMARCB1
Extrarenal rhabdoid tumor	Deletion 22q	SMARCB1/INT1 mutation
Synovial sarcoma	▪ t(X;18)(p11,q11) ▪ t(X;20)(p11,q11)	▪ SSI8-SSX1 ▪ SSI8-SSX2
Extraskeletal myxoid chondrosarcoma	Chromosome 9 translocation	Multiple chromosomal translocations involving NR4A3
Angiomatoid fibrous histiocytoma	▪ t(12;22)(q11,q25) ▪ t(12;16)(q13,q11) ▪ t(2;22)(q33,q12)	▪ EWSR1-ATF1 ▪ FUS-ATF1 ▪ FUS-CRAB1
Myoepithelioma of soft tissue	▪ t(6;22)(p22;q12) ▪ t(19;22)(q13;q12)	▪ EWSR1-POU5F1 ▪ EWSR1-ZNF444

CLINICAL HISTORY

Before interpretation of the biopsy, attention should be given to the age of the patient, size, duration, location of soft tissue tumor including anatomical plane (e.g. cutaneous, subcutaneous, fascial or deep seated). Most benign soft tissue tumors occur in the superficial region. Myxofibrosarcoma, leiomyosarcoma, myxoinflammatory fibroblastic sarcoma and epithelioid sarcoma are less frequently demonstrated in the superficial region. Conversely, soft tissue tumor located in the deep tissue beneath fascia is most likely to be sarcoma. Radiological studies can additionally suggest the composition of soft tissue tumor.

HISTOPATHOLOGIC EXAMINATION

In children, majority of soft tissue tumors are benign. Diagnostic spectrum of benign and malignant tumors is different. Benign soft tissue tumors have pushing margins and less cellularity with pale uniform nuclei, fine chromatin and few mitoses but not atypical; but absence of necrosis. Some benign cellular lesions composed of pleomorphic neoplastic cells and numerous mitoses may simulate sarcoma. Malignant soft tissue tumors have invasive border, more cellularity with pleomorphic hyperchromatic neoplastic cells, abnormal mitoses, vascular invasion, foci of metastases, and presence of necrosis.

- Some benign soft tissue tumors located in the superficial regions can simulate malignant tumors (e.g. cellular fibrous histiocytoma, cellular myxoma, spindle cell lipoma, pleomorphic lipoma, chondroid lipoma, solitary fibrous tumor, extraskeletal chondroma and pleomorphic hyalinizing angiectatic tumor). Conversely, benign soft tissue tumors located in deep region poses diagnostic challenge to distinguish from cellular schwannoma, inflammatory myofibroblastic tumor and fibromatosis.
- Some malignant soft tissue tumors can be under-diagnosed due to their resemblance to benign counterpart (e.g. atypical lipomatous tumor and leiomyosarcoma). Similarly, some malignant tumors with deceptively bland nuclei can also be under-diagnosed (e.g. fibromyxoid sarcoma and myxofibrosarcoma).
- Many soft tissue tumors exhibit different cytomorphologic patterns (e.g. spindle cell, epithelioid cell, small round cell or pleomorphic), which need to be characterized further. Features of tumor architecture, cytomorphology, tumor–stromal interface, immunohistochemical, genetic and electron microscopic analysis are essential in establishing diagnosis.

Table 28.4 French Federation of Comprehensive Cancer Center Grading System of soft tissue tumors

Criterion	Score
Tumor differentiation according to Table 28.1	
Well-differentiated tumor closely resembling normal adult mesenchymal tissue	
▪ Sarcoma closely resembling normal adult mesenchymal tissue	▪ Score 1
▪ Moderately differentiated tumor of well-defined certain histogenetic types	▪ Score 2
▪ Poorly differentiated (undifferentiated) tumors and undefined histogenetic type	▪ Score 3
Mitotic count	
▪ 0–9/10 per high power fields	▪ Score 1
▪ 10–19/10 per high power fields	▪ Score 2
▪ ≥20/10 per high power fields	▪ Score 3
Tumor necrosis on histologic examination	
▪ No necrosis	▪ Score 0
▪ ≤50% tumor necrosis	▪ Score 1
▪ >50% tumor necrosis	▪ Score 2
Grades on histologic examination	**Sum of the above scores**
▪ Grade 1	▪ Total score 2 or 3
▪ Grade 2	▪ Total score 4 or 5
▪ Grade 3	▪ Total score 6, 7 or 8

- French Federation of Comprehensive Cancer Center Grading System is based on tumor differentiation, mitotic count, tumor necrosis and histologic grade of soft tissue tumor (Tables 28.4 and 28.5). Grade 1 bone tumors have better prognosis than grade 3. Grade 2 bone tumors have intermediate prognosis. French Federation of Comprehensive Cancer Center Grading System is based on multivariable analysis of tumor differentiation, mitotic count, tumor necrosis and grade on histologic examination.

IMMUNOHISTOCHEMISTRY TECHNIQUE

Immunohistochemistry technique is an essential adjunct in diagnosing soft tissue tumors. Some tumors express

Table 28.5 French Federation of Comprehensive Cancer Center Grading System of soft tissue sarcomas

Grade	Malignant Soft Tissue Tumors (Histologic Type)
Grade 1 soft tissue sarcomas	▪ Well-differentiated liposarcoma ▪ Well-differentiated fibrosarcoma ▪ Well-differentiated malignant peripheral nerve sheath tumor ▪ Extraskeletal well-differentiated chondrosarcoma
Grade 2 soft tissue sarcomas	▪ Myxoid liposarcoma ▪ Conventional fibrosarcoma ▪ Conventional malignant peripheral nerve sheath tumor ▪ Well-differentiated malignant hemangiopericytoma ▪ Myxofibrosarcoma ▪ Malignant fibrous histiocytoma with storiform pattern ▪ Extraskeletal myxoid chondrosarcoma ▪ Conventional angiosarcoma
Grade 3 soft tissue sarcomas	▪ Round cell liposarcoma ▪ Pleomorphic liposarcoma ▪ Dedifferentiated liposarcoma ▪ Poorly differentiated liposarcoma ▪ Epithelioid malignant schwannoma ▪ Malignant Triton tumor ▪ Conventional malignant hemangiopericytoma ▪ Giant cell and inflammatory malignant fibrous histiocytoma ▪ Poorly differentiated epithelioid/pleomorphic leiomyosarcoma ▪ Synovial sarcoma ▪ Rhabdomyosarcoma ▪ Mesenchymal chondrosarcoma ▪ Poorly differentiated/epithelioid angiosarcoma ▪ Extraskeletal osteosarcoma ▪ Extraskeletal Ewing sarcoma/PNET ▪ Alveolar soft part sarcoma ▪ Malignant rhabdoid tumor ▪ Clear cell sarcoma of soft tissue ▪ Undifferentiated sarcoma of soft tissue

specific antigens. However, a panel of antibodies (e.g. first line and second line) is required according to the broad cell-lineage. Monoclonal antibodies are almost never 100% specific or sensitive in soft tissue tumors. Hence, overreliance of a single marker needs to be avoided. The initial panel of antibodies depends on the morphologic features of tumor. First and second line panel of markers is given in Table 28.6. Immunohistochemistry of soft tissue tumors is given in Table 28.7.

Table 28.6 First and second line panel of markers

Line of Marker Purpose	Panel of Markers		
First line panel of markers			
Diagnosis of spindle cell sarcoma and pleomorphic sarcoma	▪ Desmin ▪ Smooth muscle actin ▪ S-100 protein ▪ CD34	▪ Epithelial membrane antigen (EMA) ▪ CK (AE1/AE3) and (CAM 5.2)	▪ MDM2 (pleomorphic sarcoma in abdomen) ▪ CDK4 (pleomorphic sarcoma in abdomen)
Clear cell sarcoma and epithelioid sarcoma	▪ Epithelial membrane antigen (EMA) ▪ CD34	▪ CD30 ▪ CD31 ▪ Desmin	▪ S-100 protein ▪ IN1 (BAF47)
Small round cell tumors	▪ CD99 ▪ S-100 protein ▪ Desmin ▪ CD99 ▪ CD56 ▪ CD45	▪ TdT ▪ Fli-1 ▪ WT1 ▪ TLE1 ▪ Myogenin	▪ NB84a ▪ Synaptophysin ▪ Chromogranin ▪ Neuron-specific enolase (NSE) ▪ Neurofilament
Second line panel of markers			
Epithelial cell, endothelial cell, smooth muscle, skeletal muscle, melanocytic, neural and other type of differentiation	▪ CK (AE1/AE3) and (CAM 5.2) ▪ Carcinoembryonic antigen (CEA) ▪ Calretinin ▪ Thrombomodulin ▪ BerErp4 ▪ TLE1 (transducin-like enhancer of split 1) ▪ Glial fibrillary acidic protein (GFAP) ▪ CD56 ▪ Chromogranin	▪ Synaptophysin ▪ Myogenin ▪ Smooth muscle myosin ▪ h-caldesmon ▪ Calponin ▪ β-Catenin ▪ BCL-2 ▪ TFE3 ▪ HMB4 ▪ Melan-A	▪ Inhibin ▪ CD31 ▪ Flt-1 ▪ HHV8 ▪ CD45 ▪ CD117 ▪ CD21 ▪ CD23 ▪ CD35

Table 28.7 Immunohistochemistry of soft tissue tumors

Immunohistochemical Marker	Expression
Adipocytic tumors	
Lipoma, lipomatosis and angiolipoma	S-100 protein, presence of abnormalities in the chromosome region 12q13–15 containing MDM2 and CDK4 genes in lipoma and lipomatosis but absent in well-differentiated liposarcoma
Neural fibrolipoma	S-100 protein (staining nerve and fat cells), EMA (staining perineural cells)
Lipoblastoma	Presence of PLAG gene mutation on chromosome 8, but absence of DD1T3 gene mutation due to chromosomal translocation t(12;16)
Hibernoma	S-100 protein
Chondroid lipoma	S-100 protein, chromosomal translocation (11;16) (q13; p12-13)
Spindle cell and pleomorphic lipoma	CD34 (hematopoietic progenitor cell antigen)

Contd...

Table 28.7 Immunohistochemistry of soft tissue tumors (*Contd...*)

Immunohistochemical Marker	Expression
Well-differentiated liposarcoma	Amplification of MDM2, CDK4 present in the region on chromosome 12(12q–15) confirmed by FISH studies
Atypical lipomatous tumor	Amplification of MDM2, CDK4 present in the region on chromosome 12(12q–15) confirmed by FISH studies
Dedifferentiated liposarcoma	Amplification of MDM2, CDK4 present in the region on chromosome 12(12q–15) confirmed by fluorescence *in situ* hybridization (FISH) studies
Myxoid and round cell liposarcoma	Translocation involving the DDT gene on chromosome 12; and FUS gene on chromosome 22 confirmed by FISH studies, and alternate translocation involving the DDT gene on chromosome 12; and EWS gene on chromosome 22 in 10% confirmed by FISH studies
Pleomorphic type liposarcoma	S-100 protein (50%)
Fibroblastic and myofibroblastic tumors	
Nodular fasciitis	SMA (smooth muscle actin), calponin, CD68, chromosomal translocation involving USP6 gene product but caldesmon is negative in leiomyoma and positive in leiomyosarcoma
Proliferative myositis and proliferative fasciitis	Vimentin, SMA (smooth muscle actin), MSA (muscle-specific actin)
Ischemic fasciitis	Vimentin, CD34, CD68, SMA (smooth muscle actin)
Postoperative spindle cell nodule	Cytokeratin (+++), vimentin, SMA (smooth muscle actin), desmin (variable staining)
Elastofibroma	Vimentin, Verhoeff-Van Gieson elastic stain
Hypertrophic scar	α-Smooth muscle actin (70%)
Fibrous hamartoma of infancy	Vimentin (fibrous and immature areas), actin (trabecular component), desmin (trabecular component sometimes)
Gardner fibroma	Cyclin D1 (100%), β-catenin (64%), c-Myc
Nuchal fibroma	CD34, CD99, vimentin
Fibromatosis colli	Vimentin, SMA (smooth muscle actin)
Calcifying aponeurotic fibroma	MSA (muscle-specific actin) (50%), SMA (smooth muscle actin), vimentin, CD68, CD69, S-100 protein
Fibroma of tendon sheath	Vimentin, MSA (muscle-specific actin), SMA (smooth muscle actin), factor XIIIa, CD34
Superficial fibromatosis	Vimentin, MSA (muscle-specific actin) (variable staining), SMA (smooth muscle actin) (variable staining)
Deep fibromatosis	Vimentin, MSA (muscle-specific actin), β-catenin, desmin (rare case)
Inclusion body fibromatosis	Desmin, cytokeratins (CKs), calponin, CD99, α-SMA, vimentin, CD117 (occasional case)
Fibromatosis (myofibroblastic differentiation)	β-Catenin, SMA (smooth muscle actin)
Myofibroblastoma (mammary type)	Desmin, SMA (smooth muscle actin), CD34
Inflammatory myofibroblastic tumor (IMT)	ALK (anaplastic lymphoma kinase) protein (35%), SMA (smooth muscle actin) (80%), desmin (40%), vimentin, ALK gene rearrangement (detected by *in situ* hybridization in 50%), cytokeratin (+/–)
Myofibroma and myofibromatosis	Vimentin, SMA (smooth muscle actin), HHF35 pan-actin
Intranodal myofibroblastoma	Vimentin, SMA (smooth muscle actin)
Myofibroblastoma sarcoma	SMA (smooth muscle actin), MSA (muscle-specific actin), calponin
Reticulohistiocytoma	CD68, CD31 (variable staining), α_1-antitrypsin (variable staining)
Juvenile xanthogranuloma	Factor XIIIa, CD68, HAM56, CD4, Ki-MIP, vimentin

Contd...

Table 28.7 Immunohistochemistry of soft tissue tumors (*Contd...*)

Immunohistochemical Marker	Expression
Benign fibrous histiocytoma	Factor XIIIa, HMGA1, HMGA2
Angiomatoid fibrous histiocytoma	Desmin (50%), CD99 (50%), EMA (epithelial membrane antigen) (40%), CD68
Plexiform fibrohistiocytic tumor	Vimentin, SMA (smooth muscle actin), CD68
Superficial acral fibromyxoma	CD34 (hematopoietic progenitor cell antigen) (75%), EMA (epithelial membrane antigen) (50%), CD99
Superficial angiomyxoma	CD34 (hematopoietic progenitor cell antigen in most cases), cytokeratins (CKs in some cases), S-100 protein in some cases), desmin (rarely positivity), SMA (smooth muscle actin—rare positivity)
Dermatofibrosarcoma protuberans (DFSP)	CD34 (hematopoietic progenitor cell antigen (diffuse staining)
Desmoplastic small round cell tumor	WT1, CKs, EMA, desmin, NSE (neuron-specific enolase)
Giant cell fibroblastoma	CD34 (hematopoietic progenitor cell antigen), α_1-antitrypsin
Fibrosarcoma arising in dermatofibrosarcoma protuberans	CD34 (hematopoietic progenitor cell antigen), COL1A (PDGF-β gene fusion), MIB-1 labeling index, p53
Fibrosarcoma adult type	Vimentin, SMA (smooth muscle antigen)
Fibrosarcoma infantile type	Vimentin, SMA (smooth muscle antigen in rare case)
Fibrosarcoma sclerosing epithelioid type	Vimentin, SMA (smooth muscle antigen) (50%)
Fibromyxoid sarcoma low-grade	Vimentin, SMA (smooth muscle antigen—focal staining in occasional case)
Atypical fibroxanthoma	Vimentin
Intramuscular myxoma	Vimentin, CD34 (hematopoietic progenitor cell antigen—variable staining), desmin (variable staining), SMA (smooth muscle actin—variable staining), mucoid matrix stains (Alcian blue, mucicarmine, colloidal iron)
Myxofibrosarcoma	Vimentin, SMA (smooth muscle actin—focal staining), MSA (muscle-specific actin—focal staining)
Fibrohistiocytic tumors	
Tenosynovial giant cell tumor	Desmin, CD68
Benign fibrous histiocytoma	CD68, factor XIIIa, HMGA1, HMGA2
Juvenile xanthogranuloma	CD68 (clones of KPI and PGM1), CD31, factor XIIIa, HAM56, HHF35, CD4, Ki-MIP, vimentin
Atypical xanthogranuloma	CD99 (+++75%), SMA (smooth muscle actin—focal staining)
Plexiform fibrohistiocytic tumor	Vimentin, SMA (smooth muscle actin), CD68
Angiomatous (malignant) fibrous histiocytoma	Desmin (50% cases), CD68 (50% cases), CD99 (50%), EMA (40%)
Soft tissue giant cell tumor with low malignant potential	CD68 (100% cases), SMA (smooth muscle actin) (75% cases)
Undifferentiated pleomorphic sarcoma	SMA (smooth muscle actin)
Smooth muscle tumors	
Angioleiomyoma	MSA (muscle-specific actin), desmin, heavy caldesmon (h-caldesmon)
Pilar leiomyoma	MSA (muscle-specific actin), desmin, heavy caldesmon (h-caldesmon)
Leiomyoma deep soft tissue	MSA (muscle-specific actin), desmin, heavy caldesmon (h-caldesmon), ER (retroperitoneal leiomyoma)
Leiomyosarcoma soft tissue	MSA (muscle-specific actin), desmin, heavy caldesmon (h-caldesmon), CAM 5.2 (aberrant reactivity in leiomyosarcoma)
Vascular leiomyosarcoma	MSA (muscle-specific actin), desmin, heavy caldesmon (h-caldesmon—more specific marker)
Epstein-Barr virus-associated smooth muscle cell tumor	MSA (muscle-specific actin), desmin, heavy caldesmon (h-caldesmon), CD3 (stains T cells), EBA-DNA (demonstrated by *in situ* hybridization)

Contd...

Table 28.7 Immunohistochemistry of soft tissue tumors (*Contd...*)

Immunohistochemical Marker	Expression
Skeletal muscle tumors	
Rhabdomyoma adult type	MSA (muscle-specific actin), desmin, myoglobin, PTAH, SMA (smooth muscle actin—rare/focal staining), S-100 protein (rare/focal staining)
Rhabdomyoma fetal type	MSA (muscle-specific actin), desmin, myoglobin, PTAH, SMA (smooth muscle actin—rare/focal staining), S-100 protein (rare/focal staining)
Genital rhabdomyoma	MSA (muscle-specific actin), desmin, myoglobin
Alveolar rhabdomyosarcoma	MSA (muscle-specific actin), desmin (patchy cytoplasmic staining), myogenin, MyoD1 (focal nuclear staining pattern) in embryonal rhabdomyosarcoma) and (diffuse staining pattern) in alveolar rhabdomyosarcoma), PAX5, PAX3-FKHR, PAX7-FKHR
Embryonal rhabdomyosarcoma	MSA (muscle-specific actin), desmin (patchy cytoplasmic staining), vimentin (patchy cytoplasmic staining), myogenin (focal nuclear staining pattern) in embryonal rhabdomyosarcoma), MyoD1 (focal nuclear staining pattern) in embryonal rhabdomyosarcoma, cytokeratins (focal staining), S-100 protein (focal staining), CD99 (15%)
Pleomorphic rhabdomyosarcoma	Myoglobin, MyoD1, myogenin, desmin, MSA (muscle-specific actin—variable staining), SMA (smooth muscle actin—variable staining), sarcomeres (Z-bands by electron microscopy)
Gastrointestinal stromal tumors (GISTs)	
Gastrointestinal stromal tumors	c-KIT (95% diffuse staining), CD34 (75%), DOG1, BCL-2, mitotic index—high
Vascular tumors of soft tissue	
Lymphangioma	D2–40 (podoplanin)
Cavernous hemangioma	CD31, CD34, Flt-1, vWF
Spindle cell hemangioma	CD31, CD34 (hematopoietic progenitor cell antigen), desmin, MSA (smooth muscle actin)
Deeply located hemangioma including angiomatosis	CD31, CD34 (hematopoietic progenitor cell antigen), Fli-1, von Willebrand factor (vWF)
Pyogenic granuloma	CD31, CD34 (hematopoietic progenitor cell antigen), Fli-1
Arteriovenous malformation	CD31, CD34 (hematopoietic progenitor cell antigen)
Kaposiform hemangioendothelioma	CD31, CD34 (hematopoietic progenitor cell antigen), Fli-1, von Willebrand factor (vWF)
Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma	CD31, CD34 (hematopoietic progenitor cell antigen), VEGFR3, D2–40 (podoplanin)
Kaposi sarcoma	CD31 (PECAM1), CD34 (hematopoietic progenitor cell antigen in 40%), D2–40 (podoplanin), factor VIII-related antigen (demonstrated by immunoperoxidase method). Ulex europaeus 1, Flt-1, Flt-4, VEGFR3 (vascular endothelial growth factor receptor 3), CD141 (thrombomodulin in some cases), HHV8 LANA protein (most sensitive marker)
Epithelioid hemangioendothelioma	CD31, CD34 (hematopoietic progenitor cell antigen), Flt-1, von Willebrand factor (vWF), cytokeratins (low-molecular weight: 25%)
Angiosarcoma	CD31 (PECAM1), CD34 (hematopoietic progenitor cell antigen), D2–40 (some cases), Flt-1, Flt4, CD141 (thrombomodulin), von Willebrand factor (vWF), factor VIII-related antigen (demonstrated by immunoperoxidase method), Ulex europaeus 1 (positive in some cases), VEGFR3 (positive in some cases), cytokeratins (low-molecular weight: 25%)
Pericytic/perivascular tumors	
Glomus tumor	SMA (smooth muscle actin), MSA (muscle-specific actin), calponin, caldesmon, pericellular type 4 collagen, desmin (positive in some cases)
Glomuvenous malformation	Vimentin, pericellular type 4 collagen, SMA (smooth muscle actin), laminin, type 4 collagen

Contd...

Table 28.7 Immunohistochemistry of soft tissue tumors (*Contd...*)

Immunohistochemical Marker	Expression
Myopericytoma	SMA (smooth muscle actin), CD34 (hematopoietic progenitor cell antigen), heavy caldesmon (h-caldesmon)
Hemangiopericytoma	CD34 (hematopoietic progenitor cell antigen), BCL-2
Atypical or malignant glomus tumor	SMA (smooth muscle actin), collagen IV (pericellular staining)
Nerve and nerve sheath tumors	
Traumatic neuroma	S-100 protein (stains Schwann cell), neurofilament (stains axons), EMA (epithelial membrane antigen) (stains perineural cells)
Schwannoma, cellular type	S-100 protein (++++), GFAP (glial fibrillary acidic protein—variable staining), Leu7 (CD57) (variable staining), laminin (pericellular staining), collagen IV (pericellular staining)
Schwannoma, ancient type	S-100 protein, GFAP (glial fibrillary acidic protein—variable staining), Leu7 (CD57) (variable staining)
Ganglioneuroma	Synaptophysin (ganglion cells), neurofilament (ganglion cells), tubulin (ganglion cells), NSE (neuron-specific enolase—ganglion cell marker), S-100 protein (ganglion cells), GFAP (glial fibrillary acidic protein—Schwann cell marker)
Neurothekeoma	NKIC3 (also positive in cellular neurothekeoma), S-100 protein (positive except in cellular neurothekeoma), PGP9.5, MITF (microphthalmia-asspcoated transcription factor), SMA (smooth muscle actin)
Cellular schwannoma	S-100 protein (++++), GFAP (glial fibrillary acidic protein—variable), Leu7 (CD57)
Neurofibroma	S-100 protein (staining less diffuse than in schwannoma), Leu7 (CD57), CD34 (hematopoietic progenitor cell antigen—variable staining), factor X111-related antigen (variable staining)
Plexiform neurofibroma	S-100 protein (staining less diffuse than in schwannoma), Leu7 (CD57), CD34 (hematopoietic progenitor cell antigen—variable staining), factor X111-related antigen (variable staining)
Perineuroma	EMA (epithelial membrane antigen), S-100 protein, GLUT1 (glucose transporter protein isoform 1), claudin, laminin (outlines the basal lamina around individual cells or small group of cells), type 4 collagen (outlines the basal lamina around individual cells or small group of cells)
Malignant peripheral nerve sheath tumor (MPNST)	S-100 protein (diffuse staining) in epithelioid variant of MPNST, Leu7 (CD57 in 30–40%)
Malignant triton tumor	S-100 protein (diffuse staining in 50%), Leu7 (CD57 in 30–40%), cytokeratins (CKs), desmin
Granular cell tumor (benign)	CD68 (++++ strong staining), S-100 protein, SMA (smooth muscle actin), inhibin, NSE (neuron-specific enolase), laminin, myelin P0, myelin P2PAS-D (cytoplasmic granules), mitotic index—low
Granular cell tumor (malignant)	S-100 protein, neuron-specific enolase (NSE), vimentin, PAS-D (cytoplasmic granules)
Neuroglial heterotopia	S-100 protein (stains glial tissue), GFAP (glial fibrillary acidic protein—stains glial tissue), synaptophysin, chromogranin, NSE (neuron-specific enolase), neurofilamaent, N-Myc amplification (FISH analysis), chromosome 1p deletion (cytogenetic analysis)
Esthesioneuroblastoma	Synaptophysin, chromogranin, NSE (neuron-specific enolase), neurofilament, S-100 protein (staining at periphery of tumor, CD56)
Paraganglioma	Synaptophysin (chief cells), chromogranin (chief cells), NSE (neuron-specific enolase—chief cells), neurofilament, peptide hormones (calcitonin, somatostatin in chief cells), S-100 protein (sustentacular cells), GFAP (glial fibrillary acidic protein in sustentacular cells)
Melanocytic neuroectodermal tumor of infancy	Cytokeratins (CKs), HMB-45, NSE (neuron-specific enolase), synaptophysin

Contd...

Table 28.7 Immunohistochemistry of soft tissue tumors (*Contd...*)

Immunohistochemical Marker	Expression
Tumors of uncertain differentiation	
Synovial sarcoma (SS)	TLE1 (novel immunohistochemical marker for synovial sarcoma), BCL-2, calponin, S-100 protein (30%), cytokeratins (CKs—focal staining), EMA (epithelial membrane antigen—focal staining)
Epithelioid sarcoma	Cytokeratins (50% strong staining), EMA (epithelial membrane antigen), CEA (carcinoembryonic antigen—focal staining), vimentin
Alveolar soft part sarcoma	TFE3 (nuclear staining), desmin (50% focal staining), SMA (smooth muscle actin in 10%), S-100 protein, PAS/diastase resistant, rhomboid cytoplasmic crystals demonstrated by electron microscopy
PEComas (neoplasms with perivascular epithelioid cell differentiation)	SMA (smooth muscle actin), Melan-A (A103), MART-1 (melanoma-associated antigen recognized by T cells 1), HMB-45 (human melanoma black 45) marker of immature melanocytes, MITF (microphthalmia-associated transcription factor), c-KIT, cathepsin K, TFE3 (rearrangements or amplification in a subset), desmin (+/–), S-100 protein (+/–)
Ewing sarcoma/PNET extraskeletal origin	CD99, cytokeratin (CKs—focal staining in 20% cases), synaptophysin, chromogranin A, NSE (neuron-specific enolase), CD56 (neuronal cell adhesion molecule)
Chondro-osseous tumors	
Extraskeletal osteosarcoma	Osteocalcin (neoplastic cells and neoplastic matrix), vimentin (neoplastic cells and neoplastic matrix), SMA (smooth muscle actin—focal staining), desmin (focal staining), S-100 protein (focal staining)
Soft tissue chondrosarcoma	Vimentin, S-100 protein
Extraskeletal myxoid chondrosarcoma	Vimentin (diffuse staining pattern), S-100 protein (<50%), NSE (neuron-specific enolase), synaptophysin
Mesenchymal chondrosarcoma	Vimentin (small blue cells and chondrocytes components), CD99 (small blue cell component), S-100 protein (cartilaginous component)

Neural and perineural tumors are described in detail in Chapter 30; Nervous System.

First Line of Panel of Monoclonal Antibodies

First line of panel of monoclonal antibodies is used to diagnose spindle cell sarcoma and pleomorphic sarcoma. Immunohistochemical markers for soft tissue tumors include desmin, smooth muscle actin, broad spectrum cytokeratins (AE1/AE3 and CAM 6.2), S-100 protein and CD34.

- **Pleomorphic liposarcoma:** Immunohistochemical markers for pleomorphic liposarcoma located in abdomen are positive for MDM2 and CDK4.
- **Clear cell and epithelioid soft tissue tumors:** Immunohistochemical markers for clear cell and epithelioid soft tissue tumors are positive for EMA (epithelial membrane antigen), CD34, CD30, CD31, desmin, S-100 protein and INI (selected cases).
- **Round cell tumors:** Immunohistochemical markers for round cell tumors are positive for cytokeratins, CD99, S-100 protein, desmin, CD99, CD56, CD45 and TdT, FLI-1, WT1, TLE1, myogenin, NB84a, synaptophysin, chromogranin, neuron-specific enolase (NSE), neurofilament and other markers as indicated.

Second Line of Panel of Monoclonal Antibodies

Second line of panel of monoclonal antibodies is specific for epithelial cell, endothelial cell, smooth muscle, skeletal muscle, melanocytic, neural and other type of differentiation. Immunohistochemical markers include: CK (AE1/AE3) and (CAM 5.2), CEA (carcinoembryonic antigen), calretinin, thrombomodulin, BerErp4, TLE, GFAP (glial fibrillary acidic protein), CD56, chromogranin, synaptophysin, myogenin, smooth muscle myosin, h-caldesmon, calponin, β-catenin, BCL-2, TFE3, HMB4, Melan-A, inhibin, CD31, FLI-1, HHV8, CD45, CD117, CD21, CD23 and CD35.

ADIPOCYTIC TUMORS

Adipocytic tumors share variable features of lipomatous differentiation. These tumors represent the most common group of mesenchymal lesions. Besides most common lipoma tumor, adipocytic tumors range from benign, intermediate and malignant neoplasms (liposarcomas). Liposarcoma is the most common soft tissue sarcoma

Table 28.8 Adipocytic tumors according to revised 2020 WHO classification of soft tissue tumors

Adipocytic Tumors	
Benign tumors	
▪ Lipoma and lipomatosis	▪ Chondroid lipoma
▪ Lipoblastoma	▪ Extra-renal angiomyolipoma
▪ Lipomatosis of nerve	▪ Extra-adrenal myelolipoma
▪ Lipoblastoma/ lipoblastomatosis	▪ Spindle cells/pleomorphic lipoma
▪ Angiolipoma	▪ Hibernoma
▪ Myolipoma	
Intermediate (locally aggressive and recurrence after surgical excision) tumors	
Atypical lipomatous tumor/well-differentiated liposarcoma	
Malignant tumors	
▪ Dedifferentiated liposarcoma	
▪ Myxoid liposarcoma/round cell liposarcoma	
▪ Pleomorphic liposarcoma	
▪ Mixed type liposarcoma	
▪ Liposarcoma, not otherwise specified	

comprising 25% of all soft tissue sarcomas. According to revised 2020 WHO classification of soft tissue tumors, the adipocytic tumors are categorized into benign, intermediate (locally aggressive) and malignant neoplasms (Table 28.8).

LIPOMA

Lipoma is the most common benign mesenchymal tumor of mature adipocytes, which most often occurs during fifth to seventh decades of life without sex predominance. Most common sites of lipoma are superficial or subcutaneous lump in the region of shoulder, back, neck, abdomen and extremities. Infrequently, large well circumscribed lipoma may be present in the intramuscular compartment of arm, thigh or thorax.

Clinical Features and Management

Patient presents with painless, soft and mobile lump in the shoulder, back, neck, abdomen or extremities. Lipoma is treated by surgical excision.

Surgical Pathology: Lipoma

Gross Morphology

Lipoma shows yellow and translucent adipose tissue. Tumor is encapsulated. Gross morphology of lipoma is shown in Fig. 28.1.

Light Microscopy

- Lipoma is indistinguishable from normal adipose tissue. Tumor is composed of lobules of well-differentiated unremarkable adipocytes with inconspicuous eccentrically located nuclei. Adipocytes lack pleomorphism.
- Sometimes, focal areas of fat necrosis are demonstrated. Adipocytes do not show nuclear atypia.
- In occasional lipoma, there may be presence of areas of bone formation (osteolipoma), abundant fibrous tissue (fibrolipoma) or cartilage (chondrolipoma). Histology of lipoma is shown in Fig. 28.2.

Immunohistochemistry Technique

Lipoma shows positivity for S-100 protein.

Marker	Expression
S-100 protein	Positive



Fig. 28.1: Lipoma. Tumor is encapsulated, well circumscribed, mass of uniform, yellowish adipose tissue with mottled tan areas and a soft consistency. Cut surface shows white/yellow, translucent, homogeneous and lobulated appearance.

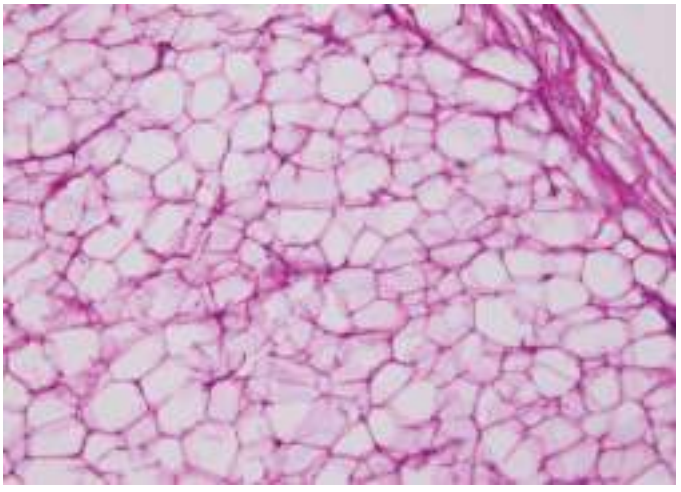


Fig. 28.2: Histology of lipoma. Tumor is indistinguishable from normal adipose tissue. It is composed of lobules of well-differentiated unremarkable adipocytes with inconspicuous eccentrically located nuclei. Adipocytes lack pleomorphism (400X).

Molecular Genetic Alterations

Conventional lipoma often shows rearrangement of HMG2 gene located on chromosome 12q13.15, 6p and 13q. Spindle cell lipoma and pleomorphic lipoma show rearrangement of 16q and 13q.

HIBERNOMA

Hibernoma is a rare benign adipocytic tumor, which arises from remnants of fetal brown adipose tissue. It is indistinguishable from malignant neoplasm taking into consideration of clinical history and radiologic findings, hence, it emphasizes its inclusion in the differential diagnosis. It usually affects young adults during third and fourth decades of life. However, cases have been reported in the age group of three months of age to fifty years. Hibernoma shows breakpoints in 11q12.21 with many chromosomal translocations.

Clinical Features

Patient presents with large painful lump in axilla, back, mediastinum, shoulder, thigh, head and neck regions; abdominal cavity, arm, breast and spermatic cord. Hibernoma is treated by surgical excision. It has no significant potential for recurrence after surgery.

Radiologic Findings

Hibernoma is indistinguishable from malignant neoplasm taking into consideration of clinical history and radiologic findings.

Surgical Pathology: Hibernoma

Gross Morphology

- Hibernoma is a lobulated well demarcated large tumor with tan-yellow to red brown in color.
- Cut surface is brown with spongy consistency. Gross morphology of hibernoma is shown in Fig. 28.3.

Light Microscopy

- Tumor is composed of large number of brown adipocytes with small centrally located nuclei and multivacuolated cytoplasm; and admixed with normal adipocytes.
- Areas of necrosis and mitoses are absent in the neoplasm. Histology of hibernoma is shown in Fig. 28.4.

Histochemistry

Oil red O and Sudan black demonstrate adipocytes in hibernoma.

Differential Diagnosis

Hibernoma should be differentiated from well-differentiated liposarcoma, conventional lipoma and residual brown fat around cervical and axillary lymph nodes.

Immunohistochemistry Technique

Hibernoma shows positivity for immunomarker S-100 protein in 85% of cases and CD31.

Markers	Expression
▪ S-100 protein	▪ Positive (85%)
▪ CD31	▪ Positive

WELL-DIFFERENTIATED LIPOSARCOMA/ATYPICAL LIPOMATOUS TUMOR

Well-differentiated liposarcoma occurs as a result of proliferation of mature adipocytes exhibiting marked variation in size with at least focal nuclear atypia. It represents 40–45% of all liposarcomas.

- Most common sites of liposarcoma are retroperitoneum, limbs, mediastinum and spermatic cord.
- Peak incidence is seen during sixth decade of life with male predominance. The tumor may affect children associated with Li-Fraumeni syndrome. It shows no potential for metastasis unless tumor undergoes dedifferentiation.



Fig. 28.3: Hibernoma. Tumor is a lobulated well demarcated large tumor with tan-yellow to red brown in color. Cut surface is brown with spongy consistency.

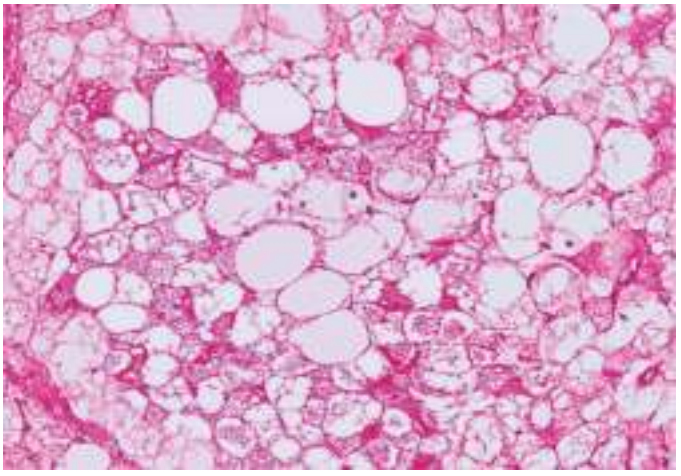


Fig. 28.4: Hibernoma. Tumor is composed of large number of brown adipocytes with small centrally located nuclei and multivacuolated cytoplasm; and admixed with normal adipocytes. Areas of necrosis and mitoses are absent in the neoplasm (400X).

- Atypical lipomatous tumor term is employed for lesion arising at surgically amenable locations in the limbs and trunk.
- Complete surgical excision of tumor is curable. Incomplete excision of well-differentiated liposarcoma without dedifferentiation and located in the mediastinum and retroperitoneal region causes recurrence and metastasis.
- According to recent 2020 WHO classification of soft tissue tumors and well-differentiated liposarcoma are of four subtypes, i.e. adipocytic, sclerosing, inflammatory and spindle cell liposarcomas.

Clinical Features

Patient presents with slow-growing painless lump in retroperitoneum, limbs, mediastinum and spermatic cord.

Surgical Pathology: Well-differentiated Liposarcoma/ Atypical Lipomatous Tumor

Gross Morphology

- Well-differentiated liposarcoma is a large well circumscribed lobulated mass. Rarely, an infiltrative growth pattern may be encountered.
- Cut surface of tumor varies from yellow to white with firm in consistency depending on the presence of variable amount of fibrous and/or myxoid components. Areas of fat necrosis are common in the large tumor (Fig. 28.5A).

Light Microscopy

According to recent 2020 WHO classification of soft tissue tumors and well-differentiated liposarcoma are of four subtypes, i.e. adipocytic, sclerosing, inflammatory and spindle cell liposarcomas. Presence of more than one morphologic pattern is common in the same lesion especially located in the retroperitoneal region.

- **Well-differentiated adipocytic subtype of liposarcoma:** Tumor is composed of mature adipocytes of variable size and shape associated with nuclear atypia in adipocytes or spindle cells in the tumor. Spindle cells containing hyperchromatic nuclei tend to be more numerous within fibrous septa. There is presence of varying number of monovacuolated or multivacuolated lipoblasts. It is worth mentioning that mere presence of lipoblasts is not essential to establish its diagnosis. Histology of well-differentiated adipocytic type of liposarcoma is shown in Fig. 28.5B.
- **Well-differentiated sclerosing subtype of liposarcoma:** Tumor tends to occur in the retroperitoneum and paratesticular region. Tumor is composed of scattered distinctive bizarre stromal cells with hyperchromatic nuclei admixed with occasional multivacuolated lipoblasts in a fibrillary collagenous background.
- **Well-differentiated inflammatory subtype of liposarcoma:** Tumor represents a rare variant of well-differentiated liposarcoma. It occurs in the retroperitoneum. Adipocytic nature of the neoplasm is obscured due to presence of chronic inflammatory infiltrate composed of polyphenotypic aggregates of lymphocytes and plasma cells. When the adipocytic component is sparse, the presence of bizarre multinucleated stromal cells represents a useful diagnostic tool.
- **Well-differentiated spindle cell subtype of liposarcoma:** Tumor is composed of bland neural-like spindle cells in a background of fibrous and/or myxoid tissue admixed with lipoblasts. Occasional tumor may contain osseous element, well-differentiated smooth or skeletal muscle component.

Differential Diagnosis

- Well-differentiated liposarcoma/atypical lipomatous tumor should be differentiated from conventional lipoma, spindle cell lipoma and pleomorphic lipoma. Presence of fat necrosis in conventional lipoma or multinucleated giant cells in spindle cell lipoma and pleomorphic lipoma may be mistaken for well-differentiated liposarcoma.
- Conventional lipoma, spindle cell lipoma and pleomorphic lipoma occur in superficial regions and lack MDM2 amplification analyzed by fluorescence *in situ* hybridization.
- Well-differentiated liposarcoma/atypical lipomatous tumor should also be differentiated from extrarenal angiomyolipoma. It occurs in the retroperitoneum. Neoplastic cells of angiomyolipoma show positivity for HMB-45 or MRT1. On the other hand, well-differentiated liposarcoma is negative for HMB-45 or MRT1.

Immunohistochemistry Technique

- Well-differentiated adipocytic type of liposarcoma shows positivity for S-100 protein, MDM2 and or CDK4. Amplification of MDM2 gene on chromosome 12q13.15 is demonstrated in well-differentiated liposarcoma by fluorescence *in situ* hybridization technique.
- Histiocytes and lipoma undergoing necrosis show positivity for MDM2.
- Well-differentiated spindle cell type of liposarcoma is negative for MDM2.

Markers	Expression
S-100 protein	Positive
MDM2	Positive
CDK4	Positive

Molecular Genetic Alterations

Quantitative profile of chromosome 12 sequences obtained by array comparative genomic hybridization (CGH) in well-differentiated liposarcoma/atypical lipomatous tumor shows discontinuous high level amplification in the region 12q14.15. Amplification of genes on chromosomes 12q13, 12q23, and 12q24.13 is also demonstrated in these cases. Amplification of MDM2 gene on chromosome 12q13.15 is demonstrated in well-differentiated liposarcoma by fluorescence *in situ* hybridization (FISH).



Fig. 28.5A: Liposarcoma in retroperitoneal region. Primary liposarcoma arises in the retroperitoneal space, but not from the retroperitoneal organs. Cut surface reveals well demarcated retroperitoneal mass having yellow cut surface. (*Courtesy*: Department of Pathology, Sher-E-Kashmir Institute of Medical Sciences, Srinagar, Jammu & Kashmir.)

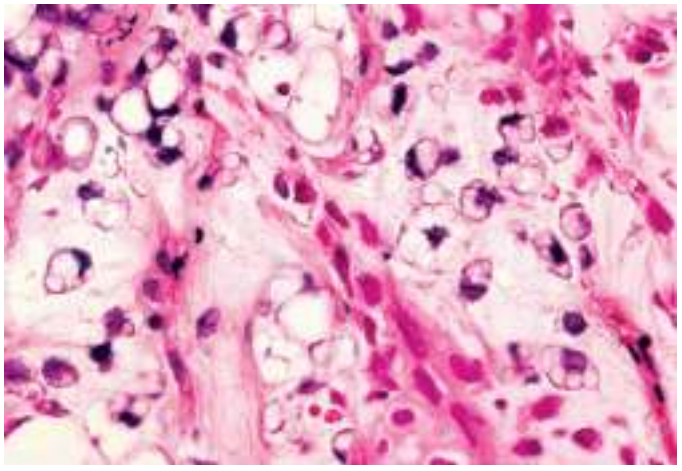


Fig. 28.5B: Well-differentiated adipocytic type of liposarcoma. Tumor is composed of mature adipocytes of variable size and shape associated with nuclear atypia in adipocytes or spindle cells in the tumor. Spindle cells containing hyperchromatic nuclei tend to be more numerous within fibrous septa. There is presence of varying number of monovacuolated or multivacuolated lipoblasts. It is worth mentioning that mere presence of lipoblasts is not essential to establish the diagnosis (400X).

Prognosis and Treatment

Anatomical location of well-differentiated liposarcoma/atypical lipomatous tumor is most important prognostic factor. Wide surgical excision of tumor located in superficial region does not recur. Surgical excision of tumor located in retroperitoneum and spermatic cord can recur or metastasize. Tumor located in retroperitoneum may transform to high-grade dedifferentiated liposarcoma.

DEDIFFERENTIATED LIPOSARCOMA

Dedifferentiated liposarcoma refers to a previously well-differentiated liposarcoma that has lost its adipocytic nature and transformed to aggressive neoplasm with metastatic potential. Peak incidence is seen during sixth decade of life with male predominance. It may affect children associated with Li-Fraumeni syndrome.

- Amplification of MDM2 gene on chromosome 12q13.15 is demonstrated in both well-differentiated liposarcoma and dedifferentiated liposarcoma by fluorescence *in situ* hybridization technique.
- Most common site of tumor is retroperitoneum. Other sites are spermatic cord, head and neck and trunk regions. Rare site is subcutaneous region.

Clinical Features

Patient presents with slow growing painless lump in retroperitoneum, head and neck and trunk regions.

Radiologic Findings

Imaging techniques demonstrate co-existence of both fatty and non-fatty solid components in the retroperitoneum.

Surgical Pathology: Dedifferentiated Liposarcoma

Gross Morphology

- Dedifferentiated liposarcoma is a large nodular yellow mass. Cut surface shows discrete solid tan gray dedifferentiated areas.
- Transformation between the lipomatous and dedifferentiated areas may sometimes be gradual (Fig. 28.6).

Light Microscopy

- In dedifferentiated liposarcoma, there is abrupt transition between well-differentiated liposarcoma and non-lipogenic high-grade areas of neoplasm. Morphology of these components of the neoplasm most often overlaps.
- Low-grade dedifferentiated liposarcoma tumor is composed of uniform fibroblastic spindle cells with nuclear atypia and arranged in a fascicular pattern.
- Dedifferentiated liposarcoma tumor may exhibit heterologous differentiation in 10% of cases. Heterologous elements include osseous, chondroid and angiomatous components. But presence of heterologous components does not affect clinical course (Fig. 28.7).

Differential Diagnosis

Dedifferentiated liposarcoma refers to previously well-differentiated liposarcoma that has lost its adipocytic nature and transformed to more aggressive neoplasm with metastatic potential. It should be differentiated from undifferentiated high-grade pleomorphic liposarcoma and high-grade liposarcoma with infiltrating benign adipose tissue component.

- Amplification of MDM2 gene on chromosome 12q13.15 is demonstrated in both well-differentiated liposarcoma and dedifferentiated liposarcoma by fluorescence *in situ* hybridization. Amplification of MDM2 gene on chromosome 12q13.15 is absent in undifferentiated liposarcoma.

- Most dedifferentiated liposarcomas retain the morphological features of residual well-differentiated liposarcoma in the tumor. This finding differentiates it from other high-grade liposarcoma infiltrating benign adipose tissue.

Immunohistochemistry Technique

Amplification of MDM2 gene on chromosome 12q13.15 is demonstrated in dedifferentiated liposarcoma by fluorescence *in situ* hybridization.

Marker	Expression
MDM2	Positive

Prognosis and Treatment

Dedifferentiated liposarcoma is treated by wide surgical excision and adjuvant chemotherapy.

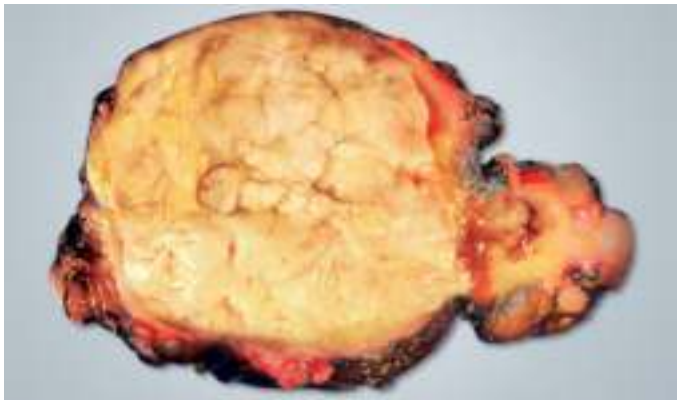


Fig. 28.6: Dedifferentiated liposarcoma. Tumor is large nodular yellow mass. Cut surface shows discrete solid tan gray dedifferentiated areas. Transformation between the lipomatous and dedifferentiated areas may sometimes be gradual.

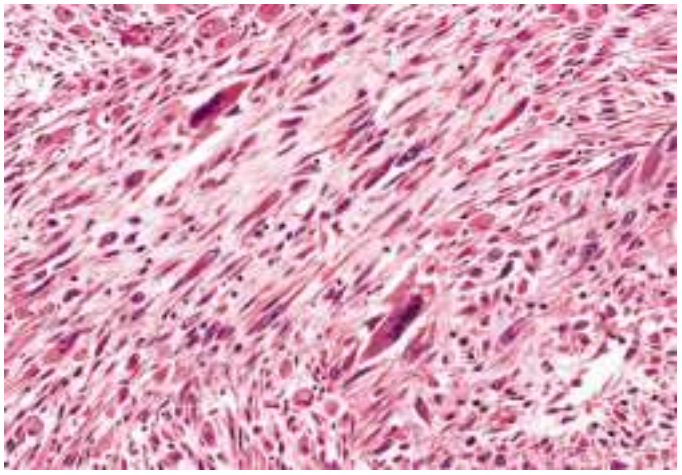


Fig. 28.7: Dedifferentiated liposarcoma. In dedifferentiated liposarcoma, there is abrupt transition between well-differentiated liposarcoma and nonlipogenic high-grade areas of neoplasm. Low-grade dedifferentiated liposarcoma is composed of uniform fibroblastic spindle cells with nuclear atypia and arranged in a fascicular pattern. Dedifferentiated liposarcoma may exhibit heterologous differentiation (osseous, chondroid and angiomatous components) in 10% of cases (400X).

MYXOID LIPOSARCOMA

Myxoid liposarcoma most often affects young adults and children with equal sex distribution and accounts for 15–20% of all liposarcomas. Most common site is deep soft tissue of the extremities especially thigh region in 66% of cases. Rare sites are retroperitoneum and subcutaneous tissue.

- Tumor is composed of uniform round to oval primitive (nonlipogenic) cells admixed with signet ring monovacuolated lipoblasts in a myxoid stroma along and plexiform vascularized myxoid stroma.
- The tumor consistently demonstrates translocation of chromosome 12 involving DDIT3 gene and chromosome 16 involving FUS gene. It may also demonstrate alternate translocation of chromosome 12 involving DDIT3 gene and chromosome 22 involving EWS gene. Fluorescence *in situ* hybridization (FISH) technique is performed to establish its diagnosis.

Clinical Features

Patient presents with large painless lump within the deep soft tissue of the extremities especially thigh region. Tumor metastasizes to retroperitoneum, opposite extremity or axilla, spine and lungs leading to clinical manifestations.

Surgical Pathology: Myxoid Liposarcoma

Gross Morphology

- Myxoid liposarcoma is well circumscribed multinodular intramuscular tumor. Gross evidence of tumor necrosis is uncommon.
- Cut surface is glistening and gelatinous in low-grade myxoid liposarcoma. It is fleshy in high-grade myxoid liposarcoma.

Light Microscopy

- Tumor is composed of uniform round to oval primitive (nonlipogenic) cells admixed with signet ring monovacuolated lipoblasts in a myxoid stroma along and plexiform vascularized myxoid stroma. Histology of myxoid liposarcoma is shown in Fig. 28.8.
- High-grade myxoid liposarcoma is composed of overlapping round cells with enlarged nuclei and prominent nucleoli arranged in solid nests.

Differential Diagnosis

Myxoid liposarcoma should be differentiated from well-differentiated liposarcoma with myxoid change, extraskeletal myxoid chondrosarcoma, lipoblastoma and myxofibrosarcoma.

- Atypical hyperchromatic tumor cells are absent in myxoid liposarcoma, but present in myxofibrosarcoma and well-differentiated liposarcoma.
- Myxoid liposarcoma never occurs during first decade of life. Morphological features of lipoblastomas and myxoid liposarcoma are identical.

- Fluorescence *in situ* hybridization (FISH) study is done to establish diagnosis showing translocation of chromosome 12 involving DDIT3 gene and chromosome 16 involving FUS gene. It may also demonstrate alternate translocation of chromosome 12 involving DDIT3 gene and chromosome 22 involving EWS gene.

Prognosis and Treatment

Myxoid liposarcoma metastasizes to distant organs in 33% of cases.

- Presence of significant population of round cells with vesicular nuclei constituting >5% of cell population in myxoid liposarcoma is associated with metastasis and poor prognosis. Patient is treated by wide surgical excision, radiotherapy and chemotherapy.
- High-grade myxoid liposarcoma with >5% round cell component has evidence of necrosis in tumor, TP53 and CDKN2A gene alterations are predictors of poor prognosis in localized myxoid liposarcoma.
- Low-grade myxoid liposarcoma has metastatic potential in <10% of cases.

PLEOMORPHIC LIPOSARCOMA

Pleomorphic liposarcoma is a high-grade neoplasm. Tumor is composed of variable number of pleomorphic lipoblasts and complex genomic profile. It most often affects adults in later life with male predominance. Peak incidence is seen during seventh decade of life. It accounts for 5% of all liposarcomas. Most common site is lower and upper extremities in decreased frequency in 66% of cases. Less frequent sites are trunk wall, retroperitoneum (25%) and spermatic cord.

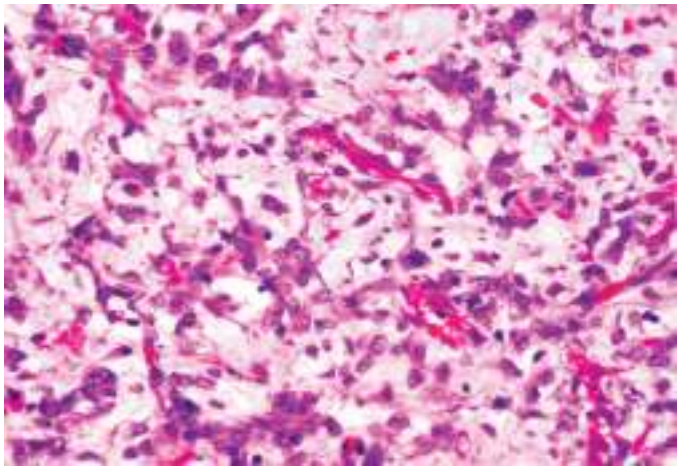


Fig. 28.8: Myxoid/round cell sarcoma. Tumor is composed of uniform round to oval primitive (nonlipogenic) cells admixed with signet ring monovacuolated lipoblasts in a myxoid stroma along and plexiform vascularized myxoid stroma (400X).

Clinical Features

Patient presents with rapidly growing mass with or without pain and symptoms related to the location of tumor over 3–6 months duration.

Surgical Pathology: Pleomorphic Liposarcoma

Gross Morphology

- Pleomorphic liposarcoma is well circumscribed tumor measuring 8–10 cm in diameter. Some tumors may be multinodular or infiltrative.
- Cut surface is white to yellow. Myxoid changes and foci of necrosis are demonstrated.

Light Microscopy

- Pleomorphic liposarcoma is composed of pleomorphic lipoblasts in variable proportion in a background of a high-grade sarcomatous element.
  - The presence of pleomorphic lipoblasts is essential for diagnosis.
  - Mitotic rate is variable. A median of 19–25 mitoses per high power field and necrosis are demonstrated in >50% of tumors.
- In most tumors, nonlipogenic component resembles undifferentiated pleomorphic liposarcoma, and shows spindle cells, multinucleated giant cells, neoplastic cells with vacuolated cytoplasm and hyaline droplets. Histology of pleomorphic liposarcoma is shown in Fig. 28.9.

Differential Diagnosis

- Pleomorphic liposarcoma should be differentiated from dedifferentiated liposarcoma, undifferentiated liposarcoma, high-grade pleomorphic liposarcoma and renal cell carcinoma.
- Pleomorphic liposarcoma and dedifferentiated liposarcoma have identical histologic features. Amplification of MDM2 gene on chromosome 12q13.15 is demonstrated in dedifferentiated liposarcoma by fluorescence *in situ* hybridization (FISH). On the other hand, pleomorphic liposarcoma lacks amplification of MDM2 gene on chromosome 12q13.15.
- Poorly differentiated carcinoma shows positivity for cytokeratins, on the other hand, pleomorphic liposarcoma lacks positivity for cytokeratins.

Immunohistochemistry Technique

Pleomorphic liposarcoma shows positivity for S-100 protein.

Markers	Expression
S-100 protein	Positive
Cytokeratin	Negative

Molecular Genetic Alterations

Pleomorphic liposarcoma is a high-grade neoplasm distinct from well-differentiated liposarcoma and myxoid liposarcoma. Like other liposarcomas, pleomorphic liposarcoma lacks translocation of chromosome 12 involving DDIT3 gene and chromosome 16 involving FUS gene analyzed by FISH. Tumor may also demonstrate alternate translocation of chromosome 12

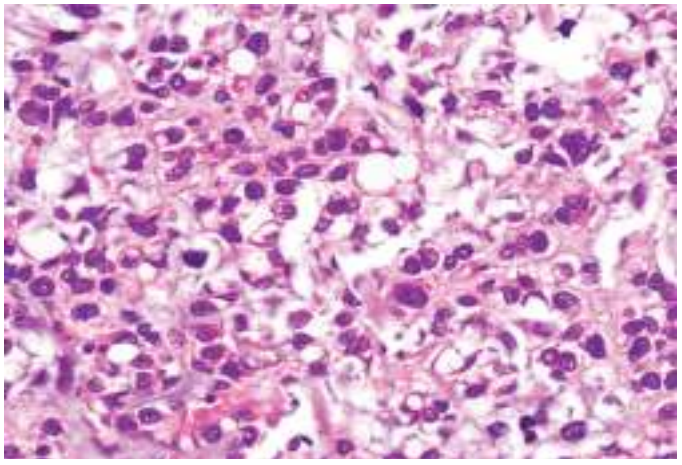


Fig. 28.9: Pleomorphic liposarcoma. Tumor is composed of pleomorphic lipoblasts in variable proportion in a background of a high-grade sarcomatous element. The presence of pleomorphic lipoblasts is essential for diagnosis. Mitotic rate is variable. A median of 19–25 mitoses per high power field and necrosis are demonstrated in >50% of tumors (400X).

involving DDIT3 gene and chromosome 22 involving EWS gene. On the other hand, these findings are present in myxoid liposarcoma.

Prognosis and Treatment

Pleomorphic liposarcoma is treated by wide surgical excision and adjuvant chemotherapy. Local recurrence and metastases in lungs and pleura are demonstrated in 30–50% of cases. Centrally located neoplasm in deep soft tissue and high mitotic index are associated with poor prognosis. Five-year survival rate is seen in 60% of cases.

Pathology Pearls: Adipocytic Tumors

- Pleomorphic lipoma and spindle cell lipoma occur in the subcutaneous tissue of neck and back regions, which may demonstrate atypical appearing multinucleated giant cells.
- Hibernoma is benign adipocytic tumor composed of brown adipocytes admixed with mature white adipose tissue.
- Angiolipoma is benign tumor composed of numerous capillaries admixed adipose tissue. Patient presents with painful nodule in subcutaneous region of young adults. It is essential to differentiate well-differentiated liposarcoma from lipoma.
- All adipocytic tumors located in retroperitoneum should be assessed for features of well-differentiated liposarcoma.
- Well-differentiated liposarcoma and dedifferentiated liposarcoma consistently demonstrate amplification of MDM2 gene located on chromosome 12 analyzed by fluorescence *in situ* hybridization.
- Pleomorphic liposarcoma is composed of bizarre lipoblasts, which lacks amplification of MDM2 gene located on chromosome 12 analyzed by fluorescence *in situ* hybridization.
- Myxoid liposarcoma and dedifferentiated liposarcoma have metastatic potential and recurrence after surgical excision.

FIBROBLASTIC/MYOFIBROBLASTIC TUMORS AND OTHER LESIONS

Fibroblastic/myofibroblastic tumors are grouped together and essentially composed of fibroblasts and myofibroblasts in varying proportion. Fibroblasts and myofibroblasts are able to contract due to the presence of myofilaments. Myofibroblasts participate in the early stages of wound healing and inflammation. According to recent 2020 World Health Organization of soft tissue tumors, fibroblastic/myofibroblastic tumors are categorized into benign tumors (cured by surgical excision), intermediate tumors (locally aggressive or rarely metastasizing) and malignant tumors (metastasizing to distant sites) in nature (Table 28.9).

Table 28.9 Fibroblastic/myofibroblastic tumors according to recent 2020 WHO classification of soft tissue tumors

Benign Tumors (Cures after Surgical Excision)

- Nodular fasciitis
- Proliferative fasciitis
- Proliferative myositis
- Myositis ossificans
- Fibro-osseous pseudotumor of digits
- Ischemic fasciitis
- Elastofibroma
- Fibrous hamartoma of infancy
- Myofibroma/myofibromatosis
- Fibromatosis colli
- Juvenile hyaline fibromatosis
- Inclusion body fibromatosis
- Fibroma of tendon sheath
- Desmoplastic fibroblastoma
- Mammary-type fibroblastoma
- Calcifying aponeurotic fibroma
- Angiomyoblastoma
- Cellular angiofibroma
- Nuchal-type fibroma
- Calcifying fibrous tumor
- Giant cell angiofibroma
- EWSR1-SMAD3 positive fibroblastic tumor (merging)

Intermediate (Locally Aggressive with Recurrence) Tumors

- Superficial fibromatosis (palmar/plantar)
- Desmoid-type fibromatosis
- Lipofibromatosis
- Giant cell fibroblastoma

Intermediate (Rarely Metastasizing) Tumors

- Dermatofibrosarcoma protuberans (DFSP)
  - Fibrosarcomatous dermatofibrosarcoma protuberans
  - Pigmented dermatofibrosarcoma protuberans
- Solitary fibrous tumor
  - Solitary fibrous tumor malignant
- Inflammatory myofibroblastic tumor
- Myxoinflammatory fibroblastic sarcoma
  - Atypical myxoinflammatory fibroblastic sarcoma
- Infantile fibrosarcoma

Malignant Tumors (Metastases Frequent)

- Adult fibrosarcoma (conventional)
- Myxofibrosarcoma
- Low-grade fibromyxoid sarcoma/hyalinizing spindle cell tumor
- Sclerosing epithelioid fibrosarcoma

KELOID

Keloid is an exaggerated response to injury to skin due to ear piercing or surgical wounds especially in dark persons. It is characterized by exuberant proliferation of fibroblasts and synthesis of abundant collagen bundles resulting in formation of large nodular scar. Keloid may recur after surgical excision.

Locations and Age Distribution

Most common sites of keloid are hands, ear lobe, neck, sternum, chest and shoulder. It most often in females on the ear lobe secondary to ear piercing during 10–30 years of age.

Clinical Features

Patient presents with large nodular scar on the upper back, shoulder, pre-sternal region and ear lobe. Keloid is most often asymptomatic. Sometimes, keloid may be tender, pruritic or painful. On clinical examination, keloid is elevated and protruding surrounding skin.

Surgical Pathology: Keloid

Gross Morphology

Keloid is nodular firm mass with curving contours with well defined borders. It varies in color, i.e. pink, red, gray or brown pigmented.

Light Microscopy

- Mature keloid is elevated and protruding surrounding skin and composed of homogenous, broad, eosinophilic fibrillary collagen bundles arranged in haphazardly array. Increased number of fibroblasts are found arrayed with an orientation that parallel the accompanying collagen bundles.
- Vascularity is diminished within keloid. Overlying epidermis is most often thinned out. Histology of keloid is shown in Fig. 28.10.

Differential Diagnosis

Keloid is differentiated from dermatofibroma, benign fibrous histiocytoma, lobomycosis (blastomycosis of skin) and hypertrophic scar. Comparison of keloid and hypertrophic scar is given in Table 28.10.

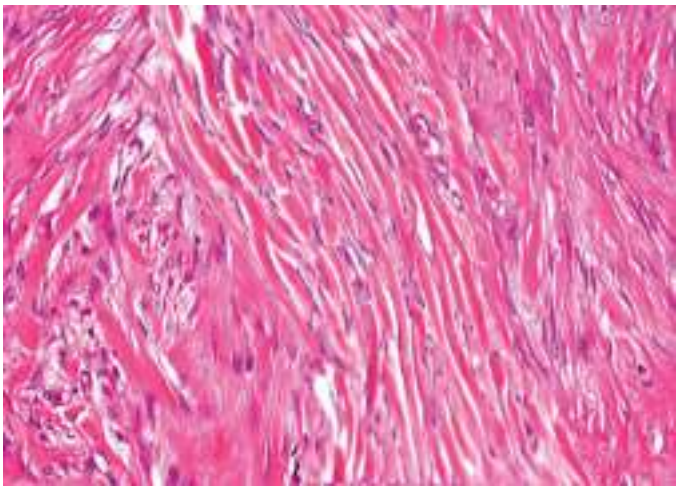


Fig. 28.10: Keloid scar. Keloid scar is composed of homogenous, broad, eosinophilic fibrillary collagen bundles arranged in haphazardly array. Increased number of fibroblasts are found arrayed with an orientation that parallel the accompanying collagen bundles. Vascularity is diminished within the lesion. It is worth mentioning that overlying epidermis is most often thinned out.

FIBROMATOSIS COLLI

Fibromatosis colli is a benign soft tissue lesion, which occurs due to proliferation of fibrous tissue resulting in thickened sternocleidomastoid muscle. It is recognized early in life as clinical torticollis (congenital muscular torticollis of sternocleidomastoid muscle tumor of infancy) possibly related to birth trauma.

Locations and Age Distribution

Most common site of fibromatosis colli is right sternocleidomastoid muscle. Diagnosis is established by the age of six months of life. Fibromatosis colli accounts for 0.4% of live births and more often affects male infants.

Clinical Features

About 20–25% present with neonatal torticollis (muscular tightening and resultant turning of the head toward the ipsilaterally involved shoulder). Within first few days to weeks of life, lesion is recognized as palpable, firm, non-tender mass at the base of the sternocleidomastoid muscle. Almost all cases show unilateral involvement of sternocleidomastoid muscle. Rarely, bilateral sternocleidomastoid muscles are involved.

Surgical Pathology: Fibromatosis Colli

Gross Morphology

- Fibromatosis colli is a small, firm gray-tan mass measuring 1–2 cm in diameter.
- Cut surface is gray white and glistening. It melts smoothly within the surrounding skeletal muscle.

Light Microscopy

- Fibromatosis colli is composed of plump fibroblasts and myofibroblasts resulting in partially displacing and replacing the skeletal muscle.
- Spindle cells contain nuclei without mitoses and arranged in long fascicles. The residual fibers show degeneration.
- Scar-like collagen fibers remain in the skeletal muscle leaving degenerating and regenerating skeletal fibers. Histology of fibromatosis colli is shown in Fig. 28.11.

Differential Diagnosis

Fibromatosis colli should be differentiated from fibromatosis, fibrodysplasia ossificans progressive and proliferative myositis.

Table 28.10 Comparison of keloid and hypertrophic scar

Features	Keloid	Hypertrophic Scar
Locations	Hands, ear lobe, neck, sternum, chest and shoulder	No predilection
Age and sex predominance	Females 10–30 years of age	No age and sex predominance
Clinical features	Asymptomatic to tender, pruritic or painful in smooth lesions	Asymptomatic
Extension beyond the margins of antecedent trauma	Present	Absent
Gross morphology	Mature keloid nodular firm mass with curving contours with well-defined borders elevated above skin and varying in color, i.e. pink, red, gray or brown pigmented	No definite gross morphology
Light microscopy	Early stage of keloid is cellular, and later mature keloid is composed of homogenous, broad, eosinophilic fibrillary collagen bundles arranged in haphazardly array. Increased number of fibroblasts are found arrayed with an orientation that parallel the accompanying collagen bundles, diminished vascularity within the lesion with thinning of overlying epidermis	Hypertrophic scar persists as a cellular fibrous tissue composed of collagen bundles but not undergoing hyalinization
Recurrence	Recurrence common after surgical excision	Recurrence after surgical excision

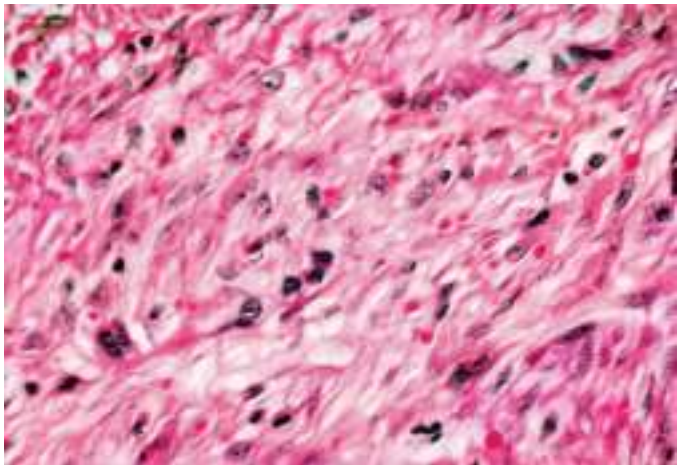


Fig. 28.11: Fibromatosis colli. Fibromatosis colli is composed of plump fibroblasts and myofibroblasts resulting in partially displacing and replacing the skeletal muscle. Spindle cells contain nuclei without mitoses and arranged in long fascicles. The residual fibers show degeneration. Scar-like collagen fibers remain in the skeletal muscle leaving degenerating and regenerating skeletal fibers.

Prognosis and Treatment

Fibromatosis colli is treated by stretching and physiotherapy resulting in resolution of lesion in 66% of cases. Some cases require surgical excision.

NODULAR FASCIITIS

Nodular fasciitis is a self-limited rapidly growing pseudosarcomatous reactive process, which occurs in the subcutaneous tissue of trunk and extremities. It affects young adults during first three decades of life. Nodular fasciitis is composed of fibroblasts and myofibroblasts, which may be associated with traumatic injury to the superficial fascia. Recurrence of nodular fasciitis is occasional observed after incomplete surgical excision.

Clinical Features

Patient presents with lump of 2–3 cm in subcutaneous tissue of trunk or extremities over a period of 3–6 weeks. Large lesion is unusual. It eventually regresses.

Surgical Pathology: Nodular Fasciitis

Gross Morphology

- Nodular fasciitis lesion is poorly circumscribed or infiltrative 2–3 cm mass without a capsule.
- Cut surface shows myxoid and fibrous tissue.

Light Microscopy

- Histologically, vast array of patterns is observed in nodular fasciitis: (a) cellular phase with myxoid changes, (b) fibrotic phase, (c) presence of ganglion-like cells, (d) presence of ossification (ossifying nodular fasciitis), and (e) presence of ischemic change (ischemic nodular fasciitis).
- Nodular fasciitis in cellular phase is composed of fibroblasts and myofibroblasts growing along fibrous septa of hypodermis. Early lesion of nodular fasciitis is composed of myxoid stroma and numerous mitoses (not abnormal mitoses). Long-standing lesion is composed of abundant collagen fibers and extracellular matrix.
- Inflammation is prominent around nodular fasciitis. Small blood vessels are numerous simulating granulation tissue. Osteoclast-like multinucleated giant cells are demonstrated in 10% of cases (Fig. 28.12).

Differential Diagnosis

Diagnostic difficulty occurs due to presence of vast array of histologic patterns in nodular fasciitis.

- Nodular fasciitis (cellular phase with myxoid change) should be differentiated from myxoma, myxofibrosarcoma, myxoid dermatofibrosarcoma and malignant nerve sheath tumor.
- Nodular fasciitis (cellular phase and without myxoid change): should be differentiated from cellular schwannoma, fibrous histiocytoma, leiomyosarcoma, fibrosarcoma, spindle cell melanoma and spindle cell carcinoma.
- Nodular fasciitis (fibrotic phase) should be differentiated from fibroma, neurofibroma and desmoplastic fibroblastoma.
- Ossifying nodular fasciitis should be differentiated from osteosarcoma.
- Nodular fasciitis with ganglion-like cells should be differentiated from rhabdomyosarcoma, ganglioneuroblastoma and pleomorphic sarcoma.
- Ischemic nodular fasciitis should be differentiated from myxofibrosarcoma and epithelioid sarcoma.

Immunohistochemistry Technique

Myofibroblasts show diffuse positivity for immunomarkers smooth muscle actin, muscle-specific actin (clone HHF35) and calponin, and focal positivity for desmin. Myofibroblasts are negative for h-caldesmon and S-100 protein.

Markers	Expression
Smooth muscle actin	Positive (diffuse staining)
Muscle-specific actin (clone HHF35)	Positive (diffuse staining)
Calponin	Positive (diffuse staining)
Desmin	Positive (focal staining)
h-caldesmon	Negative
S-100 protein	Negative

SUPERFICIAL FIBROMATOSIS (DUPUYTREN'S DISEASE OR CONTRACTURE)

Superficial fibromatosis is a benign recurring but non-metastasizing, infiltrative lesion as a result of fibroblastic proliferation in the soft tissue of palmar, plantar or penile regions. Immunohistochemistry technique and electron microscopy indicate that lesion is composed of myofibroblasts.

Locations and Age Distribution

Superficial fibromatosis most often occurs in the soft tissue of palmar, plantar or penile regions. It affects middle to advanced aged persons with male predominance. Superficial fibromatosis in plantar region is demonstrated in children and adolescents. It is more common in North Europeans.

Clinical Features

Patient presents with slow-growing subcutaneous nodules, plaques or cord-like lesions involving dermis or underlying fascia of ulnar side of palm resulting in contracture of fourth and fifth fingers. Superficial fibromatosis in plantar region is demonstrated in children and adolescents. Dupuytren's disease or contracture may be bilateral in 50% of cases with involvement of soles of feet, known as 'Ledderhose disease'.

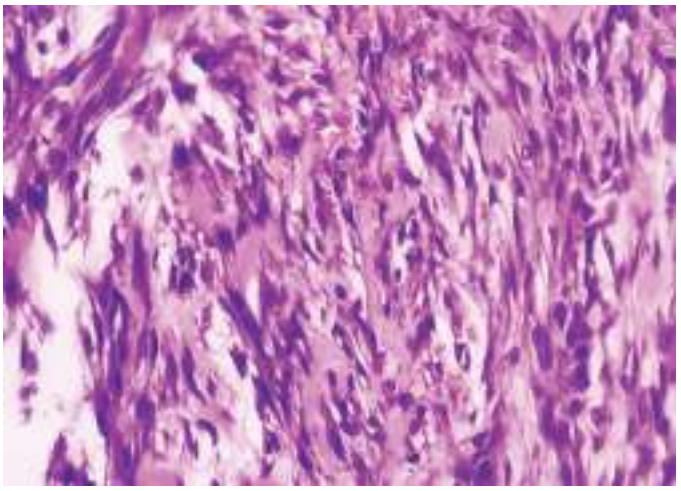


Fig. 28.12: Nodular fasciitis. Nodular fasciitis in cellular phase is composed of fibroblasts and myofibroblasts growing along fibrous septa of hypodermis. Early lesion of nodular fasciitis is composed of myxoid stroma and numerous mitoses (not abnormal mitoses). Long-standing lesion is composed of abundant collagen fibers and extracellular matrix. Inflammation is prominent around nodular fasciitis. Small blood vessels are numerous simulating granulation tissue. It is worth mentioning that osteoclast-like multinucleated giant cells are demonstrated in 10% of cases.

Surgical Pathology: Superficial Fibromatosis

Gross Morphology

- Superficial fibromatosis may be single or multiple measuring 0.5–2 cm in diameter.
- Cut surface is gray white with firm in consistency.

Light Microscopy

Superficial fibromatosis is composed of uniform nontypical fibroblasts arranged in monotonous fascicular fashion surrounded by abundant collagen fibers in long-standing lesion. Mitotic figures may be demonstrated. Hypercellularity is frequent in plantar superficial fibromatosis.

Differential Diagnosis

Superficial fibromatosis should be differentiated from synovial sarcoma, malignant peripheral nerve sheath tumor (MPNST), fibrosarcoma and desmoid type fibromatosis.

Immunohistochemistry Technique

Superficial fibromatosis shows positivity for vimentin and smooth muscle actin, and negative for CD34, cytokeratins, epithelial membrane antigen (EMA) and S-100 protein.

Markers	Expression
▪ Vimentin	▪ Positive
▪ Smooth muscle actin (SMA)	▪ Positive (focal staining)
▪ CD34	▪ Negative
▪ Cytokeratins	▪ Negative
▪ EMA	▪ Negative
▪ S-100 protein	▪ Negative

Molecular Genetic Alterations

Trisomy of 7 and 8; and loss of Y are demonstrated in Dupuytren's disease or contracture. Trisomy 8 and trisomy 14 are demonstrated in 'Ledderhose disease'.

Prognosis and Treatment

Superficial fibromatosis is treated by fasciectomy/aponeurectomy followed by skin grafting. It has strong tendency to recur after surgical intervention.

DEEP FIBROMATOSIS (DESMOID TYPE)

Deep fibromatosis (desmoid type) is a benign infiltrative fibroblastic/myofibroblastic neoplasm, which tends to recur after surgery. It does not metastasize.

Locations and Age Distribution

Deep fibromatosis is divided into abdominal, extra-abdominal and intra-abdominal types.

- **Abdominal wall deep fibromatosis:** It develops from musculoaponeurotic structures of the abdominal wall. It occurs in young women during pregnancy or within one year of childbirth, which suggests role of hormones in the pathogenesis of deep fibromatosis.
- **Extra-abdominal deep fibromatosis:** It most often occurs in skeletal muscles and aponeurosis of shoulder girdle, pelvic girdle, chest wall, thigh, head and neck regions affecting children, young and middle-aged persons. In children, most common sites are head and neck regions. The lesion tends to be more cellular and aggressive in children. Lesion may be multicentric in 5% of cases.
- **Intra-abdominal deep fibromatosis:** Trauma plays an important role in the pathogenesis of intra-abdominal deep fibromatosis in patient with prior abdominal surgery. Most common sites of deep fibromatosis are mesentery, retroperitoneum and pelvis. Pelvic deep fibromatosis is most often misdiagnosed as an ovarian tumor. Mesenteric deep fibromatosis is the commonest, which may be sporadic or associated with Gardner's syndrome.

Clinical Features

Patient presents with slow-growing painless deep-seated lump measuring 5–10 cm. Compression of structures by the tumor produce other symptoms. Abdominal deep fibromatosis occurs in young women during pregnancy or within one year of childbirth. Intra-abdominal deep fibromatosis may be sporadic or associated with Gardner's syndrome.

Radiologic Findings

Radiologic studies in deep fibromatosis reveal homogenous mass with infiltrative borders. Erosion of the underlying bone may be demonstrated. Myxoid change in the lesion may be demonstrated in some lesions.

Surgical Pathology: Deep Fibromatosis

Gross Morphology

- Deep fibromatosis is usually a solitary well circumscribed mass with infiltrative margin and soft in consistency measuring 5–10 cm in diameter.
- Cut surface shows fascicular white surface. Myxoid and cystic areas may be demonstrated in intra-abdominal deep fibromatosis. Areas of necrosis are absent.

Light Microscopy

- Deep fibromatosis is composed of spindle shape fibroblasts and myofibroblasts arranged in uniform fascicular architecture. Storiform growth pattern may be demonstrated in focal areas of the lesion.
- Abundant collagenous extracellular matrix is present. Blood vessels in muscle are well formed and showing gaping.
- Mesenteric deep fibromatosis may sometimes demonstrate keloid collagen. Myxoid change may be demonstrated in an occasional lesion (Fig. 28.13).

Differential Diagnosis

Deep fibromatosis should be differentiated from nodular fasciitis, fibrosarcoma, neurofibroma, low-grade malignant peripheral nerve sheath tumor, monophasic synovial sarcoma and scar tissue.

Immunohistochemistry Technique

Deep fibromatosis shows diffuse positivity for smooth muscle actin (SMA), variable expression of desmin and β-catenin. Lesion is negative for CD34, cytokeratins, EMA (epithelial membrane antigen) S-100 protein, and CD117.

Markers	Expression
▪ SMA	▪ Positive (diffuse staining)
▪ Vimentin	▪ Positive (focal staining)
▪ β-Catenin	▪ Positive (focal staining)
▪ CD34	▪ Negative
▪ Cytokeratins	▪ Negative
▪ EMA	▪ Negative
▪ S-100 protein	▪ Negative
▪ CD117	▪ Negative

Molecular Genetic Alterations

Trisomy 8 or 20 or both is/are demonstrated in 30% cases of deep fibromatosis. APC gene mutation is

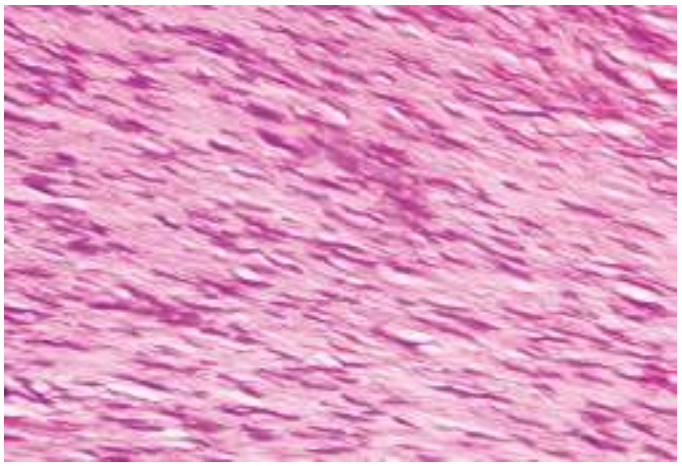


Fig. 28.13: Deep fibromatosis (desmoid type). Deep fibromatosis is composed of spindle shape fibroblasts and myofibroblasts arranged in uniform fascicular architecture. Storiform growth pattern may be demonstrated in focal areas of the lesion. Abundant collagenous extracellular matrix is present. Blood vessels in muscle are well formed and showing gaping. Mesenteric deep fibromatosis may sometimes demonstrate keloid collagen. Myxoid change may be demonstrated in an occasional lesion.

demonstrated in deep fibromatosis associated with Gardner's syndrome. Cellular abnormality of APC/β-catenin pathway, RB gene and TP53 gene mutations may be demonstrated in deep fibromatosis.

Prognosis and Treatment

Deep fibromatosis is a benign recurring but non-metastasizing lesion treated by wide surgical excision. Symptomatic patients are treated by low dose of chemotherapy and adjuvant irradiation.

INFANTILE FIBROSARCOMA (CONGENITAL FIBROSARCOMA)

Infantile fibrosarcoma (congenital fibrosarcoma) is an unusual neoplasm affecting infants and young children. Histology of infantile fibrosarcoma resembles classic adult fibrosarcoma. Tumor predominantly involves distal region of upper and lower extremities.

Locations and Age Distribution

Most common sites of infantile fibrosarcoma are superficial and deep tissues of lower extremity (foot, ankle, lower leg) and upper extremity (wrist, hand and forearm). Rare sites are trunk, head and neck region. Tumor is observed at birth in 30–40% of cases. It affects infants and children with slight male predominance.

Clinical Features

Patient presents with rapidly growing large painless mass in the distal regions of upper and lower extremities. Local examination reveals overlying skin reddened or ulcerated.

Surgical Pathology: Infantile Fibrosarcoma

Gross Morphology

- Infantile fibrosarcoma is a poorly circumscribed lobular mass measuring 5–15 cm in diameter.
- Cut surface shows grayish fleshy appearance. Areas of hemorrhage, necrosis and/or myxoid change may be demonstrated.

Light Microscopy

- Tumor is composed of densely packed monotonous spindle cells arranged in intersecting bundles. Nuclear pleomorphism is usually absent. Neoplastic cells are infiltrating the surrounding tissues.
- Numerous atypical mitoses and interstitial lymphocytes are demonstrated in the tumor. Hemangiopericytoma-like vascular pattern is most often present.

Differential Diagnosis

Infantile fibrosarcoma should be differentiated from conventional adult fibrosarcoma, malignant tumor of peripheral nerve sheath tumor, infantile fibromatosis (cellular variant) and spindle cell rhabdomyosarcoma.

Immunohistochemistry Technique

Infantile fibrosarcoma tumor cells show positivity for vimentin (diffuse staining), smooth muscle actin (variable staining), desmin (occasional case), CD34 (occasional case), S-100 protein (occasional case) and cytokeratins (occasional case). It is negative for epithelial membrane antigen.

Markers	Expression
Vimentin	Positive (diffuse staining)
SMA	Positive (variable staining)
Desmin	Positive (occasional case)
CD34	Positive (occasional case)
S-100 protein	Positive (occasional case)
Cytokeratins	Positive (occasional case)
EMA	Negative

Molecular Genetic Alterations

Reciprocal chromosomal translocation t(12;15) (p13;26) involves ETV6 gene on chromosome 12 and NTRK3 gene (a receptor tyrosine kinase gene) on chromosome 15q resulting in formation of ETV6-NTRK3 fusion protein; demonstrated in paraffin-embedded tissue or frozen tissue using RT-PCR (reverse transcriptase-polymerase chain reaction) or fluorescence *in situ* hybridization (FISH) technique. Trisomy of chromosomes 11, 20, 17 and 8 are demonstrated in infantile fibrosarcoma.

Prognosis and Treatment

Infantile fibrosarcoma is treated by complete surgical excision with or without adjuvant chemotherapy. Local recurrence after surgical excision is demonstrated in 5–50% of cases. Distant metastases are observed in <10% of case. Median five-year survival rate is observed in 75–80% of cases.

DERMATOFIBROSARCOMA PROTUBERANS

Dermatofibrosarcoma protuberans (DFSP) is a solitary slow growing highly infiltrative, locally aggressive and rarely metastasizing neoplasm. However, wide surgical excision with tumor margins is most often curative.

Locations and Age Distribution

Frequent sites of dermatofibrosarcoma protuberans are trunk, proximal upper extremity and lower extremity. It usually affects 20–50 years of age group.

Clinical Features

Patient presents with plaque-like thickening of skin and steadily progressing to form subcutaneous nodular mass over the course of several years.

Surgical Pathology: Dermatofibrosarcoma Protuberans

Gross Morphology

- Dermatofibrosarcoma protuberans is a solitary multinodular mass measuring several centimeters in diameter with average 5 cm.
- Although the neoplasm is highly infiltrative, yet it may appear as circumscribed mass (Fig. 28.14A).

Light Microscopy

- Dermatofibrosarcoma protuberans is composed of bland-appearing spindled fibroblasts arranged in an irregularly whorled or storiform pattern, which infiltrates into the periphery of tumor entrapping fat resulting in thinned out epidermis.
- Overlying epidermis may be normal, atrophic or ulcerated. plastic cells show mild atypia and occasional mitosis.
- Occasional tumor can have a more aggressive component, where neoplastic spindle cells are arranged in fascicular pattern. This variant of neoplasm is known as fibrosarcomatous variant of dermatofibrosarcoma protuberans (Fig. 28.14B).

Differential Diagnosis

- Dermatofibrosarcoma protuberans should be differentiated from fibrous histiocytoma (dermatofibroma).
- Unlike dermatofibrosarcoma protuberans, fibrous histiocytoma tends to be limited to the dermis.
- Analysis of COLLA1-PDGFB fusion protein by fluorescence *in situ* hybridization (FISH) technique can be helpful in diagnosing neoplasm in difficult cases.
- It is worth mentioning that storiform pattern may be demonstrated in dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, liposarcoma, peripheral nerve sheath tumor and nodular fasciitis.

Immunohistochemistry Technique

Dermatofibrosarcoma protuberans shows positivity for CD34.

Marker	Expression
CD34	Positive



Fig. 28.14A: Dermatofibrosarcoma protuberans. Tumor is multinodular mass measuring several centimeters with average 5 cm. Although, tumor is highly infiltrative, however it may appear as circumscribed mass. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

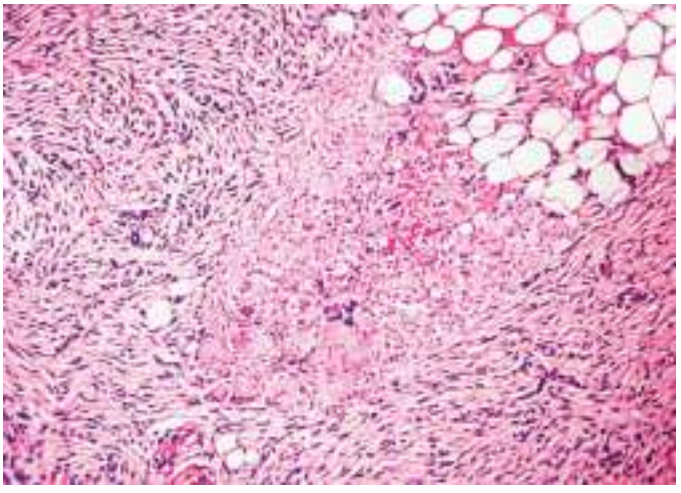


Fig. 28.14B: Dermatofibrosarcoma protuberans (DFSP). Dermatofibrosarcoma protuberans is composed of bland-appearing spindled fibroblasts arranged in an irregularly whorled or storiform pattern, which infiltrate into the periphery of tumor entrapping fat resulting in thinned out epidermis. Overlying epidermis may be normal, atrophic or ulcerated. Neoplastic cells show mild atypia and occasional mitosis. Occasional tumor can have a more aggressive component, where neoplastic spindle cells are arranged in fascicular pattern. This variant of neoplasm is known as fibrosarcomatous variant of dermatofibrosarcoma protuberans (400X).

Molecular Genetic Alterations

- Dermatofibrosarcoma protuberans exhibits chromosomal translocation t(17;22) (q22;q13) resulting in formation of COLLA1-PDGFB fusion protein analyzed by fluorescence *in situ* hybridization.
- Supernumerary ring chromosome in dermatofibrosarcoma protuberans may contain centromere of chromosome 22 and sequences from chromosomes 17 and 22.

Prognosis and Treatment

Prognosis of dermatofibrosarcoma protuberans is excellent after surgical excision of tumor. Recurrence of tumor is demonstrated in 11–33% of cases. Tumor may metastasize to lymph node in 1% and distant organs in 5% of cases.

CONVENTIONAL ADULT FIBROSARCOMA AND OTHER HISTOLOGIC VARIANTS

Conventional adult fibrosarcoma is a malignant neoplasm composed of fibroblasts and variable amount of collagen synthesis giving 'herringbone' architecture.

- Histologic variants of adult fibrosarcoma are conventional fibrosarcoma, low-grade fibromyxoid sarcoma, sclerosing epithelioid fibrosarcoma and myxofibrosarcoma.
- Low-grade fibromyxoid sarcoma shows reciprocal chromosomal translocation t(7;16) or ring chromosomes with material from chromosomes 7 and 17.

Incidence, Age Distribution and Clinical Features

- **Conventional adult fibrosarcoma:** Most common sites of tumor in decreased frequency are lower extremity (thigh region) and trunk. It most affects occurs young adults during 30–60 years of age with male predominance. Patient presents with large slow growing, deep-seated solitary lump at the site of prior burns, injury or radiotherapy site.
- **Low-grade fibromyxoid sarcoma variant of adult fibrosarcoma:** Most common sites of tumor are lower extremities and trunk. It most often affects young adults with male predominance. Patient presents with deep-seated slow-growing painless lump in the lower extremities and trunk.
- **Sclerosing epithelioid fibrosarcoma variant of adult fibrosarcoma:** Most common sites of tumor are lower extremities and limb girdles. It most often affects elderly persons during 50–70 years of age without sex predominance. Patient presents with deep-seated painless/painful lump in the lower extremities and limb girdles.
- **Myxofibrosarcoma variant of adult fibrosarcoma:** Most common sites of tumor are deep dermis, subcutaneous region of extremities and limb girdles. It most often affects adults with/without sex predominance. Patient presents with slow growing painless mass in lower dermis or deep to fascia in extremities and limb girdles.

Surgical Pathology: Adult Fibrosarcoma

Gross Morphology

- **Conventional adult fibrosarcoma:** Tumor is well circumscribed. Cut surface gives whitish and fleshy appearance with areas of hemorrhage and necrosis.
- **Low-grade fibromyxoid sarcoma variant of adult fibrosarcoma:** Tumor is well circumscribed or lobulated. Cut surface shows homogenous appearance.
- **Sclerosing epithelioid fibrosarcoma variant of adult fibrosarcoma:** Tumor is well circumscribed or lobulated measuring 7–10 cm in diameter. Cut surface shows whitish appearance with firm in consistency and areas of calcification or cystic/myxoid change.
- **Myxofibrosarcoma variant of adult fibrosarcoma:** Tumor is multinodular in the subcutaneous region. Cut surface shows multiple whitish glistening nodules. Deep-seated tumor is solitary poorly circumscribed with areas of hemorrhage and necrosis.

Light Microscopy

- **Conventional adult fibrosarcoma:** Tumor is composed of monotonous uniform bipolar neoplastic spindle cells with scanty cytoplasm, tapered nuclei and variable collagen stroma. Neoplastic cells are arranged in long, sweeping bundles showing 'herringbone' architecture. Mitotic activity is moderate in the neoplasm (Fig. 28.15).
- **Low-grade fibromyxoid sarcoma variant of adult fibrosarcoma:** Tumor is composed of an admixture of collagenous and myxoid zones. Tumor is composed of bland spindle cells with oval nuclei and eosinophilic cytoplasm arranged in fascicles or whorls in the collagenous zone. Myxoid zone of tumor contains plexiform blood vessels. Mitotic activity is low.
- **Sclerosing epithelioid fibrosarcoma variant of adult fibrosarcoma:** Tumor is composed of epithelioid or clear cells with uniform round or oval nuclei arranged in strands and nests. Dense collagenous stroma is present in the background. Tumor may be cellular and resembling conventional fibrosarcoma. Mitotic activity is low.
- **Myxofibrosarcoma variant of adult fibrosarcoma:** Tumor is composed of neoplastic cells containing hyperchromatic pleomorphic nuclei. Variable amount of myxoid areas is present in the background. There is presence of thin-walled blood vessels. Mitotic activity is low.

Immunohistochemistry Technique

- Conventional adult fibrosarcoma, low-grade fibromyxoid sarcoma, myxofibrosarcoma variants show positivity for vimentin and smooth muscle actin, and negative for cytokeratins and epithelial membrane antigen.
- Sclerosing epithelioid fibrosarcoma shows positivity for vimentin, BCL-2, p53, S-100, cytokeratins and epithelial membrane antigen.

Markers	Conventional Adult Fibrosarcoma Variant	Low-grade Fibromyxoid Sarcoma Variant	Myxofibrosarcoma Variant	Sclerosing Epithelioid Fibrosarcoma
▪ Vimentin	▪ Positive (constant staining)	▪ Positive (constant staining)	▪ Positive (constant staining)	▪ Positive (constant staining)
▪ Smooth muscle actin	▪ Positive	▪ Positive	▪ Positive	▪ Positive (90% cases)
▪ Cytokeratins	▪ Negative	▪ Negative	▪ Negative	▪ Positive (few cases)
▪ Epithelial membrane antigen	▪ Negative	▪ Negative	▪ Negative	▪ Positive (few cases)
▪ BCL-2	▪ Negative	▪ Negative	▪ Negative	▪ Positive (few cases)
▪ p53	▪ Negative	▪ Negative	▪ Negative	▪ Positive (few cases)
▪ S-100 protein	▪ Negative	▪ Negative	▪ Negative	▪ Positive (few cases)

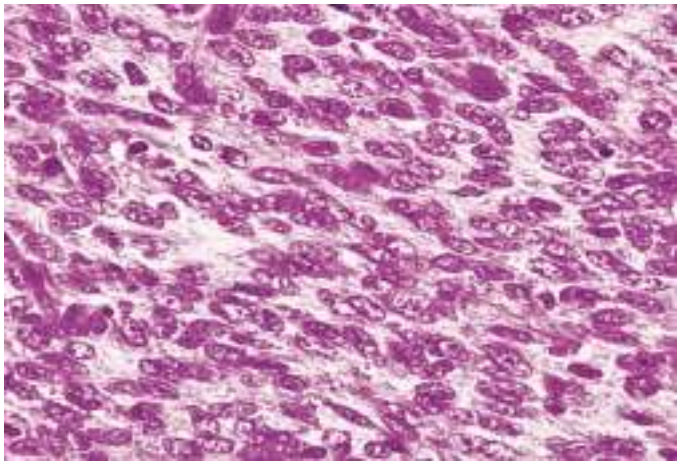


Fig. 28.15: Fibrosarcoma. Conventional fibrosarcoma is composed of monotonous uniform bipolar neoplastic spindle cells with scanty cytoplasm, tapered nuclei and variable collagen stroma. Neoplastic cells are arranged in long, sweeping bundles showing 'herringbone' architecture. Mitotic activity is moderate in the neoplasm.

Prognosis and Treatment

All histologic variants of adult fibrosarcoma are treated by wide surgical excision with tumor-free margins, radiotherapy and chemotherapy. Local recurrence and distant metastasis are demonstrated. Patients have five-year survival.

- **Conventional adult fibrosarcoma:** Adverse prognostic factors include high cellularity, high histologic grade, numerous atypical mitoses (>20 mitoses per 10 high power field), tumor size >5 cm in diameter, deep-seated location and presence of necrosis in the neoplasm.
- **Low-grade fibromyxoid sarcoma variant of adult fibrosarcoma:** Adverse prognostic factors include high-grade tumor and recurrence due to marginal surgical resection.
- **Sclerosing epithelioid fibrosarcoma variant of adult fibrosarcoma:** Adverse prognostic factors include large tumor, proximal location of tumor and male sex distribution.
- **Myxofibrosarcoma variant of adult fibrosarcoma:** Adverse prognostic factors include deep-seated location of tumor, high-grade of tumor and local recurrence within one year of treatment.

MYXOFIBROSARCOMA

Myxofibrosarcoma originates from fibroblasts in the dermis or subcutaneous tissue. Low-grade myxofibrosarcoma is composed of pleomorphic neoplastic cells admixed with myxoid stroma. Poorly differentiated high-grade myxofibrosarcoma resembles unclassified pleomorphic sarcoma. Low-grade myxofibrosarcoma shows t(17;16) cytogenetic abnormality.

Locations and Age Distribution

Most common sites of myxofibrosarcoma are shoulder and pelvic girdles followed by lower and upper extremities. It usually affects adults during sixth to eighth decades of life.

Clinical Features

Patient presents with soft tissue mass in shoulder girdle, pelvic girdle and extremities. Lung and bone metastases may occur.

Surgical Pathology: Myxofibrosarcoma
Gross Morphology
Myxofibrosarcoma demonstrates mucoid appearance.
Light Microscopy
Myxofibrosarcoma tumor can be low-grade, intermediate grade and high-grade. ▪ **Low-grade myxofibrosarcoma:** Tumor is composed of myxoid material that surrounds a conspicuous network of curved blood vessels and pleomorphic neoplastic cells containing hyperchromatic nuclei and poorly defined vacuolated cytoplasm. ▪ **High-grade myxofibrosarcoma:** Tumor is composed of numerous pleomorphic neoplastic cells with hyperchromatic nuclei arranged in clusters or sheets-like pattern.
Differential Diagnosis
Myxofibrosarcoma should be differentiated from low-grade fibromyxoid sarcoma, myxoid liposarcoma and intramuscular myxoma.

Prognosis and Treatment

Low-grade myxofibrosarcoma located in the superficial soft tissue of cutis has good prognosis. However, low-grade myxofibrosarcoma tumor >5 cm in diameter has metastatic potential. Incomplete surgical excision of tumor may recur. Intermediate/high-grade myxofibrosarcoma is most likely to recur after surgical resection. High-grade myxofibrosarcoma located in deep-seated region is more likely to metastasize to distant organs.

FIBROHISTIOCYTIC TUMORS

Fibrohistiocytic tumors according to revised 2020 WHO classification of tumors are given in Table 28.11.

BENIGN FIBROUS HISTIOCYTOMA

Benign fibrous histiocytoma is a benign tumor and characterized by proliferation of fibroblasts and histiocytes in the dermis or subcutis or both. Tumor is composed of fibroblasts and histiocytes arranged in sheets or short fascicles admixed with chronic inflammatory cells, multinucleated giant cells and hemosiderin laden macrophages. Most common sites of tumor are dermis or subcutis or both. It affects early to middle-aged persons during 20–40 years of age with female predominance.

Clinical Features

Patient presents with solitary slow-growing papule measuring <1 cm in size in the dermis or subcutis. Lesion in the deep soft tissue presents with painless measuring >5 cm in size.

Table 28.11 Fibrohistiocytic tumors according to revised 2020 WHO classification of tumors

Fibrohistiocytic Tumors	
Benign tumors	

▪ Tenosynovial giant cell tumor (giant cell tumor of tendon sheath)
  • Localized tenosynovial giant cell tumor
  • Diffuse tenosynovial giant cell tumor
  • Malignant tenosynovial giant cell tumor
▪ Deep benign fibrous histiocytoma
▪ Juvenile xanthogranuloma
▪ Xanthogranuloma	

Intermediate (rarely metastasizing) tumors	
▪ Atypical fibroxanthoma ▪ Plexiform fibrohistiocytic tumor	▪ Angiomatoid fibrous histiocytoma ▪ Giant cell tumor of soft tissue
Malignant tumors	
▪ Undifferentiated pleomorphic sarcoma ▪ Undifferentiated pleomorphic sarcoma with giant cells ▪ Undifferentiated pleomorphic sarcoma with prominent inflammation	

Surgical Pathology: Benign Fibrous Histiocytoma

Gross Morphology

- Benign fibrous histiocytoma is a plaque or nodule well circumscribed usually measuring <1 cm in diameter.
- Cut surface shows circumscribed yellow or whitish mass. Focal areas of hemorrhage may be demonstrated.

Light Microscopy

Histologic variants of benign fibrous histiocytoma are polymorphic, cellular, lipidized and variant located in the deep tissue, aneurysmal and epithelioid types.

- **Benign fibrous histiocytoma polymorphic variant:** Tumor is composed of monomorphic population of spindle cells arranged in fascicular and storiform patterns admixed with multinucleated giant cells (Touton giant cells), foamy macrophages, hemosiderin laden macrophages and interstitial fibrosis. Cutaneous lesion tends to be confined to dermis and sparing epidermis and subcutis.
- **Benign fibrous histiocytoma cellular variant:** Tumor is composed of numerous spindle cells arranged in fascicular pattern rather than storiform pattern. Multinucleated giant cells and hemosiderin laden macrophages are absent. Tumor shows numerous mitoses >3 per 10 high power fields.
- **Benign fibrous histiocytoma lipidized variant:** Tumor is composed of numerous xanthogranuloma cells admixed with a smaller number of spindle cells.
- **Benign fibrous histiocytoma in deep tissue variant:** Tumor is composed of numerous xanthogranuloma cells and few multinucleated giant cells arranged in diffusely storiform pattern.
- **Benign fibrous histiocytoma aneurysmal variant:** Tumor is composed of numerous large blood-filled spaces lined by discohesive neoplastic fragments, abundant hemosiderin deposits and mitotic activity near areas of hemorrhage. Growth of tumor is rapid due to hemorrhage.
- **Benign fibrous histiocytoma epithelioid variant:** Tumor is composed of large round to polygonal cells with abundant eosinophilic cytoplasm resembling the cells of reticulocytoma.

Differential Diagnosis

Benign fibrous histiocytoma should be differentiated from nodular fasciitis, neurofibroma, epithelioid sarcoma, dermatofibrosarcoma protuberans and Spitz nevus (epithelioid variant).

Immunohistochemistry Technique

Benign fibrous histiocytoma tumor cells show positivity for immunomarkers CD68, factor XIIIa, HMGA1, and HMGA2. It is negative for CD34, S-100 protein, CD1a and cytokeratins.

Markers	Expression
▪ CD68	▪ Positive
▪ Factor XIIIa	▪ Positive
▪ HMGA1	▪ Positive
▪ HMGA2	▪ Positive
▪ CD34	▪ Negative
▪ S-100 protein	▪ Negative
▪ CD1a	▪ Negative
▪ Cytokeratins	▪ Negative

Prognosis and Treatment

Benign fibrous histiocytoma located in dermis and subcutis regions is treated by surgical resection. Recurrence is demonstrated in <5% of cases. Tumor located in the deep soft tissue may recur after surgical resection in 50% of cases.

TENOSYNOVIAL GIANT CELL TUMOR LOCALIZED TYPE

Tenosynovial giant cell tumor localized type is also known as giant cell tumor of tendon sheath. It is well circumscribed benign lesion arising from hands and feet close to interphalangeal joints or synovium of tendon sheath.

- On the other hand, tenosynovial giant cell tumor diffuse type is known as pigmented villonodular tenosynovitis, which originates from articular space of knee or hip joints and soft tissue of wrists, thigh and feet in persons <40 years of age. Tenosynovial giant cell tumor diffuse type is more aggressive and tender than localized type.
- Histology of both localized and diffuse types is identical. It affects adults during fourth to sixth decades of life.
- Tenosynovial giant cell tumor localized type is treated by surgical excision. Recurrence may occur in 10% of cases. Forms of pigmented villonodular synovitis are given in Table 28.12.

Locations and Age Distribution

Tenosynovial giant cell tumor localized type is well circumscribed benign lesion arising from hands and feet close to interphalangeal joints or synovium of tendon sheath. It most often affects adults between 30 and 50 years of age.

Clinical Features

Patient presents with well circumscribed nodule in the interphalangeal joints of hands and feet in proximity to tendon sheath.

Surgical Pathology: Tenosynovial Giant Cell Tumor Localized Type

Gross Morphology

- Tenosynovial giant cell tumor localized type is well circumscribed and lobulated mass.
- Cut surface is whitish to variably yellow tan.

Light Microscopy

- Tenosynovial giant cell tumor localized type is composed of mononuclear cells with eosinophilic cytoplasm, osteoclast-like multinucleated giant cells and foamy histiocytes.
- Sometimes, cholesterol clefts may be demonstrated. Hemosiderin pigment may be identified (Fig. 28.16).

Differential Diagnosis

Tenosynovial giant cell tumor localized type should be differentiated from fibroma of tendon sheath.

Immunohistochemistry Technique

Mononuclear cells of tenosynovial giant cell tumor localized type show positivity for desmin and CD68. But immunohistochemistry is rarely used to establish diagnosis.

Markers	Expression
▪ Desmin	▪ Positive
▪ CD68	▪ Positive

JUVENILE XANTHOGRANULOMA

Juvenile xanthogranuloma is a self-limited benign histiocytic disorder, which arises from non-Langerhans histiocytes present in dermis or superficial cutis or both on head region; or deep soft tissue, viscera and eyes (iris).

Table 28.12 Forms of pigmented villonodular synovitis

Location and Types	Comments
Intra-articular location of pigmented villonodular synovitis	
Diffuse type	Diffuse involvement of joint synovium
Localized type	Localized (polypoid or nodular) joint synovial lesion
Extra-articular location of pigmented villonodular synovitis	
Diffuse type	Diffuse multifocal involvement of extra-articular synovial membrane (tendon)
Localized type	Localized extra-articular nodular lesion, also referred to as giant cell tumor of the tendon sheath

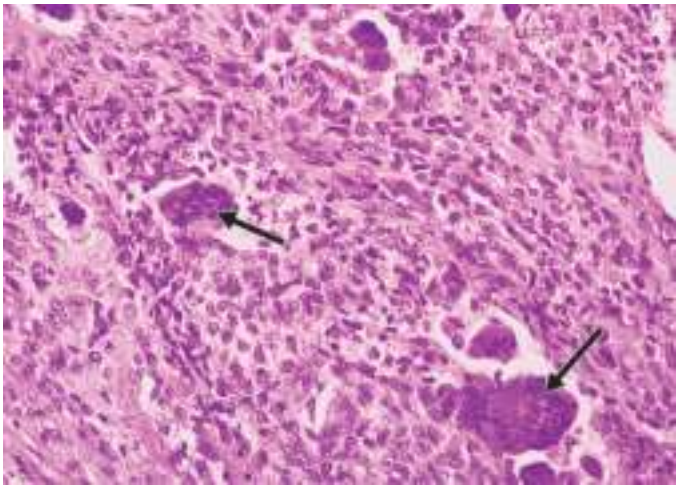


Fig. 28.16: Giant cell tumor of tendon sheath. Tenosynovial giant cell tumor localized type is composed of mononuclear cells with eosinophilic cytoplasm, osteoclast-like multinucleated giant cells and foamy histiocytes. Sometimes, cholesterol clefts may be demonstrated. Hemosiderin pigment may be identified (arrows) (400X).

Locations and Age Distribution

Most common sites of juvenile xanthogranuloma are dermis or superficial cutis or both on head region; or deep soft tissue, viscera and eyes (iris). Disorder usually affects infants and children. Cutaneous lesions have slight male predominance. Extracutaneous lesions are demonstrated in 4% of cases. Congenital juvenile xanthogranuloma is demonstrated in 10–35% of cases. Most lesions resolve by the age of five years.

Clinical Features

Most patients remain asymptomatic. Patient presents with multiple slightly raised skin papules on head region, or ocular lesion involving iris. Approximately 50% of patients with systemic involvement are accompanied by cutaneous involvement.

Surgical Pathology: Juvenile Xanthogranuloma

Gross Morphology

Juvenile xanthogranuloma is red yellow or brown papule or nodule measuring ≤1.5 cm in dimeter.

Light Microscopy

- **Cutaneous juvenile xanthogranuloma:** It is composed of histiocytes laden with lipid, eosinophils and Touton multinucleated giant cells in variable proportions. Fibrosis is demonstrated in long-standing cases.
- **Deep-seated juvenile xanthogranuloma:** It is more cellular neoplasm composed of monomorphic histiocytes laden with less lipid and a few Touton multinucleated giant cells.

Differential Diagnosis

Juvenile xanthogranuloma should be differentiated from dermatofibroma especially lipidized variant, Langerhans cell histiocytosis, mastocytosis, Spitz nevus and cutaneous xanthogranuloma.

Immunohistochemistry Technique

Juvenile xanthogranuloma shows positivity for CD68 (clones of KPI and PGM1), CD31, factor XIIIa, HAM56, HHF35, CD4, Ki-MIP, vimentin. It is negative for CD1a and S-100 protein.

Markers	Expression
CD68 (clones of KPI and PGM1)	Positive
CD31	Positive
Factor XIIIa	Positive
HAM56	Positive
HHF35	Positive
CD4	Positive
Ki-MIP	Positive
Vimentin	Positive
CD1a	Negative
S-100 protein	Negative

Prognosis and Treatment

Juvenile xanthogranuloma is self-limited benign disorder. Cutaneous, ocular lesions and systemic lesions are treated by surgical excision. Systemic corticosteroids can be used to shrink problematic visceral lesions.

XANTHOMA

Xanthoma represents a localized yellow lesion due to accumulation of lipid (cholesterol) within skin. It is associated with familial and secondary hyperlipidemia.

Locations and Age Distribution

- **Cutaneous xanthoma:** Lesions can be eruptive, plain and tuberous. Tuberous lesions present as nodules on the elbow, knee and buttock, associated with familial dysbetalipoproteinemia. These lesions resolve by treating underlying cause of hyperlipidemia. Disorder may occur in persons of any age.
- **Xanthoma in eyelid:** It is known as xanthelasma and not associated with hyperlipidemia. Xanthelasmas usually occur in persons older than 50 years.
- **Xanthoma in deep tissue:** It occurs in tendon sheath or synovium of fingers, wrist, and ankle (Achilles tendon) as a painless lesion and associated with hyperlipidemia. It may occur in persons of any age.
- **Xanthoma disseminatum:** Systemic lesions are demonstrated. Disorder occurs before the age of 25 years in 66% of cases.

Clinical Features

Patient presents with well circumscribed most often multiple masses in cutaneous regions, eyelid, deep tissues such as tendon sheath or synovium, elbow, knee and buttock regions.

Surgical Pathology: Xanthoma

Gross Morphology

Xanthoma is well circumscribed white or yellow to brown masses.

Light Microscopy

- Tuberous xanthoma and xanthogranuloma in tendons are composed of admixture of foamy macrophages, cholesterol clefts, foreign body of giant cells, lymphoid aggregates and fibrosis.
- Xanthelasma is predominantly of xanthogranuloma cells with little fibrosis.

Differential Diagnosis

Deep-seated xanthoma may be mistaken clinically for a sarcoma. However, histologic features and existence of hyperlipidemia are diagnostic of xanthogranuloma.

Prognosis and Treatment

Surgical excision of xanthoma is indicated in case of compromised joint function. Hyperlipidemia should be treated. Recurrence may occur unless hyperlipidemia is treated.

ATYPICAL FIBROXANTHOMA

Atypical fibroxanthoma is a low-grade superficial sarcoma that frequently presents as a red nodule or plaque on sun-exposed skin in the region of head and neck. Ultraviolet light appears to play a significant role since most lesions appear on the sun-exposed head and neck regions. Genetic factors are also linked to development of atypical fibroxanthoma. Other predisposing factors include trauma to the skin, radiation therapy and immunosuppression.

Locations and Age Distribution

Atypical fibroxanthoma almost occurs on sun-exposed skin in the region of head and neck. It affects elderly persons without sex predominance.

Clinical Features

Patient presents with small nodule <2 cm in diameter. Lesion may undergo ulceration.

Surgical Pathology: Atypical Fibroxanthoma

Gross Morphology

Atypical fibroxanthoma is solitary and small cutaneous nodule with or without ulceration measuring <2 cm in diameter.

Light Microscopy

- Atypical fibroxanthoma is composed of spindle to pleomorphic cells with hyperchromatic nuclei set in a variably collagenized matrix.
- Mononucleated and multinucleated tumor giant cells and mitoses including atypical mitoses are demonstrated. Solar elastosis of adjacent skin is present. Tumor necrosis is absent. Lesion is not extending into subcutis.

Differential Diagnosis

Atypical fibroxanthoma should be differentiated from spindle cell/sarcomatoid carcinoma, malignant fibrous histiocytoma, melanoma and spindle cell angiosarcoma (scalp region).

Immunohistochemistry Technique

Atypical fibroxanthoma shows positivity for CD99 and smooth muscle actin. It is negative for desmin, CD34, S-100 protein and cytokeratins.

Markers	Expression
CD99	Positive (diffuse staining in 75%)
Smooth muscle actin	Positive (focal staining)
Desmin	Negative
CD34	Negative
S-100 protein	Negative
Cytokeratins	Negative

Prognosis and Treatment

Atypical fibroxanthoma is treated by surgical excision. Local recurrence may occur in lesion invading deep soft tissue. Metastasis may be observed in exceptional case.

PLEXIFORM FIBROHISTIOCYTIC TUMOR

Plexiform fibrohistiocytic tumor is a mesenchymal tumor with intermediate malignant potential. Tumor is composed of fibroblasts, myofibroblasts and histiocytes. Most common site of tumor is deep dermis or subcutis of upper and lower extremities. It exclusively affects children and young adults with female predominance.

Clinical Features

Patient presents with painless slow-growing relatively ill-defined lump measuring 3 cm in diameter.

Surgical Pathology: Plexiform Fibrohistiocytic Tumor

Gross Morphology

Plexiform fibrohistiocytic tumor is multinodular firm and poorly defined dermal or subcutaneous mass measuring ≤3 cm in diameter.

Light Microscopy

- Plexiform fibrohistiocytic tumor is composed of fibroblasts, myofibroblasts and histiocytes arranged in short fascicular pattern. Multinucleated giant cells may be present.
- Spindle cell component branches out into the soft tissue and creating a plexiform or infiltrative architecture.
- Nuclear atypia, mitotic activity and necrosis are absent in the tumor. Vascular invasion may be demonstrated in minority of cases.

Differential Diagnosis

Plexiform fibrohistiocytic tumor should be differentiated from giant cell tumor of soft tissue, granulomatous inflammation, fibrous histiocytoma and fibromatosis.

Immunohistochemistry Technique

Plexiform fibrohistiocytic tumor shows positivity for vimentin, smooth muscle actin and CD68. It is negative for NK1/C.

Markers	Expression
Vimentin	Positive (spindle cell component)
Smooth muscle actin (SMA)	Positive (spindle cell component)
CD68	Positive (mononuclear cells and multinucleated osteoclasts like giant cells)
NK1/C	Negative

Prognosis and Treatment

Plexiform fibrohistiocytic tumor is treated by surgical excision. Local recurrence after marginal or incomplete surgical excision is demonstrated in 10–50% of cases. There is limited risk for metastases in the regional lymph nodes or distant organs. Metastasis may be demonstrated in the lymph node and lung in <1% of cases.

ANGIOMATOID FIBROUS HISTIOCYTOMA

Angiomatoid fibrous histiocytoma is a slow-growing tumor of intermediate malignant potential with a partially myxoid phenotype. Tumor occurs in the deep dermis and subcutis of extremities, trunk, head and neck. Most common sites of tumor are in the deep dermis and subcutis of extremities, trunk, head and neck in decreased frequency. Tumor may also originate from lymph node. Tumor affects children and young adults with mean age 20 years without sex predominance.

Clinical Features

Patient presents with painless lump in the deep dermis and subcutis of extremities, trunk, head and neck; and lymph node in decreased frequency.

Surgical Pathology: Angiomatoid Fibrous Histiocytoma

Gross Morphology

- Angiomatoid fibrous histiocytoma is a nodular cystic and hemorrhagic mass measuring 7–12 cm with median size >3 cm in diameter.
- Cut surface shows blood filled cystic spaces and red brown color due to hemosiderin deposit.

Light Microscopy

- Angiomatoid fibrous histiocytoma consists of a dense fibrous capsule with lymphoid infiltrate in the periphery of tumor.
- Tumor is composed of neoplastic cells with bland histoid to spindle cells arranged in short fascicles, whorls and sheets. Neoplastic cells contain hemosiderin deposits. Blood filled pseudovascular spaces are demonstrated in >50% of cases. Cellular atypia may be demonstrated in occasional tumor.

Differential Diagnosis

Angiomatoid fibrous histiocytoma should be differentiated from aneurysmal bone cyst, benign fibrous histiocytoma, metastatic tumor in lymph node, follicular dendritic tumor and rhabdomyosarcoma.

Immunohistochemistry Technique

Angiomatoid fibrous histiocytoma tumor cells show positivity for immunomarkers desmin, CD68, CD99 and epithelial membrane antigen (EMA). Tumor is negative for smooth muscle actin (SMA), myogenin, CD21, CD31, CD34, S-100 protein, HMB-45 and cytokeratins.

Markers	Expression
▪ Desmin	▪ Positive (50% cases)
▪ CD68	▪ Positive (50% cases)
▪ CD99	▪ Positive (50% cases)
▪ EMA	▪ Positive (40% cases)
▪ SMA	▪ Negative
▪ Myogenin	▪ Negative
▪ CD21	▪ Negative
▪ CD31	▪ Negative
▪ CD34	▪ Negative
▪ S-100 protein	▪ Negative
▪ HMB-45	▪ Negative
▪ Cytokeratins	▪ Negative

Molecular Genetic Alterations

Majority of cases with angiomatoid fibrous histiocytoma harbor one of three chromosomal translocations, i.e. t(12;16) (FUS-ATF1), t(12;22) (EWS:ATF1) and t(2;22) (EWS:CREB4).

Prognosis and Treatment

Angiomatoid fibrous histiocytoma is treated by wide surgical excision. Local recurrence is demonstrated in 2–10% of cases. It may metastasize to regional lymph nodes in 1% of cases.

SOFT TISSUE GIANT CELL TUMOR WITH LOW MALIGNANT POTENTIAL

Soft tissue giant cell tumor with low malignant potential is the counterpart of giant cell tumor of bone. It is now considered a fibrohistiocytic tumor of intermediate malignant potential according to recent 2020 WHO classification of soft tissue tumors.

Locations and Age Distribution

Soft tissue giant cell tumor with low malignant potential occurs as a multinodular mass in the skin or subcutis of the extremities. Tumor may also present as deep-seated mass in children. It most often affects young and middle-aged adults. It may also affect children.

Clinical Features

Patient presents with multinodular lump in dermis or subcutis of extremities. Children may present with deep-seated mass.

Surgical Pathology: Soft Tissue Giant Cell Tumor with Low Malignant Potential

Gross Morphology

- Soft tissue giant cell tumor with low malignant potential appears as small nodules in the dermis and subcutis measuring <5 cm in diameter.
- Cut surface shows foci of hemorrhage with gritty in consistency.

Light Microscopy

- Histology of soft tissue giant cell tumor with low malignant potential is identical to giant cell tumor of bone.
- Tumor is composed of bland mononuclear cells, spindle cells admixed with osteoclast-like giant cells. Spindle cells are arranged in short fascicular pattern. Mitoses without cytologic atypia are demonstrated in the tumor. Vascular invasion by neoplastic cells is demonstrated.

- Other histologic changes present in the tumor include metaplastic bone, vascular spaces, proliferation of fibroblasts and histiocytes and rarely cartilage.

Differential Diagnosis

Soft tissue giant cell tumor with low malignant potential should be differentiated from osteoclast-rich sarcomas, carcinoma, melanoma, lymphomas, tenosynovial giant cell tumor and intravascular nodular fasciitis.

Immunohistochemistry Technique

Soft tissue giant cell tumor with low malignant potential shows positivity for CD68 and smooth muscle actin (SMA). It is negative for immunomarkers CD31, CD34, S-100 protein, desmin and cytokeratins.

Markers	Expression
▪ CD68	▪ Positive (100% cases)
▪ SMA	▪ Positive (75% cases)
▪ CD31	▪ Negative
▪ CD34	▪ Negative
▪ S-100 protein	▪ Negative
▪ Desmin	▪ Negative
▪ Cytokeratins	▪ Negative

Prognosis and Treatment

Soft tissue giant cell tumor with low malignant potential is treated by surgical excision with wide tumor-free margins. Local recurrence may be demonstrated due to incomplete surgical excision of tumor in 10–50% of cases. Distant metastasis is demonstrated in <1% of cases.

UNDIFFERENTIATED PLEOMORPHIC SARCOMA

Undifferentiated pleomorphic sarcoma is the term designated for those tumors, in which melanotic and lymphoid differentiation have been excluded. Histologic variants of undifferentiated pleomorphic sarcoma include: (a) undifferentiated pleomorphic sarcoma with giant cells (so-called giant cell malignant fibrous histiocytoma) and (b) undifferentiated pleomorphic sarcoma with prominent inflammation (known as inflammatory malignant fibrous histiocytoma).

Locations and Age Distribution

- Undifferentiated pleomorphic sarcoma with giant cells variant (so-called giant cell malignant fibrous histiocytoma) occurs in deep soft tissue of extremities and trunk. It affects middle and advanced aged persons with male predominance.
- Undifferentiated pleomorphic sarcoma with prominent inflammation variant (known as inflammatory malignant fibrous histiocytoma) occurs in the retroperitoneum. This histologic variant is now considered as inflammatory form of dedifferentiated liposarcoma.

Clinical Features

Patient presents with deep-seated lump in extremities and trunk. Rapidly growing tumor is painful.

Surgical Pathology: Undifferentiated Pleomorphic Sarcoma

Gross Morphology

- Undifferentiated pleomorphic sarcoma is well circumscribed tumor measuring 5–15 cm in diameter.
- Cut surface shows areas of hemorrhage, myxoid and/or necrosis.

Light Microscopy

- Cellularity of undifferentiated pleomorphic sarcoma is variable. Tumor is composed of admixture of spindle cells and pleomorphic cells with nuclear atypia, multilobulated nuclei, mitoses, tumor giant cells, histiocytic-like foamy cells and areas of necrosis. Extracellular matrix may be collagenous to myxoid.
- Undifferentiated pleomorphic sarcoma with giant cells variant shows numerous osteoclasts like multinucleated giant cells.
- Undifferentiated pleomorphic sarcoma with prominent inflammation variant shows numerous xanthogranuloma cells, neutrophils and few tumor cells.

Differential Diagnosis

- Undifferentiated pleomorphic sarcoma should be differentiated from metastatic carcinoma or melanoma, lymphoma, dedifferentiated liposarcoma in retroperitoneum, rhabdomyosarcoma, leiomyosarcoma, malignant tumor of peripheral nerve sheath tumor and high-grade myxofibrosarcoma.
- Undifferentiated pleomorphic sarcoma with giant cells variant should be differentiated from giant cell-rich extraskeletal osteosarcoma, giant cell-rich leiomyosarcoma and giant cell tumor of soft tissue.
- Undifferentiated pleomorphic sarcoma with prominent inflammation variant should be differentiated from lymphoma, inflammatory myoblastic tumor and abscess.

Immunohistochemistry Technique

Undifferentiated pleomorphic sarcoma shows positivity for smooth muscle actin. It is negative for desmin, h-caldesmon, S-100 protein, epithelial membrane antigen and cytokeratins.

Markers	Expression
▪ Smooth muscle actin (SMA)	▪ Positive
▪ Desmin	▪ Negative
▪ h-caldesmon	▪ Negative
▪ S-100 protein	▪ Negative
▪ Epithelial membrane antigen (EMA)	▪ Negative
▪ Cytokeratins (CKs)	▪ Negative

Prognosis and Treatment

Undifferentiated pleomorphic sarcoma is treated by wide surgical excision followed by radiotherapy.

Adjuvant chemotherapy may be administered in selected young persons. Undifferentiated pleomorphic sarcoma with prominent inflammation variant shows a more indolent course. Five-year survival is observed in 30–50% of patients.

SMOOTH MUSCLE TUMORS

Smooth muscle tumors according to revised 2020 WHO classification of tumors are given in Table 28.13.

SOFT TISSUE LEIOMYOMA

Leiomyoma is a benign mesenchymal tumor with smooth muscle differentiation. Extra-uterine leiomyomas are classified according to their site of origin, i.e. pilar leiomyoma, genital leiomyoma, vascular leiomyoma, deep soft tissue leiomyoma and retroperitoneal leiomyoma. Diagnostic criteria of leiomyomas in non-gynecologic sites include minimal cytologic atypia, absence of coagulative necrosis, and mitoses not more than 1 per 50 high power fields. Complete surgical excision of leiomyoma is curative.

Locations, Age Distribution and Clinical Features

- **Pilar leiomyoma:** It is relatively a rare tumor, which arises from arrector pili smooth muscle is the subcutis region. Most common sites are extremities and trunk. Familial leiomyoma is associated with increased incidence of uterine leiomyoma and renal cell carcinoma. It affects adolescents and young adults without sex predominance. Patient presents with single or multiple painful red brown papules in the cutaneous regions of extremities and trunk regions.

Table 28.13 Smooth muscle tumors according to revised 2020 WHO classification of tumors

Smooth Muscle Tumors
Benign tumors
▪ Leiomyoma
• Genital leiomyoma
• Pilar leiomyoma
• Leiomyoma of deep soft tissue
• Vascular leiomyoma (angioleiomyoma)
• Leiomyoma of retroperitoneal region
Malignant tumors
▪ Leiomyosarcoma
• Leiomyosarcoma of deep soft tissue
• Cutaneous leiomyosarcoma
• Vascular leiomyosarcoma
• Leiomyosarcoma of peritoneum
• Epstein-Barr-associated smooth muscle tumor

- **Genital leiomyoma:** Tumor occurs in the dartos muscle in scrotum and deep muscles of vulva. It most often affects adults with female predominance. Patient presents with lump in labia majora most often misinterpreted as Bartholin cyst.
- **Deep soft tissue leiomyoma:** Tumor is extremely rare. Most common site of tumor is extremities. It affects young and middle-aged persons without sex predominance. Patient presents with solitary nodular lump in the extremities of young and middle-aged persons.
- **Vascular leiomyoma (angioleiomyoma):** Tumor most often occurs in the subcutis region. Rarely, it may occur in dermis.
  - **Solid variant of vascular leiomyoma:** It affects middle-aged to elderly adults with female predominance. Patient with solid variant of vascular leiomyoma presents with painful solitary nodule in the extremities.
  - **Venous variant of vascular leiomyoma:** It occurs in adults with male predominance. Patient with venous variant of vascular leiomyoma presents with painless lump in the head region.
- **Retroperitoneal leiomyoma:** Tumor is relatively common in the retroperitoneal region in perimenopausal women. It also occurs in retroperitoneal region in males. Patient presents with painful lump in the retroperitoneal region.

Surgical Pathology: Soft Tissue Leiomyoma

Gross Morphology

- Leiomyoma is a well circumscribed to infiltrative tumor with rubbery texture.
- Cut surface shows gray white and whorled surface. Areas of hemorrhage, cystic change, myxoid change and calcification may be demonstrated in the tumor.

Light Microscopy

- **Pilar leiomyoma:** Tumor may be infiltrative or well-circumscribed. Tumor is composed of bundles and fascicles of well-differentiated smooth muscle cells containing eosinophilic cytoplasm and blunt ended cigar-shaped nuclei arranged in an irregular manner in the reticular dermis (Fig. 28.17).
- **Genital leiomyoma, deep soft tissue leiomyoma, vascular leiomyoma (angioleiomyoma) and retroperitoneal leiomyoma:** Tumor is well-circumscribed to focally infiltrative having identical features in these regions.
- **Mitotic index in extra-uterine leiomyomas:** All type leiomyomas except vulvar leiomyoma show no significant nuclear atypia and mitotic index ≤1 mitosis per 50 high power fields. Vulvar leiomyoma may show up to 3 mitoses per 50 high power fields or coagulative necrosis in the tumor.

Differential Diagnosis

- Pilar leiomyoma should be differentiated from benign fibrous histiocytoma.
- Genital leiomyoma should be differentiated from cellular angiofibroma and hyperplasia of scrotal smooth muscle tumor.
- Deep soft tissue leiomyoma should be differentiated from schwannoma and leiomyosarcoma.
- Vascular leiomyoma (angioleiomyoma) should be differentiated from glomangiopericytoma.
- Retroperitoneal leiomyoma should be differentiated from leiomyosarcoma and cellular leiomyoma.

Immunohistochemistry Technique

Tumor cells leiomyoma shows positivity for immunomarkers desmin, smooth muscle actin, muscle-specific actin, calponin, h-caldesmon and smooth muscle myosin heavy chain.

Markers	Expression
Desmin	Positive
Smooth muscle actin (SMA)	Positive
Muscle-specific actin (MSA)	Positive
Calponin	Positive
h-caldesmon	Positive
Smooth muscle myosin heavy chain (SMMHC)	Positive

Prognosis and Treatment

Complete surgical excision of extra-uterine leiomyoma is curative.

SOFT TISSUE LEIOMYOSARCOMA

Extra-uterine leiomyosarcoma is a malignant mesenchymal tumor with smooth muscle differentiation. It accounts for 10% of soft tissue sarcomas. Depending on the site of origin of tumor, extra-uterine leiomyosarcomas are categorized into various groups: cutaneous leiomyosarcoma, deep soft tissue leiomyosarcoma, vascular leiomyosarcoma, retroperitoneal leiomyosarcoma, and EBV (Epstein-Barr virus) infection associated smooth muscle tumors.

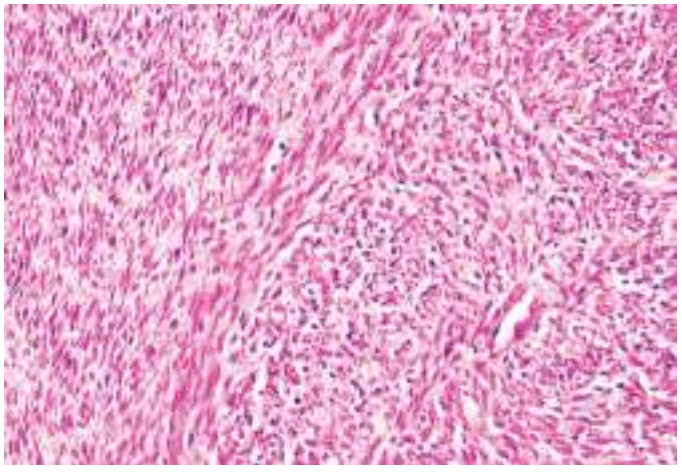


Fig. 28.17: Soft tissue leiomyoma. Pilar leiomyoma may be infiltrative or well circumscribed. Tumor is composed of bundles and fascicles of well-differentiated smooth muscle cells containing eosinophilic cytoplasm and blunt ended cigar-shaped nuclei arranged in an irregular manner in the reticular dermis (400X).

Locations and Age Distribution

- **Cutaneous leiomyosarcoma:** Tumor arises from the arrector pili muscle of skin on the extensor surface of extremities. It may also arise from dartos muscle of scrotum. It most often affects young adults with male predominance. Patient presents with slow growing lumps in these regions.
- **Deep soft tissue leiomyosarcoma:** Tumor occurs in the lower extremities. It most often affects middle-aged persons without sex predominance. Patient presents with slow growing lumps in these regions.
- **Vascular leiomyosarcoma:** Tumor arises from inferior vena cava (IVC) and large veins of the lower extremities. It most often affects adults with female predominance. Patient presents with tumor obstructing inferior vena cava and edema of lower extremities.
- **Retroperitoneal leiomyosarcoma:** Tumor originates from retroperitoneum. It most often affects adults with female predominance. Patient presents with slow growing lumps in these regions.
- **Epstein-Barr virus (EBV) infection associated smooth muscle tumors:** Immunocompromised AIDS patients and patients undergoing organ transplantation may acquire EB virus infection and develop multifocal smooth muscle cell tumors in endocrine glands, central nervous system, lung and liver. These can affect all age groups without sex predominance. These are slow growing tumor. These may be detected as incidental finding related to surveillance.

Surgical Pathology: Soft Tissue Leiomyosarcoma

Gross Morphology

- Leiomyosarcoma is poorly circumscribed fleshy red-tan.
- Cut surface shows areas of necrosis, cystic areas myxoid change (Fig. 28.18A).

Light Microscopy

Mitotic index in leiomyosarcoma is >4 per high power fields.

- **Cutaneous leiomyosarcoma:** Tumor is well to moderately differentiated and composed of atypical spindle cells and brisk mitoses. It can be either well-differentiated of diffusely infiltrative.
- **Deep soft tissue leiomyosarcoma:** Tumor is moderately differentiated and composed of atypical spindle cells arranged intersecting fascicles and brisk mitoses. Mitotic index is as low as 1 per 50 high power fields.

- **Vascular leiomyosarcoma:** Tumor is well to poorly differentiated and highly cellular with areas of necrosis. Cytoplasmic and perinuclear vacuolation are demonstrated in the neoplastic cells. Extensive myxoid change is uncommon in the tumor.
- **Retroperitoneal leiomyosarcoma:** Tumor is composed of spindle cells arranged in intersecting fascicles exhibiting a variable degree of cytologic atypia, mitotic activity and necrosis. Areas of cytoplasmic vacuolation in neoplastic cells are frequent (Fig. 28.18B).
- **EBV infection associated smooth muscle tumor:** Tumor is composed of dual population of well-differentiated spindle cells and small primitive round cells with prominent lymphocytic infiltrate. Areas of necrosis are uncommon.

Differential Diagnosis

- Leiomyosarcoma should be differentiated from spindle cell tumors, i.e. leiomyoma, gastrointestinal stromal tumors, cellular schwannoma and undifferentiated high-grade pleomorphic sarcoma. Immunohistochemistry plays important role to establish diagnosis.
- Leiomyosarcoma shows positivity for desmin, smooth muscle actin, muscle-specific actin, calponin, h-caldesmon, smooth muscle myosin heavy chain and EBV-RNA.
- Leiomyosarcoma has more cytologic atypia, mitotic index and areas of necrosis than leiomyoma.
- Unlike leiomyosarcoma, gastrointestinal stromal tumors show positivity for CD117. Schwannoma shows positivity for S-100 protein.
- Retroperitoneal leiomyosarcoma is very aggressive tumor with metastatic potential and poor prognosis.

Immunohistochemistry Technique

Leiomyosarcoma shows positivity for desmin, smooth muscle actin, muscle-specific actin, calponin, h-caldesmon, smooth muscle myosin heavy chain and EBV-RNA.

Markers	Expression
Desmin	Positive
Smooth muscle actin	Positive
Muscle-specific actin	Positive
Calponin	Positive
h-caldesmon	Positive
Smooth muscle myosin heavy chain	Positive
EBV-RNA	Positive

Prognosis and Treatment

Leiomyosarcoma is treated by wide surgical excision and radiotherapy. Chemotherapy is administered for metastatic disease.

SKELETAL MUSCLE TUMORS

Skeletal muscle tumors are classified into benign (rhabdomyoma) and malignant (rhabdomyosarcoma). Rhabdomyosarcoma affects children and adults. Accurate subclassification of rhabdomyosarcoma is essential in view of prognostic implications. Skeletal muscle tumors according to revised 2020 WHO classification of tumors are given in Table 28.14.



Fig. 28.18A: Leiomyosarcoma in retroperitoneal region. Primary leiomyosarcoma arises in the retroperitoneal space, but not from the retroperitoneal organs. Cut surface reveals poorly demarcated tumor having variegated appearance with areas of hemorrhage. (*Courtesy*: Department of Pathology, Sher-E-Kashmir Institute of Medical Sciences, Srinagar, Jammu & Kashmir.)

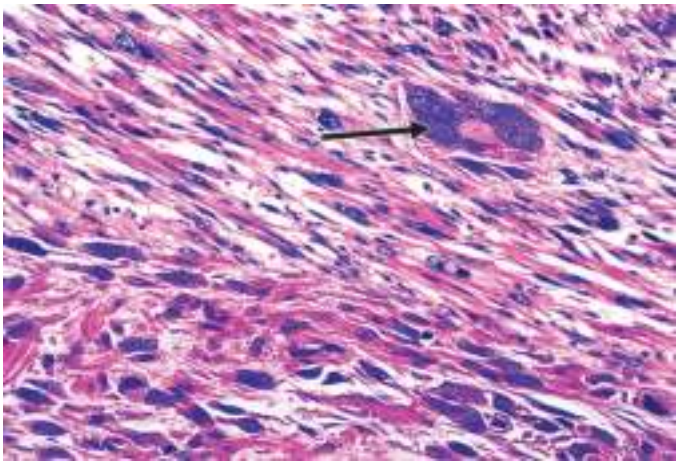


Fig. 28.18B: Leiomyosarcoma in retroperitoneal region. Tumor is composed of spindle cells arranged in intersecting fascicles exhibiting a variable degree of cytologic atypia, mitotic activity and necrosis. Areas of cytoplasmic vacuolation in neoplastic cells are frequent (arrow) (400X).

RHABDOMYOMA

Rhabdomyoma is a rare mesenchymal tumor. It is classified into cardiac and noncardiac types. Depending on the degree of differentiation, noncardiac rhabdomyoma is further categorized into adult rhabdomyoma, fetal rhabdomyoma, and genital rhabdomyoma. Genital rhabdomyoma is well-differentiated tumor. It is difficult to differentiate rhabdomyoma and rhabdomyosarcoma. Cardiac rhabdomyoma is discussed in Chapter 16. Noncardiac rhabdomyoma is treated by surgical excision.

Table 28.14 Skeletal muscle tumors according to revised 2020 WHO classification of tumors

Skeletal Muscle Tumors
Benign tumors
▪ Rhabdomyosarcoma • Adult rhabdomyosarcoma • Fetal rhabdomyosarcoma • Genital rhabdomyosarcoma
Malignant tumors
▪ Embryonal rhabdomyosarcoma • Conventional embryonal rhabdomyosarcoma • Spindle cell rhabdomyosarcoma (pediatric) • Botryoid rhabdomyosarcoma • Anaplastic rhabdomyosarcoma ▪ Alveolar rhabdomyosarcoma including solid alveolar rhabdomyosarcoma ▪ Pleomorphic rhabdomyosarcoma ▪ Spindle cell/sclerosing rhabdomyosarcoma (adult)

Locations and Age Distribution

Most common sites of adult and fetal rhabdomyoma are head and neck regions, mucosal surface of oral cavity, larynx and pharynx.

- **Adult rhabdomyoma:** Tumor is multinodular or even multicentric. It most often affects adults. Mean age is 60 years with male predominance.
- **Fetal rhabdomyoma:** Tumor may be associated with nevoid basal cell carcinoma syndrome. It affects children. Median age is 4 years. It may also affect adults.
- **Genital rhabdomyoma:** Tumor resembles a benign polyp in vagina. It exclusively affects women between 30–48 years of age.

Clinical Features

Patient presents with mass and obstruction with signs and symptoms related to location of tumor.

Surgical Pathology: Rhabdomyoma

Gross Morphology

- **Adult rhabdomyoma:** Tumor is well delineated, nodular mass measuring 1.5–8 cm in diameter. Cut surface is glistening.
- **Fetal rhabdomyoma:** Tumor is also well delineated solitary mass measuring 1–13 cm in diameter. Cut surface is more glistening than adult rhabdomyoma.

Light Microscopy

- **Adult rhabdomyoma:** Tumor is composed of lobules of closely packed large polygonal cells with abundant eosinophilic granular/or vacuolated cytoplasm and vesicular nuclei. Cell outlines are well defined. Intercellular stroma is usually scant. Mitotic figures are absent.
- **Fetal rhabdomyoma:** Tumor has two histologic variants: (a) classic immature fetal rhabdomyoma, and (b) intermediate fetal rhabdomyoma. Classic immature fetal rhabdomyoma is composed of primitive spindle cells, myotubules and myxoid stroma. Intermediate fetal rhabdomyoma is composed of numerous spindled rhabdomyoblasts arranged in fascicular pattern. Mitoses are absent in both histologic variants.
- **Genital rhabdomyoma:** Tumor is composed of rhabdomyoblasts haphazardly arranged embedded in fibrous stroma.

Histochemistry

In adult rhabdomyoma, PAS stain demonstrates abundant glycogen in the cytoplasm of neoplastic cells. Masson trichrome or phosphotungstic acid hematoxylin (PTAH) stain shows striations/inclusions in the cytoplasm of neoplastic cells.

Differential Diagnosis

- Adult rhabdomyoma should be differentiated from granular cell tumor, crystal-storing histiocytes and alveolar soft part sarcoma.
- Fetal rhabdomyoma should be differentiated from embryonal rhabdomyosarcoma.
- Genital rhabdomyoma should be differentiated from vagina; stromal polyp and embryonal rhabdomyosarcoma.

Immunohistochemistry Technique

Rhabdomyoma shows always positivity for desmin, muscle-specific actin, MyoD1 and myoglobin.

Markers	Expression
▪ Desmin	▪ Positive
▪ Muscle-specific actin	▪ Positive
▪ MyoD1	▪ Positive
▪ Myoglobin	▪ Positive

EMBRYONAL RHABDOMYOSARCOMA

Embryonal rhabdomyosarcoma is a primitive soft tissue sarcoma showing phenotypic and variable degree of embryonic skeletal muscle differentiation. Histologic variants of embryonal rhabdomyosarcoma include conventional, botryoid, spindle cell and anaplastic types.

Locations and Age Distribution

Embryonal rhabdomyosarcoma affects children during 5–15 years of age with male predominance.

- **Conventional embryonal rhabdomyosarcoma:** Most common sites of tumor are orbit, eyelid, oropharynx, parotid gland, middle ear, auditory canal, nose, paranasal sinuses, tongue and cheek.
- **Botryoid rhabdomyosarcoma:** Most common sites of tumor are wall of hollow organs or beneath mucosal lining of urinary bladder, vagina, nasopharynx and middle ear. Cystoscopy shows grape-like polypoidal masses in the urinary bladder.
- **Spindle cell rhabdomyosarcoma:** It occurs in the scrotal region.

Clinical Features

Patient presents with painful or painless mass, obstruction and/or bleeding depending on the site of involvement.

Surgical Pathology: Embryonal Rhabdomyosarcoma

Gross Morphology

- **Embryonal rhabdomyosarcoma:** It is poorly circumscribed fleshy tumor measuring 1–32 cm in diameter.
- **Botryoid rhabdomyosarcoma:** It resembles cluster of grapes.
- **Spindle cell rhabdomyosarcoma:** It is relatively firm and more fibrous in consistency.

Light Microscopy

- **Conventional embryonal rhabdomyosarcoma:** Tumor is composed of variable density neoplastic cells ranging from undifferentiated to classic rhabdomyoblasts arranged in sheets. Neoplastic cells contain round eccentric nuclei and eosinophilic cytoplasm. Interstitial matrix may be sparse or abundant, and myxoid in nature (Fig. 28.19).
- **Botryoid rhabdomyosarcoma:** Tumor is composed of round to oval cells admixed with spindle cells in loose myxoid stroma. A cambium layer of tightly packed undifferentiated neoplastic cells is demonstrated beneath mucosal lining (Fig. 28.20).
- **Spindle cell rhabdomyosarcoma:** Tumor is composed of spindle cells with eosinophilic cytoplasm, cigar-shaped blunt ended nuclei. Tumor contains >50% of spindle cells are arranged in whorls, fascicles and storiform pattern.
- **Anaplastic rhabdomyosarcoma:** Tumor is composed of anaplastic cells with hyperchromatic nuclei and numerous mitoses, the size of nuclei three times larger than the surrounding cells. Anaplastic cells may be arranged in diffuse or multifocal pattern.

Differential Diagnosis

- Embryonal rhabdomyosarcoma is a blue round cell tumor. It should be differentiated from other blue round cell tumors, e.g. Ewing sarcoma/primitive neuroectodermal tumor (PNET), neuroblastoma, nephroblastoma, desmoplastic small round cell tumor, small cell synovial sarcoma, lymphoma, rhabdoid tumor, rhabdomyoma and alveolar rhabdomyosarcoma.
- Spindle cell variant of embryonal rhabdomyosarcoma should be differentiated from leiomyosarcoma, myofibromatosis and infantile fibromatosis.

Immunohistochemistry Technique

- Embryonal rhabdomyosarcoma shows positivity for desmin, muscle-specific actin (MSA), myogenin and α-sarcomere actin.
- Desmin is universal immunohistochemical marker of smooth and striated muscle. Its expression in rhabdomyoblasts is variable.
- Muscle-specific actin (MSA) is also universal immunohistochemical marker of smooth and striated muscle.
- MyoD1 and myogenin are transcriptional factors specific to striated muscle. These immunomarkers are applied to differentiate rhabdomyosarcoma (positive for immunohistochemical markers) from leiomyosarcoma (negative for immunohistochemical markers). Embryonal rhabdomyosarcoma shows focal positivity.

Markers	Expression
Desmin	Positive (cytoplasmic staining)
Muscle-specific actin (MSA)	Positive
MyoD1 (specific immunohistochemical markers for striated muscles)	Positive (nuclear staining)
Myogenin (specific immunohistochemical markers for striated muscles)	Positive (nuclear staining)
PAX3-FOXO1A fusion transcript	Absent
PAX7-FOXO1A fusion transcript (better prognosis)	Absent

PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts are demonstrated in alveolar rhabdomyosarcoma. On the other hand, embryonal rhabdomyosarcoma lacks these PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts.

Molecular Genetic Alterations

Embryonal rhabdomyosarcoma does not show consistent cytogenetic abnormality. Extra copies of chromosomes 2, 8 and 13 are frequent. Allelic loss in the chromosomal region 11p15 has also been reported.

Prognosis and Treatment

- Embryonal rhabdomyosarcoma is treated by surgery, chemotherapy and radiotherapy. It may metastasize to lungs and lymph nodes.

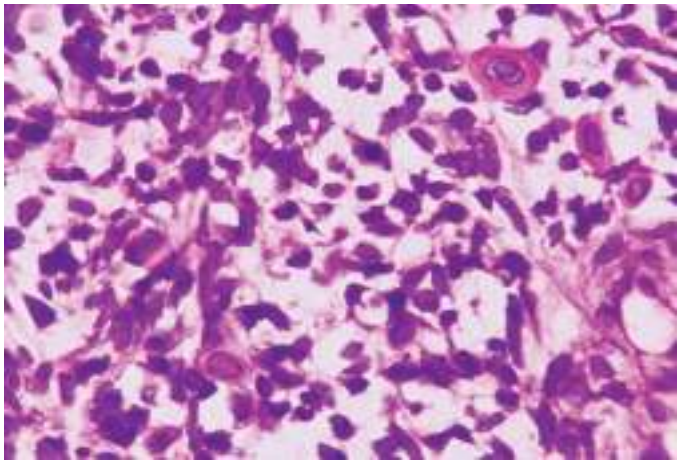


Fig. 28.19: Conventional embryonal rhabdomyosarcoma. Tumor is composed of variable density neoplastic cells ranging from undifferentiated to classic rhabdomyoblasts arranged in sheets. Neoplastic cells contain round eccentric nuclei and eosinophilic cytoplasm. Interstitial matrix may be sparse or abundant, and myxoid in nature (400X).

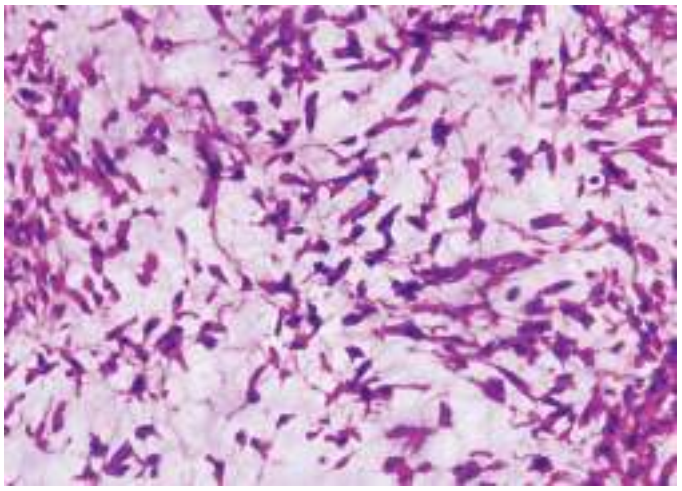


Fig. 28.20: Botryoid rhabdomyosarcoma. Tumor is composed round to oval cells admixed with spindle cells in loose myxoid stroma. A cambium layer of tightly packed undifferentiated neoplastic cells is demonstrated beneath mucosal lining (400X).

- Botryoid rhabdomyosarcoma and spindle cell rhabdomyosarcoma in paratesticular region have good prognosis in >90% of cases.
- Conventional embryonal rhabdomyosarcoma and spindle cell rhabdomyosarcoma in non-paratesticular region have intermediate prognosis in >70% of cases.
- Embryonal rhabdomyosarcoma in head and neck, orbit and genitourinary regions have better prognosis.

ALVEOLAR RHABDOMYOSARCOMA

Alveolar rhabdomyosarcoma is a high-grade round cell sarcoma, which shows alveolar and solid growth patterns with variable skeletal muscle differentiation. Most common sites of tumor are deep soft tissues of extremities. Other sites are paraspinal region, perineal region, head and neck, trunk and pelvis. It affects young adults during 2–25 years of age without sex predominance.

Clinical Features

Patient presents with rapidly growing painless lump in deep soft tissues of extremities, paraspinal region, perineal region, head and neck, trunk and pelvis. Other signs and symptoms, i.e. compression and obstruction depend on location of tumor.

Surgical Pathology: Alveolar Rhabdomyosarcoma

Gross Morphology

- Alveolar rhabdomyosarcoma is fleshy tumor measuring 2–8 cm in diameter.
- Cut surface shows variable amount of fibrous tissue and hemorrhage.

Light Microscopy

Alveolar rhabdomyosarcoma is a round cell sarcoma. Histologic variants of alveolar rhabdomyosarcoma are conventional alveolar rhabdomyosarcoma and solid variant of alveolar rhabdomyosarcoma.

- **Conventional alveolar rhabdomyosarcoma:** Tumor is composed of large neoplastic cells with large nuclei, eosinophilic cytoplasm, showing rhabdomyoblastic differentiation admixed with multinucleated giant cells. Nests of neoplastic cells are separated by fibrovascular septa that separate nests of neoplastic cells. Neoplastic cells line or cover fibrovascular septa and tend to shed in a discohesive manner into the center of alveolar spaces. Rhabdomyoblasts occasionally show clear cytoplasm, anaplastic features or rhabdoid features (Fig. 28.21).
- **Solid variant of alveolar rhabdomyosarcoma:** Tumor poses diagnostic challenge as fibrovascular septa in the tumor are hardly demonstrated. Neoplastic cells lack discohesive growth pattern, but show identical cell morphology demonstrated in conventional alveolar rhabdomyosarcoma.

Fine Needle Aspiration Cytology

Alveolar rhabdomyosarcoma is composed of neoplastic cells dispersed individually and loosely cohesive nests. Most often, a striking uniformity of the round neoplastic cells is evident. Rhabdomyoblastic differentiation may be demonstrated.

Differential Diagnosis

- Alveolar rhabdomyosarcoma should be differentiated from Ewing sarcoma, desmoplastic round cell tumor, alveolar soft part sarcoma and poorly differentiated carcinoma. Immunohistochemistry plays important role to establish diagnosis.
- PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts are demonstrated in alveolar rhabdomyosarcoma. On the other hand, embryonal rhabdomyosarcoma lacks these PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts. Prognosis of alveolar rhabdomyosarcoma showing PAX7-FOXO1A fusion transcript is better than PAX3-FOXO1A fusion transcript.
- Ewing sarcoma/primitive neuroendocrine tumor (PNET) and desmoplastic round cell tumor show EWSR1 gene mutation.
- Poorly differentiated carcinoma shows positivity for cytokeratins but lacks immunoreactivity for desmin, myogenin, MyoD1 and muscle-specific actin (MSA).
- Alveolar soft part sarcoma also has alveolar pattern but lacks multinucleated giant cells. Tumor shows positivity for desmin, myogenin, MyoD1 and MSA. PAS-diastase stain shows characteristic crystalline cytoplasmic inclusions.

Immunohistochemistry Technique

- Alveolar rhabdomyosarcoma cells show positivity for desmin, myogenin, MyoD1 and muscle-specific actin (SMA). Myogenin expression is more diffuse in alveolar rhabdomyosarcoma than embryonal rhabdomyosarcoma.

- PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts are demonstrated in alveolar rhabdomyosarcoma. On the other hand, embryonal rhabdomyosarcoma lacks PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts. Prognosis of alveolar rhabdomyosarcoma showing PAX7- FOXO1A fusion transcript is better than PAX3-FOXO1A fusion transcript.

Markers	Expression
▪ Desmin	▪ Present (cytoplasmic staining)
▪ Myogenin	▪ Present (nuclear diffuse staining)
▪ MyoD1	▪ Present (nuclear staining)
▪ Muscle-specific actin (MSA)	▪ Present
▪ PAX3-FOXO1A fusion transcript	▪ Present
▪ PAX7-FOXO1A fusion transcript (better prognosis)	▪ Present

PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts are demonstrated in alveolar rhabdomyosarcoma. On the other hand, embryonal rhabdomyosarcoma lacks these PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts.

Molecular Genetic Alterations

- About 60% of alveolar rhabdomyosarcomas demonstrate chromosomal translocation t(2;13)(q35;q14) resulting in formation of PAX3-FOXO1A fusion transcript.
- About 20% of alveolar rhabdomyosarcomas manifest chromosomal translocation t(1;3)(p36,q14) resulting in formation of X7-FOXO1A fusion transcript.

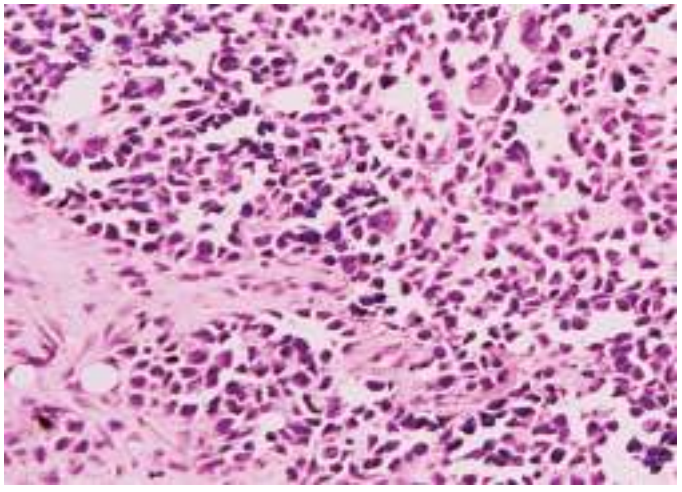


Fig. 28.21: Conventional alveolar rhabdomyosarcoma. Tumor is composed of large neoplastic cells with large nuclei, eosinophilic cytoplasm, showing rhabdomyoblastic differentiation admixed with multinucleated giant cells. Nests of neoplastic cells are separated by fibrovascular septa that separate nests of neoplastic cells. Neoplastic cells line or cover fibrovascular septa and tend to shed in a discohesive manner into the center of alveolar spaces. Rhabdomyoblasts occasionally show clear cytoplasm, anaplastic features or rhabdoid features (400X).

Prognosis and Treatment

No effective therapy is available to treat alveolar rhabdomyosarcoma. Prognosis depends on the stage of tumor. Tumor is more aggressive than embryonal rhabdomyosarcoma. It may metastasize to lungs and lymph nodes. Prognosis of patient with alveolar rhabdomyosarcoma showing PAX7-FOXO1A fusion transcript is better than PAX3-FOXO1A fusion transcript.

PLEOMORPHIC RHABDOMYOSARCOMA

Pleomorphic rhabdomyosarcoma is a high-grade soft tissue sarcoma, which most often displays skeletal muscle differentiation demonstrated by immunohistochemistry and electron microscopy. Most common site of tumor is deep soft tissues of lower extremities. It usually affects adult men during sixth and seventh decades of life.

Clinical Features

Patient presents with rapidly growing painful lump in the lower extremities.

Surgical Pathology: Pleomorphic Rhabdomyosarcoma

Gross Morphology

- Pleomorphic rhabdomyosarcoma is well circumscribed enclosed in pseudocapsule.
- Cut surface shows fleshy appearance with areas of hemorrhage and necrosis (Fig. 28.22).

Light Microscopy

- Pleomorphic rhabdomyosarcoma is composed of spindle to bizarre polygonal atypical neoplastic cells with abundant eosinophilic cytoplasm and vesicular hyperchromatic nuclei arranged in sheets, arranged in storiform pattern or without definite pattern.
- Atypical mitoses and necrosis are frequently demonstrated in the tumor. Neoplastic cells lack cross striations in the cytoplasm (Fig. 28.23).

Electron Microscopy

Electron microscopy demonstrates rudimentary sarcomere formation indicative of skeletal muscle differentiation.

Differential Diagnosis

Pleomorphic rhabdomyosarcoma should be differentiated from embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, pleomorphic leiomyosarcoma, dedifferentiated liposarcoma and malignant triton tumor.

- Embryonal rhabdomyosarcoma and alveolar rhabdomyosarcoma can have focal areas of pleomorphic cells limited to a portion of tumor.
- Dedifferentiated liposarcoma shows positivity for MDM2.

- Malignant triton tumor is relatively rare aggressive neoplasm composed of both schwannoma cells and malignant rhabdomyoblasts. It classified as malignant peripheral nerve sheath tumor with rhabdomyosarcomatous differentiation.

Immunohistochemistry Technique

Pleomorphic rhabdomyosarcoma shows focal positivity for desmin, myogenin, MyoD1 and muscle-specific actin (MSA).

Markers	Expression
▪ Desmin	▪ Present (cytoplasmic staining)
▪ Myogenin	▪ Present (nuclear diffuse staining)
▪ MyoD1	▪ Present (nuclear staining)
▪ Muscle-specific actin	▪ Present

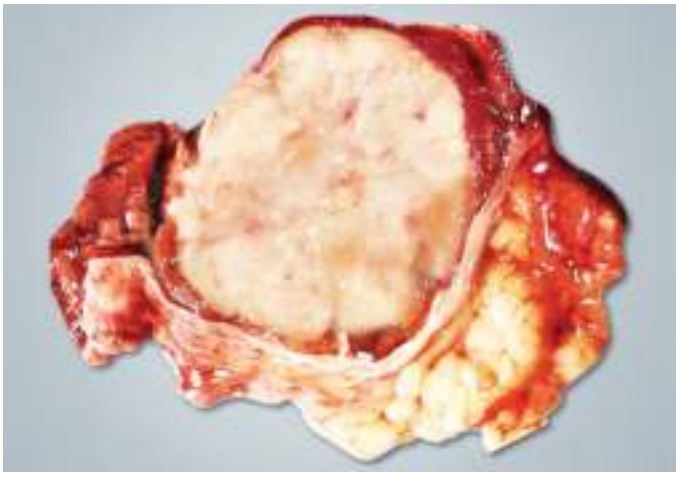


Fig. 28.22: Pleomorphic rhabdomyosarcoma. Tumor is well circumscribed enclosed in pseudocapsule. Cut surface shows fleshy appearance with areas of hemorrhage and necrosis.

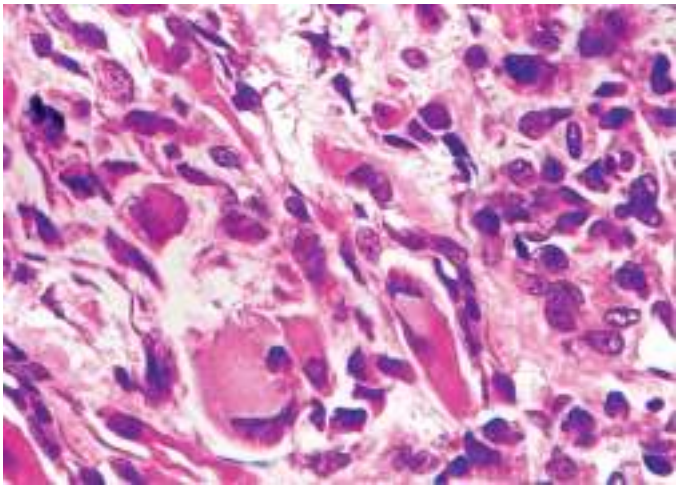


Fig. 28.23: Pleomorphic rhabdomyosarcoma. Tumor is composed of spindle to bizarre polygonal atypical neoplastic cells with abundant eosinophilic cytoplasm and vesicular hyperchromatic nuclei arranged in sheets, arranged in storiform pattern or without definite pattern. Mitoses and necrosis are frequently demonstrated in the tumor. Neoplastic cells lack cross striations in the cytoplasm (400X).

Prognosis and Treatment

Pleomorphic rhabdomyosarcoma has poor prognosis. Majority of patients die of disseminated disease especially to lungs within one year of diagnosis.

ADULT SCLEROSING RHABDOMYOSARCOMA

Recently, seven cases of rhabdomyosarcoma have been reported under the named 'adult sclerosing rhabdomyosarcoma' and pseudovascular rhabdomyosarcoma. It is not clear that this tumor either represent separate entity or unusual presentation of embryonal rhabdomyosarcoma. The tumor lacks cytogenetic changes, which are demonstrated in alveolar rhabdomyosarcoma such as chromosomal translocation t(2;13)(q35;q14) with formation of PAX3-FOXO1A fusion transcript and chromosomal translocation t(1;3)(p36,q14) resulting in formation of X7-FOXO1A fusion transcript. Differences among various histologic variants of rhabdomyosarcomas are given in Table 28.15.

Locations and Age Distribution

Most common sites of adult sclerosing rhabdomyosarcoma are extremities, orbit, nasopharynx, jaw and sacrum. It affects persons during 18–56 years of age.

Clinical Features

Patient presents with rapidly growing mass in extremities, orbit, nasopharynx, jaw and sacrum and related symptoms depending on location of tumor.

Surgical Pathology: Adult Sclerosing Rhabdomyosarcoma

Gross Morphology

- Adult sclerosing rhabdomyosarcoma is poorly circumscribed fleshy tumor.
- Cut surface is firm in consistency and showing fibrous appearance.

Light Microscopy

- Adult sclerosing rhabdomyosarcoma is composed of neoplastic cells arranged in nests, and abundant hyaline matrix resembling primitive osteoid or chondroid elements.
- Neoplastic cells may show focal areas of alveolar or pseudoalveolar pattern.

Immunohistochemistry Technique

Pleomorphic rhabdomyosarcoma shows focal positivity for desmin, myogenin, MyoD1 and muscle-specific actin (MSA).

Markers	Expression
▪ Desmin	▪ Present (cytoplasmic focal staining)
▪ Myogenin	▪ Present (nuclear diffuse staining)
▪ MyoD1	▪ Present (nuclear staining)
▪ Muscle-specific actin (MSA)	▪ Present

Table 28.15 Differences among various histologic variants of rhabdomyosarcomas

Histologic Variants	Age Group	Features	Immunohistochemistry
Embryonal rhabdomyosarcoma	Children	Some resemblance to developing muscle	Desmin, vimentin, MyoD1, myoglobin
Botryoid rhabdomyosarcoma	Children	Term botryoid (grape-like) polypoid mass with myxoid consistency in mucosa lined organs	Desmin, MyoD1, myoglobin
Alveolar rhabdomyosarcoma	Children and teenagers (10–20 years)	Located in extremities. Small cells arranged in sheets or nests separated by fibrous septa	Myogenin positivity
Pleomorphic rhabdomyosarcoma	6th–7th decades	Lower extremities (usually intramuscular)	Desmin, MyoD1, myogenin

Prognosis and Treatment

Adult sclerosing rhabdomyosarcoma is treated by surgical excision with wide margin. It has tendency to recur and metastasize.

GASTROINTESTINAL STROMAL TUMORS

Gastrointestinal stromal tumors (GISTs) according to revised 2020 World Health Organization are given in Table 28.16.

GASTROINTESTINAL STROMAL TUMORS (BENIGN/LOW-GRADE AND MALIGNANT)

Gastrointestinal stromal tumors are most common mesenchymal neoplasms that originate primarily in the wall of gastrointestinal tract. Clinical behavior ranges from benign/low-grade to malignant tumors. Malignant gastrointestinal stromal tumors can disseminate to liver and intra-abdominal structures resulting in fatal outcome. Instead of dividing GISTs into benign/low-grade to malignant; risk for progression to malignant is based on anatomic location, size and mitotic activity of gastrointestinal stromal tumors.

Locations and Age Distribution

Most common sites of gastrointestinal stromal tumor are stomach (60%), small intestine (30%), colon, appendix (10%), rectum and gallbladder. Rare sites of extra-gastrointestinal stromal tumors are omentum, mesentery, retroperitoneum and pelvis. It usually affects adult males with median age of 58 years. It can affect children and young adults in the setting of Carney's syndrome.

Table 28.16 Gastrointestinal stromal tumors according to revised 2020 World Health Organization

Gastrointestinal Stromal Tumors
▪ Benign tumors • Benign gastrointestinal stromal tumor
▪ Intermediate (locally aggressive) tumors • Gastrointestinal stromal tumor of uncertain malignant potential
▪ Malignant tumors • Malignant gastrointestinal stromal tumor

Clinical Features

Patient presents with anemia due to ulceration and bleeding of tumor, fatigue, pain abdomen and distension abdomen. GIST is detected as incidental finding in surgical specimens such as gastrectomy for gastric carcinoma. Carney's syndrome is characterized by Carney's triad (gastric GIST, extra-adrenal paraganglioma and pulmonary chondroma) in children.

Radiologic Findings

CT scan and MRI are performed to diagnose gastrointestinal stromal tumor. Endoscopy and ultrasonography are useful diagnostic techniques.

Surgical Pathology: Gastrointestinal Stromal Tumor

Gross Morphology

- Gastrointestinal stomal tumor is either unilobular or multilobular with smooth circumscribed borders. Ulceration is commonly demonstrated. Pedunculated lesion in serosa may be demonstrated.
- Cut surface is fleshy and cystic with areas of hemorrhage and necrosis.

Light Microscopy

Gastrointestinal stomal tumors (GISTs) are composed of either epithelioid or spindle-shaped cells. Majority of GISTs show combined cytomorphologic features of epithelioid and spindle-shaped cells. Cardinal diagnostic features of GISTs include lack of pleomorphism and low mitoses.

- **Epithelioid GIST:** Tumor is composed of epithelioid neoplastic cells with round-elongated nuclei, fine chromatin, inconspicuous nucleoli and abundant fibrillary cytoplasm; and arranged in either in sheets or nests.
- **Spindle cell GIST:** Tumor is composed of spindle-shaped neoplastic cells with round-elongated nuclei, fine chromatin, inconspicuous nucleoli, abundant fibrillary cytoplasm, and arranged in fascicular pattern. Spindle cell GIST may show nuclear palisading resembling schwannoma.

Electron Microscopy

Analysis of gastrointestinal stromal tumors shows lack of differentiation, i.e. incomplete differentiation or neuroaxonal differentiation.

Histochemistry

A small set of GISTs has acellular collagen bundles known as 'skeinoid fibers', that are demonstrated by PAS stain.

Differential Diagnosis

Gastrointestinal stromal tumor should be differentiated from leiomyoma, leiomyosarcoma, schwannoma, desmoid fibromatosis, malignant melanoma and carcinoma.

Immunohistochemistry Technique

Gastrointestinal stomal tumors show positivity for c-KIT (CD117), CD34 (75%), and smooth muscle actin (40%). c-KIT (CD117) staining is most often diffuse and strong membranous, cytoplasmic or paranuclear globular staining pattern. About 5% of GISTs are negative for c-KIT (CD117). Majority of these tumors are of epithelioid GISTs showing PDGFRA gene mutations.

Markers	Expression
▪ c-KIT (CD117)	▪ Positive (90%)
▪ CD34	▪ Positive (75%)
▪ Smooth muscle actin (SMA)	▪ Positive (40%)
▪ DOG1	▪ Positive
▪ BCL-2	▪ Positive
▪ Mitotic index	▪ High
▪ Desmin	▪ Negative
▪ S-100 protein	▪ Negative

Molecular Genetic Alterations

Loss of material from the short arm of chromosome 1 and monosomy for chromosome 14 and chromosome 22 is most common cytogenetic profile in gastrointestinal stromal tumor. Loss of material from the short arm of chromosomes 9 and 11 is less common and associated with aggressive clinical course.

Prognosis and Treatment

Majority of GISTs exhibit benign clinical course. Recurrence and metastases are demonstrated in 50% of cases. GISTs have tendency for intra-abdominal spread and metastasize to liver. Lymph node metastasis is extremely rare. Lungs are the most common site of metastasis outside the abdomen.

VASCULAR TUMORS OF SOFT TISSUE

Vascular tumors of soft tissue occur in the deep subfascial soft tissue and/or subcutis. Some vascular tumors occurring in the dermis may involve deep soft tissue. Vascular tumors according to revised 2020 World Health Organization classification of soft tissue tumors are given in Table 28.17. Kindly refer to Chapter 15 for vascular tumors discussed in details.

Table 28.17 Vascular tumors according to revised 2020 WHO classification of soft tissue tumors

Vascular Tumors

- Reactive vascular hyperplasia
  - Papillary endothelial hyperplasia
  - Pyogenic granuloma (lobular capillary hemangioma)
  - Bacillary angiomatosis
  - Glomeruloid hemangioma
- Benign tumors
  - Capillary hemangioma
  - Cavernous hemangioma
  - Hemangioma of subcutaneous/deep soft tissue
  - Angiomatosis
  - Epithelioid hemangioma (angiolymphoid hyperplasia with eosinophilia)
  - Intramuscular hemangioma
  - Spindle cell hemangioma
  - Lymphangioma
- Intermediate (borderline) malignant tumors
  - Kaposiform hemangioendothelioma
  - Dabska-type (papillary intralymphatic angioendothelioma) and retiform hemangioendothelioma
  - Kaposi sarcoma
- Malignant tumors
  - Angiosarcoma
  - Epithelioid hemangioendothelioma

PERICYTIC/PERIVASCULAR TUMORS

Pericytic/perivascular tumors according to revised 2020 WHO classification of soft tissue tumors are given in Table 28.18. Pericytic/perivascular tumors may be benign, intermediate (borderline) and malignant.

HEMANGIOPERICYTOMA, SOLITARY FIBROUS TUMOR OF SOFT TISSUE AND RELATED LESIONS

According to revised 2020 World Health Organization of soft tissue tumors, hemangiopericytoma and solitary fibrous tumor of soft tissue are considered closely related. Hemangiopericytoma is a benign neoplasm showing uncertain line of differentiation (not true microvascular pericytes). Solitary fibrous tumor of soft tissue is also a benign tumor.

Locations and Age Distribution

Both hemangiopericytoma and solitary fibrous tumor of soft tissue affect adults without male predominance.

- Most common sites of hemangiopericytoma are retroperitoneum and abdomen.

Table 28.18 Pericytic/perivascular tumors according to revised 2020 WHO classification of soft tissue tumors

Pericytic/Perivascular Tumors
Benign tumors
▪ Hemangiopericytoma and related tumors • Giant cell angiofibroma • Lipomatous hemangiopericytoma ▪ Glomus tumor • Solid glomus tumor • Extra-tumoral perivascular sheathing by neoplastic glomus cells • Glomangiomyoma • Multiple glomus tumors • Glomus tumor with marked hemangiopericytic vessels • Symplastic glomus tumor • Glomus tumor with epithelioid cells • Glomus tumor with oncocytes • Glomus tumor with extensive myxoid change • Vascular invasion in otherwise benign glomus tumor • Glomangiomatosis ▪ Myopericytic family of tumors • Infantile myofibromatosis • Solitary myofibroma in infants and children • Myopericytoma • Glomangiopericytoma
Intermediate tumors
▪ Glomus tumor of uncertain malignant potential ▪ Atypical glomus tumor
Malignant tumors
Malignant glomus tumor/glomangiosarcoma

- Solitary fibrous tumor of soft tissue occurs in the thoracic cavity and abdomen.

Clinical Features

Patient presents with non-specific soft tissue mass and hypoglycemia induced by the tumor.

Surgical Pathology: Hemangiopericytoma and Solitary Fibrous Tumor

Gross Morphology

- Both hemangiopericytoma and solitary fibrous tumor of soft tissue are large well delineated soft tissue mass measuring 5–8 cm in superficial, deep soft tissue and mesothelial lining surface.
- Cut surface is gray white and firm in consistency.

Light Microscopy

- **Hemangiopericytoma:** Tumor is composed of staghorn vasculature with perivascular hyalinization, around which are present bland spindle to ovoid cells arranged without a definite pattern. Occasional tumor may demonstrate pseudovascular spaces lined by multinucleated giant cells and termed giant cell angiofibroma. It shows mitoses 1–3 per 10 high power fields.
- **Solitary fibrous tumor of soft tissue:** Histologic features are identical to hemangiopericytoma. In addition, tumor shows broad areas of collagenization and proliferation of bland fibroblastic spindle cells arranged without a definite pattern. Occasional tumor may demonstrate pseudovascular spaces lined by multinucleated giant cells and termed giant cell angiofibroma. It shows mitoses 1–3 per 10 high power fields.
- **Malignant hemangiopericytoma and malignant solitary fibrous tumor of soft tissue:** Tumors exhibit hypercellularity with pleomorphic neoplastic cells containing hyperchromatic nuclei, areas of necrosis and >4 mitoses per high power fields including atypical mitoses.

Differential Diagnosis

Both hemangiopericytoma and solitary fibrous tumor of soft tissue should be differentiated from synovial sarcoma, low-grade fibromyxoid sarcoma, malignant peripheral nerve sheath tumor, desmoplastic mesothelioma, well-differentiated liposarcoma and giant cell fibroblastoma (giant cell rich hemangiopericytoma and solitary fibrous tumor of soft tissue).

Immunohistochemistry Technique

Both hemangiopericytoma and solitary fibrous tumor of soft tissue neoplastic cells show positivity for CD34, CD99, epithelial membrane antigen (EMA), BCL-2 and smooth muscle actin. These are negative for desmin, CD117 and cytokeratins.

Markers	Expression
▪ CD34	▪ Positive (90–95%)
▪ CD99	▪ Positive (70%)
▪ Epithelial membrane antigen (EMA)	▪ Positive (33%)
▪ BCL-2	▪ Positive (33%)
▪ Smooth muscle actin	▪ Positive (33%)
▪ Desmin	▪ Negative
▪ CD117	▪ Negative
▪ Cytokeratins	▪ Negative

Prognosis and Treatment

Both hemangiopericytoma and solitary fibrous tumor of soft tissue are treated by wide surgical excision.

- Tumors with uncertain malignant potential may metastasize in occasional case.
- Malignant hemangiopericytoma and malignant solitary fibrous tumor of soft tissue metastasize to distant organs in 15–20% of cases. These patients are treated by wide surgical excision, radiotherapy or chemotherapy or both.

MYOPERICYTOMA FAMILY OF TUMORS

Myopericytoma denotes group of histologically overlapping lesions described as infantile myofibromatosis,

infantile myofibroma and adult myofibroma. These are usually benign tumors derived from true microvascular pericytes related to glomus tumor.

Locations and Age Distribution

Most common sites of myofibroma are subcutaneous region of head and neck, soft tissue, bone and viscera. Lesions may be either solitary or multiple.

- Infantile myofibroma affects children with male predominance.
- Adult myofibroma and myopericytoma affect adults of any age with sex predominance.

Clinical Features

Infantile myofibromatosis presents as multicentric lesions in soft tissue, skin, bone and viscera. Solitary myofibroma and myopericytoma present as small lesions in the skin.

Surgical Pathology: Myopericytoma Family of Tumors

Gross Morphology

- Infantile myofibromatosis, infantile myofibroma and adult myofibroma are soft tissue masses.
- Cut surface of myofibroma shows areas of necrosis and calcification.

Light Microscopy

- **Myofibroma:** Tumor shows central and peripheral zones. Peripheral zone of the tumor is composed of hyalinized myxoid appearing spindled cells. Central zone of the tumor is composed of primitive appearing round to short spindled cells with vasculature of hemangiopericytoma, areas of necrosis and calcification resembling sarcoma.
- **Adult myofibroma:** Tumor shows central and peripheral zones. It most often shows reversal of zones. Peripheral zone of the tumor is composed of primitive appearing round to short spindled cells with vasculature of hemangiopericytoma, areas of necrosis and calcification resembling sarcoma. Central zone of the tumor is composed of hyalinized myxoid appearing spindled cells.
- **Infantile myofibroma:** Histologic features correspond to the central zone of tumor with minimal atypia. Central zone of the tumor is composed of primitive appearing round to short spindled cells with vasculature of hemangiopericytoma, areas of necrosis and calcification resembling sarcoma.
- **Myopericytoma:** Tumor is composed of myxoid appearing short spindled cells arranged around small blood vessels, vascular pattern of hemangiopericytoma and glomus-like areas (glomangiopericytoma).

Differential Diagnosis

Myopericytoma family of tumors should be differentiated from infantile fibrosarcoma, glomus tumor, hemangiopericytoma and angioleiomyoma.

Immunohistochemistry Technique

Infantile myofibromatosis, infantile myofibroma and adult myofibroma show positivity for smooth muscle actin. These are negative for desmin and CD34.

Markers	Expression
▪ Smooth muscle actin	▪ Positive
▪ Desmin	▪ Negative
▪ CD34	▪ Negative

Prognosis and Treatment

Myopericytoma family of tumors is treated by surgical excision. Solitary myofibroma in children may spontaneously regress. Malignant myopericytoma is locally aggressive tumor. Multicentric myofibroma in infants involving viscera has fatal outcome. Low dose of chemotherapy may be beneficial in treating these tumors.

GLOMUS TUMOR

Glomus tumor is a mesenchymal neoplasm that differentiates along the lines of glomus cells, which resemble modified smooth muscle of normal glomus having thermoregulatory body.

Locations and Age Distribution

Most common sites of glomus tumor are subungual region, hands, wrist and foot. Rare sites are deep soft tissue and viscera in young adults without sex predominance. Glomus tumor in subungual region most often affects females. Glomuvenous malformation (glomangioma) affects children.

Clinical Features

Clinical manifestations are variable depending on location of benign glomus tumor. Cutaneous tumor usually presents as red-blue colored small nodule. Subungual tumor presents as discoloration of beneath nail associated with pain and cold sensitivity. Deep soft tissue tumor is asymptomatic.

Surgical Pathology: Glomus Tumor

Gross Morphology

- Surgical specimen of glomus tumor from cutaneous and subungual regions is red-blue colored nodule <1 cm in diameter.
- Surgical specimen of glomus tumor from deep soft tissue appears as non-specific soft tissue mass.

Light Microscopy

Typical glomus tumors are classified depending on the relative prominence of glomus cells, smooth muscle and blood vessels described as under:

- **Solid glomus tumor:** It is the most common histologic variant accounting for 75% of glomus tumors.

- Tumor is composed of small, round and uniform glomus cells with centrally placed round nucleus and amphophilic to lightly eosinophilic cytoplasm.
- Glomus cells are arranged in nests surrounding capillary sized blood vessels.
- Each glomus cell is surrounded by basal lamina demonstrated by PAS and toluidine blue stains.
- The stroma of the tumor may show hyalinization or myxoid change (Fig. 28.24).

- **Glomuvenous malformation:** Tumor is poorly circumscribed lesion and composed of proliferation of cavernous hemangioma-like vascular channels surrounded by small cluster of glomus cells.
- **Glomangiomyoma:** Tumor shows a transition from typical glomus cells to elongated mature smooth muscle cells.

Histochemistry

In glomus tumor, glomus cell is surrounded by basal lamina demonstrated by PAS and toluidine blue stains.

Differential Diagnosis

Typical glomus tumor should be differentiated from myopericytic tumors, hemangiopericytoma, solitary fibrous tumor, spindle cell malignant glomus tumor and other round cell sarcomas.

Immunohistochemistry Technique

Glomus tumor cells show positivity for smooth muscle actin and pericellular collagen IV. It is negative for desmin, CD34, S-100 protein and cytokeratins.

Markers	Expression
▪ Smooth muscle actin	▪ Positive
▪ Pericellular collagen IV	▪ Positive
▪ Desmin	▪ Negative
▪ CD34	▪ Negative
▪ S-100 protein	▪ Negative
▪ Cytokeratins (CKs)	▪ Negative

Molecular Genetic Alterations

Multiple inherited glomuvenous malformations occur due to glomulin gene mutation on chromosome 1p21-22. During embryogenesis, glomulin gene is normal component of vascular smooth muscle.

Prognosis and Treatment

Benign glomus tumor and glomuvenous malformations are treated by wide surgical excision. Glomus tumor located in the deep soft tissue and viscera are of uncertain malignant potential and treated by complete surgical excision. Patients should be followed closely for lung metastasis.

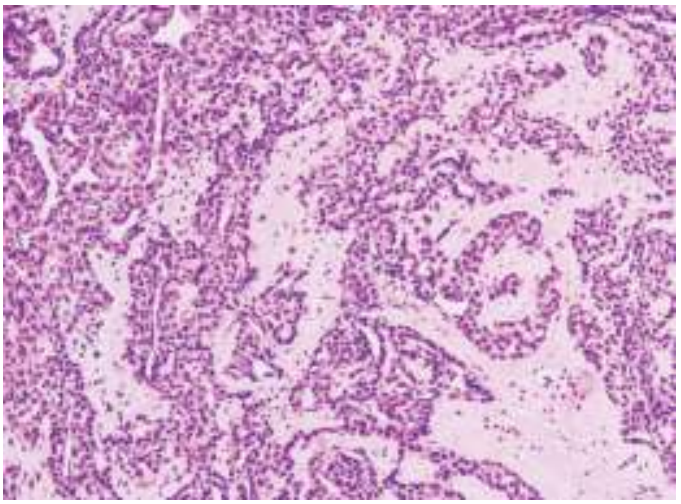


Fig. 28.24: Glomus tumor. Tumor is composed of small, round and uniform glomus cells with centrally placed round nucleus and amphophilic to lightly eosinophilic cytoplasm. Glomus cells are arranged in nests surrounding capillary sized blood vessels. Each glomus cell is surrounded by basal lamina demonstrated by PAS and toluidine blue stains. The stroma of the tumor may show hyalinization or myxoid change (400X).

ATYPICAL AND MALIGNANT GLOMUS TUMORS

Unusual glomus tumors display unique features such as large size tumor in deep location, infiltrative growth, mitotic activity, nuclear pleomorphism and necrosis. Authors studied previously diagnosed as atypical or malignant glomus tumors by virtue of nuclear atypia and mitotic activity; and evaluated size of tumor, depth of tumor, growth pattern, cellularity, nuclear grade, number of mitoses per 50 high power fields, atypical mitotic figures, vascular invasion and necrosis to define criteria for malignancy in glomus tumor.

Classification scheme of atypical and malignant glomus tumors based on histologic features is given in Table 28.19.

Table 28.19 Classification scheme of atypical and malignant glomus tumors based on histologic features

Atypical and Malignant Glomus Tumor Variants	Histologic Features
Malignant glomus tumor	Neoplastic cells with high nuclear grade plus mitotic activity or atypical mitotic activity
Symplastic glomus tumor	Neoplastic cells showing marked nuclear atypia (mitoses <1 per 50 high power fields)
Glomus tumor of uncertain malignant potential	Neoplastic cells not fulfilling diagnostic criteria for malignancy, i.e. absence of atypia or mitoses, but presence of large size tumor measuring >2 cm in diameter in the deep soft tissue

Surgical Pathology: Atypical and Malignant Glomus Tumors

Light Microscopy

Histologic variants of atypical and malignant glomus tumors are malignant glomus tumor (type 1 and 2), symplastic glomus tumor and glomus tumor of uncertain malignant potential.

- **Malignant glomus tumor type 1:** Tumor is composed of a component of previously pre-existing benign glomus tumor in the center of the tumor, surrounded by a nodule of cytologically atypical mitotically active spindle cells resembling features of fibrosarcoma or leiomyosarcoma.
- **Malignant glomus tumor type 2:** Tumor is usually lacking a component of previously pre-existing benign glomus tumor. Tumor retains the histologic features similar to benign glomus tumor. Malignant component of the tumor is composed of highly malignant pleomorphic neoplastic cells with high nuclear grade and numerous mitoses.
- **Symplastic glomus tumor:** Tumor is composed of marked nuclear atypia believed to be degenerative phenomenon. Mitoses >1 per 50 high power fields are demonstrated in the tumor.
- **Glomus tumor of uncertain malignant potential:** Tumor does not fulfill the diagnostic criteria of malignancy without cytologic atypia and mitotic activity. Characteristic feature is deep-seated large size tumor.

Immunohistochemistry Technique

Benign, atypical and malignant glomus tumors show positivity for smooth muscle actin and pericellular collagen IV. It is negative for desmin, CD34, S-100 protein and cytokeratins.

Markers	Expression
Smooth muscle actin (SMA)	Positive
Pericellular collagen IV	Positive
Desmin	Negative
CD34	Negative
S-100 protein	Negative
Cytokeratins (CKs)	Negative

Prognosis and Treatment

Glomus tumor of uncertain malignant potential is treated by wide surgical excision. Malignant glomus tumors metastasize to distant organs resulting in fatal outcome.

NEURAL AND PERINEURAL TUMORS AND OTHER LESIONS

Nerve sheath and neuroectodermal tumors are derived from the neural crest or neuroectodermal tissue of the developing embryo.

- Normal nerve is composed of axons surrounded by Schwann cells. Endoneurium is composed of axons, Schwann cells, fibroblasts, mast cells, capillaries and collagen fibers. Group of axons and their Schwann cells are surrounded by perineurium forming a fascicle. A group of fascicles is surrounded by epineurium. Normal structure of a nerve helps to understand origin of tumors from nerve.
- Neuromas are mainly reactive lesions of existing nerves. Neurofibromas arise from within the nerve or axon. Schwannomas are derived from Schwann cells than encase of the axons. Neural and perineural tumors and lesions of soft tissue according to revised 2020 WHO classification of soft tissue tumors are given in Table 28.20.

Table 28.20 Neural and perineural tumors and lesions of soft tissue according to revised 2020 WHO classification of soft tissue tumors

Neural and Perineural Tumors and Lesions of Soft Tissue
Benign tumors
▪ Neuromuscular hamartoma (benign triton tumor)
▪ Neuromas
• Traumatic neuroma
• Morton neuroma
▪ Granular cell tumor
▪ Schwannoma
• Conventional schwannoma
• Cellular schwannoma
• Plexiform schwannoma
• Melanotic schwannoma
• Microcytic/reticular schwannoma
• Other schwannomas
▪ Neurofibroma
• Circumscribed neurofibroma
• Diffuse and massive soft tissue neurofibroma
• Plexiform neurofibroma
• Other neurofibromas
▪ Perineuroma
• Intraneural perineuroma
• Soft tissue perineuroma
• Sclerosing perineuroma
• Reticular perineuroma
▪ Hybrid peripheral nerve sheath tumor
Intermediate tumors
▪ Nerve sheath myxoma
▪ Atypical granular tumor
Malignant tumors
▪ Malignant peripheral nerve sheath tumor (MPNST)
▪ Low-grade malignant peripheral nerve sheath tumor
▪ High-grade malignant peripheral nerve sheath tumor
▪ Epithelioid peripheral nerve sheath tumor
▪ Malignant peripheral nerve sheath tumor ex-schwannoma
▪ Malignant granular cell tumor
▪ Malignant perineuroma
Benign to malignant tumors
Paraganglioma of head and neck region
Intermediate to malignant tumors
Ependymoma of soft tissue

TRAUMATIC NEUROMA

Traumatic neuroma occurs in response to injury or surgical amputation of limb. It is characterized by painful disorganized proliferation of nerves. It can affect persons of all age groups.

Locations

Traumatic neuroma occurs as a painful nodule in response to injury or surgical amputation of limb and digits.

Clinical Features

Patient presents with firm painful nodule at the site of nerve injury.

Radiologic Findings

Traumatic neuroma is demonstrated by the presence of nerve terminating into a bulbous mass.

Surgical Pathology: Traumatic Neuroma
Gross Morphology
Traumatic neuroma is firm gray white nodule measuring <5 cm in diameter.
Light Microscopy
▪ Traumatic neuroma is composed of haphazardly arranged small nerve fascicles containing Schwann cells, perineural cells surrounded by fibrous tissue. ▪ Myxoid traumatic neuroma is composed of collagen bundles, cellular architecture in the center of lesion and scattered mast cells.
Differential Diagnosis
Traumatic neuroma should be differentiated from neurofibroma, Morton's neuroma of toe and mucosal neuroma.

Prognosis and Treatment

Traumatic neuroma is treated by surgical excision and prior reapproximating of nerve endings or re-embedding of proximal nerve stump in an area far away from scar.

GRANULAR CELL TUMOR

Granular cell tumor is a benign tumor rarely with malignant behavior. Tumor is derived from Schwann cell. Neoplastic cells contain cytoplasmic granules representing aggregates of lysosomes.

Locations and Age Distribution

Most common sites of granular cell tumor are dermis/subcutaneous region of upper extremities, gastrointestinal tract and respiratory tract. Granular cell tumor affects persons during second to fourth decades of life. However, it may affect any age group with female predominance.

Clinical Features

Patient presents with painless solitary mass in the dermis/subcutaneous region of upper extremity, gastrointestinal tract and respiratory tract over <6 months of duration. Multiple tumors may be demonstrated in 15–20% of cases.

- Benign granular cell tumor occurs in the superficial or deep tissue of upper extremity.
- Malignant cell tumors arise in *de novo*, but not from benign granular cell tumor.

Surgical Pathology: Granular Cell Tumor	
Gross Morphology	
▪ **Benign granular cell tumor:** Tumor is poorly delineated measuring <3 cm in diameter. Cut surface shows pale yellow color. ▪ **Malignant granular cell tumor:** Tumor is poorly delineated measuring of large size with mean 4 cm in diameter. Cut surface shows pale yellow color.	
Light Microscopy	
▪ **Benign granular cell tumor:** Tumor is composed of large polygonal cells containing eosinophilic granular cytoplasm, dark ovoid nuclei, inconspicuous nucleoli and showing minimal mitotic activity. Distinctive cytoplasmic aggregates of lysosomes surrounded by a clear halo are demonstrated in the neoplastic cells. ▪ **Atypical granular cell tumor:** Tumor should have one or two of the histologic features such as sarcomatoid spindling, necrosis, mitoses >2 per high power fields, high nuclear to cytoplasmic ratio, pleomorphic neoplastic cells, vesicular nuclei with macronucleoli. ▪ **Malignant granular cell tumor:** Tumor should have at least three and most often five to six of the histologic features: sarcomatoid spindling, necrosis, mitoses >2 per high power field (HPF), high nuclear to cytoplasmic ratio, pleomorphic neoplastic cells, vesicular nuclei with macronucleoli.	
Electron Microscopy	
Neoplastic cells of granular cell tumor contain cytoplasmic granules representing aggregates of lysosomes. Some neoplastic cells contain large boat-shaped cytoplasmic crystals.	
Differential Diagnosis	
Granular cell tumor should be differentiated from other granular cell tumors.	
Immunohistochemistry Technique	
Granular cell tumor shows positivity for immunomarkers S-100 protein, CD68 and α-inhibin.	
Markers	**Expression**
▪ S-100 protein ▪ CD68 ▪ α-Inhibin	▪ Positive ▪ Positive ▪ Positive

Prognosis and Treatment

Benign granular cell tumor is treated by surgical excision. Malignant granular cell tumor behaves like high-grade sarcoma.

NEUROFIBROMA, NEUROFIBROMATOSIS TYPE 1 AND MALIGNANT CHANGE IN NEUROFIBROMA

Neurofibroma is a benign tumor, which arises from within a peripheral nerve. Solitary neurofibroma is relatively common. It can be associated with neurofibromatosis type 1. Neurofibromatosis type 1 presents with multiple neurofibromas. Café au lait spots, optic iris hamartoma, sphenoid bone dysplasia, macrocephaly, short stature and mental retardation.

Diagnostic Criteria of Neurofibromatosis Type 1

Patient presents with two or more neurofibromas or one plexiform neurofibroma, six or more café au lait macules >5 mm (pubertal) or >15 mm (post-pubertal), ocular iris hamartomas, sphenoid bone dysplasia and first degree relative with neurofibromatosis type 1. Out of these clinical manifestations, patient must have at least two or more clinical features.

Locations and Age Distribution

Solitary neurofibroma is relatively a common tumor. Plexiform neurofibroma associated with neurofibromatosis type 1 is relatively uncommon. Both tumors originate from peripheral nerve especially in the scalp region and lower extremities. Neurofibroma usually affects persons of 20–40 years of age. Neurofibroma associated with neurofibromatosis type 1 affects children and adolescents with male predominance.

Clinical Features

Patient with neurofibromatosis type 1 presents with solitary or multiple neurofibromas, Café au lait spots, optic iris hamartoma, sphenoid bone dysplasia, macrocephaly, short stature and mental retardation. Clinical photograph of patient is shown in Fig. 28.25.

Surgical Pathology: Neurofibroma, Neurofibromatosis Type 1 and Malignant Change in Neurofibroma

Gross Morphology

- **Solitary neurofibroma:** Tumor is well delineated and fusiform shaped mass measuring 1–2 cm in diameter.
- **Diffuse neurofibroma:** Tumor is gray plaque-like thickening of dermis with soft in consistency.
- **Plexiform neurofibroma:** Tumor is large soft tissue mass giving 'bag of worms' appearance.

Light Microscopy

- **Neurofibromatosis type 1:** Cellular and stromal milieu in neurofibromatosis type 1 includes Schwann cells, intraneural fibroblasts, collagen bundles and myxoid change and increased cellularity in the center of neurofibroma, when compared with edematous appearance in the peripheral region of neurofibroma.
- **Solitary neurofibroma:** Tumor is composed of spindle cells arranged in storiform pattern with abundant intervening collagen fibers. Intraneural growth is extending into the surrounding tissue (Fig. 28.26).
- **Diffuse neurofibroma:** Tumor is composed of small round to spindle cells associated with fibrillar and myxoid background. It infiltrates subcutaneous fat in a honeycomb pattern. Presence of 'Wagner-Meissner bodies' is the hallmark of diffuse neurofibroma.
- **Plexiform neurofibroma:** Tumor is tortuous distension of peripheral nerve showing plexiform architecture demonstrated on imaging technique, gross morphology and histologic features with multiple intraneural nodules of tumor separated by epineurium and stroma. It can involve the subcutis or skeletal structures or both. Clinicopathologic diagnosis is established due to its 'bag of worms' appearance.
- **Low-grade malignant peripheral nerve sheath tumor (low-grade MPNST):** Neurofibroma may undergo early malignant transformation. Low-grade malignant peripheral nerve sheath tumor resembles neurofibroma but with increased cellularity with pleomorphism and arranged in fascicular architecture and scattered mitotic activity in the deep area of tumor.

Differential Diagnosis

Neurofibroma should be differentiated from schwannoma, malignant peripheral nerve sheath tumor (MPNST), benign fibrous histiocytoma and desmoplastic malignant melanoma.

Immunohistochemistry Technique

Neurofibroma and neurofibromatosis type 1 may undergo early malignant transformation show positivity for S-100 protein (Schwann cells), epithelial membrane antigen (EMA), CD34 (intraneural fibroblasts), neurofilament protein (NFP) and glial fibrillary acidic protein (GFAP).

Markers	Expression
S-100 protein	Positive
Epithelial membrane antigen	Positive
CD34	Positive
NFP	Positive
GFAP	Positive (occasional)

Molecular Genetic Alterations

Neurofibromatosis type 1 occurs due to germline mutation of NF1 tumor suppressor gene located on



Fig. 28.25: Neurofibroma. On clinical examination, patient showed multiple neurofibromas.

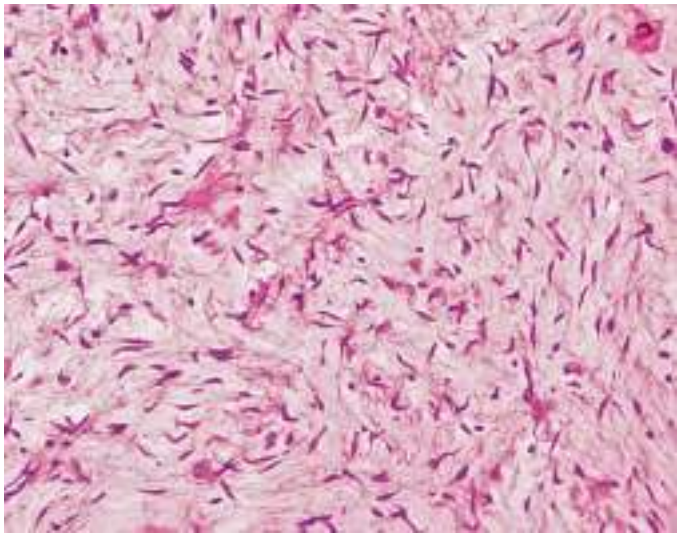


Fig. 28.26: Neurofibroma. Solitary neurofibroma is composed of spindle cells arranged in storiform pattern with abundant intervening collagen fibers. Intraneural growth is extending into the surrounding tissue (400X).

chromosome 17. NF1 gene encodes protein product known as 'neurofibromin', homologous to the GTPase-activating (GAP) for members of the p21 (RAS) family protein that affects cell cycle by downregulating the p21 (RAS) gene. Malignant transformation of neurofibroma is common in these patients. Neurofibromatosis type 1 presents with multiple neurofibromas, café au lait spots, optic iris hamartoma, sphenoid bone dysplasia, macrocephaly, short stature and mental retardation.

Prognosis and Treatment

Solitary neurofibroma, diffuse neurofibroma and plexiform neurofibroma may be associated with neurofibromatosis type 1. These tumors are treated by surgical excision. Recurrence is common in diffuse neurofibroma after surgical excision. Early malignant peripheral nerve sheath tumor in a case of neurofibromatosis type 1 is treated similar to low-grade sarcoma. High-grade malignant transformation in neurofibroma is treated similar to high-grade sarcoma.

SCHWANNOMA AND NEUROFIBROMATOSIS TYPE 2

Schwannoma is a benign tumor of peripheral nerve sheath tumor, which commonly involves the vestibular division of eighth cranial nerve.

- Schwannoma accounts for 8% of intracranial tumors and 28% of primary spinal tumors. Most common sites are head and neck region. Other sites are extensor aspect of extremities, vestibular division of eighth cranial nerve, bone and gastric region.
- Schwannoma usually affects persons of 20–50 years of age except in the setting of neurofibromatosis type 2 (NF2), which occurs due to germline NF2 gene mutation located on chromosome 22 encoding aberrant protein 'Merlin'.

Diagnostic Criteria of Neurofibromatosis Type 2

Diagnostic criteria of neurofibromatosis type 2 include bilateral vestibular schwannomas or family history of neurofibromatosis type 2 in first degree relative and unilateral vestibular schwannoma in below 30 years of age and any two of the lesions, i.e. meningioma, schwannoma, glioma, posterior subcapsular lenticular opacities/juvenile cortical cataract. Comparison of neurofibromatosis type 1 and neurofibromatosis type 2 is given in Table 28.21.

Clinical Features

Patient with neurofibromatosis type 2 presents with tinnitus, hearing loss, acoustic bilateral schwannoma and facial paresthesia. Patient with schwannoma in peripheral region presents with mass.

Radiologic Findings

Radiograph demonstrates intracranial calcification in neurofibromatosis type 2. Radiologic study demonstrates mass eccentric to nerve involved in a case of schwannoma.

Table 28.21 Comparison of neurofibromatosis type 1 and neurofibromatosis type 2

Parameters	Neurofibromatosis Type 1 (NF1)	Neurofibromatosis Type 2 (NF2)
Germline gene mutation	NF1 gene mutation on chromosome 17p11 by deletion	NF2 gene mutation on chromosome 22q12.2 by deletion or nonsense mutation
Encoded protein by NF1 and NF2 genes	Neurofibromin 1	Merlin
Encoded protein function by NF1 and NF2 genes	Neurofibromin type 1 homologous to GAP protein, that influences cell cycle by downregulating p21 RAS gene (RAS/MAP-kinase signaling pathway)	Merlin stabilizes skeleton membrane linkage
Diagnostic criteria of NF1 and NF2	Patient presents with two or more neurofibromas or one plexiform neurofibroma, six or more café au lait macules >5 mm (pubertal) or >15 mm (postpubertal), ocular iris hamartomas, sphenoid bone dysplasia and first degree relative with neurofibromatosis type 1	Diagnostic criteria of neurofibromatosis 2 include bilateral vestibular schwannomas or family history of neurofibromatosis type 2 in first degree relative and unilateral vestibular schwannoma in below 30 years of age and any two of the lesions, i.e. meningioma, schwannoma, glioma, posterior subcapsular lenticular opacities/juvenile cortical cataract

Surgical Pathology: Neurofibroma and Neurofibromatosis Type 2

Gross Morphology

- Classic schwannoma is encapsulated tan-yellow mass measuring 2–10 cm in diameter.
- Sinonasal and intracranial schwannomas are not encapsulated.

Light Microscopy

Classic schwannoma is encapsulated and composed of alternating Antoni A cellular component with Verocay bodies (cellular areas surrounded by nuclear palisading), and Antoni B hypocellular component with myxoid architecture.

- **Antoni A cellular component of schwannoma:** It is cellular component and composed of monomorphic spindle-shaped Schwann cells containing poorly defined cytoplasm, pointed basophilic nuclei set in a variably collagenous stroma showing nuclear palisading surrounding pink areas known as 'Verocay bodies'.
- **Antoni B cellular component of schwannoma:** It is composed of Schwann cells containing inconspicuous cytoplasm and nuclei suspended in abundant myxoid microcystic matrix.

Differential Diagnosis

Schwannoma should be differentiated from neurofibroma, nevomelanocytic lesions, leiomyosarcoma, gastrointestinal stromal tumor and malignant peripheral nerve sheath tumor (MPNST).

Immunohistochemistry Technique

Schwannoma shows strong positivity for S-100 protein.

Marker	Expression
S-100 protein	Positive

Molecular Genetic Alterations

NF2 gene located on chromosome 22 encodes protein 'Merlin'. Merlin stabilizes skeleton membrane linkage. Neurofibromatosis type 2 is caused by germline NF2 gene mutation due to deletion or nonsense mutation, which encodes aberrant protein '**Merlin**'.

Prognosis and Treatment

Benign schwannoma is treated by surgical excision. Neurofibromatosis type 2 due to germline NF2 gene mutation is difficult to treat.

PSAMMOMATOUS MELANOTIC SCHWANNOMA

Psammomatous melanocytic schwannoma is a melanin pigmented neural neoplasm, which arises from sympathetic nervous system. It differs from conventional schwannoma. About 50% of psammomatous melanotic schwannoma have been associated with autosomal dominant Carney's complex, which is characterized by spotty skin pigmentation, Cushing's syndrome, acromegaly, sexual precocity, atrial myxomas and angiomyxomas.

Locations and Age Distribution

Most common sites of psammomatous melanotic schwannoma are spinal nerve roots, soft tissue of extremities and trunk region. Uncommon sites are intestine, skin, bronchus, heart, liver and bone. It affects persons of 20–40 years of age.

Clinical Features

About 50% of psammomatous melanotic schwannoma have been associated with autosomal dominant

Carney's complex,which is characterized by spotty skin pigmentation, Cushing's syndrome, acromegaly, sexual precocity, atrial myxomas and angiomyxomas.

Surgical Pathology: Psammomatous Melanotic Schwannoma

Gross Morphology

- Psammomatous melanocytic schwannoma is a well-circumscribed fusiform shaped gray-black tumor measuring 1–10 cm in diameter with mean 5 cm in size.
- Cut surface shows cystic appearance.

Light Microscopy

- Psammomatous melanocytic schwannoma is composed of tightly packed spindled cells containing dark brown melanin pigment obscuring nuclear and cytoplasm morphology.
- Calcified psammomatous bodies are demonstrated in nearly all cases.
- Spindled cells are arranged in fascicular, solid or whorled growth patterns. Nuclei may show mild atypia. Presence of marked nuclear atypia, mitotic activity and necrosis are indicative of malignant phenotype.

Differential Diagnosis

- Psammomatous melanocytic schwannoma should be differentiated from malignant melanoma and blue nevus.
- Psammoma bodies are not demonstrated in malignant melanoma.
- Blue nevus shows small cutaneous lesions with lesser degree of nuclear atypia and absence of psammomatous bodies.

Immunohistochemistry Technique

Psammomatous melanocytic schwannoma shows positivity for S-100 protein, HMB-45 (human melanoma black 45) and glial fibrillary acidic protein (GFAP). It is essential to bleach the sections to observe immunoreactivity.

Markers	Expression
S-100 protein	Positive
HMB-45	Positive
GFAP	Positive (occasional)

Prognosis and Treatment

Benign psammomatous melanocytic schwannoma is treated by wide surgical resection. Malignant psammomatous melanocytic schwannoma may metastasize to lungs, pleura, liver and spleen in 10% of cases.

PERINEUROMA (SCLEROSING, INTRANEURAL, SOFT TISSUE AND RETICULAR PERINEUROMAS)

Perineuroma is usually a benign tumor composed of perineural cells counterpart of meningeal cells of intracranial pia–arachnoid membranes.

- Tumor may arise in the superficial or deep regions. Cytogenetic analysis reveals monosomy of chromosome 26.
- Perineuroma is of following types in decreased frequency:
  - Sclerosing perineuroma of soft tissue is the most common histologic type of perineuroma.
  - Intraneural perineuroma usually involves peripheral nerves of the extremities.
  - Soft tissue perineuroma occurs in soft tissue. It does not involve peripheral nerves.
  - Reticular perineuroma is uncommon.

Incidence and Locations

- Intraneural perineuroma usually involves peripheral nerves of the extremities.
- Soft tissue perineuroma occurs in soft tissue. It does not involve peripheral nerves.

Age and Sex Predilection

- Intraneural perineuroma affects adolescents and young adults without sex predominance.
- **Soft tissue perineuroma** affects adults with female predominance.

Clinical Features

- Intraneural perineuroma: Patient with intraneural perineuroma presents with muscular weakness as a result of nerve involvement.
- Soft tissue perineuroma does not involve nerve. Patient presents with soft tissue mass.

Surgical Pathology: Perineuroma

Gross Morphology

- **Intraneural perineuroma:** Tumor shows segmental enlargement of nerves up to 10 cm or longer. Cut surface is gray white and gelatinous with firm in consistency.
- **Soft tissue perineuroma:** Tumor is solitary well-circumscribed mass measuring 1.5–2 cm in greater dimension. Cut surface is gray-white to gelatinous with firm in consistency.

Light Microscopy

- **Intraneural perineuroma:** Tumor is composed of bulb-like perineural cells arranged in concentric pattern giving 'onion skin' appearance. Perineural cells are present around residual nerve twigs. The lesion may be hyalinized.
- **Soft tissue perineuroma:** Sclerosing perineuroma of soft tissue is the most common histologic type of perineuroma. Tumor is composed of uniform perineural cells arranged in whorls embedded collagenized stroma. But typical soft tissue perineuroma is composed of spindled shaped, waxy, perineural cells embedded in collagenized stroma. Some cells are arranged in whirling pattern.

Differential Diagnosis

Intraneural perineuroma has quite distinctive histology.

- Typical soft tissue perineuroma should be differentiated from neurofibroma, low-grade fibromyxoid sarcoma, solitary fibrous tumor, dedifferentiated liposarcoma with meningothelial like whorls and malignant peripheral nerve sheath tumor (MPNST).
- Sclerosing soft tissue perineuroma should be differentiated from benign fibrous histiocytoma, glomus tumor and myoepithelial tumor.

Immunohistochemistry Technique

- Intraneural perineuroma: It demonstrates epithelial membrane antigen (EMA) positive perineural cells. Surrounding residual Schwann cells show positivity for S-100 protein. Axons of the nerve fibers show positivity for neurofilament protein (NFP).
- Soft tissue perineuroma (typical and sclerosing types): Both histologic variants of tumor show positivity for EMA. This tumors are negative for S-100 protein. Both histologic variants can be positive for claudin 1 and GLUT-1 (glucose transporter protein 1).

Component of Perineuroma	Markers	Expression
Intraneural perineuroma		
▪ Perineural cells ▪ Schwann cells ▪ Residual axons of nerve fibers	▪ Epithelial membrane antigen (EMA) ▪ S-100 protein ▪ Neurofilament protein (NFP)	▪ Positive ▪ Positive ▪ Positive
Soft tissue perineuroma (typical and sclerosing types)		
Perineural cells	▪ Epithelial membrane antigen (EMA) ▪ Claudin 1 ▪ Glucose transporter protein 1 (GLUT-1) ▪ S-100 protein	▪ Positive ▪ Positive ▪ Positive ▪ Negative

Molecular Genetic Alterations

Cytogenic analysis reveals monosomy of chromosome 26 in perineuroma.

Prognosis and Treatment

Most histologic variants of benign perineuroma are treated by surgical excision. Malignant perineuroma should be treated similar to sarcomas.

NERVE SHEATH MYXOMA

Nerve sheath myxoma is a benign tumor not associated with neurofibromatosis type 1 or type 2.

Locations and Age Distribution

Most common sites of nerve sheath myxoma are in the subcutaneous and dermis of extremities, head and neck region. It rarely involves deep soft tissue. It affects children and young adults without sex predominance.

Clinical Features

Patient with nerve sheath myxoms presents with small mass of 0.5–2 cm size in the dermis or subcutaneous region of extremities, head and neck region.

Surgical Pathology: Nerve Sheath Myxoma

Gross Morphology

Nerve sheath myxoma is small multinodular mucoid mass measuring 0.5–2 cm.

Light Microscopy

- Nerve sheath myxoma is composed of well demarcated myxoid lobules of variable size. Myxoid lobules are separated by fibrous septa containing stellate, spindled cells and rarely epithelioid cells with dark nuclei. Cells are showing little pleomorphism or mitotic activity.
- Occasional nerve sheath myxoma may demonstrate scattered enlarged cells with hyperchromatic nuclei.

Differential Diagnosis

Nerve sheath myxoma should be differentiated from angiomyxoma, myxofibroma, myxoid schwannoma and myxoid neurofibroma.

Immunohistochemistry Technique

Nerve sheath myxoma shows positivity for S-100 protein, collagen type 4 and epithelial membrane antigen (EMA).

Markers	Expression
▪ S-100 protein ▪ Collagen type 4 ▪ EMA	▪ Positive ▪ Positive ▪ Positive

Prognosis and Treatment

Nerve sheath myxoma is treated by surgical excision. Prognosis is excellent.

MALIGNANT PERIPHERAL NERVE SHEATH TUMOR

Malignant peripheral nerve sheath tumor (MPNST) can originate in *de novo* or in the setting of neurofibroma and neurofibromatosis type 1.

Locations and Age Distribution

Most common sites of malignant peripheral nerve sheath tumor are buttock, thigh, brachial plexus, upper arm and paraspinal regions. Sporadic malignant nerve sheath tumor affects adults during second to fifth decades of life. On the other hand, malignant nerve sheath tumor originating in the setting of neurofibromatosis type 1 affects persons during third to sixth decades of life.

Clinical Features

Patient presents with signs and symptoms depending on location of malignant nerve sheath tumor.

Radiologic Findings

Malignant peripheral nerve sheath tumor poses diagnostic difficulty to differentiate from benign nerve sheath tumor on radiologic techniques. Tumor size >5 cm with irregular borders and presence of necrosis are suggestive of MPNST.

Surgical Pathology: Malignant Peripheral Nerve Sheath Tumor

Gross Morphology

- Tumor is creamy gray and fusiform shaped measuring >5 cm in diameter.
- Cut surface shows areas of hemorrhage and necrosis.

Light Microscopy

- Tumor exhibits diverse histologic patterns resembling marble-like appearance with alternating zones of hypercellularity and hypocellularity.
- Tumor is composed of neoplastic cells with waxy or buckled hyperchromatic nuclei and moderate amount of lightly eosinophilic cytoplasm.
  - Neoplastic cells are arranged in long fascicles.
  - In occasional area of the tumor, neoplastic cells may be arranged in storiform pattern.
  - Hemangiopericytoma like vascular pattern may also be demonstrated.
- Heterologous differentiation, e.g. skeletal muscle, cartilage, bone, blood vessels and glands is demonstrated in few cases of neurofibromatosis type 1 related MPNST.

Differential Diagnosis

Malignant peripheral nerve sheath tumor (MPNST) should be differentiated from synovial sarcoma, fibrosarcoma, malignant solitary fibrous tumor and malignant melanoma.

Electron Microscopy

Malignant peripheral nerve sheath tumor (MPNST) shows melanosomes combined with schwannian features of well-developed basement membranes and complex processes.

Immunohistochemistry Technique

- Spindled malignant peripheral nerve sheath tumor shows positivity for HMB-45 and S-100 protein (weak and focal staining).
- Epithelioid malignant peripheral nerve sheath tumor shows positivity for HMB-45 and S-100 protein (strong staining).
- Malignant peripheral nerve sheath tumor with rhabdoblastic differentiation shows positivity for desmin, myogenin, MyoD1 and HMB-45.

Markers	Expression
Spindled malignant peripheral nerve sheath tumor	
▪ HMB-45 ▪ S-100 protein	▪ Positive ▪ Positive (weak and focal staining)
Epithelioid malignant peripheral nerve sheath tumor	
▪ S-100 protein ▪ HMB-45	▪ Positive (strong staining) ▪ Positive
Malignant peripheral nerve sheath tumor with rhabdoblastic differentiation	
▪ Desmin ▪ Myogenin ▪ MyoD1 ▪ HMB-45	▪ Positive ▪ Positive ▪ Positive ▪ Positive

Prognosis and Treatment

- Malignant peripheral nerve sheath tumor arising *de novo* and in the setting of neurofibromatosis type 1 has five-year survival rate in 85% of cases.
- Malignant peripheral nerve sheath tumor of head and neck region is a low-grade tumor associated with best prognosis.

MISCELLANEOUS SOFT TISSUE TUMORS OF UNCERTAIN LINEAGE

Soft tissue tumors of uncertain differentiation or uncertain lineage are given in Table 28.22.

MYXOMA (INTRAMUSCULAR MYXOMA AND JUXTA-ARTICULAR MYXOMA)

Myxoma is a benign mesenchymal tumor. Tumor is composed of bland stellate to spindled cells embedded in hypovascular abundant myxoid matrix.

Locations and Age Distribution

- Intramuscular myxoma arises from large skeletal muscles of thigh and buttock region in women. Mazabraud syndrome is characterized by myxoma and fibrous dysplasia.

Table 28.22 Soft tissue tumors of uncertain differentiation or uncertain lineage

Benign Tumors
▪ Acral fibromyxoma ▪ Intramuscular myxoma including cellular variant ▪ Juxta-articular myxoma ▪ Deep aggressive angiomyxoma ▪ Ectopic hamartomatous thymoma
Intermediate (Locally Aggressive) Malignant Tumors
Hemosiderotic fibrolipomatous tumor
Intermediate (Rarely Metastasizing) Malignant Tumors
▪ Atypical fibroxanthoma ▪ Angiomatoid fibrous histiocytoma ▪ Ossifying fibromyxoid tumor malignant ▪ Mixed tumor, not otherwise specified ▪ Mixed tumor, not otherwise specified, malignant ▪ Myoepithelioma ▪ Myoepithelial carcinoma ▪ Phosphaturic mesenchymal tumor, benign ▪ Phosphaturic mesenchymal tumor, malignant ▪ Pleomorphic hyalinizing angiotectic tumor ▪ Hemosiderotic fibrolipomatous tumor
Malignant Tumors
▪ Synovial sarcoma • Synovial sarcoma, spindle cell variant • Synovial sarcoma, biphasic ▪ Epithelioid sarcoma ▪ Alveolar soft part sarcoma ▪ Clear cell sarcoma of soft tissue ▪ Extraskeletal myxoid chondrosarcoma ▪ Extraskeletal Ewing sarcoma ▪ Desmoplastic small round cell tumor ▪ Extra-renal rhabdoid tumor ▪ Neoplasms with perivascular epithelioid cell differentiation (PEComa) • PEComa, not otherwise specified, benign • PEComa, not otherwise specified, malignant ▪ Intimal sarcoma

- Juxta-articular myxoma occurs in the region of knee joint, shoulder and hip regions. It affects men especially in region of degenerative joint disease.

Clinical Features

Patient presents with painless soft tissue mass in the skeletal muscles of thigh, buttock, knee, shoulder and hip regions.

Surgical Pathology: Myxoma

Gross Morphology

- Myxoma is a well circumscribed soft tissue mass.
- Cut surface is gelatinous with areas of cystic change.

Light Microscopy

- Tumor is composed of stellate to spindled cells embedded in hypovascular abundant myxoid matrix.
- Cellular myxoma located in juxta-articular region is a highly cellular tumor composed of stellate to spindled cells arranged in fascicular pattern.
- Numerous blood vessels are present in the tumor.

Differential Diagnosis

Myxoma should be differentiated from myxofibrosarcoma, myxoid liposarcoma, myxoid chondrosarcoma, myxoid desmoid-type fibromatosis and myxoid nodular fasciitis.

Immunohistochemistry Technique

Myxoma cells show strong positivity with vimentin. It is negative for smooth muscle actin.

Markers	Expression
▪ Vimentin ▪ Smooth muscle actin (SMA)	▪ Positive ▪ Negative

Prognosis and Treatment

Myxoma is cured by surgical excision.

SYNOVIAL SARCOMA

Synovial sarcoma is a highly malignant mesenchymal tumor, which displays variable degree of epithelial differentiation and formation of glandular structures. Tumor originates in the region of a joint rather within joint cavity, which has no etiologic or direct anatomic relation to the synovium. Demonstration of chromosomal translocation t(X;18)(p11;q11) is characteristic finding in synovial sarcoma in 95% of cases.

Locations and Age Distribution

Most common sites of synovial sarcoma are deep soft tissue of lower and upper extremities in the juxta-articular regions in 70% of cases. Other sites are trunk region (15%) and head and neck region (7%). Unusual sites are sex organs, kidney, adrenal glands, retroperitoneum, viscera, mediastinum, bone, central nervous system and peripheral nerves. Tumor usually affects adolescents and young adults during 20–50 years of age without sex predominance.

Clinical Features

Patient presents with painful and tender slowly growing mass in the vicinity of knee joint and parapatellar regions. Tumor may occur in other unusual sites.

Surgical Pathology: Synovial Sarcoma

Gross Morphology

- Synovial sarcoma is a well circumscribed tumor measuring 3–10 cm in diameter. Tumor in hands and feet measures 1 cm in diameter.
- Cut surface is gray-yellow or pink with soft in consistency depending on proportion of cellularity, collagenization, myxoid change or hemorrhage, multinodular and multicystic areas. Tumor may show areas of calcification, metaplastic ossification and necrosis. Synovial sarcoma in amputated leg is shown in Fig. 28.27.

Light Microscopy

Synovial sarcoma shows two histologic patterns depending on the presence of epithelial and spindle cell components in varying proportions: (a) monophasic variant (containing spindle cell component without epithelial differentiation) or (b) biphasic (containing both epithelial and spindle cell components). Biphasic synovial sarcoma usually shows areas of calcification and ossification and contains both epithelial and spindle cell components.

- **Epithelial component of biphasic synovial sarcoma:** Epithelial component of biphasic synovial sarcoma resembles that predominates in epithelial malignancy.
  - Epithelial component cells are arranged in solid cords or nests or glandular, tubular, sometimes alveolar or papillary architecture.
  - Glandular architecture is lined by epithelial cuboidal or columnar cells containing vesicular nuclei and abundant pale eosinophilic cytoplasm.
  - Mucin is demonstrated in glandular lumen.
  - Epithelial keratinization, metaplasia and glandular change may be demonstrated in occasional tumor.
  - Epithelial cells show positivity for cytokeratins (AE1/AE3) and epithelial membrane antigen.
- **Spindle cell component of biphasic synovial sarcoma:** Spindle cell component of biphasic synovial sarcoma resembles that predominates in monophasic synovial sarcoma.
  - Tumor is composed of small and uniform neoplastic spindle cells with sparse cytoplasm and ovoid hyperchromatic nuclei, granular chromatin, inconspicuous nucleoli, and high nucleocytoplasmic ratio.
  - Spindle cells are arranged in dense cellular sheets or vague fascicles, with occasional nuclear palisading or herringbone pattern.
  - Collagen is present in variable proportions. Poorly differentiated synovial sarcoma shows severe nuclear atypia and high mitotic activity >15 per high power field (HPF) in the spindle cell component. Histology of biphasic synovial sarcoma is shown in Fig. 28.28.

Histochemistry

Epithelial cell component of synovial sarcoma is outlined by PAS stain, Alcian blue stain and colloidal iron.

Electron Microscopy

- Epithelial cell component of synovial sarcoma shows microvilli, tonofilaments and cell junctions; surrounded by basement membrane similar to demonstrated in adenocarcinoma. These ultrastructural features are frequently demonstrated in biphasic synovial sarcoma.
- Monophasic synovial sarcoma shows fewer specific features.

Immunohistochemistry Technique

Epithelial cell component of biphasic synovial sarcoma shows positivity for CD99, BCL-2, cytokeratins, epithelial membrane antigen (EMA) and TLE-1. It is negative for CD34. Spindle cell component of synovial sarcoma shows positivity for vimentin.

Markers	Expression
Epithelial cell component of synovial sarcoma	
▪ CD99 (membranous staining) ▪ BCL-2 ▪ Cytokeratins (7, 8, 14, 18, 19) by AE1/AE3 ▪ Epithelial membrane antigen (EMA) ▪ TLE-1 (nuclear staining) ▪ CD34	▪ Positive ▪ Positive ▪ Positive (70–80%) ▪ Positive (70–80%) ▪ Positive (80%) ▪ Negative
Spindle cell component of synovial sarcoma	
Vimentin	Positive

Molecular Genetic Alterations

Demonstration of chromosomal translocation t(X;18)(p11,q11) is characteristic finding in 95% of synovial



Fig. 28.27: Synovial sarcoma. Amputated surgical specimen of synovial sarcoma. Cut surface revealed gray-yellow or pink with soft in consistency depending on proportion of cellularity, collagenization, myxoid change or hemorrhage. It showed multinodular and multicystic areas. It may show areas of calcification, metaplastic ossification and necrosis.

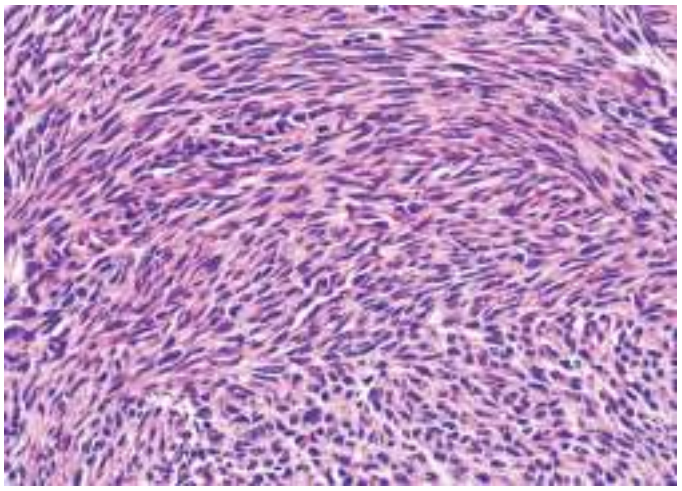


Fig. 28.28: Synovial sarcoma. Tumor shows two histologic patterns depending on the presence of epithelial and spindle cell components in varying proportions: (a) monophasic variant (containing spindle cell component without epithelial differentiation) and (b) biphasic (containing both epithelial and spindle cell components). Biphasic synovial sarcoma usually shows areas of calcification and ossification (400X).

sarcoma cases. As a result of chromosomal translocation t(X;18) (p11,q11), SS18 gene (also known as SYT or SSXT) fuses with one of the SSX genes (SSX1, SSX2, SSX4) on X chromosome. Molecular genetic alterations in synovial sarcoma are given in Table 28.23.

Prognosis and Treatment

Synovial sarcoma has variable prognosis depending on the age of patient, size of the tumor, histologic features, i.e. differentiation of tumor, mitotic activity, nuclear grading, vascular invasion and clinical stage. Prognostic factors of synovial sarcoma are given in Table 28.24.

- **Poor prognostic factors:** Synovial sarcoma >5 cm in diameter with necrosis in trunk, head and neck region, presence of 10 mitoses per 10 high power fields in a patient above 40 years of age most often metastasizes to lungs and bone resulting in fatal outcome. Synovial sarcoma with >20% poorly differentiated histology has aggressive behavior. Prognosis is poor in synovial sarcoma showing vascular invasion on histologic examination.
- **Good prognostic factors:** Prognosis is excellent in patients with small size synovial sarcoma measuring <1 cm in diameter in extremities and tumor <5 cm in diameter without necrosis and <10 mitoses per 10 high power fields. Prognosis is better in children than adults.

Table 28.23 Molecular genetic alterations in synovial sarcoma

Fusion Protein	Frequency
SS18-SSX1	66%
SS18-SSX2	33%
SS18-SSX4	1%

EPITHELIOID SARCOMA

Epithelioid sarcoma is a malignant mesenchymal tumor showing epithelial differentiation with epithelioid cytomorphology. Two clinicopathological subtypes of epithelioid sarcoma have been described in Table 28.25.

Locations and Age Distribution

- **Conventional or classic distal subtype of synovial sarcoma:** Common sites of tumor are upper extremity (fingers and hands) in dermis or subcutis region or deep soft tissue. It affects children and young adults (10–40 years).
- **Proximal subtype of synovial sarcoma:** Common sites of tumor are pelvis, perineum, genital, inguinal region, buttock, hip, thigh, head, neck, distal extremity and axilla in dermis or subcutis region or deep soft tissue. It affects children and adults.

Table 28.24 Prognostic factors of synovial sarcoma

Parameters	Good Prognostic Factors	Poor Prognostic Factors
Clinical features		
▪ Age of the patient ▪ Location of tumor ▪ Size of tumor ▪ Stage	▪ Children ▪ Extremities ▪ <5 cm (excellent if <1 cm) ▪ Stage I	▪ Adults ▪ Trunk, head and neck ▪ >5 cm ▪ Advanced stage with distant metastasis in lungs and bone
Gross morphology		
Necrosis in large tumor	Absent	Present
Histologic features		
▪ Tumor differentiation ▪ Vascular invasion ▪ Calcification in tumor ▪ Mitoses	▪ Well-differentiated tumor ▪ Absent ▪ Present ▪ <10 mitoses per 10 high power fields	▪ Tumor with >20% poorly differentiated histology ▪ Present ▪ Absent ▪ >10 mitoses per 10 high power fields

Table 28.25 Clinicopathological subtypes of epithelioid sarcoma

Parameters	Conventional or Classic Distal Subtype of Synovial Sarcoma	Proximal Subtype of Synovial Sarcoma (Large Cell Variant)
Clinical history		
Location of tumor	Upper extremity (fingers and hands) in dermis or subcutis region or deep soft tissue	Pelvis, perineum, genital, inguinal region, buttock, hip, thigh, head, neck, distal extremity and axilla in dermis or subcutis region or deep soft tissue
Age group	Children and young adults (10–40 years)	Children and young adults
Gross morphology		
Tumor	Few mm to 5 cm in diameter in superficial region (solitary or multiple nodules) and up to 15 cm in deep soft tissue	1–20 cm in diameter (solitary or multiple nodules)
Cut surface	Gray-white or gray tan, glistening surface	Gray-white with areas of hemorrhage and necrosis

Clinical Features

Patient presents with slow-growing painless firm solitary or multiple nodules in dermis or subcutis region or deep soft tissue. Tumor usually metastasizes to regional lymph nodes.

Surgical Pathology: Epithelioid Sarcoma

Gross Morphology

- **Conventional or classic distal subtype of synovial sarcoma:** Tumor excised from dermis and subcutaneous region is ill-defined nodule measuring few mm to 5 cm in diameter.
  - Tumor excised from tendon and fascia in the deep-seated region is multinodular measuring 1–15 cm in diameter.
  - Cut surface is gray-white or gray-tan with glistening surface.
- **Proximal subtype of synovial sarcoma:** Tumor is solitary or multiple whitish nodule measuring 1–20 cm in diameter. Cut surface shows areas of hemorrhage and necrosis.

Light Microscopy

- Epithelioid sarcoma is composed of epithelioid cells admixed with distinct elongated spindle cells. Epithelioid cells small to large contain uniform hyperchromatic nuclei. Presence of bland nuclear features in association with central necrosis may closely resemble a granulomatous process termed necrobiotic granuloma. There is presence of lymphocytes surrounding neoplastic cells.
- Some epithelioid sarcomas are entirely composed of spindle cells resembling benign fibrous histiocytoma and fibrosarcoma.

Differential Diagnosis

Epithelioid sarcoma should be differentiated from necrobiotic granuloma, cutaneous squamous cell carcinoma and adnexal carcinoma, benign fibrous histiocytoma, fibrosarcoma and epithelioid angiosarcoma (pseudoalveolar variant).

Immunohistochemistry Technique

Epithelioid sarcoma shows co-expression of cytokeratins and vimentin. It shows positivity for CD34 and epithelial membrane antigen (EMA). Loss of INI-1 is demonstrated in 90% of cases.

Markers	Expression
▪ Cytokeratins (low and high molecular weight)	▪ Positive
▪ Vimentin	▪ Positive
▪ CD34	▪ Positive (50%)
▪ Epithelial membrane antigen (EMA)	▪ Positive
▪ Loss of INI-1	▪ Positive (90%)

Molecular Genetic Alterations

Genetic chromosomal alterations of 22q11.12 have been implicated in the pathogenesis of epithelioid sarcoma. Alterations in tumor suppressor gene SMARCB1 (HSSn5, INI-1) have been demonstrated in epithelioid sarcoma.

Prognosis and Treatment

Epithelioid sarcoma is treated by surgical excision, i.e. amputation of extremity, radiotherapy and chemotherapy.

- Local recurrence after surgery occurs in 70% of cases. Tumor metastasizes to regional lymph nodes and lungs in 50% of cases.
- Adverse prognostic factors are elderly persons, deep-seated tumor, tumor size >5 cm in diameter with extensive necrosis, multifocal involvement, high mitotic activity, lymph node and distant metastasis and proximal type of epithelioid sarcoma associated with presence of rhomboid cells.

ALVEOLAR SOFT PART SARCOMA

Alveolar soft part sarcoma (ASPS) is a rare slow-growing malignant tumor. Tumor is composed of large uniform epithelioid cells with well-defined borders, centrally placed bland nuclei and abundant eosinophilic cytoplasm somewhat granular and rod-shaped intracytoplasmic inclusions.

- Neoplastic cells are arranged in solid nests and/or alveolar structures and separated by delicate

fibrovascular septa. Presence of ASPSCR1-TFE3 fusion protein is highly specific for alveolar soft part sarcoma. However, it is also demonstrated in renal cell carcinoma affecting young persons.

- Alveolar soft part sarcoma is defined by a specific cytogenetic alteration, der(17) t(X;17) (p11;q25) resulting in the formation of fusion protein ASPSCR1-TFE3. ASPSCR1 located on 17q25 fuses with TFE transcription factor gene located on Xp11.

Locations and Age Distribution

Most common sites of alveolar soft part sarcoma are deep soft tissue of thigh, buttock region, popliteal region, chest and forearm in decreased frequency in adults.

- In children and infants, most common sites are head and neck, tongue and orbit.
- Unusual sites are lung, breast, stomach, liver, bone, larynx, heart, urinary bladder, uterine cervix and another region of female genital tract.
- Alveolar soft part sarcoma most often affects 15–35 years of age. However, it can occur at any age. Tumor rarely affects children below 5 years and adults above 50 years of age. Males are more affected than females during first three decades of life. But after 30 years of age, females are more affected than males.

Clinical Features

Patient presents with slow-growing painless mass within soft tissue in the region of thighs, buttock, popliteal region, chest, trunk, head and neck, and female genital system. Orbital lesion presents with proptosis and lid swelling. Vaginal bleeding occurs due to tumor in female genital tract.

Surgical Pathology: Alveolar Soft Part Sarcoma

Gross Morphology

- Alveolar soft part sarcoma is a poorly circumscribed tumor.
- Cut surface is pale gray with soft in consistency.
- Areas of hemorrhage and necrosis are demonstrated in large tumor.

Light Microscopy

- Alveolar soft part sarcoma is composed of large uniform epithelioid cells with well-defined borders, centrally placed bland nuclei and abundant eosinophilic cytoplasm somewhat granular and rod-shaped intracytoplasmic inclusions.
- Tumor cells are arranged in solid nests and/or alveolar structures and separated by delicate fibrovascular septa (Fig. 28.29).
- Loss of cellular cohesion and necrosis of the neoplastic cells in the central region of nests of tumor cells gives pseudoalveolar pattern.

Histochemistry

Neoplastic cells of alveolar soft part sarcoma demonstrate rhomboid or rod-shaped intracytoplasmic inclusions, and variable amount of glycogen and diastase resistant granules demonstrated by PAS stain.

Electron Microscopy

- Most distinctive feature of alveolar soft part sarcoma is the presence of membrane bound rhomboid or rectangular crystals composed of periodic lattice work of rigid fibrils with 5–7 nm in diameter.
- Cytoplasmic granules associated with formation of crystals contain monocarboxylate transporter protein 1 and its cellular chaperone CD147.

Differential Diagnosis

Alveolar soft part sarcoma.

Immunohistochemistry Technique

Alveolar soft part sarcoma shows strong nuclear positivity for TFE3 retained in the fusion protein.

Marker	Expression
TFE3	Positive

Molecular Genetic Alterations

- Alveolar soft part sarcoma is defined by a specific cytogenic alteration, der(17) t(X;17) (p11;q25) resulting in the formation of fusion protein ASPSCR1-TFE3. ASPSCR1 located on 17q25 fuses with TFE transcription factor gene located on Xp11.

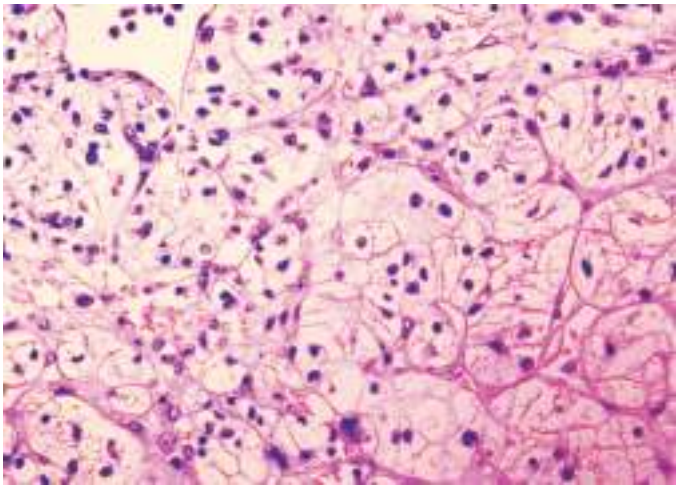


Fig. 28.29: Alveolar soft part sarcoma. Tumor is composed of large uniform epithelioid cells with well-defined borders, centrally placed bland nuclei and abundant eosinophilic cytoplasm somewhat granular and rod-shaped intracytoplasmic inclusions. Neoplastic cells are arranged in solid nests and/or alveolar structures and separated by delicate fibrovascular septa. Loss of cellular cohesion and necrosis of the neoplastic cells in the central region of nests of tumor cells gives pseudoalveolar pattern (400X).

- Presence of ASPSCR1-TFE3 fusion transcript is highly specific for alveolar soft part sarcoma. However, it is also demonstrated in renal cell carcinoma affecting young persons.

Prognosis and Treatment

Alveolar soft part sarcoma is treated by surgical excision. Tumor may recur after surgical resection of tumor. Before detection of primary tumor, metastases can be observed in lung, bone and brain. Metastases in lymph nodes are uncommon. Factors influencing prognosis are age of the patient, tumor size and metastases to distant organs.

CLEAR CELL SARCOMA OF SOFT TISSUE

Clear cell sarcoma of soft tissue is also known as malignant melanoma of soft tissue, which most often occurs in the deep soft tissue of the extremities in proximity to tendons and aponeurotic structures.

- Tumor shows nested growth pattern of neoplastic cells, consistent melanocytic differentiation and recurrent EWSR1-ATF1 fusion protein in majority of cases.
- Reciprocal chromosomal translocation t(12;22)(q13,q12) leads to formation of EWSR1-ATF1 fusion transcript in >90% of cases.

Locations and Age Distribution

Most common site of clear cell sarcoma is foot/ankle of deep region intimately associated with tendons and aponeurosis without involving skin in 40% of cases. Uncommon sites are head and neck, trunk, retroperitoneum, viscera, bone and gastrointestinal tract especially small intestine in decreased frequency. It usually affects young adults with equal sex distribution. Peak incidence of tumor is during third and fourth decades of life.

Clinical Features

Patient presents with slow-growing mass most often in foot/ankle region over weeks to several years' duration. Pain and tenderness is present in 50% of cases. Lymph node metastasis is frequent.

Surgical Pathology: Clear Cell Sarcoma of Soft Tissue

Gross Morphology

- Tumor is well-circumscribed soft tissue mass measures <5 cm in diameter. However, tumor >10 cm has been reported.
- Cut surface shows a lobulated gray white tumor with pushing margin. Rarely, areas of pigmentation, necrosis and cystic areas are demonstrated.

Light Microscopy

- Under low magnification, tumor shows nested growth pattern with collagenous fibrous bands dividing the tumor into distinct compartments. Neoplastic cells display predominantly epithelioid morphology.
- Tumor is composed of epithelioid cells containing vesicular nuclei and amphophilic cytoplasm.
- Scattered wreath-like multinucleated giant cells are most often demonstrated. Melanin is demonstrated in 66% of tumors. Average 4 mitoses per high power fields are demonstrated.

Histochemistry

Melanin is demonstrated in clear cell sarcoma of soft tissue by Masson-Fontana stain in 66% of cases.

Electron Microscopy

Melanosomes are demonstrated in majority of cases in clear cell sarcoma of soft tissue.

Immunohistochemistry Technique

Clear cell sarcoma of soft tissue shows positivity for S-100 protein, HMB-45 (human melanoma black 45) and MITF (microphthalmia transcription factor).

Markers	Expression
S-100 protein	Positive
HMB-45 (human melanoma black 45)	Positive
MITF (microphthalmia transcription factor)	Positive

Molecular Genetic Alterations

Genetic hallmark of clear cell sarcoma of soft tissue is reciprocal chromosomal translocation t(12;22)(q13,q12) resulting in the formation of EWSR1-ATF1 fusion transcript in >90% of cases.

Prognosis and Treatment

Clear cell sarcoma of soft tissue is treated by wide margin surgical excision. Local recurrence of tumor occurs after surgical excision. Poor prognostic factors include tumor size >5 cm, presence of necrosis, local recurrence after surgical excision, and metastases to regional lymph nodes, lungs and bones. Clear cell sarcoma of gastrointestinal tract usually metastasizes to lymph nodes, peritoneum and liver.

CHONDRO-OSSEOUS TUMORS AND OTHER LESIONS

Normally, somatic soft tissue lacks mature osteoblasts and chondrocytes. Therefore, somatic soft tissue does not contain bone or cartilage. However, under certain circumstances, undifferentiated mesenchymal cells are stimulated to develop into bone or cartilage producing tumors. Chondro-osseous tumors according to recent 2020 WHO classification of soft tissue tumors are shown in Table 28.26.

Table 28.26 Chondro-osseous tumors according to recent 2020 WHO classification of soft tissue tumors

Chondro-osseous Tumors
Benign tumors/lesions
▪ Soft tissue chondroma ▪ Myositis ossificans ▪ Fibro-osseous pseudotumor of digits
Malignant tumors
▪ Primary soft tissue osteosarcoma ▪ Mesenchymal chondrosarcoma

MYOSITIS OSSIFICANS

Myositis ossificans is a non-neoplastic reactive bone and cartilage producing pseudotumor developing in the skeletal muscle.

Locations and Age Distribution

Most common sites of myositis ossificans are large muscles of the proximal extremities of persons during second and third decades of life. Males and females are equally affected.

Clinical Features

Patient presents with short history of pain and swelling of the affected extremity. Localized erythema is rarely seen.

Radiologic Findings

Conventional radiograph and magnetic resonance imaging initially demonstrate a soft tissue mass. In mature lesion of myositis ossificans demonstrates areas of ossification.

Surgical Pathology: Myositis Ossificans

Gross Morphology

- Myositis ossificans most often measures <5 cm in diameter.
- Early lesion of myositis ossificans is ill-defined soft tissue mass, that may exhibit focal gritty calcifications of mineralized bone.
- As the lesion matures, mass is completely ossified appearing solid bone and easily demarcated from the skeletal muscle.

Light Microscopy

- **Early lesion of myositis ossificans:** It is composed of plump myofibroblastic cells arranged in sheets and fascicles.
  - Myofibroblastic cells contain oval nuclei with vesicular chromatin and eosinophilic nucleoli; embedded within a myxoid to collagenous ground substance.
  - Mitotic figures are usually demonstrated. Necrosis is absent.
- **Mature lesion of myositis ossificans:** It is composed of well-organized more mature bone (lamellar cortical and lamellar bone containing hematopoietic marrow).
  - Intersecting fibrillary eosinophilic lace-like woven bone trabeculae appear to originate from the spindle cells.
  - Transition of immature foci of woven bone to thicker mineralized woven bone trabeculae showing prominent osteoblastic rimming is termed 'zoning phenomenon' (Fig. 28.30).

Differential Diagnosis

- Myositis ossificans should be differentiated from fibro-osseous pseudotumor (non-muscular soft tissue mass) of hand, nodular fasciitis and extraskeletal osteosarcoma.
- Extraskeletal osteosarcoma affects adults during fifth and six decades of life. Tumor measures >5 cm in diameter. Tumor is composed of pleomorphic cells with cytologic atypia.

Immunohistochemistry Technique

Early lesion of myositis ossificans composed of spindle cells shows positivity for smooth muscle actin and muscle-specific actin reflecting their myofibroblastic morphology.

Markers	Expression
▪ Smooth muscle actin (SMA) ▪ Muscle-specific actin (MSA)	▪ Positive ▪ Positive

Prognosis and Treatment

Myositis ossificans is cured by surgical excision. Recurrence is extremely uncommon, even when resection margins are positive.

FIBRO-OSSEOUS PSEUDOTUMOR OF DIGITS

Fibro-osseous pseudotumor of digits is a non-neoplastic reactive bone and cartilage producing pseudotumor in the soft tissue of digits. It is clinicopathologic analogous to myositis ossificans.

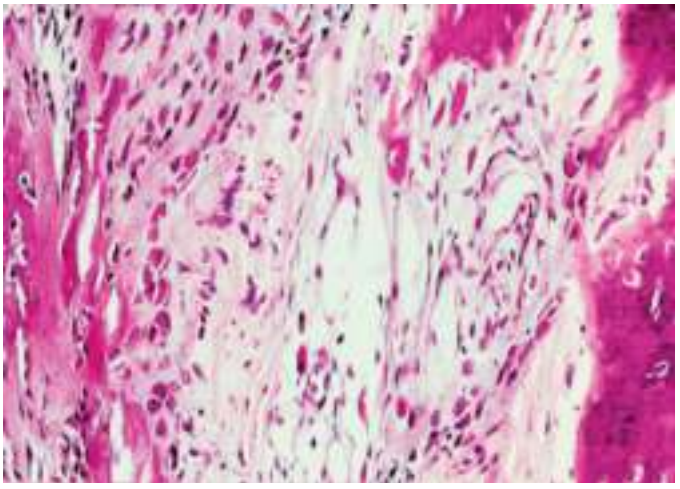


Fig. 28.30: Myositis ossificans. Myositis ossificans is a non-neoplastic reactive bone and cartilage producing pseudotumor developing in skeletal muscle. As the lesion matures, it becomes less cellular and shows osteoid and chondroid formation in the skeletal muscle (400X).

Locations and Age Distribution

Most common sites of fibro-osseous pseudotumor are soft tissue surrounding the small tubular bones of hands and feet in decreased frequency. It most common affects adults during second and third decades of life. Males and females are equally affected.

Clinical Features

Patient presents with swelling digits over short duration especially history of trauma in <50%.

Radiologic Findings

Radiograph demonstrates soft tissue swelling with or without linear periosteal bone formation affecting the underlying bone.

Surgical Pathology: Fibro-osseous Pseudotumor of Digits
Gross Morphology
Fibro-osseous pseudotumor is firm, tan and rubbery measuring <3 cm in diameter.
Light Microscopy
▪ Histology of fibro-osseous pseudotumor of digits is identical to myositis ossificans. Fibro-osseous pseudotumor is composed of plump spindle cells admixed with osseocartilaginous tissue. Zoning phenomenon, i.e. transition of immature foci of woven bone to thicker mineralized woven bone trabeculae with prominent osteoblastic rimming is absent in fibro-osseous pseudotumor. ▪ Typical mitotic figures are demonstrated. High-grade nuclear atypia and necrosis are absent.
Differential Diagnosis
Fibro-osseous pseudotumor of digits should be differentiated from myositis ossificans, soft tissue chondroma and fracture callus.

Prognosis and Treatment

Fibro-osseous pseudotumor of digits is cured by surgical excision. Recurrence is uncommon, even when margins are positive. Aggressive destructive growth does not occur.

EXTRASKELETAL OSTEOSARCOMA

Extraskeletal osteosarcoma originates in somatic soft tissue, in which the neoplastic cells produce osteoid and/or bone matrix.

Locations and Age Distribution

Extraskeletal osteosarcoma accounts for 1–2% of soft tissue sarcomas. Most common sites of extraskeletal osteosarcoma are the deep soft tissue of the thigh and buttock regions. It usually affects adults during fifth and sixth decades with male predominance.

Clinical Features

Patient presents with soft tissue painless mass in the thigh or buttock regions.

Radiologic Findings

Most of extraskeletal osteosarcomas have nonspecific imaging features identical to other high grade soft tissue sarcomas. Mineralization tends to be minimal.

Surgical Pathology: Extraskeletal Osteosarcoma	
Gross Morphology	
▪ Tumor is large heterogenous well circumscribed tumor measuring >10 cm in diameter. ▪ Mineralized bone is not demonstrated on gross examination. ▪ Cut surface shows areas of hemorrhage and necrosis.	
Light Microscopy	
▪ Tumor is composed of spindle to epithelioid neoplastic cells forming neoplastic lace-like eosinophilic osteoid and/or bone. Mineralized osteoid (bone) is relatively uncommon. ▪ Lobules of cellular atypical hyaline cartilage may be demonstrated. ▪ All histologic patterns of osseous osteosarcoma including small cell, telangiectatic and undifferentiated spindle cell sarcoma patterns may be demonstrated in extraskeletal osteosarcoma.	
Differential Diagnosis	
Extraskeletal osteosarcoma should be demonstrated from osseous osteosarcoma (primary or metastatic and recurrent) and other non-matrix producing soft tissue sarcomas.	
Immunohistochemistry Technique	
▪ Tumor cells show identical pattern of staining to skeletal osteosarcoma. ▪ Diffuse strong vimentin staining is demonstrated in extraskeletal osteosarcoma. ▪ Focal staining for S-100 protein, desmin, and smooth muscle actin may also be demonstrated. ▪ Antibody to the bone matrix osteocalcin is specific for both neoplastic cells and neoplastic bone/osteoid matrix.	
Markers	**Expression**
▪ Osteocalcin	▪ Positive (neoplastic cells and neoplastic matrix)
▪ Vimentin	▪ Positive (diffuse strong staining)
▪ Smooth muscle actin	▪ Positive (focal staining)
▪ Desmin	▪ Positive (focal staining)
▪ S-100 protein	▪ Positive (focal staining)

Prognosis and Treatment

Extraskeletal osteosarcoma is a high-grade sarcoma treated by wide surgical excision with systemic

chemotherapy. Tumor has high rate of metastases to distant organs.

SOFT TISSUE CHONDROMA

Soft tissue chondroma is a benign tumor composed of mature chondrocytes, associated with well-formed hyaline cartilage.

Locations and Age Distribution

Most common sites of soft tissue chondroma are distal extremities especially fingers and wrist region. It most affects adults during fourth to seventh decades of life with male predominance.

Clinical Features

Patient presents with painless soft tissue mass in fingers or wrist.

Radiologic Findings

Radiograph shows nonspecific appearance. Lesion is not attached to the underlying skeleton.

Surgical Pathology: Soft Tissue Chondroma

Gross Morphology

- Tumor is well circumscribed tumor measuring <2 cm in diameter. It is easily removed from the surrounding structures.
- Cut surface shows pearly white, chalk-like areas of calcification.

Light Microscopy

- Tumor is composed of mature chondrocytes arranged in clusters embedded within well formed hyaline cartilage. Chondrocytes may demonstrate mild nuclear irregularity and nucleoli. Mitotic figures are rarely demonstrated. Overall cellularity of chondroma is greater than normal hyaline cartilage.
- Tumor is lobulated and well demarcated from adjacent soft tissue. It shows microscopic calcification with or without enchondral ossification.
- Few chondromas show increased cellularity, and are composed of more plump epithelioid chondrocytes and clusters of multinucleated osteoclast-like giant cells. Such tumors are designated as 'chondroblastoma-like' soft tissue chondromas.

Differential Diagnosis

Chondroma should be differentiated from synovial chondromatosis, fibro-osseous pseudotumor of digits and soft tissue chondrosarcoma.

Immunohistochemistry Technique

Chondroma shows positivity for vimentin and S-100 protein.

Markers	Expression
▪ Vimentin	▪ Positive
▪ S-100 protein	▪ Positive

Prognosis and Treatment

Soft tissue chondroma is treated by surgical excision. Recurrence is demonstrated in 20% of cases.

EXTRASKELETAL MYXOID CHONDROSARCOMA

Extraskeletal myxoid chondrosarcoma is a soft tissue tumor composed of spindle and epithelioid neoplastic cells associated with extracellular matrix, that resembles cartilage on histological and histochemical examination. The majority of neoplasms exhibit the chromosomal translocation t(9;22)(q22;q12) involving the EWS and CHN genes. These tumors are distinct from osseous chondrosarcomas on histological, histochemical and immunohistochemical analysis.

Locations and Age Distribution

Extraskeletal myxoid chondrosarcoma accounts for 2.5% of all soft tissue sarcomas. Most common sites of tumor are deep soft tissue of proximal extremities and trunk. However, it may occur at any site. It affects adults during fourth to seventh decades of life with male predominance.

Clinical Features

Patient presents with painless soft tissue mass in the deep soft tissue of the proximal extremity or trunk. Rarely, patient may present with lung metastasis and an occult primary tumor.

Surgical Pathology: Extraskeletal Myxoid Chondrosarcoma

Gross Morphology

- Tumor is a lobulated mass measuring >5 cm in diameter.
- Cut surface is gray and gelatinous with soft in consistency.

Light Microscopy

- Tumor demonstrates a lobular growth pattern under low power examination.
- Tumor is composed of uniform oval to spindled cells with uniform dark staining nuclei and indistinct nucleoli arranged in nests, cords and clusters, and embedded within flocculent basophilic matrix.
- Individual lobules of the tumor are separate by fibrous bands. Blood vessels are indistinct.
- In recent years, the existence of cellular matrix-poor regions of extraskeletal chondrosarcoma has been recognized. In these areas, there is presence of large neoplastic cells with hyperchromatic nuclei, nuclear atypia, high nuclear/cytoplasmic ratio and mitoses.

Histochemistry

Alcian blue histochemical staining is positive at pH 4.0 and 1.0, suggesting the presence of chondroitin sulfate in the tumor.

Differential Diagnosis

Extraskeletal myxoid chondrosarcoma should be differentiated from soft tissue sarcoma, osseous chondrosarcoma with soft tissue extension, ossifying fibromyxoid tumor and myoepithelioma/mixed tumor of soft tissue.

Immunohistochemistry Technique

Extraskeletal myxoid chondrosarcoma cells show diffuse positivity for vimentin, S-100 (<50%), neuron-specific enolase (NSE) and synaptophysin.

Markers	Expression
▪ Vimentin	▪ Positive (diffuse staining pattern)
▪ S-100 protein	▪ Positive (<50%)
▪ Neuron-specific enolase	▪ Positive
▪ Synaptophysin	▪ Positive

Prognosis and Treatment

Extraskeletal myxoid chondrosarcoma is treated by surgical excision. Five-year survival rate is demons trated in >80% of cases. Late metastasis may occur in lungs.

EXTRASKELETAL MESENCHYMAL CHONDROSARCOMA

Extraskeletal mesenchymal chondrosarcoma is a high-grade biphasic sarcoma composed of undifferentiated small blue cells and islands of well-formed hyaline cartilage.

Locations and Age Distribution

Extraskeletal mesenchymal chondrosarcoma accounts for <1% of soft tissue sarcomas. Most common sites of tumor are craniospinal axis soft tissues, meninges and paraspinal musculature, which may occur at any age, although most cases are diagnosed in adults during second to fourth decades of life. Males and females are equally affected.

Clinical Features

Patient with extraskeletal mesenchymal chondrosarcoma presents with painless within soft tissues of craniospinal axis or paraspinal musculature. Patient with meningeal mesenchymal chondrosarcoma presents with focal neurologic signs depending on location of intracranial tumor.

Radiologic Findings

Radiograph demonstrates soft tissue mass and focal cartilaginous-type calcification.

Surgical Pathology: Extraskeletal Mesenchymal Chondrosarcoma

Gross Morphology

- Tumor is well defined measuring <5 to >30 cm in diameter.
- Cut surface is tan gray. Focal areas of hemorrhage or necrosis are demonstrated. Gritty areas representing the calcified cartilage are usually demonstrated.

Light Microscopy

- Tumor is composed of undifferentiated 'small blue cell' component.
- Neoplastic cells are arranged in sheets, vague nests or both. Characteristic feature of tumor is the presence of a supporting capillary network that has acute angle branching resulting in a 'hemangiopericytoma-like' growth pattern.
- The blue cells are associated with bands of eosinophilic hyalinized collagen fibers that may resemble the 'rope-like' collagen of solitary fibrous tumor.
- Islands of well-formed hyaline cartilaginous matrix with embedded well-differentiated chondrocytes exhibiting granular purple mineralization of the matrix are demonstrated.

Differential Diagnosis

- Extraskeletal mesenchymal chondrosarcoma should be differentiated from other blue cell sarcomas such as Ewing sarcoma/primitive neuroectodermal tumor (PNET) and small cell osteosarcoma.
- Ewing sarcoma is characterized by recurrent balanced chromosomal translocations in almost all cases. Reciprocal translocation between chromosomes 11 and 22 results in fusion of the amino terminus of EWSR1 gene and Flt-1 gene, which encodes a nuclear transcription factor resulting in unrestricted cellular proliferation.
- Solitary fibrous tumor and synovial sarcoma demonstrate sheets of small, rather undifferentiated appearing cells often associated with a hemangiocytoma-like vascular pattern. These tumors do not show hyaline cartilage.

Immunohistochemistry Technique

Small blue cells and chondrocytes of extraskeletal mesenchymal chondrosarcoma show positivity for vimentin. Small blue cells are positive for CD99. Cartilaginous component of the tumor shows positivity for S-100 protein.

Markers	Expression
▪ Vimentin	▪ Positive (small blue cells and chondrocytes components)
▪ CD99	▪ Positive (small blue cell component)
▪ S-100 protein	▪ Positive (cartilaginous component)

Prognosis and Treatment

Extraskeletal mesenchymal chondrosarcoma is highly aggressive tumor treated by a combination of surgery and chemotherapy. Five-year survival is demonstrated in 50% of cases.

NON-NEOPLASTIC DISORDERS OF SKELETAL MUSCLE

SKELETAL MUSCLE: STRUCTURE

Skeletal muscle is a specialized tissue of mesodermal origin, which has special properties like excitability, contractibility and elasticity.

- Each skeletal muscle is composed of numerous muscle fibers bundled together and wrapped in a connective tissue sheath called epimysium. Fascia, connective tissue outside the epimysium surrounds and separates the skeletal muscle fibers. Portions of the epimysium project inward to divide the skeletal muscle into compartments. Each bundle of skeletal muscle fiber is called fasciculus and surrounded by a connective tissue called perimysium. Within the fasciculus, each skeletal muscle fiber is surrounded by connective tissue called endomysium.
- Skeletal muscles have an abundant supply of blood vessels and nerves. Skeletal muscle fiber contains a specialized smooth endoplasmic reticulum called sarcoplasmic reticulum, that stores, releases and retrieves calcium ions (Ca^{++}).
- Skeletal muscle fiber is composed of many myofibrils, which contain sarcomeres with light and dark regions that give the cell, its striated appearance, and surrounded by a plasma membrane called sarcolemma (Greek word '*sarco*' means 'flesh'), which contains cytoplasm called sarcoplasm.
- The sarcomere, the region from one Z line to the next Z line, is the fundamental functional unit of a skeletal muscle fiber. When sarcomere contracts, the Z lines move close together and the I band (light band) becomes smaller. The A band (dark band) stays the same width. At full contraction, the thick filaments and thin overlap. Contractile thick and thin filaments are present in the sarcoplasm.
  - Contractile thick filaments possess myosin component, which has a tail and head (actin binding site). Myosin head has contractile property as well as ATPase like action. Contractile thin filaments possess troponin, actin and tropomyosin. Troponin masks the active sites on the F-actin.
- Skeletal muscle fibers contain two types of fibers: slow-twitch (type 1) and fast twitch (type 2). Slow-twitch skeletal muscle fibers support long distance endurance activities like marathon running. Fast-twitch skeletal muscle fibers support quick, powerful movements such as sprinting or weight-lifting. Comparison of type 1 and type 2 skeletal muscle fibers is given in Table 28.27.

Table 28.27 Comparison of type 1 and type 2 skeletal muscle fibers

Features	Type 1 (Red) Skeletal Muscle Fibers	Type 2 (White) Skeletal Muscle Fibers
Prototype muscle	Soleus muscle	Pectoral muscle
Action and duration	Sustained movement with force and prolonged duration action	Sudden movement and short duration action
Contraction rate	Contraction rate slow	Contraction rate fast
Glycogen content	Scant amount of glycogen	Abundant amount of glycogen
Lipid content	Abundant amount	Scant amount
Staining properties	▪ ATPase at pH 4.2 dark staining ▪ ATPase at pH 9.4 light staining ▪ NADH-tetrazolium reductase dark staining	▪ ATPase at pH 4.2 light staining ▪ ATPase at pH 9.4 dark staining ▪ NADH-tetrazolium reductase light staining
Myoglobin	Present	Absent
Ultrastructure findings	▪ Many mitochondria ▪ Z-band wide	▪ Lesser mitochondria ▪ Z-band narrow
Physiology	Slow-twitch	Fast-twitch
Aerobic energy production	Aerobic energy production high	Aerobic energy production low
Anaerobic energy production	Low	High
Fatigue	Slow-onset fatigue	Rapid-onset fatigue
Energy utilization	Energy utilization low	Energy utilization high
Color of muscle fiber	Red color	White color
Function	Weight bearing	Purposeful motion

NADH-TR: Nicotinamide adenine dinucleotide tetrazolium reductase.

MYOPATHIES

Myopathy refers to skeletal and cardiac muscle dysfunction unrelated to any disorder of innervation or neuromuscular junction. Disruption of the structural integrity and metabolic processes of skeletal muscle cells can result from genetic abnormalities, inflammation, infection, toxins and hormonal and electrolyte imbalance.

- **Clinical features:** Patients typically present with proximal skeletal muscle weakness of legs more than arms with motor nerve involvement and without sensory nerve involvement. Myopathy can also affect the respiratory muscles, which can lead trouble breathing. Other symptoms of myopathy may include: skeletal muscle cramps, stiffness, muscle pain, low energy and fatigue and exercise intolerance. Over time, patients may also experience skeletal muscle wasting.
- **Classification of myopathies:** Age of clinical presentation, duration of illness, and distribution of weakness of skeletal muscles are helpful in determining the classification and etiology of myopathy. Myopathies can be divided into two main categories: inherited and acquired myopathies. Clinical course, the pattern of muscle weakness, and the absence of a family history of myopathy help in distinguishing between the inherited and acquired types.
  - **Inherited myopathies:** An early age of onset with a relatively longer duration of the disease suggests an inherited myopathy, which occurs due to transmission of an abnormal gene mutation from parents to children resulting in disruption of structural integrity and function of skeletal muscles, which can be further subclassified as muscular dystrophies, congenital myopathies, mitochondrial myopathies and metabolic myopathies. Inherited myopathies are given in Table 28.28.
  - **Acquired myopathies:** Acquired myopathies can be subclassified as inflammatory myopathies, toxic myopathies, and myopathies associated with systemic disorders. Treatment options for myopathy depends on the type and severity. Acquired myopathies are given in Table 28.29.

Table 28.28 Inherited myopathies

Skeletal Muscle Dystrophy
Duchenne muscular dystrophy (severe dystrophinopathy)
Becker muscular dystrophy (less severe dystrophinopathy)
Myotonic dystrophy
Emery-Dreifuss muscular dystrophy
Facioscapulohumeral dystrophy
Oculopharyngeal muscular dystrophy
Limb girdle muscular dystrophy
Congenital Myopathy
Nemaline myopathy
Central core myopathy
Centronuclear myopathy
Myotubular myopathy
Congenital fiber-type disproportion myopathy
Hyaline body myopathy
Mitochondrial Myopathy
Myoclonic epilepsy and ragged red fibers (MERRF)
Mitochondrial myopathy, lactic acid and strokes (MELAS)
Mitochondrial neurogastrointestinal encephaolpathy (MNGIE)
Progressive external ophthalmoplegia (PEO)
Metabolic Myopathy
Acid maltase or acid alpha-1,4-glucosidase deficiency (Pompe disease)
Glycogen storage disorders 1–11
Carnitine palmitoyl transferase deficiency

Table 28.29 Acquired myopathies

Autoimmune Myopathy
Polymyositis
Dermatomyositis
Myositis associated with antisynthetase syndrome
Immune-mediated necrotizing autoimmune myopathy
Inclusion body myositis
Infectious Myopathy
Viral infections (Epstein-Barr virus, influenza virus, human immunodeficiency virus)
Bacterial infections (pyomyositis due to *Staphylococcus aureus* and Streptococci)
Spirochete (Lyme disease)
Parasitic infections (trichinosis, cysticercosis, toxoplasmosis)
Fungal infections (Candida, Coccidioidomycosis)
Toxic Myopathy
Medications (corticosteroids, checkpoint inhibitor immunosuppressive agents, cholesterol-lowering medicines, i.e. statins, amiodarone, colchicine chloroquine, tryptophan, omeprazole)
Toxic agents (alcohol consumption, toluene vapor in spray paint inhalation)
Myopathy Associated with Systemic Disorders
Endocrine disorders (dysfunction of thyroid, parathyroid, pituitary and adrenal glands)
Systemic autoimmune disorders (systemic lupus erythematosus, rheumatoid arthritis, scleroderma, Sjögren's syndrome, sarcoidosis and mixed connective tissue disease)
Amyloidosis [primary amyloidosis, familial amyloidosis (TTR mutation)]
Critical illness myopathy (nonpolarizing neuromuscular blocking agents, steroids)

- **Laboratory evaluation of myopathy:** Screening testing which indicates skeletal muscle damage, includes creatine phosphokinase (CPK), aldose, lactate dehydrogenase (LDH), and liver enzymes.
  - **Screening panel of laboratory tests:** Serologic testing, electromyography, skeletal muscle biopsy and genetic testing are helpful diagnostic tools in identifying the presence of myopathy and determining the etiology. A screening panel of laboratory tests should be performed to rule out more common causes of myopathy, which include: identification of etiology (complete blood count, metabolic panel, thyroid function tests, parathormone assay, C-reactive protein), identification of inflammation etiology (myositis-specific autoantibodies, anti-double-stranded DNA antibody, anti-Scl70 antibody, anti-SSA and SSB antibodies, rheumatoid factor, anti-PM1 antibody, angiotensin-converting enzyme levels), and identification of mitochondrial pathway, or metabolic pathway (serum lactate, pyruvate, ammonia, carnitine levels).
  - **Commercially available genetic tests:** Genetic tests are performed to detect gene mutation to diagnose myopathy, which include: (a) mutation in dystrophin gene in 'Duchenne muscular dystrophy' and Becker muscular dystrophy, (b) mutation in emerin gene in 'Emery-Dreifuss muscular dystrophy', (c) mutation in gene coding for lamin A/C, calpain, dysferlin and Fukutin-related protein in limb–girdle muscular dystrophy, (d) mutation in D4Z4 gene (deletion) in 'facio-scapulohumeral muscular dystrophy', (e) mutation in poly(A)-binding protein 2 gene coding for GCG repeat expansion in 'oculopharyngeal muscular dystrophy', (f) mutation in DMK gene for type 1 and CNBP (ZNF3) gene for type 2 are demonstrated in 'myotonic muscular dystrophy' type 1 and 2, (g) specific mutation in mitochondrial DNA (mitochondrial DNA**)** gene in 'mitochondrial myopathy', (h) mutation in transthyretin gene in 'familial amyloid myopathy', and (i) mutation in SLCO1B1 gene indicates increased susceptibility to 'statin myopathy'.
- **Management:** Management is largely supportive for an inherited myopathy. In acquired myopathies, treatment is targeted toward the underlying etiology. Treatment of statin myopathy is dependent on creatine phosphokinase levels and degree of skeletal muscle symptoms. Consider and lower doses when initiation of statin therapy.

INHERITED MYOPATHIES

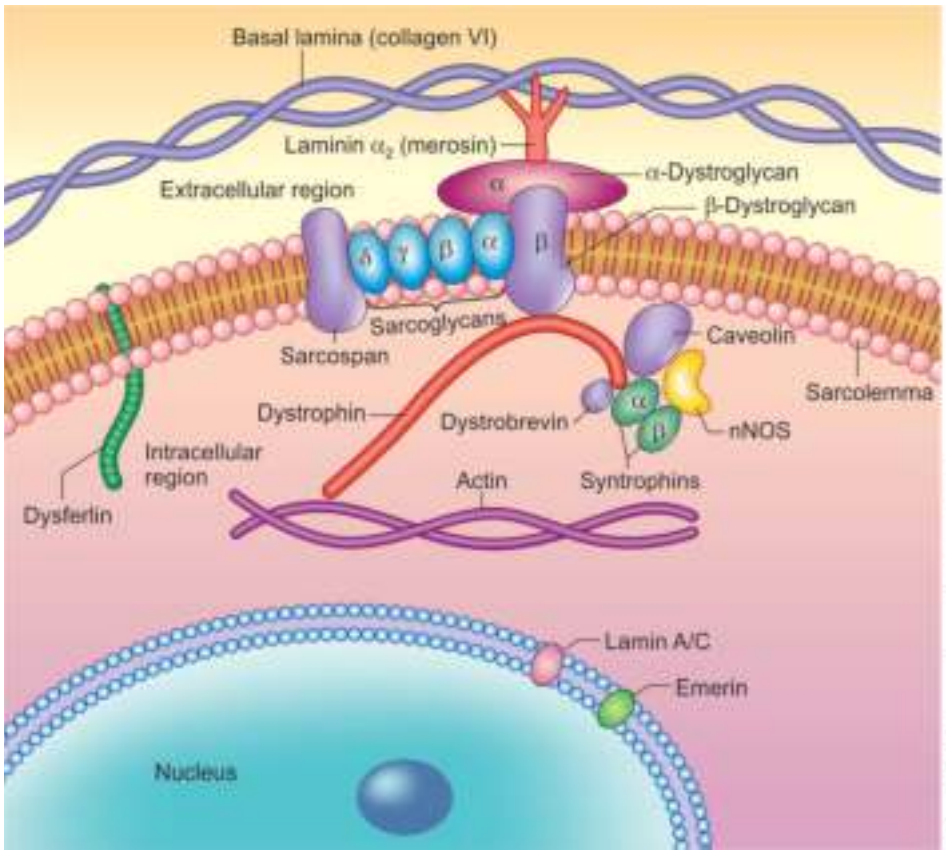
Genetic abnormalities cause inherited myopathies that cause progressive weakness and degeneration of skeletal muscles used during voluntary movement. Inherited myopathies are differentiated by age of onset, skeletal muscle groups involved, and mode of inheritance.

- Inherited myopathies primarily affect the skeletal muscles caused by mutations in different genes coding for proteins that normally maintains skeletal muscle structure and function. Skeletal muscle weakness and hypotonia are classic clinical presentations in most common inherited myopathies.
- Some inherited myopathies result from irregularities in the mitochondria of the cells, and these are called mitochondrial myopathies, which can cause weakness of skeletal muscles of face and neck muscles including slurred speech, difficulty in swallowing and drooping upper eyelids (ptosis).
- Metabolic myopathies affect the genes that normally code for the enzymes responsible for normal skeletal muscle function. Patient presents with exercise intolerance and exertional muscle pain. Skeletal muscle biopsy is helpful in distinguishing muscle dystrophies from abnormalities secondary to denervation.

Muscular Dystrophies

Muscular dystrophy is a group of diseases that cause progressive weakness of skeletal muscles and loss of muscle mass. In muscular dystrophy, abnormal genes (mutations) interfere with the production of proteins needed for normal structure and function of muscles. The main sign of muscular dystrophy is progressive muscle weakness. Specific signs and symptoms begin at different ages and in different skeletal muscle groups depending on the type of muscular dystrophy. Having a family history of myopathy increases the likelihood to inherit an abnormal gene (mutation) that causes inherited myopathy. Muscular dystrophies include dystrophinopathy (Duchenne muscular dystrophy, Becker muscular dystrophy), 'myotonic dystrophy', 'facioscapulohumeral dystrophy, 'oculopharyngeal muscular dystrophy' and 'limb–girdle muscular dystrophy'. Patterns of inheritance of myopathies can be X-linked, autosomal dominant and autosomal recessive. Dystrophin associated protein complex and other proteins involved in skeletal muscular dystrophies is shown in Fig. 28.31. Salient features of skeletal muscular dystrophies are given in Table 28.30.

- **X-linked (sex-linked) recessive inheritance:** X-linked (sex-linked) recessive inheritance of muscular dystrophies occurs when as mother carries the defective gene on one of her two X chromosomes and transmitting it to her sons, as males always inherit an X chromosome from mother and the Y chromosome from their father, while daughters inherit an X chromosome from each parent.



Protein alteration	Muscular dystrophies
Dystrophin	DMD (Duchenne muscular dystrophy)
Dystrophin	BMD (Becker muscular dystrophy)
Laminin α_2 (merosin)	CMD (congenital muscular dystrophy)
Sarcoglycan subtypes	LGMD (limb–girdle muscular dystrophy)
α	LGMD
β	LGMD
γ	LGMD
δ	LGMD
Caveolin	LGMD1C
Dysferlin	LGMD2B (Miyoshi myopathy)
Collagen IV	CMD
Lamin A/C	Emery-Dreifuss MD (AD) Emery-Dreifuss MD (AR)
Emerin	Emery-Dreifuss MD (X-linked)

- **DMD:** Duchenne muscular dystrophy
- **CMD:** Congenital muscular dystrophy
- **LGMD:** Limb–girdle muscular dystrophy

Fig. 28.31: Dystrophin associated protein complex and other proteins involved in skeletal muscular dystrophies.

Table 28.30 Salient features of skeletal muscular dystrophies

Myopathy	Pattern of Inheritance	Genetic Defects	Distribution of Muscular Weakness	Other Systemic Manifestations
Duchenne muscular dystrophy	X-linked recessive	Dystrophin gene (Xp21)	Symmetrical proximal shoulder and pelvic girdles weakness, calf pseudohypertrophy, ankle contractions	Low intelligence, cardiomyopathy, gastrointestinal tract, muscle involvement, kyphoscoliosis, cognitive impairment
Becker muscular dystrophy	X-linked recessive	Dystrophin gene (Xp21)	Slowly progressive weakness of girdle muscles (minor variant of Duchenne's type)	Cardiomyopathy
Emery-Dreifuss muscular dystrophy	X-linked recessive, autosomal dominant or autosomal recessive	Emerin gene	Slowly progressive contractures of the Achilles tendon, elbow, neck and spine	Cardiomyopathy
Limb–girdle muscular dystrophy	Autosomal dominant or autosomal recessive	Lamin A/C, calpin, dysferlin, Fukutin-related protein	Slowly progressive weakness of proximal pelvic girdle followed by shoulder girdle, calf hypertrophy, scapular winging	Cardiomyopathy, cardiac arrhythmias, respiratory muscle weakness
Facioscapulohumeral dystrophy	Autosomal dominant	D4Z4 gene	Slowly-progressive weakness of facial, scapular and upper arm muscles, scapular winging	Hearing loss, retinal telangiectasis, hypertension

Contd...

Table 28.30 Salient features of muscle dystrophies (*Contd...*)

Myopathy	Pattern of Inheritance	Genetic Defects	Distribution of Muscular Weakness	Other Systemic Manifestations
Oculopharyngeal muscular dystrophy	Autosomal dominant	Short GCG repeat expansion in the poly(A)-binding protein 2 gene (PABP2 gene)	Slowly progressive weakness of extraocular eyelids, face and pharyngeal muscles	Ophthalmoparesis, bulbar weakness, dysarthria and dysphagia
Myotonic dystrophy type 1 and type 2 (DM1, DM2)	Autosomal dominant	Myotonic dystrophy type 1 (DMPK gene), type 2 (CNBP, i.e. CNF9 gene)	▪ Slowly progressive myotonic stiffness or spasm after a forceful, muscle contraction ▪ Myotonic dystrophy type 1 (distal muscle weakness) ▪ Myotonic dystrophy type 2 (proximal muscle weakness)	Cataracts, frontal balding, cardiac conduction defects, cardiac arrhythmias, mental impairment, diabetes mellitus, pregnancy and delivery related complications, eyelid ptosis without extra-articular muscle weakness, gonadal atrophy
Mitochondrial myopathy	Maternally inherited (some autosomal dominant or autosomal recessive)	Specific point mutation analysis for diseases like MELAS, POLG1 sequencing for MERRF available and South blot analysis for mtDNA deletions and mtDNA sequencing	Skeletal muscle weakness, hypotonia, exercise intolerance, feeding problems ▪ Infant symptoms (encephalomyopathy, respiratory muscle weakness, hypotonia and feeding difficulty) ▪ Children symptoms (progressive external ophthalmoplegia, drooping of upper eyelids, mild visual impairment)	Extraocular muscle weakness (drooping of upper eyelids), peripheral neuropathy, ataxia, deafness, cardiac conduction system defects
Acid alpha glucosidase, i.e. acid maltase deficiency (glycogen storage disorder type 2, i.e. Pompe disease)	Autosomal recessive	Gene mutation causing acid alpha glucosidase (acid maltase deficiency)	Proximal girdle weakness	Macroglossia, hepatomegaly in infancy, severe ventricular muscle weakness with adult presentation, cardiomyopathy
Statin-associated myopathy	Unknown	SLCO1B1 gene	Mild-to-moderate muscular weakness, muscle pain	Potentially life-threatening rhabdomyolysis

MELAS: Mitochondrial myopathy, lactic acid and strokes
MERRF: Myoclonic epilepsy and ragged red fibers
MNGIE: Mitochondrial neurogastrointestinal encephalopathy
PEO: Progressive external ophthalmoplegia

- Sons of carrier mothers have a 50% chance of inheriting the X-linked recessive disorder. Daughters also have a 50% chance of inheriting the defective gene, but usually are not affected since the healthy X chromosome they receive from their father can offset (less noticeable) the defective gene received from their mother.
- Affected fathers cannot transmit an X-linked recessive disorder to their sons but their daughters will be carriers of the disorder. Carrier females occasionally can exhibit milder symptoms of skeletal muscular dystrophy.

▪ **Autosomal dominant inheritance:** Autosomal dominant inheritance of muscular dystrophies occurs when as child receives a normal gene from one parent and defective gene from the other parent. Autosomal dominant inheritance means only one parent needs to transmit an abnormal gene to the child in order to cause the disorder. In families, where one parent carries a defective gene, each child has a 50% chance of inheriting the gene and therefore suffers from this disorder. Both males and females are equally at risk and the severity of the disorder can differ from person to person.

▪ **Autosomal recessive inheritance:** Autosomal recessive inheritance of muscular dystrophies means that both parents must carry faulty gene and transmit to their children. The parents each have one defective gene but are not affected by genetic disorder. Children in these families have a 25% chance of inheriting both copies of defective gene at a 50% chance of inheriting one gene and therefore becoming a carrier, able to transmit along the defective gene to their children. Children of

either sex can be affected by autosomal recessive inheritance.

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is most common and severe form of X-linked recessive genetic disorder in boys characterized by progressive skeletal muscle degeneration and weakness. Although girls can be carriers and mildly affected.

- **Molecular genetic alterations:** Duchenne muscular dystrophy is caused by mutations of the dystrophin gene (DMD gene) mapped on chromosome Xp21 due to in-frame deletion of one or many exons leading to incomplete translation of dystrophin protein.
  - Loss of dystrophin protein leads to recurrent myofiber damage, progressive muscle fiber fibrosis and weakness, and dysfunction of muscle stem cells during muscle contractions.
  - Normal dystrophin protein links the subsarcolemmal cytoskeleton to the exterior of the cell of muscle fiber through a transmembrane complex of proteins that normally helps in keeping skeletal muscle cells intact against the mechanical and contractile stresses by inhibiting calcium (Ca^{++}) from entering the cell membrane of muscle fiber, affecting signaling of the cell, and water enters the mitochondria that causes muscular cell burst.
- **Clinical features:** Skeletal muscle weakness of arms, shoulder girdle, pelvic girdle and thighs may present initially with difficulty in independent ambulation but progressively advances to such an extent that affected patients are unable to carry out activities of daily living and must use wheelchairs. Patient has difficulty in rising from the floor, climbing stairs, maintaining balance and raising the arms. Orthopedic and cardiac complications are common and death occurs in the twenties due to respiratory insufficiency caused by muscular weakness or cardiac arrhythmia due to myocardial involvement. Duchenne skeletal muscle dystrophy involving proximal muscles of the extremities is shown in Fig. 28.32.
- **Laboratory diagnosis:** Laboratory diagnosis of Duchenne muscular dystrophy can usually be made after careful review of clinical history and examination of affected boys presenting with developmental delay, proximal skeletal muscle weakness, and marked elevated serum levels of creatine phosphokinase (CPK) plus confirmation by muscle biopsy and genetic testing (dystrophin gene mutation).
- **Management:** Current therapy is focused on treatment of Duchenne muscular dystrophy with glucocorticoids and physiotherapy to prevent onset of orthopedic complications. New emerging treatment includes viral-mediated microdystrophin gene replacement, exon skipping to restore frame, and nonsense expression therapy to allow translation and production of modified dystrophin protein.

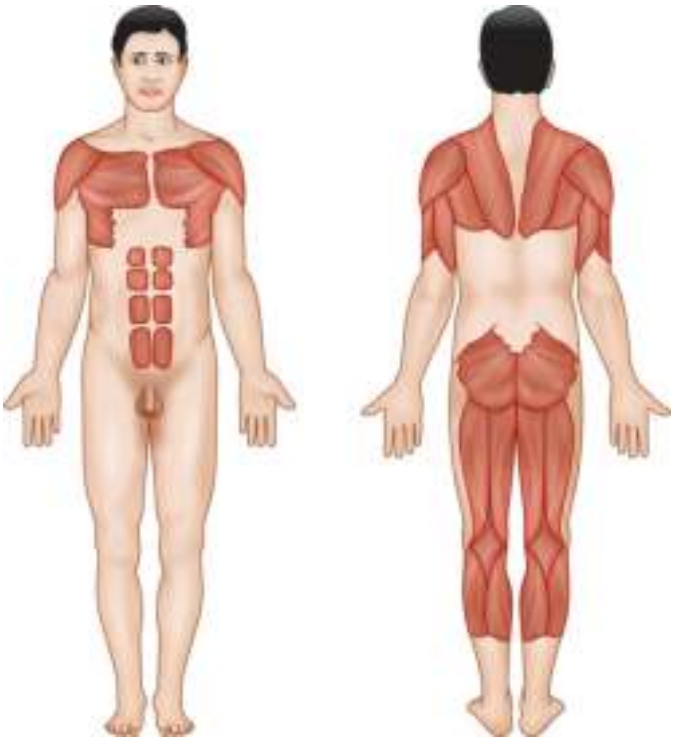


Fig. 28.32: Duchenne skeletal muscular dystrophy. It is X-linked recessive disorder involving proximal muscles of the extremities, progressing to muscle necrosis, wasting, muscle contracture with fatal outcome in their teens.

Becker Muscular Dystrophy

Becker muscular dystrophy (BMD) is an X-linked recessive inherited disorder characterized by slowly progressing skeletal muscle weakness of the legs and pelvis. BMD is generally milder and less severe muscle weakness than Duchenne skeletal muscular dystrophy. The affected maternal uncles with BMD continue to be ambulatory after age 15–20 years.

- **Molecular genetic alterations:** Becker muscular dystrophy is caused by mutation in the dystrophin gene located on the short arm of the X chromosome (Xp21) as a result of segmental deletion of dystrophin gene. It does not cause a coding frameshift, which encodes dystrophin protein.
- **Clinical features:** Signs and symptoms of Becker muscular dystrophy are clinically similar to those of Duchenne muscular dystrophy in boys, but tend to be milder, less severe, slower, less predictable and progress more slowly. Symptoms generally begin in the teens and might not occur until the mid-20s or later. Generalized weakness first affects skeletal muscles of hips, pelvis, thighs and shoulders. Calves are enlarged. There can be significant cardiac involvement. Patients can usually live at least for

30 years. The principal cause of death in patients with BMD is cardiac failure due to dilated cardiomyopathy.

- **Management:** Physical, occupational, speech and recreational therapy can all play in management of Becker muscular dystrophy.

Myotonic Muscular Dystrophy

Myotonic muscular dystrophy is an adult-onset, most common autosomal dominant disorder characterized by an inability to relax muscles after use following prolonged muscle contractions, progressive wasting of skeletal muscle, smooth muscles (gastrointestinal tract), cardiac electric conduction system defects, testes, atrophy and cataract.

- **Molecular genetic alterations:** Myotonic muscular dystrophy is caused by an increased number of trinucleotide repeats (50–1000) in the myotonin protein kinase gene (normal is <30 nucleotides).
- **Major types of myotonic muscular dystrophy:** There are two major types of myotonic muscular dystrophy, i.e. type 1 (mild and congenital variants) and type 2 (milder variant).
  - **Type 1 (mild and congenital) variant myotonic muscular dystrophy:** Type 1 mild variant of myotonic muscular dystrophy becomes apparent in mid-to-late adulthood with features of mild myotonia (lower legs, hands, neck and face) and cataract. Type 1 congenital variant of myotonic muscular dystrophy is apparent at birth with features of myotonia (lower legs, hands, neck and face), cataract clubfoot, breathing problems, delayed development and intellectual disability and other life-threatening health problems.
  - **Type 2 (milder) variant of myotonic muscular dystrophy:** It is caused by mutations in different genes, which involve skeletal muscles of the neck, shoulders, elbows and hips.
- **Clinical features:** Primary symptom of myotonic muscular dystrophy is myotonic stiffness or spasm following muscle contraction. Patient is unable to relax muscles once contracted.

Facioscapulohumeral Muscular Dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder characterized by slowly-progressive skeletal muscle weakness and wasting of the face, scapular region, and upper arm with normal life expectancy. The shoulder blade might stick out like wings when arms are raised. Signs and symptoms usually appear in adolescence.

- **Molecular genetic alterations:** Facioscapulohumeral muscular dystrophy may be inherited through either father or mother or it may occur without a family history. FSHD is caused by insufficient repeat-mediated epigenetic repression of the D_4Z_4 gene microsatellite repeat array on chromosome 4q35 resulting in variegated expression of the D_4Z_4 retrogene, encoding double homeobox protein 4 (transcription factor) in skeletal muscle.
- **Clinical features:** However, onset and severity of FSHD disorder varies widely. Milder cases may not become noticeable until later in life. Severe cases present with muscular weakness with a descending progression starting in the face and scapular muscles then progressing to humeral muscles, foot dorsiflexor muscles and finally, proximal lower extremity of skeletal muscles during infancy or early childhood. Facioscapulohumerous skeletal muscular dystrophy is shown in Fig. 28.33.

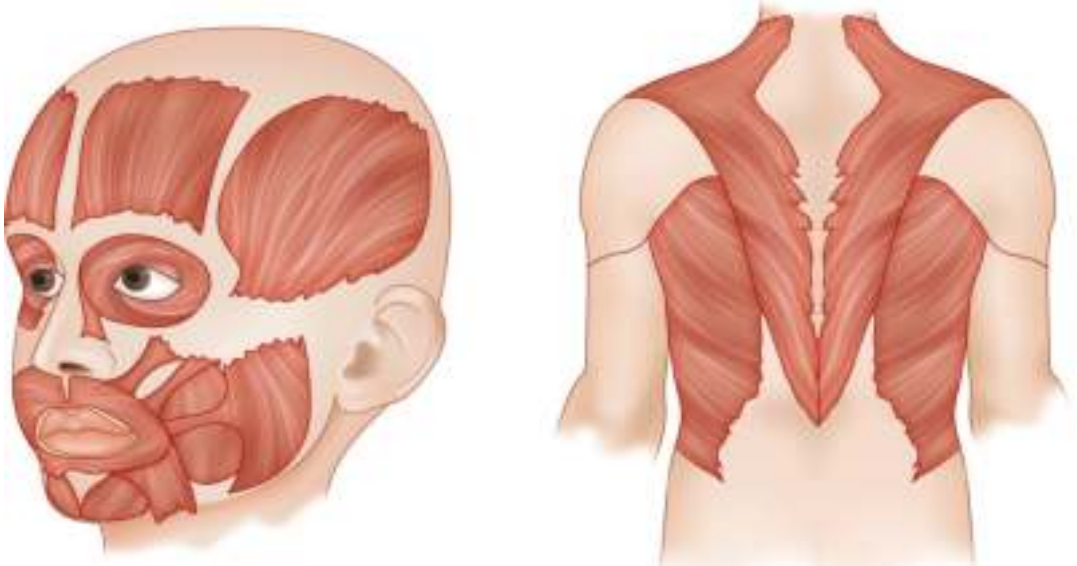


Fig. 28.33: Facioscapulohumerous skeletal muscular dystrophy. It is an autosomal dominant disorder involving skeletal muscles of the face, scapular region and upper arm.

Limb–Girdle Muscular Dystrophy

Limb–girdle muscular dystrophy is a genetic disorder (autosomal dominant or autosomal recessive) characterized by progressive weakness of skeletal muscles of upper arms, shoulder girdle, pelvic girdle and thighs during childhood or adolescence.

- **Molecular genetic alterations:** Limb–girdle muscular dystrophy is caused by different genes coding for aberrant proteins needed for maintenance and repair of skeletal muscles. Type 1 limb–girdle muscular dystrophy is an autosomal dominant disorder. Type 2A and 2B limb–girdle muscular dystrophy is an autosomal recessive disorder (CAPN3 gene mutation in type 2A and DYFS gene mutation in type 2B). Sarcoglycanopathies are forms of limb–girdle muscular dystrophy caused by mutations in the SGCA, SGCB, SGCG and SGCD genes.
- **Clinical features:** Patients might have difficulty in lifting the front part of the foot and so might trip frequently and may eventually require wheelchair assistance. Patients may develop cardiomyopathy. However, clinical manifestations remain mild in some cases. Limb–girdle muscular dystrophy involves skeletal muscles of the upper arms, shoulder, pelvic girdle and thighs is shown in Fig. 28.34.
- **Laboratory diagnosis:** Laboratory diagnosis of limb–girdle muscular dystrophy is based on clinical history, family history, physical examination, and supporting laboratory studies.



Fig. 28.34: Limb–girdle muscular dystrophy. It is genetic disorder involving skeletal muscles of the upper arms, shoulder, pelvic girdle and thighs.

Emery-Dreifuss Muscular Dystrophy

Emery-Dreifuss muscular dystrophy (EDMD) is a genetic disorder with inheritance patterns (X-linked, or autosomal dominant or autosomal recessive), that primarily affects skeletal muscles of the elbows, ankle joints and cardiac muscle. Among the earliest features of the disorder are joint deformities called contractures of elbows, ankle joints (Achilles tendon), neck and spine during childhood.

- **Molecular genetic alterations:** Emery-Dreifuss muscular dystrophy is caused by absence of some protein functions in the cell's nucleus (emerin, lamin A, lamin C). Small protein called emerin is normally present in the cell membrane that surrounds each cell's nucleus. Untreated patients have fatal outcome as a result of defects in cardiac electric conduction.
- **Clinical features:** Patient presents with joint contractures that begin in early childhood, slowly progressive muscle weakness and wasting initially in humeroperoneal muscles that later extends to the scapular muscles and pelvic girdle muscles. There may be cardiac involvement in these cases.
- **Diagnosis:** Genetic testing is commercially available for the genes associated with Emery-Dreifuss muscular dystrophy: EMD, LMNA, HSH1, SYN1, SYNE2, TMEM43, SUN1, SUN2 and TTN.

Oculopharyngeal Muscular Dystrophy

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant disorder characterized by weakness of skeletal muscles of upper eyelids (drooping of upper eyelids) and pharynx (dysphagia) in both men and women. OPMD is more common in people of French-Canadian descent, Hispanic people in Northern New Mexico and Ashkenazi Jewish. Patient presents with problems related to vision swallowing and talking.

- **Molecular genetic alterations:** Oculopharyngeal muscular dystrophy is caused by mutation in PABPN1 gene that codes for protein called polyadenylate-binding protein nuclear 1 (PABPN1). Either one copy or two copies (one from each parent) of defective gene is transmitted from parents to their children. Mutation in gene leads to buildup of aberrant PABPN1 protein in skeletal muscle cells. The PABPN1 clumps inside the skeletal muscle cells and may cause cells to die leading to skeletal muscle weakness.

- **Clinical features:** Patient presents with weakness of skeletal muscles of upper eyelids (drooping of upper eyelids) and pharynx (dysphagia) in both men and women.
- **Laboratory diagnosis:** Oculopharyngeal muscular dystrophy is diagnosed by family history, DNA analysis, swallowing tests, nerve conduction studies and electromyography and skeletal muscle biopsy for OPMD-PABPN1 clumps.

Congenital Myopathy

Congenital myopathy is term applied to distinct primary neuromuscular disorders that may be present at birth in both boys and girls. Symptoms may not appear until adolescence. Three common types of congenital myopathy include central core myopathy, nemaline myopathy and centronuclear myopathy. Other types include microtubular myopathy, multi-mini core myopathy, congenital muscle-fiber type disproportion myopathy and hyaline body myopathy. Different types of congenital myopathy are distinguished primarily by their clinical manifestations, skeletal muscle biopsy, MRI of skeletal muscles and DNA testing. Treatment of congenital myopathy is supportive and includes physiotherapy.

Nemaline Myopathy

Nemaline myopathy is a genetic disorder (autosomal dominant or autosomal recessive) caused by mutations in about 10 genes that encode thin filament proteins. Severe disease results in weakness of respiratory muscles and respiratory failure. Moderate disease manifests with progressive weakness of skeletal muscles of the face, trunk, and feet, but life expectancy may be normal. Mild disease is nonprogressive and life expectancy is normal. Skeletal muscle biopsy demonstrates tangles of small rod-shaped granules, predominantly in type 1 fibers.

Central Core Myopathy

Central core myopathy is a genetic disorder (autosomal recessive) caused by mutation in PYR_1 gene. Sporadic cases are also observed.

- Neonates develop hypotonia and mild proximal skeletal muscle weakness, but sometimes clinical manifestations do not manifest until adulthood. The affected infants eventually become ambulatory. Many patients also have nonprogressive skeletal muscle weakness of face.
- Life expectancy is normal, but some patients are severely affected and require a wheelchair. The gene mutation associated with central core myopathy is also associated with increased susceptibility to malignant hyperthermia.
- Skeletal muscle biopsy specimen lacks oxidative enzyme staining in the region (core). The regions may be peripheral, or central, focal, multiple, or extensive. A loss of mitochondria and other organelles occur in the central portion of type 1 muscle fibers.

Centronuclear Myopathy

Centronuclear myopathy is a genetic disorder (autosomal dominant or autosomal recessive or X-linked recessive inheritance) caused by mutations in genes that code for membrane-trafficking proteins. Skeletal muscle biopsy demonstrates an abundance of central nuclei.

- Autosomal dominant form of centronuclear myopathy typically manifests in adolescence or adulthood with exercise-induced myalgia, bifacial weakness, ptosis (drooping of upper eyelids) and external ophthalmoplegia.
- X-linked recessive inheritance form of centronuclear myopathy is most common and severe affecting children, which do not survive beyond the first year of life.

Myotubular Myopathy

Myotubular myopathy is a rare form of congenital myopathy affecting boys. Mother notices reduced movements of the weak and floppy baby in her womb during pregnancy.

- Floppy infant syndrome is marked by hypotonia and skeletal muscle weakness at birth or during the neonatal period and in some cases, delayed motor development later in childhood.
- Newborns usually have significant difficulties in respiration and swallowing. Many children do not survive. This order is also associated with osteopenia.

Congenital Fiber-type Disproportion Myopathy

Congenital fiber-type disproportion myopathy is a rare form of congenital myopathy that begins with floppiness, limb and facial weakness and respiratory problems.

Hyaline Body Myopathy

Hyaline body myopathy is a rare form of congenital myopathy characterized by the specific appearance on the microscopic examination of skeletal muscle tissue. Hyaline body myopathy has several different causes. Because of this, the clinical manifestations are quite variable.

Mitochondrial Myopathy

Mitochondrial myopathy is mediated by maternally transmitted mitochondrial DNA (mtDNA) abnormalities (most often deletions in mitochondrial DNA) affecting predominantly the skeletal muscle.

- Mitochondria regulate the production of cellular energy and carry the genetic blueprints for this process within their own unique DNA (mtDNA).
- Mitochondrial myopathy often affects more than one organ system of the body such as high energy tissues like skeletal muscle, cardiac muscle and brain.
- Kearns-Sayre syndrome is characterized by ophthalmoplegia, pigmentary retinopathy, heart conduction system block, cerebellar ataxia, and an exclusively maternal mode of transmission.
- Skeletal muscle biopsy demonstrates ragged red appearance due to various mitochondrial enzyme or coenzyme defects. In most mitochondrial disorders, abnormally high numbers of defective mitochondria are present in the cells of the body.

Metabolic Myopathies

Metabolic myopathies are disorders of muscle energy metabolism arising from specific enzymatic defects due to defective genes that result in skeletal muscle dysfunction.

- Enzymatic defects adversely affect glycogen breakdown, glucose utilization (glycolysis), fatty acid transport and oxidation to maintain adequate energy and adenosine triphosphate (ATP) production along the mitochondrial pathway.
- Cardiac and systemic metabolic dysfunction may coexist. Symptoms are often intermittent and provoked by exercise or changes in the supply of carbohydrate and lipid fuels.
- Evaluation of metabolic myopathies often requires provocative testing, which may include ischemic forearm exercise aerobic cycle exercise (failure to elevate lactate in blood confirming an abnormality of glycolytic metabolism), and 31P magnetic resonance spectroscopy with exercise.

Carnitine Palmitoyltransferase Deficiency Associated Myopathy

Patients with carnitine palmitoyltransferase deficiency cannot metabolize long-chain fatty acids because of an inability to transport these lipids into the mitochondria, where they undergo β-oxidation.

- **Clinical features:** Patient presents with skeletal muscular pain after prolonged exercise, which may progress to myoglobinuria. After such an episode, fibers regenerate and restore skeletal muscle structure.
- **Laboratory diagnosis:** Laboratory diagnosis depends on the biochemical assay for carnitine palmitoyltransferase activity. Skeletal muscle biopsy shows no microscopic abnormalities.

Pompe Disease Associated Myopathy

Pompe disease is an autosomal recessive glycogen storage disorder characterized by an inability to degrade glycogen due to acid maltase (acid alpha-1,4-glucosidase) deficiency. It is the most severe form of disease, which adversely affects skeletal muscle, cardiac muscle, central nervous system, and liver in the neonates or infants. Patient presents with severe hypotonia and absence of reflex (areflexia). Sometimes, the patient has an enlarged tongue and cardiomegaly and die of cardiac failure. Child usually dies within the first 2 years of life.

ACQUIRED MYOPATHIES

Acquired myopathies can be subclassified as inflammatory myopathies, toxic myopathies, and myopathies associated with systemic disorders such as autoimmune etiology (systemic lupus erythematosus, rheumatoid arthritis) or inflammation, infectious, endocrine hormonal imbalance etiology (hypothyroidism, hyperthyroidism, hyperparathyroidism, Cushing syndrome) and toxic agents. An electrolyte imbalance (hypokalemia, hyperkalemia) can also interfere with a muscle's ability to contract and relax as it should.

Inflammatory Myopathies

Inflammatory myopathies represent a heterogeneous group of acquired disorders, which are immune-mediated process triggered by environmental factors (tobacco smoke, infections, ultraviolet radiation) in a genetically susceptible person. Infectious myopathies occur as a result of infections that affect skeletal muscle function due to viruses (Epstein-Barr virus, human immunodeficiency virus, and influenza virus), bacteria, parasites (trichinosis, toxoplasmosis, cysticercosis) and fungi (Candida and Coccidioidomycosis) and Lyme disease.

- **Pathology:** Inflammatory myopathies are characterized by symmetric proximal skeletal muscle weakness, increased serum levels of muscle-derived enzymes, and nonsuppurative inflammation, necrosis and phagocytosis of skeletal muscle fibers, and a mixture of regenerating and atrophic fibers, and fibrosis.
- **Clinical features:** Patient presents with subacute to chronic onset of proximal muscle weakness manifested by difficulty with rising from a chair, climbing stairs, lifting objects and combing hair.
- **Laboratory diagnosis:** Laboratory diagnosis of inflammatory myopathies is based on clinical history, physical examination, electrodiagnosis and blood samples for skeletal muscle enzymes analysis. Muscle biopsy remains the gold standard for diagnosis. Skin biopsy can show changes in the skin associated with dermatomyositis. Goals of treatment are to eliminate inflammation, restore muscle performance, reduce morbidity and improve quality of life. Most

commonly used medicines are corticosteroids, immunosuppressants and immunoglobulin therapy.

Endocrine Myopathies

Endocrine myopathies occur when hormones interfere with skeletal muscle function in an overt endocrinopathy, i.e. disorders of thyroid gland (hyperthyroidism or hypothyroidism), parathyroid glands (hyperparathyroidism), and adrenal glands (Cushing syndrome and Addison disease). All the patients with neuromuscular complaints should be investigated for endocrine causes. In endocrine myopathy type 2, fast twitch skeletal muscle fibers are more severely affected than type 1 muscle fibers. Myopathies caused by endocrine disorders are mainly due to alterations in the protein and carbohydrate metabolism. Either deficiency or excess of hormones produced by the endocrine glands can cause muscle dysfunction that can be reversed by starting hormone replacement therapy.

Thyroid Gland Dysfunction Associated Myopathy

Thyrotoxicosis produces myopathy caused by protein catabolism, accelerated basal metabolic rate and impaired carbohydrate metabolism.

- In thyrotoxicosis, shortening of skeletal muscle contraction time may result from accelerated myosin ATPase activity and increased calcium uptake by the sarcoplasmic reticulum. Depolarization of the muscle fiber and impaired Na^+-K^+ activity in muscle may predispose to thyrotoxic paralysis. Neuromuscular presynaptic impairment may account for worsening of myasthenia gravis by thyrotoxicosis.
- In hypothyroidism, impaired energy metabolism, deduction in myosin ATPase activity and impaired Na^+-K^+ activity, impaired calcium uptake by sarcoplasmic reticulum in skeletal muscle may limit the force generation leading to slow contraction and relaxation of muscles. Treatment of thyroid disorders associated myopathy is restoration of euthyroid state.

Parathyroid Glands Dysfunction Associated Myopathy

Primary hyperparathyroidism and secondary hyperparathyroidism are always associated with proximal muscle weakness and fatigability. Myopathy is improved with restoration of normal parathormone levels and vitamin D replacement.

Adrenal Gland Dysfunction Associated Myopathy

Corticosteroid therapy and excess of ACTH (Cushing syndrome) cause myopathy having a pattern of proximal muscle weakness affecting the legs more than the arms. Steroid myopathy is not apparent until other signs of excess corticosteroid are present. Adrenal gland insufficiency (Addison disease) produces generalized skeletal muscle weakness, muscle cramps and fatigue in about 50% of cases.

Toxic Myopathies

Toxic myopathy is characterized by skeletal muscle weakness, myalgia, blood creatine kinase elevation or myoglobinuria that can occur in patients without muscle disease when they are exposed to certain drugs (corticosteroids, check point inhibitor immunosuppressive agents, cholesterol-lowering medicines, i.e. statins, amiodarone, colchicine chloroquine, tryptophan, omeprazole) and toxins (alcohol consumption, toluene vapor in spray paint inhalation). Early recognition of toxic myopathies is important as they potentially are reversible on removal of the offending drug or toxin, with greater likelihood of complete resolution the sooner this is achieved.

Autoimmune Myopathies

Autoimmune myopathies consist of dermatomyositis (most common in children), polymyositis (more common in females), myositis associated with antisynthetase syndrome, immune-mediated necrotizing autoimmune myopathy and inclusion body myositis (males) in the settings of systemic lupus erythematosus, rheumatoid arthritis, and sarcoidosis.

- In patients with suspected autoimmune myopathy, muscle imaging, electrophysiologic examination and serologic studies can be performed to confirm diagnosis. When present, dermatomyositis-specific autoantibodies may help to identify patients with different disease subtypes.
- Histologic features of dermatomyositis are perifascicular atrophy and perivascular inflammation. Histologic features of immune-mediated necrotizing myopathy are prominent myofiber necrosis. Histologic features of polymyositis are non-necrotic muscle fibers surrounded by lymphocytes.

MISCELLANEOUS NON-NEOPLASTIC DISORDERS OF SKELETAL MUSCLE

Skeletal Muscle Atrophy

Skeletal muscle atrophy is defined as a decrease in muscle mass and occurs when protein degradation exceeds protein synthesis leading to adverse functional consequences. Skeletal muscle atrophy occurs due to disuse of skeletal muscles (e.g. denervation, prolonged immobilization), starvation, aging and cachexia.

- Denervation skeletal muscle atrophy involves both type 1 and type 2 fibers. Disuse skeletal muscle atrophy fibers in prolonged immobilization involves type 2. Decrease in protein synthesis and increase in

protein degradation have been observed to contribute to skeletal muscle protein loss due to disuse.

- Recent studies have delineated elements of both synthetic and proteolytic processes. It has become evident that interactions among known proteolytic pathways (ubiquitin-proteasome, lysosomal, and calpain) are involved in skeletal muscle proteolysis during skeletal muscle atrophy.
- Tumor necrosis factor-α, glucocorticoids, myostatin and reactive oxygen species can induce skeletal muscle protein loss under specified conditions. It has become apparent that transcription factor NF-κB is a key intracellular signal inducer in disuse skeletal muscle atrophy.

Myasthenia Gravis

Myasthenia gravis is an autoimmune disorder more frequent in women than in men. Disorder is characterized by breakdown of normal communication between nerves and muscles arising from production of autoantibodies to postsynaptic acetylcholine receptors of the neuromuscular junction (motor end-plate) involving extraocular and facial muscles, muscles of the extremities, and other muscle groups.

- **Pathogenesis:** Thymoma or thymic hyperplasia plays an important role in the pathogenesis of myasthenia gravis. Surgical removal of thymoma tumor is often curative in 40% of patients.
- **Clinical features:** Patient presents with drooping of one or both upper eyelids (ptosis), double vision (diplopia), difficulty in chewing, swallowing or speaking, and weakness of neck, arm and legs. Myasthenia gravis can be complicated by respiratory failure due to diaphragmatic weakness. Patient improves dramatically with administration of drugs with anticholinesterase activity.

Lambert-Eaton Myasthenic Syndrome

Lambert-Eaton myasthenic syndrome is a paraneoplastic disorder is characterized by muscular weakness of pelvic girdles, muscular wasting, and fatigability of the proximal limbs and trunk. Although it is most commonly associated with small cell lung carcinoma, yet it may also occur in patients with other malignant diseases. The pathogenic IgG autoantibodies recognize voltage sensitive calcium channels that are expressed both in motor nerve terminals and in the cells of the lung cancer. The calcium (Ca^{++}) channels, which are necessary for release of acetylcholine, are greatly reduced in the presynaptic membrane in these patients, thereby interfering with neuromuscular transmission. Patients respond to treatment with corticosteroids.

Myositis Ossificans

Myositis ossificans most often affects young persons. It is a benign condition but mimics a malignant neoplasm. The lesion typically results from blunt trauma to the muscle and soft tissues, usually of the lower limb. Over a short time, peripheral revascularization occurs in the hematoma results in formation of bone spicules in the soft tissue. It often occurs near a bone on radiography and may be misdiagnosed as a malignant bone-forming tumor.

Rhabdomyolysis

Rhabdomyolysis refers to the dissolution of skeletal muscle fibers and the release of myoglobin into the circulation, an event that may result in myoglobinuria and acute renal failure.

- Rhabdomyolysis may occur due to heat stroke or malignant hyperthermia after administration of an anesthetic such as halothane.
- Skeletal muscle biopsy shows active, non-inflammatory myopathy, with scattered necrosis of muscle fibers and varying degrees of degeneration and regeneration.
- Rhabdomyolysis may be acute, subacute, or chronic. During acute phase, the muscles are swollen, tender, and profoundly weak.

Skeletal Muscle Hypertrophy

Skeletal muscle hypertrophy of type 2 fibers occurs due to androgenic steroids. Stimulation of skeletal muscle type 2 fibers elicits a faster, shorter, and more powerful contraction than occurs in type 1 fibers.

- Skeletal muscle type 2 fibers rapidly contract for brief duration and react to strength training with hypertrophy.
- Skeletal muscle hypertrophy does not respond to an increased workload by increasing the number of fibers (hyperplasia).

Soft Tissue Tumor Specimen Handling in Surgical Pathology

- Soft tissues of the body connect, support, and other body parts and organs, which include: bone, cartilage, skeletal muscles, tendons, adipose tissue, blood vessels, lymphatic channels, nerves, and synovial tissue.
- According to WHO classification, soft tissue tumors are classified into three categories: benign tumors, intermediate tumors (locally aggressive and rarely metastasizing potential), and malignant tumors.
- Benign soft tissue tumors are common than malignant tumors. Soft tissue sarcomas can form anywhere in the body in the head, neck, arms, legs, trunk, abdomen, and retroperitoneum.

- Soft tissue tumors are diagnostically challenging group of neoplasms with a diverse spectrum and overlapping clinical features, radiologic and histologic features that can have varying lines of differentiation. Accurate diagnosis is essential for appropriate treatment and prognostication.
- Benign soft tissue tumors are common and treated with surgery alone. Surgery (wide surgical excision) is a common treatment for soft tissue sarcoma. External radiation therapy and internal radiation therapy, targeted therapy with receptor tyrosine kinase inhibitor (RTKI), and chemotherapy may be used to treat soft tissue sarcoma. Advances in molecular oncology have improved diagnostic, prognostic, and treatment protocols for patients with soft tissue high-grade sarcomas.
- Risk factors for soft tissue sarcoma include various inherited disorders: Li-Fraumeni syndrome (TP53 mutation), von Recklinghausen disease (neurofibromatosis type 1 (NF1) mutation), Gardner's syndrome (APC, nevoid basal cell carcinoma syndrome (Gorlin syndrome, PTCH1 mutation), and Werner syndrome (adult progeria, WRN mutation). Acquired risk factors for soft tissue sarcomas include: prior treatment with radiotherapy, persistent lymphedema in the arms or legs, exposure to thorium dioxide, vinyl chloride or arsenic, and being infected with HIV and human herpesvirus 8 (HHV8).
- Tests and procedures used to diagnose soft tissue sarcomas include imaging diagnostic tools (X-rays, CT scan, MRI scan and positron emission tomography), might help show the size and location of the tumor, biopsy procedures (core needle biopsy and surgical biopsy such as marginal resection, wide resection, and radical resection), molecular genetic tests, gross morphology of surgical specimen, histologic examination, and immunohistochemistry technique.
- Upon receipt of the surgical specimen in the pathology laboratory, it is essential to know clinical history, radiologic findings, staging, and prior chemotherapy or radiotherapy. Pathologist must evaluate gross morphologic features of surgical specimen such as unifocal/multifocal tumor, tumor site, tumor size in greatest dimension, cut surface appearance of tumor. Following the neutral buffered formalin fixation, representative thin slice of surgical specimen is processed and ultimately immersed in paraffin wax, termed a 'paraffin block'. Tissue sections are stained with hematoxylin and eosin stain and examined under the light microscope.
- Pathologist must follow reporting protocol such as histologic examination, tumor grading, mitotic rate, macroscopic or macroscopic necrosis, lymphovascular invasion, tumor margin status, regional lymph node status and distant metastasis status. Special studies are performed such as immunohistochemistry, cytogenetics, and molecular genetics.

CHAPTER

29

Skin

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

SKIN HISTOLOGY AND SKIN DISORDERS

- Skin histology
  - Epidermis
  - Dermis and subcutaneous tissue
  - Nails structure
- Skin disorders

INFECTIOUS DERMATITIS

- Bacterial skin infections
  - Eczematous dermatitis
  - Impetigo
  - Neurodermatitis
  - Scabies
  - Leprosy (Hansen's disease)
- Viral skin infections
  - Molluscum contagiosum
  - Common warts (verrucous vulgaris)
  - Dermatitis herpetiformis (herpes simplex virus II skin infection)
  - Varicella zoster virus skin infection (chickenpox)
- Fungal skin infections
  - Dermatophytosis (superficial fungal skin infections)
  - *Candida albicans* skin infection
  - Deep subcutaneous fungal infections (mycetoma, mucormycosis)

INFLAMMATORY DERMATITIS

- Inflammatory dermatitis: overview
- Inflammatory dermatitis: clinical entities
  - Psoriasis
  - Erythema multiforme
  - Lichen planus
- Pemphigus vulgaris
- Bullous pemphigoid

NON-NEOPLASTIC MELANOCYTIC DISORDERS AND MELANOCYTIC NEOPLASMS

- Non-neoplastic melanocytic disorders
  - Albinism
  - Vitiligo
  - Freckle (ephelis)
  - Lentigo simplex
- Melanocytic neoplasms
  - Congenital melanocytic nevi
  - Melanocytic nevus (common mole)
    - Dysplastic melanocytic nevus
    - Lentigo maligna
    - Blue nevus
    - Spitz nevus
  - Melanoma

NONMELANOCYTIC CUTANEOUS NEOPLASMS

- Carcinogenesis of skin cancers
  - Genomic instability
  - Chemical carcinogenesis
  - Ultraviolet radiation in carcinogenesis
- Epidermis tumors
  - Epithelial precancerous lesions
    - Actinic keratosis
    - Arsenical keratosis
    - Viral-associated epithelial precancerous lesions (bowenoid papulosis, epidermodysplasia verruciformis)
  - Bowen disease (squamous cell carcinoma *in situ*)
  - Squamous cell carcinoma of skin
  - Keratoacanthoma of skin
  - Basal cell carcinoma of skin
  - Basal cell nevus syndrome
  - Seborrheic keratosis
- Neuroendocrine tumors
  - Merkel cell carcinoma
- Skin appendageal tumors and hamartomatous of the skin
  - Nodular hidradenoma
  - Eccrine spiradenoma
  - Chondroid syringoma
  - Pilomatrixoma
- Dermis and subcutaneous fat: tumors
  - Dermatofibroma
  - Dermatofibrosarcoma protuberans
- Vascular tumors of skin
  - Capillary hemangioma
  - Kaposi sarcoma
  - Cutaneous angiosarcoma
- Neural tumors of skin
  - Plexiform neurofibroma
  - Traumatic neuroma
- Cutaneous metastases

MISCELLANEOUS SKIN DISORDERS

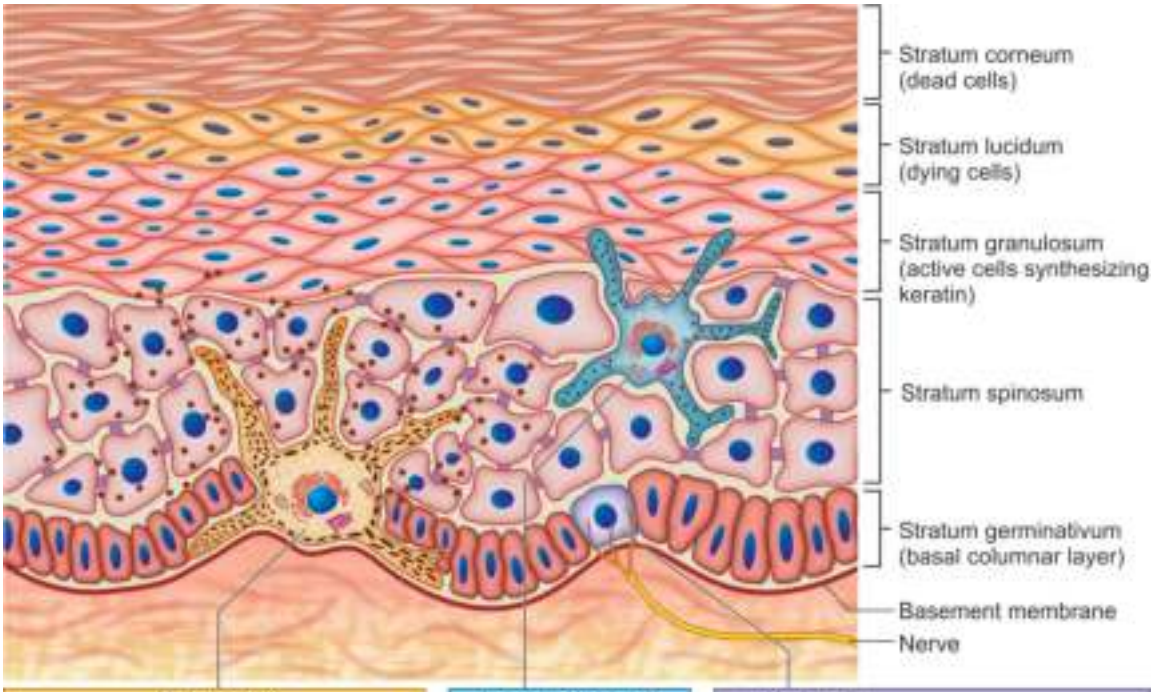
- Acne vulgaris
- Fibroepithelial polyp
- Epidermal keratinous cyst
- Acanthosis nigricans
- Xanthomas
- Granuloma pyogenicum
- Keloid

SKIN HISTOLOGY AND SKIN DISORDERS

SKIN HISTOLOGY

Skin consists of three layers: epidermis (outermost layer), dermis, and hypodermis (Fig. 29.1). Skin performs many functions: sensation, thermoregulation, protection, and metabolism (Table 29.1). Cells in the epidermis and dermis are given in Table 29.2.

- Epidermis is lined by stratified squamous epithelium that contains 4–5 layers depending on location: stratum basalis (basal layer close to dermis), stratum



Cells and structures in the epidermis and dermis

Skin layer	*Cells*	*Other structures*
Epidermis	• Keratinocytes • Melanocytes • Langerhans' cells • Merkel cell	Hair follicles, apocrine glands and sweat glands are derived from epidermis and extended to dermis
Dermis	• Fibroblasts • Mast cells • Histiocytes • Macrophages • Lymphocytes	Matrix of polysaccharides and proteins

Fig. 29.1: Schematic representation of structure of epidermis and dermis. Epidermis shows stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. The basal cells in the stratum germinativum undergo mitosis producing keratinocytes that change their size as they move upward, replacing cells that are lost during normal cell shedding. Schematic representation demonstrates melanocytes, Langerhans' cells and Markel cells in epidermis.

spinosum (prickle cell layer), stratum lucidum (present only in soles and palms), and stratum corneum (keratinized layer). Keratinocytes make up about 85% of cells in the epidermis.

- Dermis is a thick layer of connective tissue consisting of collagen, elastin, nerve endings, blood vessels, and adnexal structures such as hair shafts, sweat glands, and sebaceous glands. Hypodermis consists of adipose tissue.

EPIDERMIS

The epidermis rests on a basement membrane, a thin, acellular film of protein, which separates the epidermis from the dermis.

- Epidermis is composed of stratum germinativum (basal cells), stratum spinosum (prickle layer), stratum granulosum and stratum corneum. Stratum lucidum is present between stratum corneum and stratum granulosum.

Table 29.1 Functions of the skin

There are four main functions of the skin: sensation, thermoregulation, protection, and metabolism:

- **Sensation:** The skin contains many types of different receptors that sense temperature, pain, pressure, and touch.
- **Thermoregulation:** Hair and sweat glands participate in the regulation of body temperature to maintain homeostasis.
- **Protection:** The skin serves as the barrier between the inside and outside of the body against infection, chemical stress, thermal stress, and UV light.
- **Metabolism:** Adipose tissue in the hypodermis is vital in the production of vitamin D and lipid storage.

Table 29.2 Cells in the epidermis and dermis

Cells in the Epidermis
Melanocytes: The epidermis also contains melanocytes, which are derived from the embryonic nervous system (neural crest), present among the basal cells on the basement membrane. These synthesize melanin. A single melanocyte associates with a number of keratinocytes forming an epidermal melanin unit. Schematic representation of signaling pathways in melanogenesis is shown in Fig. 29.2
Langerhans' cells (dendritic cells): These specialized immune system cells are voracious phagocytes, which trap microbes and other alien materials that manage to cross the basement membrane. Langerhans' cells then present captured antigen to immune system lymphocytes. Schematic representation of Langerhans' cell functions is shown in Fig. 29.3
Merkel cells: Merkel cells are found in normal epidermis and act as transducers for fine touch
Cells in the Dermis
Fibroblasts: The main cell in the dermis is the fibroblast, that produces the collagen and elastin fibers and account for skin toughness and resiliency
Dermal dendritic cells: These are similar to epidermal Langerhans' cells

- As the epidermal cells mature, these cells undergo flattening with accumulation of keratin to form stratum corneum, which acts as barrier to infectious agents. Epidermis is very thin in most of regions, which may be 10–20 cells thick in some. It is considerably thicker on the palms and soles because of the extra thickness of the surface layer of dead cells of the stratum corneum.

Stratum Germinativum (Basal Cell Layer)

Stratum germinativum layer, the deepest mitotically active layer, that rests on a basement membrane, which attaches it to the underlying dermis. It contains melanin producing melanocytes, a single row of keratinocytes, and epidermal stem cells with progressive keratinization.

- Stratum germinativum provides the germinal cells necessary for the regeneration of the layers of epidermis.
- Keratinocytes from basal cell layer evolve and mature as they travel outward/upward to create the remaining layers of epidermis. Stratum germinativum is characterized by cuboidal or low columnar cells with basophilic cytoplasm and melanocytes in H&E-stained sections.
- Melanocytes are present as rounded cell bodies with clear cytoplasm and slender cytoplasmic processes.

Stratum Spinosum (Prickle Cell Layer)

Stratum spinosum layer comprises most of the epidermis, which contains several layers of keratinocytes connected by desmosomes. The desmosomes allow cells to remain tightly bound to one another and resemble 'spines' architecturally.

- Desmosomes are composed of transmembrane desmocollin-desmoglein pairs, which bind to the tonofilaments via desmoplakins, plakoglobin (Dsg-1 and Dsg-3) and plakophilin-1. Desmoglein-1 (Dsg-1) is expressed in the upper epidermis, while desmoglein-3 (Dsg-3) is mostly expressed in the basal epidermis.
- Stratum spinosum layer comprises desmosomes attached to polyhedral-shaped keratinocytes with round to oval nuclei and prominent nucleoli. Desmosome junctions appear as spines between cells of stratum spinosum in H&E-stained sections.

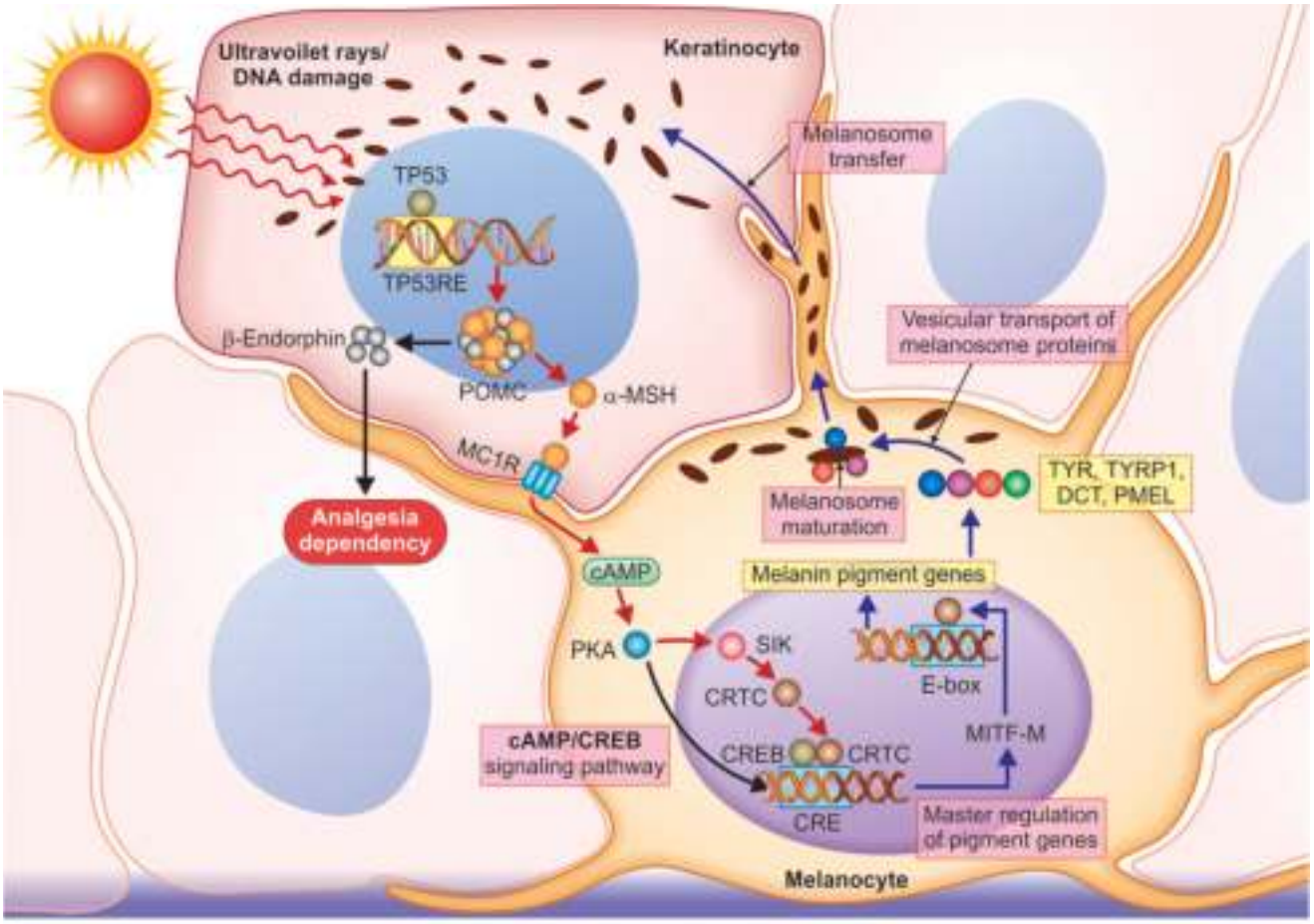
Stratum Granulosum (Granular Cell Layer)

Stratum granulosum layer consists of several layers of cells, which contain lipid-rich lamellar granules.

- Stratum granulosum layer synthesizes glucosylceramides, phospholipids, cholesterol, free fatty acids, which help in the barrier function. In this layer, cells begin to immortalize and lose their nuclei, as they move away from the nutrients located in the deeper tissue. Stratum granulosum also participates in process of keratinization.
- The process of maturation of cells with change in quality and type of keratins is called keratinization. Keratins (from the Greek *keras* meaning horn) are the main intermediate filaments in epithelial cells, which are of two types—basic and acidic and are always in pairs.
- Different keratins are found at different levels of the epidermis depending on the stage of differentiation and disease. Normal basal cells make keratins 5 and 14, but terminally differentiated suprabasal cells make keratins 1 and 10. Keratins 6 and 16 become prominent in hyperproliferative states, i.e. psoriasis. Stratum granulosum layer is characterized by keratinocytes with dense, ovoid, and basophilic keratohyalin granules in H&E-stained sections.

Stratum Lucidum

Stratum lucidum layer only exists in the thick skin of soles and palms and consists of mostly immortalized dead skin cells in the epidermis, and is named for its translucent appearance. Stratum lucidum contains a clear substance called eludin, which eventually becomes keratin. This layer is only found on the soles and palms. Stratum lucidum layer is characterized by a thin homogenous eosinophilic zone that is difficult to identify in H&E sections.



Signaling pathways in melanogenesis

1. Melanin is produced in the melanosomes (pigment granules or organelles) within melanocytes derived from neural crest.
2. Exposure of keratinocytes to UV light causes DNA mutations.
3. DNA damage stimulates p53 protein, which increases expression of proopiomelanocortin (POMC), a precursor protein to α-melanocyte-stimulating hormone (α-MSH).
4. α-MSH binds MCIR, which increases cAMP and microphthalmia associated transcription factor (MITF), which is transcriptional activator of several genes (TYR, TYRP1, DCT and PMEL) that encode melanosome localized proteins involved in synthesis of melanin and melanosomes and in melanoma biogenesis and transport.
5. Dopamine tautomerase (DCT) is a type 1 membrane protein involved in melanin synthesis.
6. Melanocyte is in contact with many keratinocytes through their processes forming an epidermal melanin unit.
7. Processes of the melanocyte 'dock' with keratinocyte, allowing transfer of the melanosome.
8. Once in the keratinocyte, the melanosome degranulates and releases melanin pigment, which protects the nucleus from UV light by dissipating the ultraviolet rays as heat (i.e. internal conversion).
9. Disorders related to melanin production include vitiligo, albinism, freckles, nevi and malignant melanoma.

Fig. 29.2: Signaling pathway in melanogenesis. Melanogenesis is a process by which the melanin pigment is produced in melanosomes by melanocytes. Cirsimaritin induces melanogenesis by stimulating the expression of TYR and TRP1 through the cyclic AMP/PKA cascade and subsequent activation of CREB and MITF in B16F10 cells.

Stratum Corneum (Keratin Layer)

Stratum corneum serves as protective outermost layer of the epidermis and contains flattened dead cells (corneocytes) without nuclei and separated by lipids and the mortar—in the intercellular space. Due to keratinization and lipid content, stratum corneum allows for the regulation of water loss by preventing internal fluid evaporation. This layer also prevents invasion by infectious agents and toxic chemical agents.

Pathology Pearls: Desmosomal Cadherins—Desmocollin and Desmoglein

- Desmosomes are intercellular junctions that tether intermediate filaments to the plasma membrane. Desmosomes contain two specific cadherins (**desmocollin** and **desmoglein**).
- Extracellular domains of desmocollin and desmoglein mediate extracellular adhesion of desmosome junctions of adjacent cells, whereas the cytoplasmic tails of desmosomal

cadherins associate with desmosomal plaque proteins such as plakoglobin, desmoplakin, and plakophilin.

- There are seven desmosomal cadherins, i.e. three desmocollins and four desmogleins. In human beings, desmocollin-2 and desmoglein-2 are the primary isoforms at the intercalated discs identified.
- Mutations in cadherins superfamily members are associated with hereditary diseases of epidermis, mucosal membranes and cardiomyocytes.

DERMIS AND SUBCUTANEOUS TISSUE

Dermis lies deep to the epidermis, which is a thick layer of connective tissue composed of collagen fibers and elastin. Collagen fibers provide strength and elastin gives elasticity to the skin.

- The dermis also contains nerve endings, blood vessels and adnexal structures such as hair shafts, sweat glands and sebaceous glands.
- The apical layer of dermis folds to form papillae that extend into the epidermis. The lower layer of dermis is referred to as the reticularis dermis.
- The hypodermis is the third deepest layer consisting mainly of adipose tissue. Below subcutaneous fat is a layer of dense fascia that sheaths the entire body and covers bone, muscle, and other deep structures.

NAILS STRUCTURE

The hard keratin of the nail plate is formed in the nail matrix, which lies in an invagination of the epidermis (nail fold) on the back of the terminal phalanx of each digit.

- The nail matrix runs from the proximal end of the floor of the nail fold to the distal margin of the lunula. From this region, the nail plate grows forward over the nail bed, ending in a free margin at the tip of the digit.
- The cuticle acts as a seal to protect the potential space of the nail fold from chemicals and from infection. The nails provide strength and protection for the terminal phalanx. The presence of nails helps with fine touch and handling of small subjects. Schematic

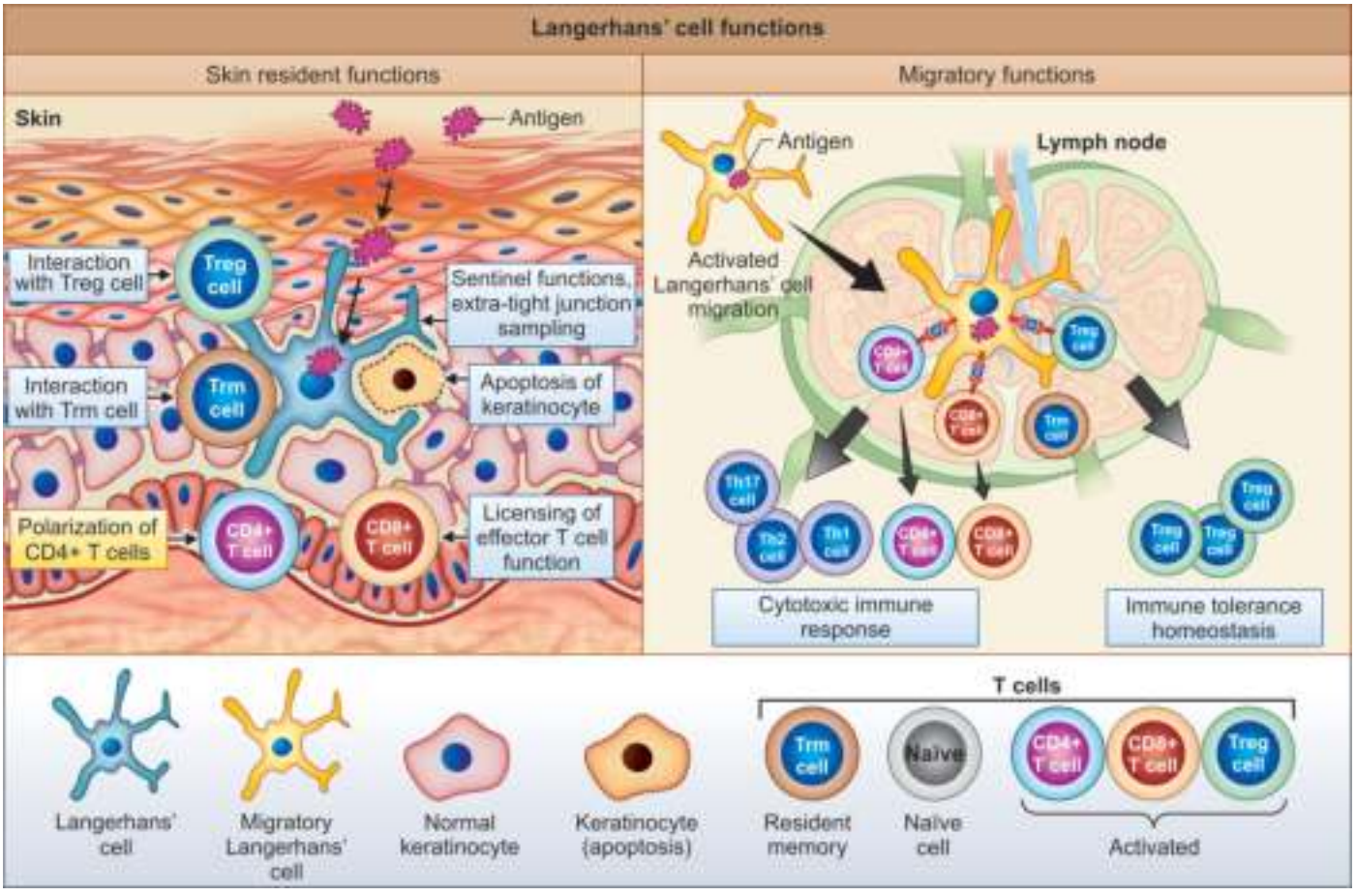


Fig. 29.3: Schematic representation of Langerhans' cells and their functions, Langerhans' cells populate across the epidermis of the skin from the early stage of development, but which have the ability to migrate from the epidermis to draining lymph nodes. These cells provide act as first line of defense against pathogens encountered via the skin.

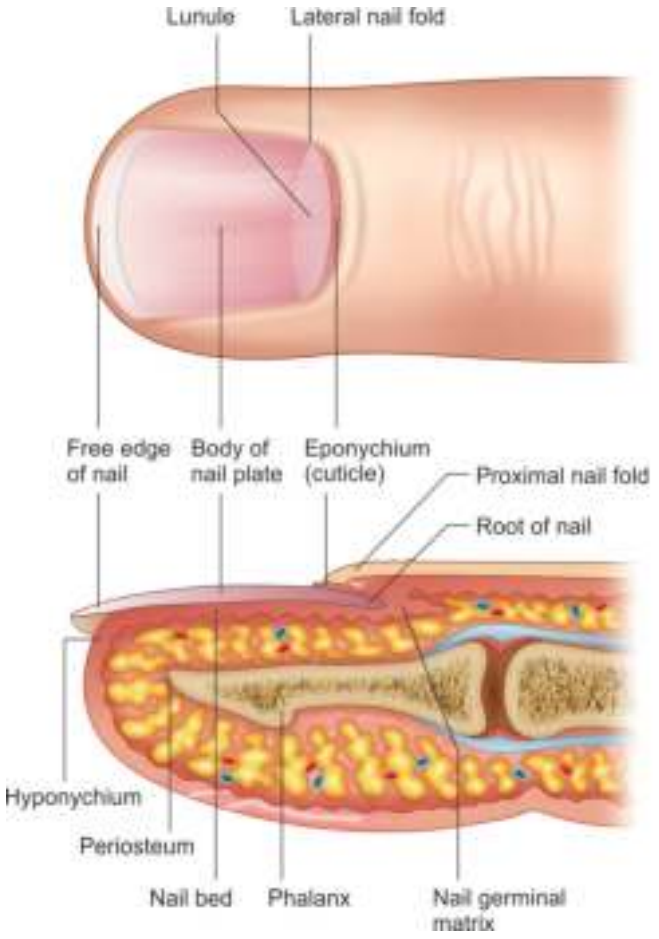


Fig. 29.4: Structure of the nail. The nail structure is divided into six parts: root, nail bed, nail plate, eponychium, paronychium and hyponychium. The nail bed contains blood vessels, nerves and melanocytes that produce melanin. The nail functions by protecting the digits and contributing to tactile sensation.

representation of structure of the nail and nail bed is shown in Fig. 29.4.

SKIN DISORDERS

Skin disorders may be primary or secondary. Primary disorders of the skin include inflammatory, pigment disorders, autoimmune disorders, dermatoses, thermal injuries, and skin cancers (squamous cell carcinoma, basal cell carcinoma and malignant melanoma). Clinical and histologic terms used in dermatopathology are given in Table 29.3. Classification of skin disorders based on histologic examination is given in Table 29.4. Categorization of skin disorders based on etiology is given in Table 29.5. Skin manifestations in systemic disorders are given in Table 29.6. Immunofluorescence patterns and skin disease association are given in Table 29.7. Revised 2024 WHO classification of skin tumors with new entities is given in Table 29.8.

Table 29.3 Clinical and histologic terms used in dermatopathology

Term	Description
Clinical terms used in dermatopathology	
Macule	Flat skin lesion recognized by discoloration <1 cm
Papule	Smaller palpable skin lesion <1 cm
Patch	Flat skin lesion recognized by discoloration >1 cm
Nodule	Elevated skin lesion, 'marble-like' >1 cm in width and depth
Induration	Firmness to palpation
Plaque	▪ Large palpable skin lesion >1 cm ▪ Coalescent papules
Lichenification	Palpable, visibly thickened skin with accentuated skin markings
Scale	▪ Visibly altered stratum corneum ▪ Scales are dry and whitish
Vesicle	Small blister filled with clear fluid <1 cm
Pustule	Visible collection of pus in blister
Bulla	Blister filled with clear fluid >1 cm
Crust formation	Dried exudate (serum, blood or pus) on the skin surface
Histologic terms used in dermatopathology	
Hyperkeratosis	Increased thickness of stratum corneum due to an abnormal increase in keratin in eczema, warts and corns
Parakeratosis	Retention of nuclei in the stratum corneum, and is a sign of increased cell turnover in psoriasis
Acanthosis	Thickening of the epidermis due to hyperplasia of stratum spinosum in 'acanthosis nigricans'
Acantholysis	Loss of keratinocyte cell-to-cell adhesion (separation of epidermal cells) resulting in round rather than polygonal appearing cells in 'pemphigus vulgaris'
Atrophy	Thinning; reduction in size
Hypergranulosis	Increased thickness of stratum granulosum in lichen planus
Spongiosis	Intercellular edema between the intercellular spaces of keratinocytes of the spinous layer in eczematous dermatitis
Lichenification	Hyperkeratosis, hypergranulosis, acanthosis and fibrosis of the capillary dermis corresponding to clinical skin thickening
Lichenoid	A band-like inflammatory infiltrate (usually lymphocytes) below the dermal-epidermal junction
Orthokeratosis	Stratum corneum without retention of nuclei
Pedunculated lesion	A papule connected to the skin by a stalk thinner than the lesion itself
Psoriasiform	Inflammatory pattern: epidermis with thickening and elongation of rete ridges
Vacuolar alteration	Alteration including vacuole formation in cells of the basal layer

Table 29.4 Classification of skin disorders based on histologic examination

Skin Layer	Skin Disorders	
Epidermis	▪ Psoriasis	▪ Pemphigus vulgaris
Dermal-epidermal interface	▪ Bullous pemphigoid ▪ Erythema multiforme	▪ Lupus erythematosus
Superficial and deep vascular bed	▪ Cutaneous vasculitis	▪ Contact dermatitis
Dermal connective tissue	Systemic sclerosis	
Panniculus adiposus	▪ Erythema nodosum	▪ Erythema induratum

Table 29.5 Categorization of skin disorders based on etiology

Categories	Skin Disorders	
Infectious disorders	▪ Impetigo ▪ Cellulitis, verruca	▪ Molluscum contagiosum
Inflammatory dermatoses	▪ Eczema ▪ Psoriasis ▪ Acne vulgaris	▪ Lichen planus ▪ Contact dermatitis
Blistering dermatoses	▪ Pemphigus vulgaris ▪ Bullous pemphigoid	▪ Dermatitis herpetiformis
Disorders of pigmentation and melanocytes	▪ Vitiligo ▪ Albinism	▪ Nevus ▪ Melanoma
Epithelial tumors arising from keratinocytes	▪ Basal cell carcinoma ▪ Basal cell nevus syndrome (BCNS) ▪ Bowen disease (*in situ* squamous cell carcinoma)	▪ Squamous cell carcinoma ▪ Keratoacanthoma ▪ Markel cell carcinoma

Table 29.6 Skin manifestations in systemic disorders

Systemic Disorders	Skin Manifestations	
Sarcoidosis	▪ Erythema nodosum	▪ Lupus pernio causing severe cosmetic disfigurement
Scleroderma	▪ Thickened tight skin especially of fingers ▪ Skin telangiectasias	▪ Calcified nodules
Hyperlipidemia	▪ Xanthelasmas (eyelids)	▪ Xanthomas (elbows, knuckles, buttock, soles, palms and Achilles tendon)
Diabetes mellitus	▪ Necrobiosis lipoidica (symmetric plaques on shins with atrophic yellow appearance and waxy feel, cutaneous)	▪ Candida infection ▪ Ulcers on feet
Cushing's syndrome	Purple striae, thin skin, easy bruising	
Inflammatory bowel disease (ulcerative colitis and Crohn's disease)	Pyoderma gangrenosum (large ulcer)	
Dermatomyositis	Edema and mauve discoloration of eyelid, erythema of the knuckles and other bony points, such as elbow and shoulder to photosensitive butterfly rash on face	
Cancers	Acanthosis nigricans—brown velvet-like thickening of skin in axilla, and groin-thickening or painless ichthyosis—fish-like skin appearance	

Table 29.7 Immunofluorescence patterns and skin disease association

Disease	Antibodies	Immunofluorescence Patterns	Histologic Manifestations
Pemphigus vulgaris	Antidesmosal antibody	Intercellular chicken-wire IgG in the epidermis	Suprabasal vesiculation
Bullous pemphigoid	▪ Antiepithelial BM antibody ▪ Anti-hemidesmosome (collage XII-BP180) antibody	Linear IgG along BM; in salt-split skin, reactivity along roof	Subepithelial vesiculation
Epidermolysis bullosa acquisita (EBA)	EBA antibody	Linear IgG along BM; in salt-split skin, reactivity along floor	Subepithelial vesiculation
Dermatitis herpetiformis	Antigluten antibody	Granular IgA, especially in tips of dermal papillae	Subepithelial vesiculation

Table 29.8 Revised 2024 WHO classification of skin tumors with new entities

Type of Skin Tumor relating to Origin	Representative Skin Tumors	
Keratinocytic/epidermal (nonmelanoma) origin	▪ Basal cell carcinoma ▪ Basal cell carcinoma with sarcomatoid differentiation ▪ Bowen disease (squamous cell carcinoma *in situ*) ▪ Squamous cell carcinoma ▪ Clear cell squamous cell carcinoma ▪ Lymphoepithelioma-like carcinoma	▪ Squamous cell carcinoma with sarcomatoid differentiation ▪ Squamous cell carcinoma with osteoclastic-like giant cells ▪ Epidermolytic actinic keratosis ▪ Merkel cell carcinoma
Melanocytic origin	▪ Combined BAP1-inactivated nevus/melanocytoma	▪ Malignant melanoma
Appendageal tumors	▪ Malignant tumors with eccrine and apocrine differentiation ▪ Malignant tumors with follicular differentiation ▪ Sebaceous carcinoma ▪ Adnexal adenocarcinoma NOS ▪ Endocrine mucin-producing sweat gland carcinoma ▪ Squamoid eccrine ductal carcinoma ▪ Syringocystadenocarcinoma papilliferum ▪ Secretory carcinoma	▪ Cribriform carcinoma ▪ Signet-ring cell histiocytoid carcinoma ▪ Trichoblastic carcinoma ▪ Trichoblastic carcinosarcoma ▪ Trichilemmal carcinoma ▪ Melanocytic matricoma ▪ Adenocarcinoma of anogenital mammary-like glands ▪ Fibroadenoma of anogenital mammary-like glands ▪ Phyllodes tumor of mammary-like glands
Soft tissue tumors	▪ Adipocytic tumors (liposarcoma) ▪ Fibroblastic, myofibroblastic and fibrohistiocytic tumors ▪ Vascular tumors (Kaposi sarcoma, angiosarcoma)	▪ Myopericytic tumors ▪ Neural tumors (malignant peripheral nerve sheath tumor) ▪ Tumors of uncertain differentiation
Uncertain differentiation	▪ Pleomorphic dermal sarcoma ▪ Epithelioid sarcoma ▪ Myxo-fibrosarcoma	▪ Dermal clear cell sarcoma ▪ Ewing sarcoma
Hematologic and lymphoid origin	▪ Mycosis fungoides ▪ CD30+ T cell lymphoproliferative disorders ▪ Skin T cell leukemia/lymphomas (primary/secondary) ▪ Skin B cell leukemia/lymphomas (primary/secondary) ▪ B-lymphoblastic leukemia ▪ Blastic plasmacytoid dendritic cell neoplasia	▪ T-lymphoblastic leukemia ▪ Skin determination in myeloid leukemia ▪ Lymphomatoid papulosis subtype D, E and DUSP22 ▪ Primary cutaneous gamma-delta T cell lymphoma ▪ Primary cutaneous acral CD8+ T cell lymphoma ▪ Erdheim-Chester disease
Inherited tumor syndromes associated with skin malignancies	▪ Familial melanoma ▪ Gorlin syndrome (nevoid basal cell carcinoma syndrome) ▪ BAP1 tumors predisposition syndrome	▪ Skin cancers linked to xeroderma pigmentosum ▪ Muir-Torre syndrome

INFECTIOUS DERMATITIS

BACTERIAL SKIN INFECTIONS

Bacterial skin infections include erythrasma and related disorders, impetigo, ecthyma, folliculitis, erysipelas and cellulitis. While there are certainly other bacterial skin infections, which are either uncommon or result from systemic illness. Bacterial skin infections are given in Table 29.9.

Table 29.9 Bacterial skin infections

Disorder	Bacteria	Age Group	Clinical Features
Impetigo	*Staphylococcus aureus, Streptococcus* pyogenes	Children and immuno-compromised persons	Nonbullous impetigo on nose associated with lymph-adenopathy to bullous impetigo 1–2 cm with fever
Staphylococcal-scalded skin syndrome	*Staphylococcus aureus*	Young children (newborn to 2 years of age)	Blistering desquamative eruption, sheet-like desqua-mation and positive Nikolsky sign, fever, malaise
Necrotizing fasciitis	*Staphylococcus aureus*	Adults (median age 40)	Rapidly progressive bacterial infection, begins as ill-defined erythema and tenderness
Ecthyma	Group A (β-hemolyticus *Streptococcus pyogenes*)	Children and elderly persons	Ulcerative skin lesions, punched out, discrete ulcer with thick overlying crust on lower extremities
Bacillary angiomatosis	*Bartonella henselae* and *Bartonella quintana* gram-negative bacilli	Immunocompromised persons	Erythematous, papules or nodules (solitary/multiple), fever, lymphadenopathy, weight loss, visceral lesions in bones, liver, spleen, lungs, and brain
Actinomycosis	*Actinomyces israelii* (filamentous gram-positive bacteria)	All ages	Fistulae, sinus tracts and multiple abscesses, fever, weight loss and five subtypes: cervicofacial (mandible), abdomen, thoracic, female pelvic and mycetoma foot (sinus tracts)
Granuloma inguinale	*Calymmatobacterium granulomatis* gram-negative bacteria (sexual transmission)	20–40 years both males and females	Painless expansive ulcer with a clean beefy red base and rolled margins, may be verrucous lesion in male (penis, scrotum, perianal, inguinal) and female (labia minora, fourchette, clitoris, cervix); and lymphadenopathy due to secondary infection
Anthrax	*Bacillus anthracis* gram-positive bacteria	Adults	Cutaneous anthrax erythematous papule with extensive surrounding edema; pulmonary anthrax occurs due to inhalation of spores with involvement of lymph nodes and systemic dissemination, gastrointestinal tract anthrax due to ingestion of infected meat
Corynebacterium infections (erythrasma and trichomycosis)	*Corynebacterium minutissimum* gram-positive filamentous bacillus	All ages	Erythrasma (asymptomatic erythematous macular eruption with distinct border in axilla, inguinal folds, interdigital web spaces); and trichomycosis (concretions yellow, red, black especially in axilla and pubic area)
Syphilis	*Treponema pallidum* spirochete (sexual transmission)	Young persons (30–40 years)	Primary syphilis (painless chancre on penis or labia), secondary syphilis (macular, erythematous, symmetrical eruptions on trunk and proximal extremities); tertiary syphilis (syphilitic aortitis, dementia, tabes dorsalis, meningitis, ataxia and skin nodules)
Lupus vulgaris	*Mycobacterium tuberculosis*	Any age	Erythematous, scaly papules and nodules, peripheral exten-sion with scarring, ulcerative and verrucous lesions may erode into cartilage (skin lesion due to lymphovascular spread)
Leprosy	*Mycobacterium leprae* with varied immunological response	All ages	▪ Tuberculoid leprosy patient has good cell-mediated granulomatous response and develops hypopigmented center and raised erythematous border ▪ Lepromatous leprosy patient has poor immunological response and develops macules, papules and plaques, but firm nodules may also be seen in the face ▪ Borderline leprosy is an intermediate form between tuberculoid and lepromatous leprosy and patient develops hypopigmented macules

ECZEMATOUS DERMATITIS

Eczematous dermatitis, also known as atopic dermatitis, is the most common form of dermatitis in children, who have history of hay fever or bronchial asthma. It can be seen in adults. Genetic as well as environmental factors (chemical agents such as hair dye) are thought to play role in its pathogenesis. Patient presents with dry skin that leads to a skin rash as a result of scratching or rubbing. The skin rash is prone to infection. The most important treatment of eczematous dermatitis is skin hydration followed by topical steroids for flare-ups.

Pathogenesis

Recent research demonstrates that there is a genetic component to eczematous dermatitis. Filaggrin gene encodes a protein responsible for maturation of skin by creating the tough, flat corneocytes tightly packed in an organized manner that form the outermost protective layer of skin. Filaggrin gene mutation has been observed in eczematous dermatitis.

- A patient with filaggrin gene mutation will have a dysfunctional skin barrier due to the haphazard organization of the skin cells, which allows water loss and decreased protection from harmful substances. Persons with eczematous dermatitis also have reduced levels of β-defensins (host defense peptides) in the skin against certain bacteria, viruses and fungi. Decreased levels of β-defensins (host defense peptides) leads to increased colonization of bacteria and infection, especially with *Staphylococcus aureus*.
- Chemical agents can directly injure skin or may act as antigens. Type 4 cell-mediated hypersensitivity reaction plays a key role in eczematous dermatitis due to contact chemical agent resulting from cooperation of skin macrophages (Langerhans' cells) and helper T cells. The skin manifestations in atopic patients are most often caused by type 4 rather than type 1 hypersensitivity reaction. Patient presents with itching, erythema and oozing following use of hair dye.

Clinical Features

Patient presents with intensely pruritic, dry scaly, skin rash with erythematous papules and small excoriations.

- Continuous rubbing of itching skin rash leads to eczematous dermatitis over face, especially the cheeks in children. Later, the children develop skin lesions on the extensor surfaces of wrist, elbows, ankles and knees; and flexor surfaces on antecubital and popliteal fossa.
- Clinical examination of face may reveal '**Dennie-Morgan lines**' (crease-like wrinkles just below the lower lid) in 25% of patients with eczematous dermatitis. Clinical photograph of eczematous dermatitis is shown in Fig. 29.5.



Fig. 29.5: Clinical photograph of eczematous dermatitis. A patient with itching, erythema and oozing following use of hair dye (arrow).

Surgical Pathology: Eczematous Dermatitis

Light Microscopy

- Morphology of the eczematous dermatitis depends on the activity of disease.
- In acute stage of eczematous dermatitis, skin lesion demonstrates spongiosis with vesicle formation. In chronic stage, skin lesion shows acanthosis, hyperkeratosis, lichenification, and focal lymphocytic dermal infiltrates.
- In subacute stage of eczematous dermatitis, skin lesion shows intermediate changes between acute and chronic; less spongiosis and vesiculation than in acute; less acanthosis and hyperkeratosis than in chronic eczematous dermatitis.

Treatment

Eczematous dermatitis is treated by hydration skin moisturizing and ointment and topical anti-inflammatory medicines. An ointment is preferred over a cream due to the high proportion of oil to water in lotions.

IMPETIGO

Impetigo is a common highly contagious infection of the superficial layers of the epidermis on sun exposed skin (face, hands) in pediatric age group (2–5 years), or any age group; and most commonly caused by gram-positive bacteria (*Staphylococcus aureus* or group A Streptococcus). Children younger than two years of age account for 90% of cases of bullous impetigo caused by *Staphylococcus aureus*. Nonbullous impetigo is caused by *Staphylococcus aureus* and group A β-hemolytic streptococci.

Pathophysiology

Impetigo can be classified as either primary or secondary. Primary impetigo involves previously normal skin by

bacterial invasion into epidermis. Secondary impetigo involves skin at a previous skin wound site.

- Disturbance in the skin barrier leads to access to *Staphylococcus aureus* and group A Streptococcus, which bind to fibronectin receptors for colonization.
- Predisposing factors for breakdown of skin barrier include trauma, insect bites, surgery, atopic dermatitis, burns and varicella. Incidence of impetigo is increased in immunocompromised persons, overcrowding, diabetes mellitus and poor hygiene.

Clinical Features

- Patient with nonbullous impetigo usually develops a vesicle or a pustule, which later ruptures resulting in purulent exudate forming honey-colored crust, which are often multiple lesions on the face and extremities in areas of disruption of skin barrier. Mild regional lymphadenopathy is a common associated finding. Systemic symptoms such as fever are typically absent in nonbullous impetigo.
- Bullous impetigo in patient begins with fewer small vesicles on nose, that become flaccid bullae 1–2 cm in diameter than in nonbullous impetigo containing a clear or yellow fluid which eventually progresses to become purulent. Surrounding erythema and edema are typically absent.
  - Bullous impetigo does not form a dried serum giving honey-colored crust. If the crust is not removed, new lesions form about the periphery and extensive epidermal damage may ensue.
  - Patient presents with skin lesions in the intertriginous regions, and unlike nonbullous impetigo, may occur in the buccal membranes.
  - Regional lymphadenopathy is absent. Systemic symptoms, such as fever, are more common than in nonbullous impetigo.
- Development of impetigo in the deep tissue is known as exthyma, that occurs due to penetration of ulcerative lesion through the epidermis and deep into the dermis. The lesions appear as 'punched out' with violaceous margins. Clinical photograph of impetigo is shown in Fig. 29.6.

Surgical Pathology: Impetigo

Light Microscopy

Impetigo lesion shows subcorneal cleft in the stratum granulosus, vesicle contains neutrophils and gram-positive bacteria, acantholytic cells, intradermal perivascular neutrophils and lymphocytes. Superficial dermal inflammation accompanies these findings.

Treatment

Treatment of impetigo involves topical and oral antibiotics and symptomatic care. Localized uncomplicated non-bullous impetigo is treated by topical antibiotics by prior removal of the crust. Bullous impetigo in skin or oral mucosa with lymphadenopathy is treated by systemic administration of antibiotics.



Fig. 29.6: Clinical photograph of impetigo. A child with lesions of impetigo on the face with the serum giving rise to the classic golden-yellow, "honey-crusted" lesion (arrows).

NEURODERMATITIS

Neurodermatitis is also known as lichen simplex chronicus, that presents as dry, patchy areas of skin that are scaly and thick. Neurodermatitis occurs in the settings of psychological stress or cutaneous pruritic diseases such as eczema or psoriasis due to chronic, habitual rubbing and scratching. This lesion is clinically indistinguishable from chronic eczematous dermatitis.

Clinical Features

Patient presents with well-defined hyperpigmented to erythematous leathery single or multiple plaques with exaggerated skin lines (lichenification) on head, scalp, posterior neck, genitalia, extensor aspect of arms and legs. The most common symptom of neurodermatitis is itching.

- Repeated scratching of the skin lesion leads to formation of leathery plaques of variable sizes (3–10 cm) and potentially thickened skin lesions.
- Center region of skin lesions has different shades of yellow or a deep reddish brown. However, with the respective age of the lesion, this color may transform into a leucodermic center with a darker zone surrounding the lesion. Clinical photograph of neurodermatitis is shown in Fig. 29.7.

Laboratory Diagnosis

Skin biopsies can be performed to exclude skin disorders such as psoriasis or mycosis fungoides. Blood tests may be performed. Elevated serum immunoglobulin E levels support the diagnosis of an underlying atopic diathesis linked to allergic disorders.



Fig. 29.7: Clinical photograph of neurodermatitis. Chronic scratching leads to a thick raised plaque with increase in surface markings (lichen simplex chronicus) (arrows).

SCABIES

Scabies is caused by female mite Sarcoptes scabiei. Its transmission occurs by close personal contact: mother–child, siblings, or sexual partners.

- Female mites burrow in the epidermis just below the stratum corneum and deposit eggs and feces in the interdigital spaces, sides of the hands and feet, flexural surface of the wrist, anterior axillary line, genital skin, and nipples. The first infestation remains asymptomatic for a period of weeks, until an immune response develops and then pruritus results.
- Upon re-infestation, the symptoms appear within a few days. Patient presents with intense skin itching at night. Immune reaction of type 4 to mites leads to both pruritus and diffuse exanthema. Typical sites of scabies are thighs, buttocks, and trunk. Clinical photograph of scabies is shown in Fig. 29.8.



Fig. 29.8: Clinical photograph of scabies. Pathognomonic involvement of the web spaces in a case of scabies (arrows).

LEPROSY (HANSEN'S DISEASE)

Leprosy, also known as **Hansen's disease**, is a chronic infectious disease caused by obligate intracellular gram-positive and weakly acid-fast *Mycobacterium leprae*, a microorganism that has a predilection for the skin and nerves. The complexity of presentation is related to the varied immunologic responses.

- *Mycobacterium leprae* are transmitted from human body by nasal discharge and digital impregnation of skin, as bacilli can be carried under nails and are inoculated under the skin by scratching. The incubation period is usually 3–5 years. Skin and peripheral nerves are commonly involved.
- *Mycobacterium leprae* target Schwann cells (SCs) leading to injury of the nerve, demyelination, loss of axonal conductance and consequent disability. The pathogen can invade the Schwann cells by a specific laminin-binding protein known as PGL-1.

Leprosy Classification

Based on clinical, histologic and immunologic status, leprosy is classified within two poles of the disease with transition into five forms: tuberculoid polar leprosy (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous polar leprosy (LL). Schematic representation of Ridley-Jopling and WHO classification of leprosy is shown in Fig. 29.9.

Indian Leprosy Classification

According to Indian classification, leprosy is classified into five categories: indeterminate, tuberculoid, borderline, lepromatous and pure neuritic leprosy. Pure neuritic leprosy (PNL) constitutes a significant proportion of cases in India. It is essential to pay more attention to this form of leprosy to diagnose and treat patients earlier to prevent deformities and sequelae of nerve damage.

Myrid Leprosy Classification

According to Myrid classification, leprosy is classified into two categories: immunological stable (lepromatous and tuberculoid leprosy) and immunological unstable (indeterminate and borderline leprosy). The most widely accepted scheme of classification is Ridley-Jopling classification.

WHO Leprosy Classification

According to WHO leprosy classification, patients are divided into two groups for therapeutic purposes: paucibacillary (TT, BT) and multibacillary (mid-borderline (BB), BL, LL).

- Paucibacillary (PB) leprosy is characterized by 1–5 skin lesions, one nerve involvement and slit skin smear negative for AFB.

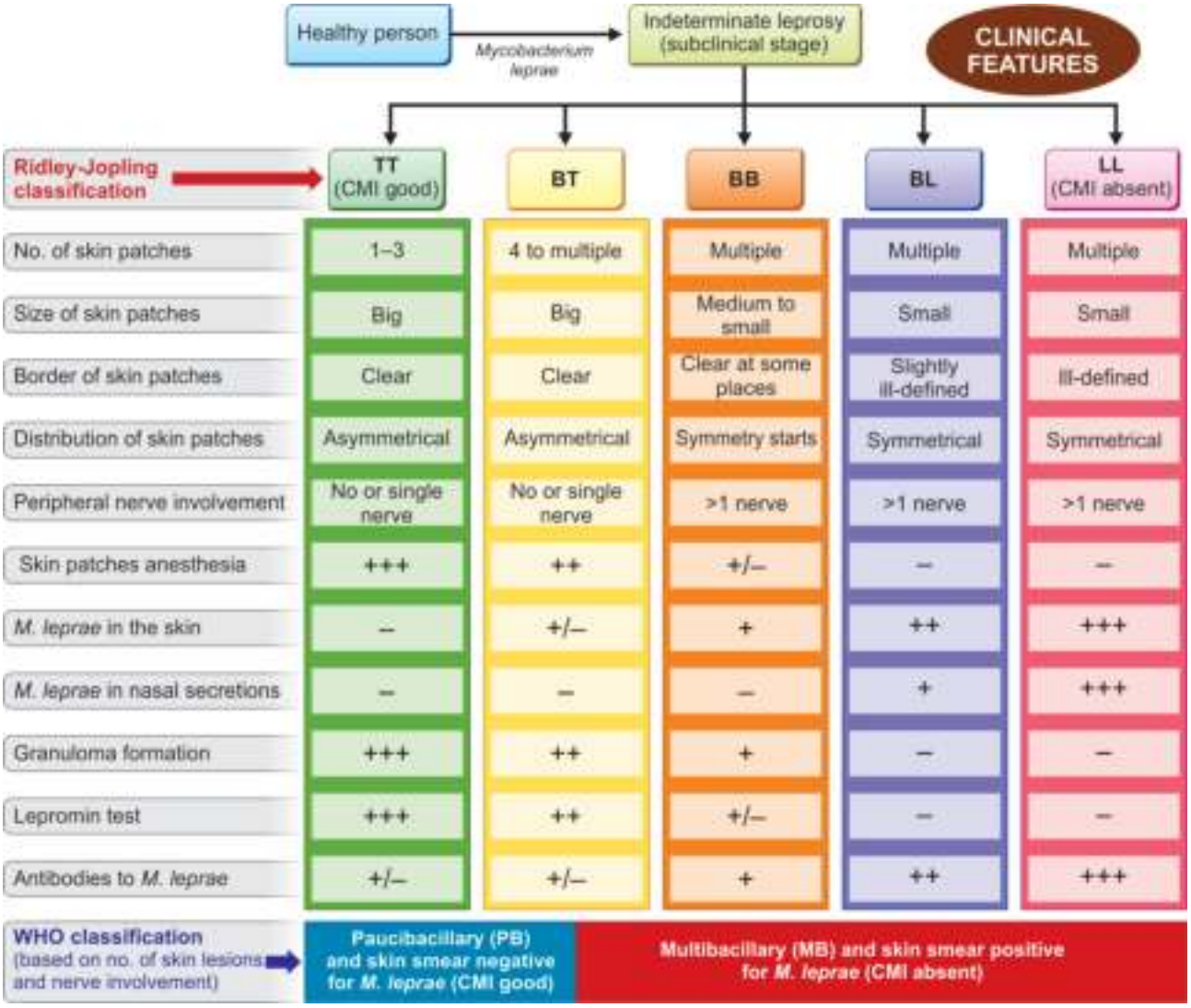


Fig. 29.9: Schematic representation of Ridley-Jopling and WHO classification of leprosy.

- Multibacillary (MB) leprosy is characterized by 6 or more skin lesions, involvement of more than two nerves and slit skin smear positive for AFB.

Ridley-Jopling Leprosy Classification

Ridley-Jopling leprosy classification is based on a combination of clinical findings, bacteriological index (BI), immunological reactivity (lepromin test) and the histologic findings. This leprosy classification ranges from a spectrum of high- to low-resistance pattern. Borderline leprosy includes borderline tuberculoid leprosy, mid-borderline leprosy, borderline lepromatous leprosy, tuberculoid leprosy, and lepromatous leprosy.

- **Borderline tuberculoid leprosy (BT):** Patient presents with multiple large anesthetics less-defined discrete; satellite papules similar to those seen in tuberculoid leprosy. Several peripheral nerve trunks are symmetrically enlarged and neuropathy often occurs. Bacterial index ranges from zero to 2.
- **Mid-borderline leprosy (BB):** Patient presents with numerous asymmetrical annular dimorphic plaques with poorly-defined outer borders and sharply defined central 'punched out' skin lesions, which exhibit both tuberculoid and lepromatous lesions. These patients may develop nerve hypertrophy and/or neuritis. Bacterial index ranges from 3 to 4.
- **Borderline lepromatous leprosy (BL):** Majority of patients have variable immunological response. BL patients do not fall into polar group. Borderline tuberculoid leprosy tends to be more towards tuberculoid leprosy and borderline lepromatous leprosy towards lepromatous leprosy. Patient presents with dimorphic, symmetrical, bilateral widespread small macules, papules and nodules of variable sizes and shapes on skin including symmetric peripheral nerve involvement. Bacterial index is 4 or 5.

- **Tuberculoid leprosy (TT):** Tuberculoid leprosy is characterized by presence of immunological response against *Mycobacterium leprae*. Patient develops large macular lesions on cooler parts especially nose, outer ears, testes and superficial nerve endings. There is presence of cell-mediated immune response. Infectivity is low in tuberculoid leprosy.
- **Lepromatous leprosy (LL):** Lepromatous leprosy has least capacity to mount immunological response against *Mycobacterium leprae*. Patient develops extensive erythematous macules, papules or nodules with extensive destruction of skin. Immune response is severely depressed. There is high infectivity in lepromatous leprosy.

Immune Status in Leprosy

Cell-mediated and humoral immunity have been implicated in leprosy. T cells participate in cell-mediated immunity. Increased titers of IgG, IgA and IgM have been demonstrated in these patients, which are not protective. Tuberculoid leprosy occurs in persons with good cell-mediated immunity; patients develop granulomatous response. Lepromatous leprosy occurs in persons with poor cell-mediated immunity; does not develop a granulomatous response. Borderline leprosy is an intermediate form between tuberculoid and lepromatous leprosy.

- **High-immune response in indeterminate, tuberculoid leprosy (TT) and borderline tuberculoid leprosy (BT):** Patient presents with few well-defined skin lesions with regular borders with asymmetrically distribution pattern and nerve enlargement. Slit skin smear may show scant *Mycobacterium leprae*. Lepromin test is positive.
- **Low-immune response in borderline lepromatous (BL) and lepromatous leprosy (LL):** Patient presents with symmetrical distribution of numerous skin lesions ranging from macules–papules with ill-defined margins. These skin lesions may be hypoanesthetic. Lepromin test is negative.
- **Mid-immune response in mid-borderline leprosy (BB):** Patient shows features of both the spectrums of leprosy. This mid-borderline leprosy is immunologically unstable, and hence prone to immune reactions.

Clinical Features

Activity of leprosy disease is assessed by various signs: erythema/infiltration, extension or appearance of new lesions, extension/new appearance of anesthesia, paresis and paralysis, tenderness and pain in nerves, morphological index and occurrence of immune reaction. Even though the nerves affected in leprosy are mixed motor and sensory nerves, the sensory loss is more marked compared to motor dysfunction.

Indeterminate Leprosy

Indeterminate leprosy is a prelude to the determinate form of leprosy. The initial cutaneous manifestations of leprosy are subtle and not specific, which are most common cutaneous lesions in Indian children. Most cases resolve, but some advance into more severe disease.

- Patient develops an ill-defined, bizarre hypopigmented macule(s) with a smooth or scaly surface. The sensations over the macule may or may not be impaired. The nerve proximal to the patch may or may not be thickened.
- Histologic examination of indeterminate leprosy shows scant lymphocytes and histiocytes in the superficial and deep in the dermis with some tendency to localize around skin appendages; and increased mast cells. Histology of indeterminate leprosy is shown in Fig. 29.10.

Tuberculoid Leprosy

Tuberculoid leprosy is a milder, less severe form of leprosy (called paucibacillary leprosy), which occurs in patients with robust cell-mediated immune response with prompt granulomatous inflammation. Tuberculoid leprosy is characterized by infiltration of dermis and subcutaneous fat by noncaseating granulomas predominantly centered around small cutaneous nerves.

- Nerves are completely destroyed in the process and replaced by noncaseating epithelioid granulomas. There is preponderance of CD4+ helper T cells over CD4+ suppressor T cells and without antibody response to *Mycobacterium leprae*. Cell-mediated

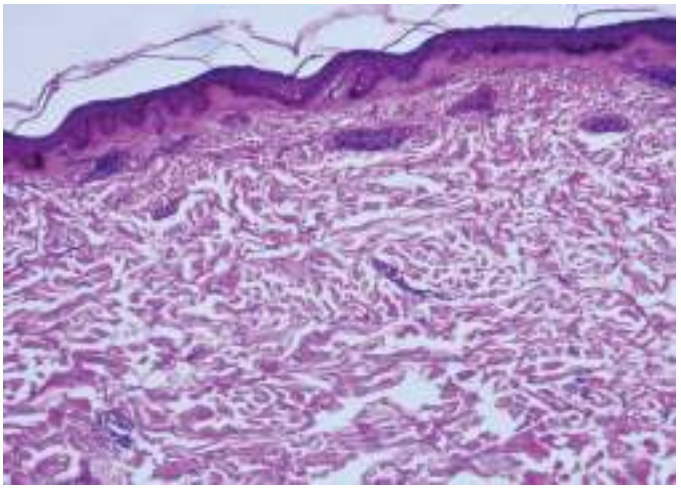


Fig. 29.10: Histology of indeterminate leprosy. Histologic examination of indeterminate leprosy shows scant lymphocytes and histiocytes around neurovascular bundles in the superficial and deep in the dermis with some tendency to localize around appendages; and increased mast cells (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

hypersensitivity reaction eradicates bacteria from the tuberculoid leprosy lesion in tissue. Therefore, the *Mycobacterium leprae* bacilli are scarce and usually not identified with modified Ziehl-Neelsen acid-fast stain (Wade-Fite stain).

- Most skin lesions have no identifiable bacteria; hence diagnosis is achieved using real-time polymerase chain reaction (PCR) to amplify *Mycobacterium leprae* specific DNA sequence and to detect serum antibodies specific to *Mycobacterium leprae* antigens.
- Patient presents with only single or multiple flat or well-circumscribed hypoanesthetic and hypopigmented plaque with raised erythematous border on extremities, buttock and face, thickened nerves, loss of sensation and diminished sweating. All patients have nerve involvement. Inflammation of Schwann cells leads to thickening of peripheral nerves. Clinical course is generally benign.
- On histologic examination, tuberculoid leprosy demonstrates noncaseating granuloma, Langhans giant cells, CD4+ helper T lymphocytic infiltration, and involvement of peripheral nerve. The close association of granulomatous response with the cutaneous nerves is a helpful diagnostic feature in tuberculoid leprosy. Tuberculoid leprosy is less contagious than other forms of leprosy. Histology of tuberculoid leprosy is shown in Fig. 29.11.

Borderline Leprosy

Borderline leprosy is an intermediate form between tuberculoid and lepromatous leprosy. Patient has hypopigmented macules and involvement of nerves exhibiting onion-skin appearance.

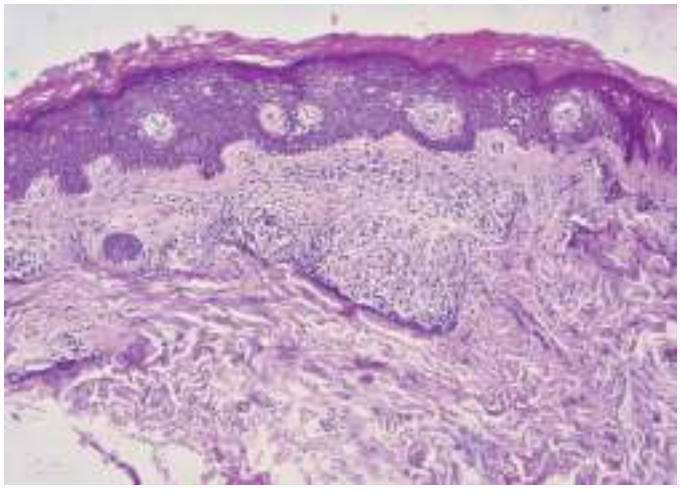


Fig. 29.11: Histology of tuberculoid leprosy. Hematoxylin and eosin-stained section shows noncaseating epithelioid granuloma, Langhans giant cells, T-helper lymphocytic infiltration, and involvement of peripheral nerve (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

- **Borderline tuberculoid leprosy (BT):** Borderline tuberculoid leprosy is characterized by granulomas and numerous lymphocytes in dermis. Diagnosis of borderline tuberuloid leprosy is achieved using real-time polymerase chain reaction (PCR) to amplify *Mycobacterium leprae* specific DNA sequence and to detect serum antibodies specific to *Mycobacterium leprae* antigens. Clinical photograph of borderline tuberculoid leprosy is shown in Fig. 29.12. Histology of borderline tuberculoid leprosy is shown in Fig. 29.13.
- **Borderline lepromatous leprosy:** Majority of borderline lepromatous leprosy patients have variable



Fig. 29.12: Clinical photograph of borderline tuberculoid leprosy. Clinical examination shows more widespread and less sharply defined lesions than in tuberculoid form. These are usually symmetrical on trunk but may be asymmetric on face. These are less likely to have scale with sharply raised border seen over right elbow. Nerve involvement less prominent. (*Courtesy*: Dr. Krishna Deb Barman, Director Professor of Dermatology, Maulana Azad Medical College and Associated Lok Nayak Hospital, New Delhi.)

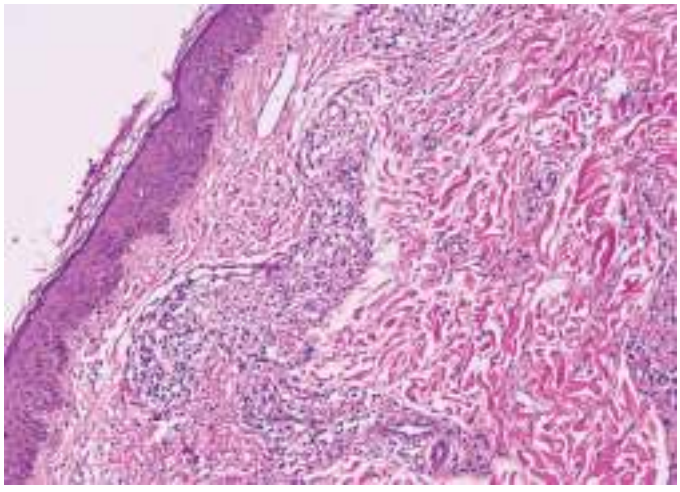


Fig. 29.13: Histology of borderline tuberculoid leprosy. Hematoxylin and eosin-stained section shows formation of epithelioid granulomas (400X).

immunological response. BL patients do not fall into polar group. Borderline tuberculoid leprosy tends to be more towards tuberculoid leprosy and borderline lepromatous leprosy towards lepromatous leprosy. Patient presents with dimorphic, symmetrical, bilateral widespread, small macules, papules and nodules of variable sizes and shapes on skin including symmetric peripheral nerve involvement. Bacterial index is 4 or 5. Clinical photograph of borderline lepromatous leprosy is shown in Fig. 29.14. Histology of borderline lepromatous leprosy is shown in Fig. 29.15.

Lepromatous Leprosy

Patients with lepromatous leprosy develop numerous macules, papules, plaques or firm nodules on nose and ears in symmetric distribution, later involve cooler parts (face, ears, wrists, elbows, buttocks, and knees) sparing inguinal and axillary regions. Facial lesions can be markedly swollen with loss of eyebrows (Lucio sign). Facial nerve involvement, hoarseness, loss of eyebrows and eyelashes, and nasal collapse (saddle nose) secondary to nasal septal perforation may occur in advanced cases of disease. Nasal secretions are rich in *Mycobacterium leprae* organism. Differences between tuberculoid and lepromatous leprosy are given in Table 29.10.



Fig. 29.14: Clinical photograph of borderline lepromatous leprosy. Clinical examination shows symmetric bilateral widespread, well-defined, hypopigmented to erythematous hypoesthetic small macules, papules and nodules of variable sizes and shapes with downward slopping edges seen over the back. Small satellite lesion is also in the periphery of skin lesions. These skin lesions are mostly lepromatous in nature but also contain aspects of tuberculoid lesions. (*Courtesy*: Dr. Krishna Deb Barman, Director Professor of Dermatology, Maulana Azad Medical College and Associated Lok Nayak Hospital, New Delhi.)

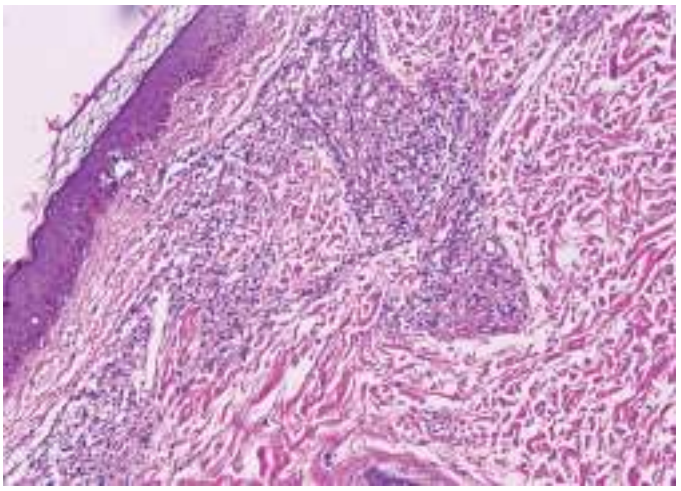


Fig. 29.15: Histology of borderline lepromatous leprosy. Hematoxylin and eosin-stained section shows formation of epithelioid granulomas (400X).

- The predominant histologic feature in lepromatous leprosy is a diffuse histiocytic infiltrate in the dermis that is not necessarily centered around nerves. A grenz zone of sparing is usually present in the papillary dermis. There are no discrete granulomas in the dermis. The histiocytes are arranged in poorly circumscribed clusters. Lymphocytes are scarce and those are present usually CD4+ suppressor T cells. The histiocytes may demonstrate vacuolated or foamy cytoplasm with a grayish-blue tinge on hematoxylin and eosin-stained sections due to clusters of *Mycobacterium leprae* bacilli. With modified Ziehl-Neelsen acid-fast (Wade-Fite) stain, *Mycobacterium leprae* bacilli can be visualized in large numbers in histiocytes surrounding cutaneous nerves, blood vessels and eccrine glands. In some cases of lepromatous leprosy, histiocytes are spindle-shaped and arranged in storiform pattern mimicking fibrous histiocytoma, which is referred to as histoid leprosy. Histology of histoid leprosy a type of lepromatous leprosy is shown in Fig. 29.16. Ziehl-Neelsen (Wade-Fite) stained section demonstrates numerous *Mycobacterium leprae* bacilli as shown in Fig. 29.17.
- Histoid leprosy is a rare variant of lepromatous leprosy characterized by unique clinical, histopathologic, and morphologic features. Histiocytes in lepromatous leprosy are spindle-shaped and arranged in a storiform pattern. This variant is called histoid leprosy. Patient presents with cutaneous and

Table 29.10 Differences between tuberculoid and lepromatous leprosy

Characteristics	Tuberculoid Leprosy	Lepromatous Leprosy
Host resistance	Present	Absent
T cell immunity	Present	Absent
Lepromin test	Strongly positive	Negative
Fernandez reaction (24–48 hours)	Positive	Negative
Mitsuda reaction (3–4 weeks)	Positive	Negative
Skin lesions	▪ Macular lesions with central depressed areas and hypopigmented margins ▪ Asymmetrical lesions not involving both sides	▪ Macular/papular/nodular lesions on hand and face (leonine face) ▪ Symmetrical lesions involving both sides
Touch sensation	Hypoanesthetic/ anesthetic lesions	Absent (anesthetic patches)
Clinical appearance	Disfigurement minimal	Disfigurement maximum (leonine facies, claw hands and pendulous ear lobes)
Nerves involved	Ulnar, facial and peroneal nerves	Ulnar and peroneal nerves
Other organs involved	Other organs are not involved	Anterior chamber eye, upper respiratory tract, testes, lymph nodes, liver, spleen and gynecomastia in males
Infectivity	Low infectivity	Very high infectivity
Skin histology	▪ Epidermis becomes nonatrophic ▪ Rete ridges are present ▪ Dermal papillae are normal ▪ Clear zone between epidermis and dermis is absent ▪ Epithelioid cell granulomas are present ▪ CD4+ helper T cells: Present at the periphery of granulomas ▪ CD4+ suppressor T cells: Very few at the center of lesion ▪ Fite stain: AFB (*M. leprae* bacilli) few (4–5) in macrophages	▪ Epidermis becomes atrophic ▪ Rete ridges are absent ▪ Dermal papillae become flattened ▪ Clear zone between epidermis and dermis is present ▪ Epithelioid cell granulomas are absent ▪ CD4+ helper T cells are absent ▪ CD4+ suppressor T cells are present in large number in diffuse manner ▪ Fite stain: AFB (*M. leprae* bacilli) abundant in macrophages
Complications	These are related to nerve damage like paralysis, distinct sensory losses	Antigen–antibody complex mediated erythema nodosum, leprosum, vasculitis, glomerulonephritis besides nerve related
Prognosis	Milder disease, hence good prognosis	Extensive progressive disease, hence poor prognosis

subcutaneous nodules. Histoid leprosy patients represent probable residual bacilli and a highly active lepromatous leprosy process. These cases may act as reservoirs of the disease and lead to further spread of leprosy. Continual occurrence of these cases does not bode well (favorable) for eradication of leprosy.

Erythema Nodosum Leprosum

Erythema nodosum leprosum (ENL) is an immune-mediated complication, which affects 50% of patients with lepromatous leprosy (LL) and 10% of borderline lepromatous leprosy (BL).

- Erythema nodosum leprosum is characterized by the presence of tender red plaques and nodules together with areas of erythema over skin, fever, malaise, arthritis, neuritis and lymphadenitis, orchitis, dactylitis, osteitis, lymphadenitis, facial mutilation, eye inflammation, neurotrophic ulcers, flexion contractures of hands, foot drop and nephritis. The eruption is generally widespread and accompanied by fever, malaise and arthralgia. Clinical photograph of erythema nodosum leprosum (ENL) is shown in Fig. 29.18.
- Erythema nodosum leprosum is treated mainly with corticosteroids. Prolonged administration of corticosteroids can cause morbidity and mortality.
- Histologic examination of H&E-stained sections demonstrates an inflammatory neutrophilic infiltrate,

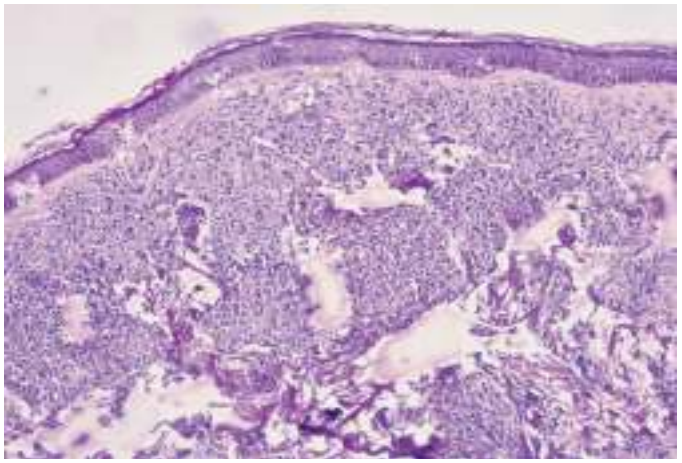


Fig. 29.16: Histology of histoid leprosy is a type of lepromatous leprosy. In some cases of lepromatous leprosy, histiocytes are spindle-shaped and arranged in storiform pattern mimicking fibrous histiocytoma around blood vessels and adnexal structures (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)



Fig. 29.18: Clinical photograph of erythema nodosum leprosum. The skin lesions are characterized clinically by tender red plaques and nodules together with areas of erythema (arrow). The eruption is generally widespread and accompanied by fever, malaise and arthralgia. (*Courtesy*: Dr. Krishna Deb Barman, Director Professor of Dermatology, Maulana Azad Medical College and Associated Lok Nayak Hospital, New Delhi.)

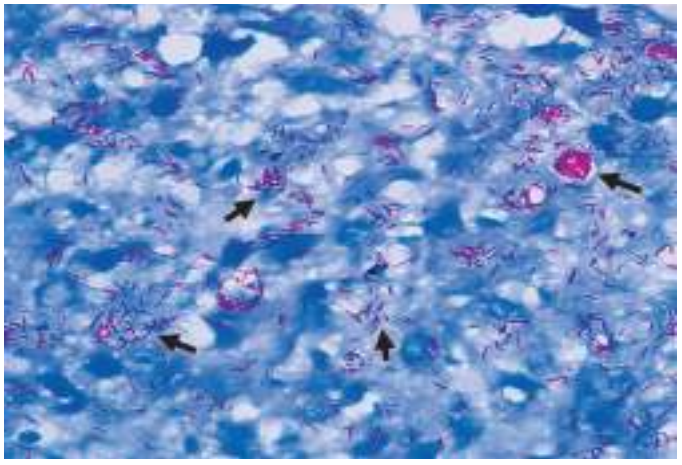


Fig. 29.17: Lepromatous leprosy. Modified Ziehl-Neelsen (Wade-Fite) stain shows macrophages laden with *Mycobacterium leprae* (arrows) (400X). This technique is used to evaluate classification of leprosy into those with low and high bacillary load, and in assessing the response to therapy. (*Courtesy:* Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

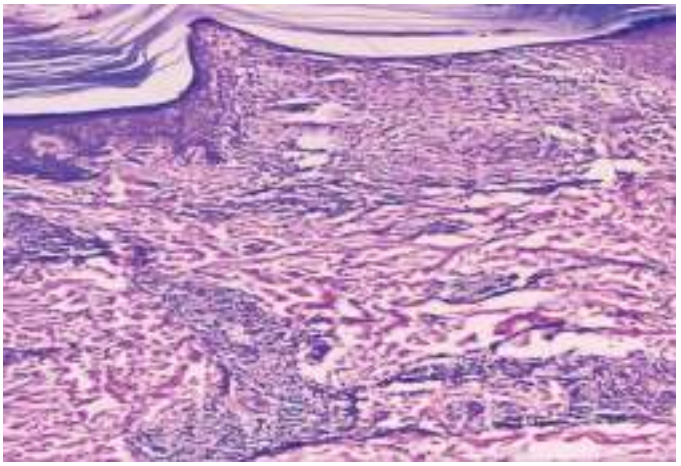


Fig. 29.19: Histology of erythema nodosum leprosum. Histologic examination of H&E-stained sections demonstrates an inflammatory neutrophilic infiltrate, vasculitis and/or panniculitis, immune-complex deposit and complement deposit associated with antigens of *Mycobacterium leprae* (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

vasculitis and/or panniculitis, immune-complex deposit and complement deposit associated with antigens of *Mycobacterium leprae*. Histology of erythema nodosum leprosum is shown in Fig. 29.19.

- The clinical course of erythema nodosum leprosum is prolonged with recurrent episodes of *Mycobacterium leprae* over a period of 12–24 months or even longer than seven years in some cases.

Lepromin Test

Lepromin test is used to demonstrate immune status in leprosy patients. Antigenic extract obtained from *Mycobacterium leprae* is administered by intradermal route. The injection site is evaluated at 1–2 days and 3 weeks. Lepromin test is positive in tuberculoid leprosy. Delayed hypersensitivity is demonstrated in tuberculoid leprosy (TT). Lepromin test is negative in lepromatous leprosy (LL). Lepromin positive reaction is of two types: Fernandez reaction and Mitsuda reaction.

- **Fernandez reaction:** Fernandez reaction occurs within first 2 days and represents a delayed hypersensitivity reaction to intradermally injected heat-killed leprosy bacilli. Patient develops skin induration within 24–48 hours at the site of injection.

- **Mitsuda reaction:** Mitsuda reaction measures the granulomatous immune response to intradermally injected heat-killed leprosy bacilli. A positive Mitsuda reaction is described as an indurated skin lesion of more than 4 mm that histologically shows epithelioid cell granuloma formation after 3–4 weeks at the site of injection. A positive reaction corresponds to the acquisition of cell-mediated immunity against the *Mycobacterium leprae* and occurs in tuberculoid leprosy patients. Borderline leprosy patients show an indurated skin lesion of less than 3 mm.

VIRAL SKIN INFECTIONS

Viral skin infections account for a significant percentage of skin disorders across world, which include verruca vulgaris, verruca plantaris, verruca plana, condyloma acuminatum, molluscum contagiosum, herpes simplex, varicella zoster. Viral skin disorders and morphologic features are given in Table 29.11.

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum is self-limited viral infection of the skin caused by molluscum contagiosum virus (MCV), a double-stranded DNA virus, that belongs to the Poxviridae family. MCV has four genotypes: MCV1 (75–95%), MCV2, MCV3 and MCV4. MCV1 is transmitted by direct skin-skin contact or sharing infected clothes. MCV2 is transmitted via sexual contact in adults and immunocompromised individuals.

- MCV infects the epidermis and replicates in the cytoplasm of cells with a variable incubation period

Table 29.11 Viral skin disorders and morphologic features

Disorder	Virus	Age Group	Clinical Features	Histologic Features
Verruca vulgaris	HPV: 2, 4 followed by types 1, 3, 27, 29 and 57	Children and adults	Discrete white to tan flat, papules or plaques (0.1 to 1.0 cm) with a rough, pebble-like surface on hands, fingers, feet and toes	Epidermal squamous differentiation with papillomatosis, hyperkeratosis, koilocytic change within stratum granulosum
Verruca plantaris (plantar wart)	HPV: 1, 2, 4, 63 (traumatic skin transmission)	Children and teenagers	Verrucous hyperkeratotic, papule, nodule of variable size (1 to 2 cm), asymptomatic/ painful	Hyperkeratosis with parakeratosis, acanthosis with papillomatosis, keratohyaline aggregates, koilocytic changes within epidermis, blood vessels dilated with thrombosis
Verruca plana (plane wart)	HPV: 3, 10, 28	Children and adults	Thin papules on face, dorsal surface of hands, arms and knees	Orthokeratosis (basket wave), hypergranulosis area, acanthosis, focal koilocytic change within epidermis prickle cell and granular cell layer
Condyloma acuminatum	HPV: 6,11 (sexual transmission)	Adolescents and young adults	Cauliflower-like pruritus, tender or bleeding lesions on genitalia and perianal regions	Bullous papillomatous acanthosis with exoendophytic growth pattern, parakeratosis, hyperkeratosis, koilocytic change within epidermis, coarse keratohyaline granules, occasional mitoses in lower epidermis
Molluscum contagiosum	Poxvirus (sexual transmission)	Children and young adults	Skin-colored to erythematous papule with central keratinous core ≤1 cm, can be associated with eczematous dermatitis	Bullous acanthosis with central crateriform invagination extending deep into the dermis, intracytoplasmic oval bodies (Henderson-Patterson bodies) within wall of squamous proliferation
Herpes simplex	HSV:1, 2 (DNA virus)	General population with immunosuppression	Recurrent painful clustered vesicles in oral, genital and anal regions	Epidermal intracytoplasmic edema, epidermal necrosis and intraepithelial vesicle, multinucleated giant cells, follicular involvement
Varicella zoster virus (VZV)	Varicella zoster (chickenpox) lies dormant within nerve ganglion	Children and older persons	Vesicles few mm to 2 cm evolving through pustular and crusted phases leaving shallow scar	Features identical to HSV infections, epidermal intracytoplasmic edema, epidermal necrosis and intraepithelial vesicle, multinucleated giant cells, follicular involvement
ORF (ecthyma contagiosum)	Parapoxvirus (infection from sheep, goat)	Male adults	Evolving lesion as macule, papule, erythematous on hands	Intraepithelial vesiculation, pseudoepitheliomatous hyperplasia, epidermal vacuolation and necrosis

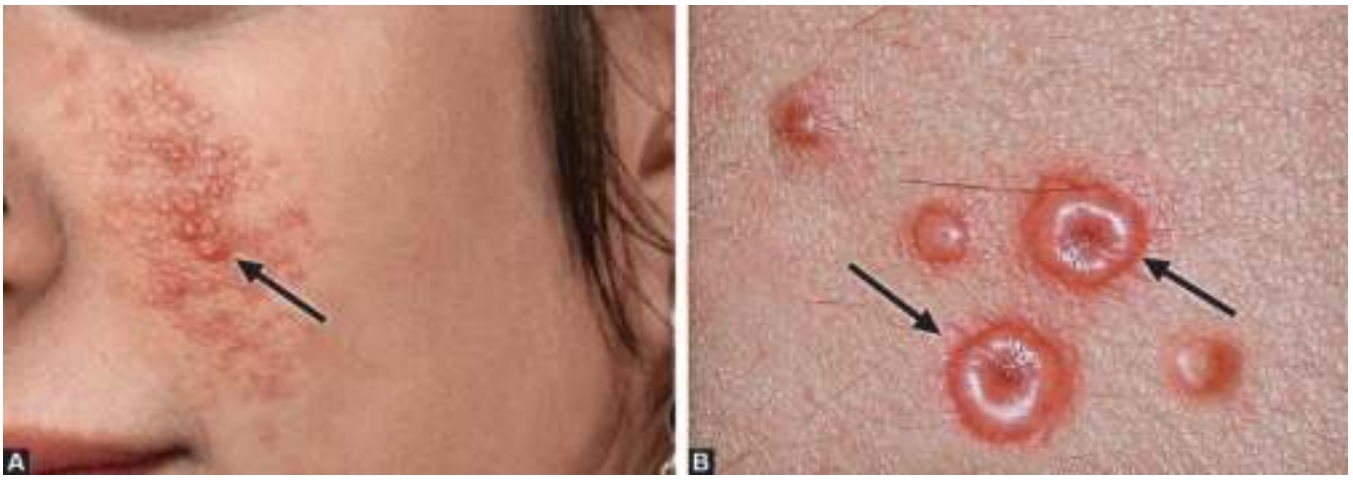


Fig. 29.20: Clinical photograph of molluscum contagiosum. Typical distribution of lesions is present on a child's face. Note the lesions in the eyelid (arrows). The lesion is a skin infection caused by a poxvirus (molluscum contagiosum), that produces benign raised lesions on the upper layer of skin. The lesion may appear anywhere on the body. (A) The lesions are distributed on a child's face. Note the eyelid lesions (arrow). (B) Close up of the lesion is dome-shaped pearly painless papule with central umbilication (arrows).

between two and six weeks. Four viral genes have been identified that code proteins that would alter the activation of the nuclear factor κB (NF-κB): MC159, MC160, MC132, and MC005. MCV most often affects children and adolescents.

- In children, the main affected areas include skin, over trunk, extremities, intertriginous regions, genitalia (mainly due to autoinoculation and not pathognomonic of sexual abuse), and face, except palms and soles; rare involvement of oral mucosa.
- In adults, lesions are most frequently located in the lower abdomen, thighs, genitalia, and perianal area. Patient presents with umbilicated, dome-shaped papules, 2–5 mm size pink or skin-colored over body, arms and legs. Pruritus may be present. Lesions may be single or multiple. Umbilication of skin lesion occurs as a result of intracytoplasmic viral inclusions extruding infected cell on the surface with central pore. Clinical photograph of molluscum contagiosum is shown in Fig. 29.20A and B.

Surgical Pathology: Molluscum Contagiosum

Light Microscopy

- In molluscum contagiosum, epidermis grows deeper down into the dermis and form closely packed lobules. Epidermal cells contain large intracytoplasmic eosinophilic stained inclusion bodies known as molluscum bodies.
- The molluscum bodies displace the nuclei at the periphery of epidermal cells. In the center of the lesion, stratum corneum disintegrates and release the molluscum bodies together with keratinous debris resulting in formation of central crater.
- Dermis shows prominent acute and chronic inflammatory infiltrate and foreign body giant cells. Histology of molluscum contagiosum is shown in Fig. 29.21.

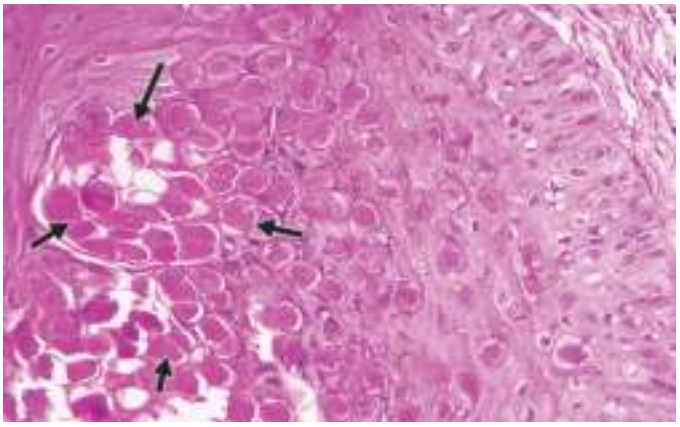


Fig. 29.21: Histology of molluscum contagiosum. Hematoxylin and eosin-stained section demonstrates epidermal cells containing large intracytoplasmic eosinophilic stained inclusion bodies containing virus particles in keratinocytes of stratum spinosum and stratum granulosum, and these are known as Henderson-Patterson bodies (molluscum bodies) shown by arrows. Skin histologic examination also reveals epidermal lobular acanthosis with inverted epidermal hyperplasia (400X).

COMMON WARTS (VERRUCOUS VULGARIS)

Common warts (verrucous vulgaris) are benign lesions that occur on mucosa and skin of persons of all ages with equal sex predilection. Verrucocus vulgaris is caused by human papillomavirus (HPV), with over 100 serotypes of HPV identified. Transmission involves direct contact between individuals, or autoinoculation.

- The primary clinical manifestations of HPV infection include common warts (HPV types 1, 2), genital warts, flat warts (HPV types 3, 10, 28), deep palmoplantar warts (HPV type 1), focal epithelial hyperplasia (HPV types 13, 32), epidermodysplasia verruciformis (HPV types 3, 5, 8, 20, and 47), butcher's wart (HPV type 7); and plantar cystic wart (HPV type 60).

- Generally, warts are self-limited lesions, which most often regress spontaneously within six months to two years. These strains are distinct from HPV strains associated with gynecologic neoplasms. Clinical photograph of common warts (verrucous vulgaris) is shown in Fig. 29.22.

Surgical Pathology: Common Warts (Verrucous Vulgaris)

Light Microscopy

- **Common warts:** Skin biopsy in common warts (verruca vulgaris) demonstrates acanthosis, digitated epidermal hyperplasia, papillomatosis, compact orthokeratosis, hypergranulosis, tortuous capillaries within the dermal papillae, and vertical tiers of parakeratotic cells with red blood cells entrapped above the tips of the digitations. Elongated rete ridges may point radially toward the center of the lesion. Koilocytic cells are pathognomonic. Koilocytes are large HPV-infected keratinocytes with prominent condensed keratohyaline granules, abundant eosinophilic cytoplasm and vacuoles; and small wrinkled-appearing pyknotic nuclei as a result of viral cytopathic effects. Histology of verruca vulgaris is sown in Fig. 29.23.
- **Filiform warts:** Filiform warts appear similar to common warts, but they may have prominent papillomatosis.
- **Warts with focal epithelial hyperplasia:** Skin biopsy shows acanthosis, blunting, hyperplastic mucosa with thin parakeratotic stratum corneum, anastomosis of rete ridges, and whiteness of epidermal cells due to intracellular edema. Some may have prominent keratohyaline granules, and vacuolated cells may be present.
- **Deep palmoplantar warts:** Histologic features of deep palmoplantar warts are similar to common warts except the lesion lies deep to the plane of the skin surface. The endophytic epidermal growth is composed of polygonal, refractile-appearing, eosinophilic, cytoplasmic inclusions made up of keratin filaments, forming ring-like structures. Basophilic parakeratotic cells loaded with virions and basophilic nuclear inclusions and may be in the upper layers of the epidermis.
- **Flat warts:** Histologic features of flat warts are similar to common warts. Skin lesion is composed of cells with prominent perinuclear vacuolization around pyknotic, basophilic, centrally located nuclei can be in the granular layer. These are referred to as 'owl's eye cells.
- **Cystic warts:** Cystic warts are filled with horny material. The wall of cystic wart is composed of basal, granular, and squamous cells. Many epithelial cells have large nuclei and clear cytoplasm with eosinophilic inclusion bodies. The cyst may rupture causing a foreign body granuloma.

DERMATITIS HERPETIFORMIS (HERPES SIMPLEX VIRUS II SKIN INFECTION)

Dermatitis herpetiformis is an intensely pruritic inflammatory cutaneous eruption disease related to gluten sensitivity caused by herpes simplex virus II (HSV-II) in 20–40 years of age and most often immunocompromised elderly patients.



Fig. 29.22: Clinical photograph of common warts (verrucous vulgaris). The lesion is caused by localized infection with human papillomavirus (DNA virus). The common warts are especially common among children and adolescents and may occur on any mucocutaneous surface. This patient has multiple common warts with flesh colored and rough surface (arrows).

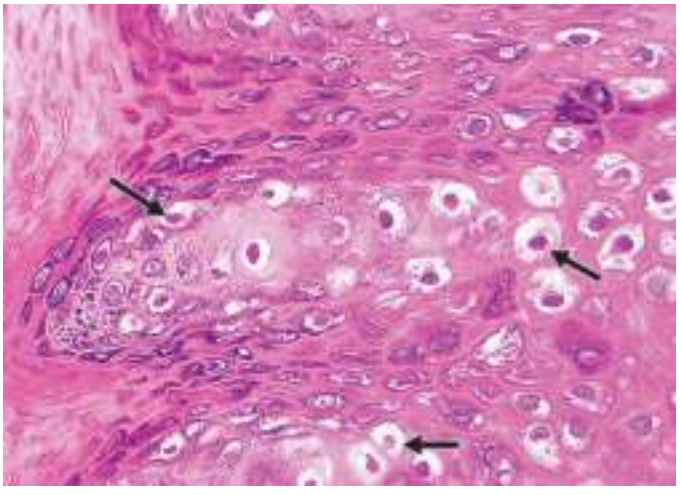


Fig. 29.23: Histology of verruca vulgaris. Hematoxylin and eosin-stained section demonstrates vacuolization of the epidermal cells (koilocytotic change) in this verruca vulgaris is seen. It indicates the viral origin of this lesion. One type of human papillomavirus (HPV) is associated with appearance of warts (arrows) (400X).

- Dermatitis herpetiformis has chronic relapsing course, pruritic polymorphic lesions, and typical histopathologic and immunopathologic findings.
- Patient presents with urticaria-like plaques and vesicles over the extensor surfaces of knees and elbows, scalp, upper back, and sacral area.
- Patient improves when patients are placed on gluten-free diets. Clinical photographs of dermatitis herpetiformis are shown in Figs 29.24 and 29.25.

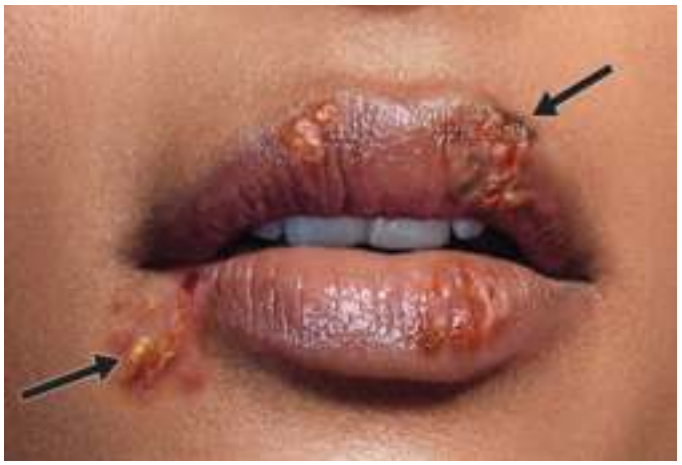


Fig. 29.24: Clinical photograph of dermatitis herpetiformis. The disease is a persistent immunobullous disease that has been linked to coeliac disease, gluten-sensitive enteropathy. The name dermatitis herpetiformis is derived from the tendency for blisters to appear in clusters resembling herpes simplex virus (HSV). However, dermatitis herpetiformis is not due to HSV infection. Dermatitis herpetiformis is also known as Duhring-Brocq disease. Clinical photograph of a female shows erythematous blisters (vesicles) on the face. Patient has recurrent HSV infection on the face in a female with grouped vesicles (arrows).



Fig. 29.25: Clinical photograph of dermatitis herpetiformis. Grouped, erythematous, vesicles are seen here in a zosteriform distribution (arrows).

Pathogenesis

IgA antibodies formed against gluten in the intestine gain access to the circulation and get deposited in the skin. Release of lysosomal enzymes by inflammatory cells cleave the epidermis from the dermis.

- According to several studies, dermatitis herpetiformis is considered the specific cutaneous manifestation of celiac disease (CD). Both dermatitis herpetiformis and celiac disease disorders occur in gluten-sensitive persons, share same HLA haplotypes (DQ2 and DQ8).
- These patients improve following the administration of a gluten-free diet. Small intestinal biopsy performed in dermatitis herpetiformis demonstrates villous atrophy to augmented presence of intraepithelial lymphocytes in all the cases, as well as the generation of circulating autoantibodies to tissue transglutaminase (tTG).
- Patient contracting herpes simplex virus I (HSV-I) infection presents with tender lip, tongue, or buccal sores. Another pattern produced by herpes zoster (also called varicella zoster virus) is the appearance of painful, clear vesicles on large areas of skin, usually on a dermatomal distribution.

Laboratory Diagnosis

Diagnosis of dermatitis herpetiformis is based on clinical history, physical examination, histologic examination, demonstration of granular IgA deposits along the dermal–epidermal junction or at the papillary tips by direct immunofluorescence (DIF) assay and positive anti-tissue transglutaminase antibody.

- Gluten-free diet for weeks to months leads to disappearance of skin lesions.
- IgA anti-endomysium antibodies (EMAs), IgA and IgG anti-deamidated synthetic gliadin-derived peptides, and IgA anti-epidermal transglutaminase (eTG) antibodies are considered specific and sensitive serologic markers for dermatitis herpetiformis.

Surgical Pathology: Dermatitis Herpetiformis (Herpes Simplex Virus II Infection)

Light Microscopy

- Histologic examination of skin lesion demonstrates subepidermal vesicles and blisters with dead keratinocyte, multinucleated giant cells with large nuclei having viral inclusions associated with accumulation of neutrophils at the papillary tips.
- Sometimes, eosinophils can be demonstrated within the inflammatory infiltrate, making difficult to the differential diagnosis with bullous. Histology of dermatitis herpetiformis is shown in Fig. 29.26.

Immunofluorescence Microscopy

- Deposits of IgA are demonstrated at the tips of dermal papillae of skin lesion.

VARICELLA ZOSTER VIRUS SKIN INFECTION (CHICKENPOX)

Chickenpox is a contagious disease of childhood caused by the varicella zoster virus (VZV). Symptoms begin 10–21 days after exposure, but the average incubation period is about two weeks. The disease is more severe in adults than in children.

- Chickenpox typically starts with vesicular eruptions (skin rashes), that form small, itchy blisters on the chest, back, and face then spreads all over body leading to scab formation. Complications of

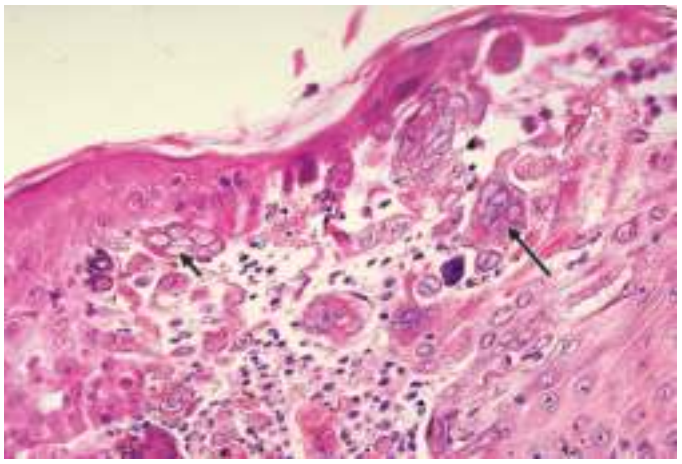


Fig. 29.26: Histology of dermatitis herpetiformis. It is caused by herpes simplex viral infection. On H&E-stained section, histologic examination of skin lesion demonstrates subepidermal vesicles and blisters with dead keratinocyte, multinucleated giant cells with large nuclei having viral inclusions (long arrow) associated with accumulation of neutrophils at the papillary tips; and steel gray to mauve inclusions (short arrow) typical for herpes simplex infection (400X).



Fig. 29.27: Clinical photographs of chickenpox (varicella zoster infection). The disease is caused by varicella zoster virus (DNA virus) especially in children. The virus stays in the sensory nerve ganglia as a latent infection. Patient develops generalized vesicular eruptions (erythematous rashes) also termed the dewdrop on a rose petal (arrows).

chickenpox include pneumonia, brain inflammation, and bacterial skin infections.

- Exposure to varicella zoster virus (VZV) causes the production of host immunoglobulins such as IgG, IgM, and IgA. IgG antibodies persist and confer life-long immunity. Cell-mediated immune responses are important in limiting the duration of primary varicella infection. Following overt varicella zoster infection, varicella zoster virus can remain latent for years in dorsal root ganglia. Clinical photographs of chickenpox or varicella zoster infection are shown in Figs 29.27 and 29.28.

FUNGAL SKIN INFECTIONS

Fungal infections of skin are categorized into dermatophytosis (superficial), angioinvasive and traumatic implanted fungi (dematiaceous fungus).

- Superficial fungal infections are caused by dermatophytes such as Microsporum, Trichophyton and Epidermophyton. These fungi reside in stratum corneum of epidermis, hair and nails.
- Tinea infections are named according to the Latin term that designates the anatomic site of infection, such as tinea capitis (scalp), tinea corporis (body), tinea manuum (hand), tinea cruris (groin), tinea pedis (foot) and tinea unguium (nail).
- Immunocompromised person with angioinvasive fungi presents with necrotic papules/plaques.
- Traumatic implanted fungus/dematiaceous fungus causes scaly and erythematous nodules at the site of trauma. Fungal infections of skin are given in Table 29.12.



Fig. 29.28: Clinical photographs of chickenpox or varicella zoster infection. The disease is caused by varicella zoster virus (DNA virus) especially in children. Patient develops generalized vesicular eruptions (erythematous rashes) also termed the dewdrop on a rose petal at higher magnification. The virus stays in the sensory nerve ganglia as a latent infection.

DERMATOPHYTOSIS (SUPERFICIAL FUNGAL SKIN INFECTIONS)

Dermatophytosis (superficial fungal infections) is caused by dermatophytes such as Microsporum, Trichophyton and Epidermophyton, which resides in stratum corneum of epidermis, hair and nails. Tinea infections are named according to the Latin term that designates the anatomic site of infection, such as tinea capitis (scalp), tinea corporis (body), tinea manuum (hand), tinea cruris (groin), tinea pedis (foot), and tinea unguium (nail). A diagnosis of tinea infection may be suspected on the basis of clinical history and examination.

Table 29.12 Fungal infections of skin

Disorder	Organism	Age Group	Clinical Features	Histologic Features
Dermatophytosis	*Trichophyton, Microsporum, Epidermophyton* species	Children and adults	Hyperkeratotic, inflamed lesions present on various sites: tinea corporis (trunk, limbs except hands and feet), tinea capitis (scalp), tinea pedis (feet), tinea magnum (hands), tinea faciei (face), tinea inguinalis/cruris (groin), tinea unguium (nails)	Key histologic feature is the presence of dermatophytes: fungal forms in the stratum corneum of the epidermis, nail plate, or hair shaft
Tinea versicolor	*Malassezia globosa* (*Malassezia furfur*)	30–40 years	Fine scaly, erythematous patches to thin plaques over trunk	Key histologic feature is the 'spaghetti and meatballs' appearance of intracorneal hyphae and yeast forms, hyperkeratosis, parakeratosis and slight acanthosis
Candidiasis	*Candida albicans*	Children and elderly persons especially immunocompromized persons	Papules, pustules and ulcers, red erosive skin lesions; satellite arrangement of papulopustular lesions classical presentation	Epidermal hyperplasia and spongiosis, *Candida albicans* pseudohyphae, acute and chronic inflammatory cells
Pityrosporum folliculitis	*Malassezia globosa* (*Malassezia furfur*)	Adolescents and middle-aged	Pruritic, erythematous papules and pustules on chest, back, neck, upper arms	Hair follicle dilated with yeast forms and keratin plugs, surrounding chronic inflammatory cells; follicular rupture with neutrophils
Mycetoma	*Pseudoallescheria boydii* and Medurella	Middle-aged persons	Cutaneous infection with 'granules' of fungi (eumycetoma) involving foot after trauma; nodules and draining abscesses	Key histologic features are collection of granules of organisms in the dermis and subcutaneous tissue surrounded by acute and chronic inflammatory cells
North American blastomycosis (Gilchrist disease)	Blastomycosis dermatitis (droplet infection)	Adult men	Usually disseminated disease (genitourinary tract, bone and skin); skin lesions such as hyperkeratotic nodules, plaques, ulcers with heaped up margins <1 to several centimeteres	Epidermal pseudoepitheliomatous hyperplasia with neutrophilic abscess; presence of a broad-based budding yeast with a thick cell wall and multiple nuclei, neutrophils, lymphocytes and histiocytes; granulomatous component with multinucleated giant cells in older lesion
Histoplasmosis	*Histoplasma capsulatum* (bird droppings)	Immunocompetent and immunocompromised persons	Disseminated disease (liver, adrenal gland, reticuloendothelial system, meninges, skin and mucous membranes), skin lesions such as papules, nodules, plaques and punched-out ulcers	The yeast small 2–4 μm, intracellular surrounded by clear space (pseudocapsule), tuberculoid caseating granulomas
Cryptococcosis	*Cryptococcus neoformans* (bird droppings)	Immunocompromised persons	Widespread papules, pustules, nodules and ulcers on head and neck regions	Histologic variants granulomatous, gelatinous and suppurative lesions. Organism is 3–6 μm yeast surrounded by thick mucoid capsule of cryptococcosis include

Contd...

Table 29.12 Fungal infections of skin (*Contd...*)

Disorder	Organism	Age Group	Clinical Features	Histologic Features
Coccidioidomycosis	*Coccidioides immitis*	Immunocompromised persons	Pustules, nodules, plaques and often verrucous after pulmonary involvement	Fungus containing numerous organisms, pseudoepitheliomatous hyperplasia, microabscesses, neutrophils, lymphocytes and histiocytes, sometimes foci of necrosis
Paracoccidioidomycosis (South American blastomycosis)	*Paracoccidioides brasiliensis*	Adults	Pulmonary infection involving skin with ulcers and plaques on face with subcutaneous involvement	Fungus resembles ship's wheel with central large thick-walled yeast form with multiple narrow based buddings, pseudoepitheliomatous hyperplasia, microabscesses and granulomatous inflammation
Chromoblastomycosis (chromomycosis)	*Cladosporium, Fonsecaea, Exophiala* and *Philophora* species	Adult men	Verrucous papules, nodules, plaques, ulcerations and scarring at subcutaneous trauma on leg and foot	Presence of small, round, thick walled, septate, brown yeast of 5–12 µm, pseudoepitheliomatous hyperplasia, neutrophils, lymphocytes, histiocytes, granulomas, microabscesses
Phaeohyphomycosis	*Cladosporium, Fonsecaea, Waniella, Bipolaris, Culvularia, Philophora* species	Healthy persons following trauma/ immunocompromised persons	Nodules, pustules and hemorrhagic plaques on extremities or cystic lesion	Mixed suppurative and granulomatous inflammation, brown yeasts and hyphae (narrow, septate and branching)
Sporotrichosis	*Sporothrix schenckii* (in decaying plants)	Adult men (gardeners)	Papules, nodules and ulcers following subcutaneous trauma	Pseudoepitheliomatous hyperplasia with neutrophilic microabscesses, mixed granulomatous and suppurative dermal inflammation, 4–6 µm yeast forms in center of microabscesses or giant cells, yeasts may be surrounded by eosinophilic material (Splendore-Hoeppli phenomenon)
Aspergillosis	*Aspergillus fumigatus* (most common), *Aspergillus flavus, Aspergillus niger* (otitis media)	Immunocompromised persons due to neutropenia	Lung involvement resulting in skin lesions such as papules, nodules and ulcers especially in venous injection site and burns	Fungus with septate hyphae with branching at 45°, intense acute chronic inflammatory infiltrate, less evident in neutropenic persons; and granulomas in primary infections
Zygomycosis	*Rhizopus, Absidia* and *Mucor* species	Immunocompromised persons	Erythematous macules/ patches with dusky center, which ulcerate	Fungus with thick nonseptate hyphae that branch at right angles, mixed chronic granulomatous suppurative inflammatory infiltrate

- Tinea infection can be diagnosed by using light microscopy and culture, which allows for fungal specification and viability assessment. Skin scrapings and nail clippings are performed on KOH (potassium hydroxide) and examined under microscope to demonstrate fungi. Fungal culture can take up to four to six weeks and but has a false-negative rate of at least 30% for nail samples. Repeat culture should be performed if there is a high index of clinical suspicion of superficial fungal infections.
- The mode of treatment depends on the extent and location of the tinea infection.
  - Topical antifungal shampoos for tinea capitis can reduce the risk for fungal transmission to others but are ineffective in treating the infection. Most cases of tinea corporis, tinea cruris and tinea pedis are

amenable to topical therapy. Recommended first-line topical therapy is terbinafine 1% cream once or twice daily for one to two weeks. Topical treatments are usually ineffective against onychomycosis.

- Onychomycosis is treated by systemic administration of antifungal therapy. Overview of various sites affected with superficial fungal infections is shown in Fig. 29.29.

Surgical Pathology: Dermatophytosis

Light Microscopy

- A skin scraping with KOH mount can be utilized to identify these fungi. Fungal cell walls are rich in mucopolysaccharides and stained with PAS stain, which gives bright pink to red appearance. Involved areas of skin and nails may fluoresce under ultraviolet light.
- The fungi are present in the anucleate cornified layer of skin, hair, or nails. Therefore, scrapings are taken for fungi culture and definite classification. Reactive epidermal changes may produce a pattern that mimics a mild eczematous dermatitis.

Tinea Capitis

Tinea capitis is a dermatophyte infection of the scalp and hair in prepubertal children. There are three main clinical presentations of tinea capitis: (a) scaly patches with alopecia, (b) alopecia with black dots at the folliular opening and (c) diffuse scalp scaling with subtle hair loss. Severe form of tinea capitis is known as 'kerion', which is characterized by a tender plaque with pustules and crusting. Cervical lymphadenopathy is a common associated finding in patients with tinea capitis.

Tinea Corporis and Tinea Cruris

Tinea corporis is commonly referred to as ringworm, annular or ring-like eruption on the skin, other than face, hands, feet or groin. It most commonly occurs in children and young adults. It may spread from other infected humans. It may be autoinoculated from other areas of the body infected by tinea pedis or tinea capitis. Another common mode of transmission of tinea corporis is noted in wrestlers.

- Tinea cruris is also known as 'jock itch' and occurs in the groin fold and is more frequent in adult men.

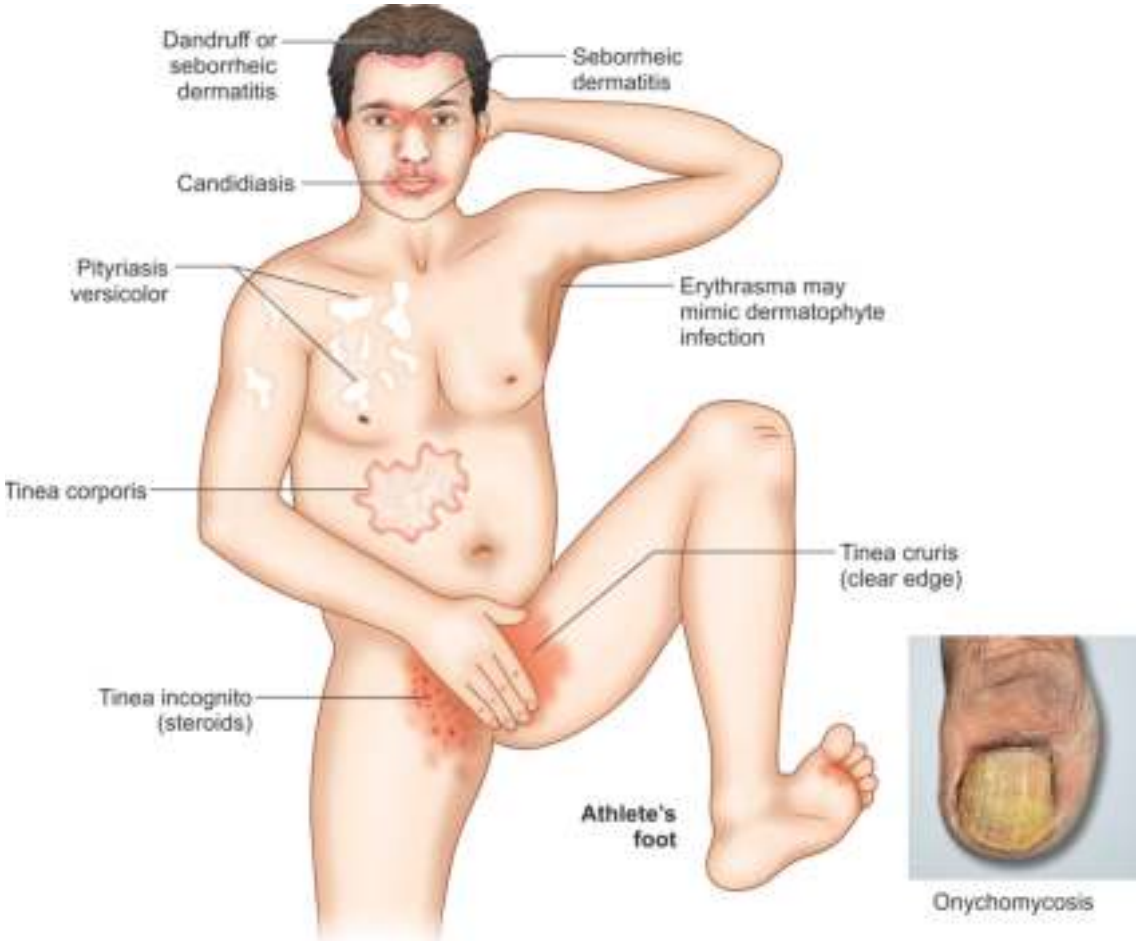


Fig. 29.29: Overview of various sites affected with superficial fungal infection.

Tinea corporis and tinea cruris classically present as annular plaques with central clearing and leading scale. Skin lesions may be single or multiple and of varying sizes, which may coalesce. Pustules or vesicles can sometimes occur at the active edge.

- While tinea may be suspected on the basis of clinical grounds, it is important to be aware of the various conditions considered in the differential diagnosis that may mimic tinea infections.
- Annular skin rash in tinea capitis and tinea cruris should be differentiated from discoid eczema, annular psoriasis, pityriasis rosea, subacute cutaneous lupus erythematosus and erythema annulare centrifugum.
- Topical and systemic antifungal modalities are available and are selected on the basis of the subtypes and severity of tinea infection. Untreated, tinea can cause significant morbidity and predispose to complications, including cellulitis and ulcers on the feet and alopecia on the scalp. Clinical photograph of tinea corporis is shown in Fig. 29.30.

Tinea Pedis

Tinea pedis, known as 'athlete foot', is the most common dermatophyte infection in young persons, but rare in children. Predisposing factors are exposure to occlusive footwear and sweating.

- The interdigital subtype is the most common form of tinea pedis, which manifests as maceration or scales between toes.



Fig. 29.30: Clinical photograph of tinea corporis. A classic case of tinea corporis showing erythematous raised scaly advancing margins with central clearing showing increased activity of the fungus at the margins and presence of host immune response which clears the fungus from the center.



Fig. 29.31: Clinical photograph of tinea unguium. It demonstrates proximal and distal pharynx onychomycosis.

- Another subtype of tinea pedis known as chronic hyperkeratotic (moccasin-type), that spares dorsal surface is characterized by chronic plantar erythema with scaling involving the lateral and plantar surfaces of the foot.
- Recurrent tinea pedis may be due to a reservoir of untreated tinea in the nails.

Tinea Unguium

Tinea unguium, also known as onychomycosis, is a dermatophyte infection of the nails in elderly persons over age of 70 years. About 50% of patients have concomitant fungal skin infections, most commonly tinea pedis.

- Distal lateral subungual onychomycosis is the most common subtype and characterized by yellowish or brownish discoloration associated with onycholysis and subungual hyperkeratosis.
- The other common subtype is the white superficial onychomycosis characterized by white spots on the nail plate that can involve the entire nail if not treated.
- Onychomycosis should be differentiated from nail psoriasis, lichen planus, yellow nail syndrome, traumatic onychodystrophy, alopecia areata and age-related nail dystrophies. Immunocompromised and diabetic persons are at greater risk for fungal and bacterial infections. Clinical photograph of tinea unguium is shown in Fig. 29.31.

CANDIDA ALBICANS SKIN INFECTION

Candida albicans is an opportunistic human fungal pathogen, which can switch from an oval yeast form to a filamentous hyphal form, which is characteristic

of filamentous fungi. It affects children and immunocompromised elderly persons.

- Patient presents with papules, pustules and ulcers, red erosive skin lesions, and satellite arrangement of papulopustular lesions.
- Histologic examination of skin lesion shows epidermal hyperplasia and spongiosis, *Candida albicans* pseudohyphae, acute and chronic inflammatory cells. Clinical manifestations of *Candida albicans* and oral candidiasis by *Candida albicans* are shown in Figs 29.32 and 29.33.

DEEP SUBCUTANEOUS FUNGAL INFECTIONS

Majority of cases in clinical practice are seen due to superficial fungal infections. Invasive subcutaneous mycoses are important to recognize and treat, as deep fungal infections often have significant morbidity and mortality. Deep fungal infections demonstrate species-specific syndromes, which may be identified by clinical and histologic features in addition to serological evaluation and culture.

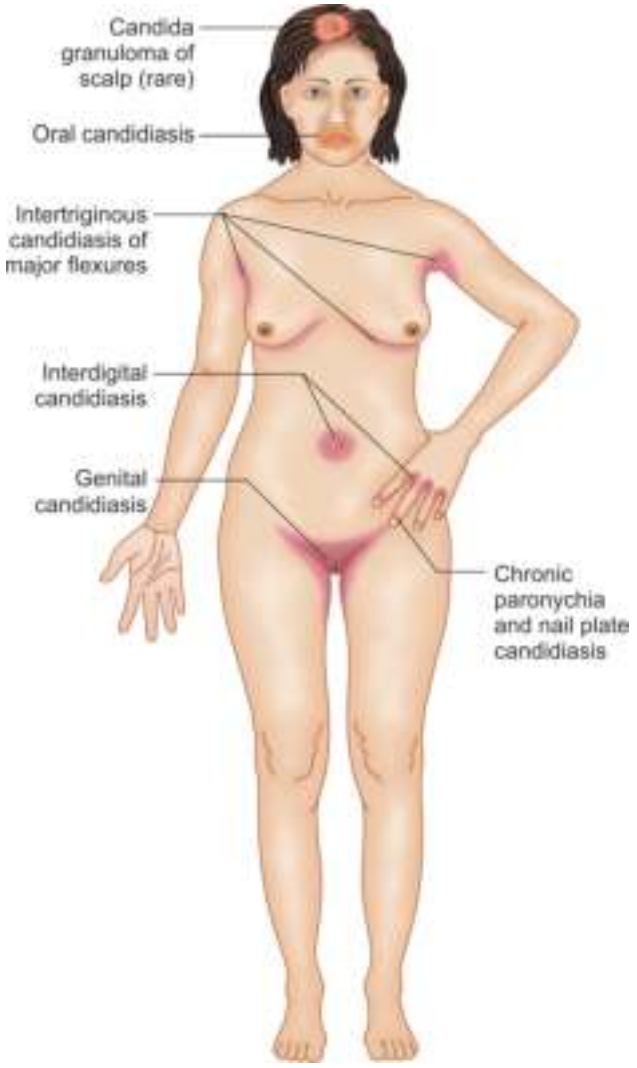


Fig. 29.32: Clinical manifestations of *Candida albicans*. It depicts the sites of involvement in candidiasis.



Fig. 29.33: Oral candidiasis by *Candida albicans*. Oral mucosa shows white deposits present on the labial mucosa and tongue, which on removal leaves an erythematous mucosal surface.

- Identification of the common inoculation subcutaneous mycoses, as well as those associated with pulmonary primary infection and dissemination to the skin is important, as treatments vary due to causative organism and clinical settings.
- Deep fungal infections include mycetoma, chromoblastomycosis, sporotrichosis, zygomycosis, mucormycosis and phaeohyphomycosis.

Mycetoma

Mycetoma is a localized chronic, suppurative, and deforming granulomatous inflammation of subcutaneous tissue, skin and bones, mainly of feet.

- Diagnosis of mycetoma can be made by the classic triad of painless soft tissue swelling, draining sinus tracts and extrusion of grains depending on causative organisms. Mycetoma is caused by various species of fungi and bacteria, which occur as saprophytes in soil or on the plants.
- Actinomycotic mycetoma is caused by aerobic species of gram-positive actinomycetes belonging to genera *Nocardia asteroides*, *Nocardia brasiliensis*, *Actinomedura medurae* and *Streptomyces somaliensis*.
- Gram-negative eumycotic mycetoma is associated with a variety of fungi, the most common being *Madurella mycetomatis*, that extrudes black to brown granules through sinus tracts. Fungi causing mycetoma and the type of granules produced are given in Table 29.13.

Table 29.13 Fungi causing mycetoma and the type of granules produced

Causative Organisms	Color of Granules
Actinomycotic mycetoma	
Actinomadura medurae	White
Actinomadura pelletieri	Red to pink
Streptomyces somaliensis	White to yellow
Nocardia asteroides	White
Nocardia brasiliensis	White
Nocardia otitidiscaviarum	White
Eumycotic mycetoma	
Madurella mycetomatis	Black to brown
Madurella grisea	Black to brown

Pathogenesis

The fungus is usually implanted after a penetrating injury while performing agricultural work barefoot or through preexisting abrasions, which leads to invasive and widespread infection. Predisposing factors are poor general health, diabetes, and malnutrition.

- Neutrophil degranulation and adherence to the grain surface, resulting in gradual disintegration of the grain. Outside the zone of neutrophils is a zone of granulation tissue containing macrophages, lymphocytes, and plasma cells.
- After disappearance of the neutrophils, macrophages participate in clearance of granules, which is followed by formation of epithelioid cell granulomas. T cell response plays an important part in the development of mycetoma. Most common sites of mycetoma are lower extremities commonly in the foot (70%) followed by hand, head, neck, chest, shoulder, and arm.

Clinical Features

Patient presents with small, painless, subcutaneous nodule at the site of injury, which over time softens and ulcerates to form draining sinuses to discharge a viscous, purulent, or serosanguineous fluid containing characteristic granules on the skin surface. Clinical photograph of mycetoma foot is shown in Figs 29.34 and 29.35.

Mucormycosis

Mucormycosis is a serious but rare fungal infection caused by a group of molds called mucoromycetes.

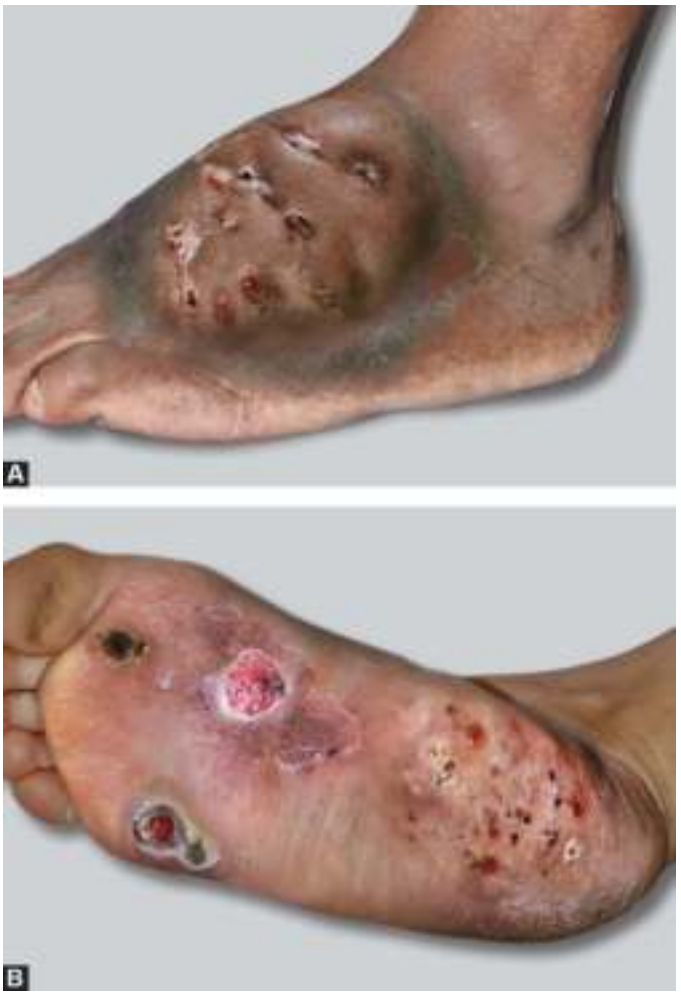


Fig. 29.34: Clinical photograph of mycetoma foot. (A) A case of eumycetoma with chronic swelling of the dorsum of the foot and sinuses, (B) a case of eumycetoma with visible black granules.



Fig. 29.35: Gross morphology of mycetoma foot. Cut surface of mycetoma foot shows black granules. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

- Mucormycosis is characterized by hyphae growing in and around blood vessels, resulting in formation of blood clots and surrounding tissue death due to ischemia, which can be potentially life-threatening in severely immunocompromised (HIV) or uncontrolled diabetic individuals, organ transplant, chronic kidney disease, long-term corticosteroid and immunosuppressive therapy.
- Angioinvasion of fungus is common and may elicit intravascular thrombus. There is often a marked inflammatory response, which may be neutrophils and formation of epithelioid cell granulomas. The fungus most commonly affects the sinuses, brain or the lungs after inhalation of fungal spores from the air, or the skin after the fungus enters the skin through a cut, burn, or other type of skin injury.
- Infection of the oral cavity or brain is the most common site; however, the fungus can also infect gastrointestinal tract, skin, and other organ system. Histology of mucormycosis is shown in Fig. 29.36.

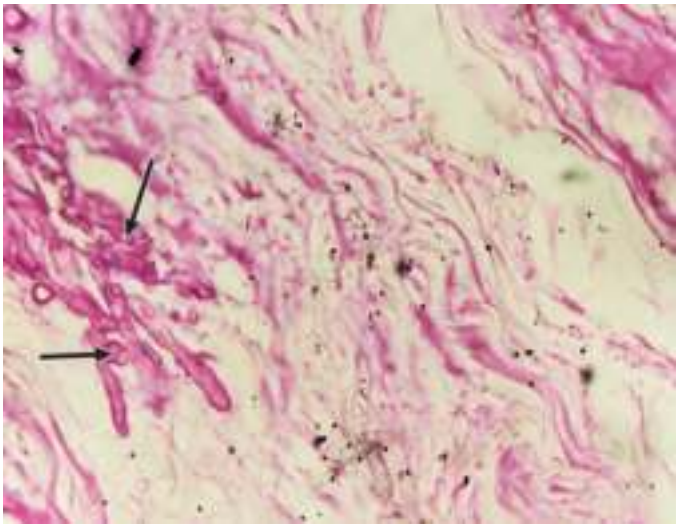


Fig. 29.36: Histology of mucormycosis. Hematoxylin and eosin-stained section demonstrates broad, irregular nonseptate hyphae of mucormycosis. Angle of the branching is greater than other organs. There is often a marked inflammatory response which may be neutrophils and granulomas (arrows) (400X).

INFLAMMATORY DERMATITIS

INFLAMMATORY DERMATITIS: OVERVIEW

Cutaneous inflammation can lead to many skin disorders. Lichenoid/interface dermatoses are characterized lymphocytic infiltration in the upper dermis leading to damage to basal keratinocytes. Psoriasiform dermatoses are characterized by expanded space between adjacent keratinocytes of the spinous layer of epidermis. Vesicobullous dermatoses are characterized by vesicles or bullae formation. Granulomatous dermatoses are characterized by granulomatous inflammation. Vasculopathic dermatoses are characterized by damage to endothelial cells, which become dysfunctional. Panniculitis dermatoses are characterized by inflammatory changes to the septa or lobules of the subcutaneous adipose tissue. Inflammatory dermatoses terminology and examples are given in Table 29.14. Nine basic patterns of inflammatory skin disorders are given in Table 29.15. Blistering diseases of the skin are given in Table 29.16.

Table 29.14 Inflammatory dermatoses terminology and examples

Histologic Features	Skin Disorders	Comments
Lichenoid/interface dermatoses		
Damage to basal keratinocytes	Lichen planus	Multiple papules and plaques on skin, hair, nails and mucous membrane
Lymphocytic infiltration in the upper dermis	▪ Lichen planus like keratosis ▪ Erythema multiforme (Stevens-Johnson syndrome) ▪ Lupus erythematosus ▪ Graft-versus-host disease	▪ Single red scaly plaque on sun-exposed regions ▪ Symmetrical cutaneous sloughing lesions on palms and soles ▪ Butterfly-shaped skin lesions and organs involvement ▪ Acute GVHD: Eruptions on palms, soles, head and neck ▪ Chronic GVHD: It may resemble lichen planus or lichen morphea

Contd...

Table 29.14 Inflammatory dermatoses terminology and examples (*Contd...*)

Histologic Features	Skin Disorders	Comments
Psoriasiform dermatoses		
Expanded space between adjacent keratinocytes of the spinous layer of epidermis	▪ Eczema/atopic dermatitis ▪ Pityriasis rosea ▪ Eosinophilic spongiosis	▪ Erythematous scaly plaques with variable distribution ▪ Erythematous scaly plaques (Christmas tree-like pattern) on back ▪ Lesions vary based on etiology
Vesicobullous dermatoses		
Vesicles or bullae formation	▪ Bullous pemphigoid ▪ Pemphigus vulgaris ▪ Porphyria cutanea tarda ▪ Epidermolysis bullosa acquisita ▪ Dermatitis herpetiformis	▪ Generalized tense blisters on erythematous base in elderly ▪ Painful oral erosions and flaccid cutaneous blisters that extend with lateral pressure (Nikolsky's sign) ▪ Blistering of skin-exposed on hand and face ▪ Blisters on site of trauma ▪ Pruritic small vesicles
Granulomatous dermatoses		
Epithelioid cell granuloma	▪ Necrobiotic granuloma (e.g. granuloma annulare) ▪ Necrobiosis lipoidica ▪ Sarcoidosis	▪ Erythematous plaques or subcutaneous nodules on localized or generalized sites ▪ Plaques with depressed yellow center in diabetes mellitus ▪ Variable lesions (annular, hypopigmented spots, ichthyosis-like, alopecia, subcutaneous nodules)
Vasculopathic dermatoses		
Damage to endothelial cells (dysfunctional endothelial cells)	▪ Acute vasculitis of small blood vessels (leukocytoclastic vasculitis) ▪ Granuloma faciale ▪ Lymphocytic vasculitis ▪ Vaso-occlusive vasculopathies ▪ Neutrophilic dermatosis (Sweet's syndrome) ▪ Urticaria ▪ Pigmented purpuric dermatosis	▪ Palpable purpura on extremities ▪ Reddish brown plaques on face ▪ Variable morphology ▪ Purpura, ulcer or infarct ▪ Abrupt onset of erythematous plaques on head, neck and upper extremities ▪ Pruritic wheals ▪ Multiple clinical variants
Panniculitis dermatoses		
Inflammatory changes to the septa or lobules of the subcutaneous adipose tissue	▪ Erythema nodosum (septal) ▪ Erythema induratum/nodular fasciitis ▪ Subcutaneous fat necrosis of the newborn ▪ Membranous lipodystrophy	▪ Single or multiple painful erythematous nodules on anterior leg (shin) ▪ Multiple erythematous plaques on posterior leg (calf) ▪ Firm, nodular areas of body ▪ Variable lesions on lower extremities

Table 29.15 Nine basic patterns of inflammatory skin disorders

▪ Superficial and deep perivascular dermatitis	▪ Intraepidermal vesicular and pustular dermatitis
▪ Vasculitis	▪ Perifolliculitis and folliculitis
▪ Nodular and diffuse dermatitis	▪ Fibrosing dermatitis
	▪ Panniculitis

Contd...

Table 29.16 Blistering diseases of the skin

Feature	Bullous Pemphigoid	Pemphigus Vulgaris	Dermatitis Herpetiformis
Epidemiology	Peak >60 years of age, most common bullous autoimmune disease	Peak incidence 40–60 years of age	Peak 15–40 years of age
Skin areas involved	Extremities, axillae and groin; oral mucosa in 10–15%	Oral mucosa (prominent >50% of cases), scalp, face and chest	Extensor surfaces; buttocks
Clinical features	Initially urticarial lesions resulting in large blisters, intensely pruritic lesions	Painful, flaccid intradermal blisters resulting in rupture, pruritus absent	Pruritic eruption on buttocks and extensor surface
Antibody target	Hemidesmosomes (bullous pemphigoid antigen of the epithelial membrane antigen)	IgG antibodies directed against desmoglein-3 and desmoglein-1 in desmosomes	Gliadin and reticulin (associated with HLA-DR3 and HLA-DQ2)
Histology	Subdermal clefting and blistering	Intradermal suprabasal clefting and blistering just above basal layer of the epidermis	Subdermal clefting and blistering
Pattern of immunofluorescence	Linear pattern of IgG and C3 along dermoepidermal junction	Deposition of IgG in intercellular spaces of the dermis giving net-like pattern	IgA positive clusters at tips of dermal papillae
Nikolsky sign	Negative	Positive	Negative
Tzanck test	Negative	Positive	Negative
Treatment	Topical steroids (first-line), systemic glucocorticoids (second-line)	Topical steroids (first-line), immunosuppressive drugs (second-line)	Gluten-free diet, dapsone (first-line), topical steroids and low intake of iodine
Prognosis	Benign disease, usually responds to treatment	Often fatal without treatment	Lifelong disorder requiring treatment

Nikolsky sign refers to slight rubbing of the skin resulting in exfoliation of the outermost layer.

INFLAMMATORY DERMATITIS: CLINICAL ENTITIES

PSORIASIS

Psoriasis is a chronic inflammatory multisystem non-pruritic disease affecting skin, nails, and musculocutaneous system. Psoriasis is characterized by persistent epidermal hyperplasia resulting in well-demarcated erythematous papules and plaques covered with silvery white scales commonly on the dorsal extensor cutaneous surfaces of the elbows and knees, as well as the scalp and sacral region.

- Psoriasis has complex pathogenesis include genetic (Cw6 gene mutation strongest association, HLA-B27, HLA-B13, and HLA-DR7), and autoimmune disorder. On histologic examination, psoriatic lesion is made of acanthosis, perivascular inflammation, dilated vessels in the papillary dermis and thinning of the suprapapillary plates.
- Scratching of silvery white scaly lesion causes rupture of small blood vessels in the suprapapillary thinned plates resulting in pinpoint hemorrhages known as 'Auspitz sign'. Clinical photograph of psoriasis is shown in Fig. 29.37.



Fig. 29.37: Clinical photograph of psoriasis. Multiple erythematous plaques of psoriasis are demonstrated (arrows). (*Courtesy*: Dr. Krishna Deb Barman, Director Professor of Dermatology, Maulana Azad Medical College and Associated Lok Nayak Hospital, New Delhi.)

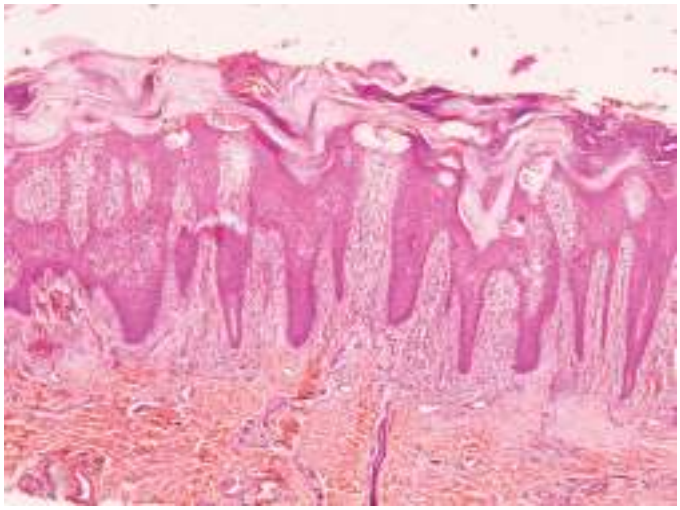


Fig. 29.38: Histology of psoriasis. Psoriasis is characterized by downward elongation of the rete ridges with thinning of overlying stratum granulosum, with parakeratosis above this. Small aggregates of neutrophils with surrounding spongiform change are seen in the superficial epidermis. Capillaries within dermal papillae are brought close to the surface (400X).

Surgical Pathology: Psoriasis

Light Microscopy

- Skin biopsy from patient with psoriasis shows thickening of epidermis, several folds, hyperkeratosis, parakeratosis, regular acanthosis and suprapapillary thinning (portion of epidermis over papillae thinned out).
- Neutrophils may become localized in the epidermal spinous layer or in small '*Munro microabscesses*' in the stratum corneum.
- The dermis below the papillae exhibits a varying number of mononuclear inflammatory cells around the superficial vascular plexuses. The capillaries of the papillae are dilated and tortuous. Histology of psoriasis is shown in Fig. 29.38.

ERYTHEMA MULTIFORME

Erythema multiforme (EM) is a cutaneous and mucosal hypersensitivity reaction with characteristic lesions in target triggered by certain antigenic stimuli. It is interface dermatitis, in which primary pathology is related to the dermoepidermal junction involving basal layer and the papillary dermis. Erythema multiforme is associated with herpes simplex virus infection.

- *Mycoplasma pneumoniae*, drugs, autoimmune disorders (concomitant connective tissue disorders) and malignancy have been implicated in pathogenesis of erythema multiforme.
- Erythema multiforme mainly involves the skin (macules, papules, bullous and necrotic lesions) and sometimes the mucosa. Skin lesions can be isolated, recurrent, or persistent. Disorder can affect young adults and children.

Clinical Features

Patient presents with symmetrical <3 cm of skin lesions on the extensor surface of the extremities (palms, and back of hands and feet without involvement of trunk) and spread centripetally.

- Typical skin lesion of erythema multiforme is round and regular three concentric circles and a well-defined border. The peripheral ring is erythematous, sometimes microvesicular, middle zone is clear, edematous and palpable; and the central zone is erythematous, covered by a blister. In erythema multiforme, skin lesions are typically fixed for a minimum of seven days.
- Mucosal lesions with fibrin-whitish coating occur in mouth (cheek, palate), ocular mucous membrane and genitalia. Initial mucosal lesions are bullous, then quickly turn into painful erosions. Mucosal lesions are frequently painful, whereas skin lesions painless.
- Pulmonary lesions occur due to *Mycoplasma pneumoniae* and manifest as cough and dyspnea. Erythema multiforme is most often associated with a target lesion resembling an archer's bull's eye. Clinical photograph of erythema multiforme is shown in Fig. 29.39.

Treatment

Erythema multiforme is treated by topical steroids or antihistamines and treating the underlying etiology. Recurrent erythema multiforme associated with the herpes simplex virus is treated by prophylactic antiviral therapy. Severe mucosal erythema multiforme can require hospitalization for intravenous fluids and repletion of electrolytes.



Fig. 29.39: Clinical photograph of erythema multiforme. Typical lesion of erythema multiforme is round and regular three concentric circles and a well-defined border. The peripheral ring is erythematous, sometimes microvesicular, middle zone is clear, edematous and palpable; and the center zone is erythematous and covered by a blister. Center cyanosis surrounded by red ring in this case (arrows).

LICHEN PLANUS

Lichen planus (LP) is an inflammatory disorder of the skin and mucous membranes of unknown etiology.

- Patient presents with pruritic, violaceous papules and plaques on the inner side of the wrist or blisters on palms, lower back, ankles and soles. A lattice-like network of white lines called Wickham striae overlies the lesions in oral mucosa, drug-induced lichen planus, or lichenoid drug eruption, may be indistinguishable from idiopathic lichen planus.
- Clinical course of lichen planus varies significantly. Cutaneous lesions clear within 1–2 years after initial presentation in majority of the patients, however, recurrences are common and the residual cutaneous lesions become hyperpigmented. While oral mucosal lesions may or may not remit.
- Drug-induced lichen planus gradually undergoes resolution after withdrawal of the causative therapeutic agents.

Pathogenesis

Pathogenesis of lichen planus is not known. It is "considered to be autoimmune disorder mediated by T cells.

- Prevailing theory is that lichen planus occurs due to exposure to viruses (HCV), drugs or contact allergens, which cause alteration of epidermal self-antigens and activation of CD8+ cytotoxic T cells. The altered self-antigens cross-react with normal self-antigens present on the basal keratinocytes resulting in CD8+ cytotoxic T cells targeting and apoptosis.
- More commonly associated drugs linked to lichen planus include antimalarials, NSAIDs, thiazide diuretics, angiotensin converting enzyme inhibitors (ACEIs), quinidine, β-blockers, tumor necrosis factor-α (TNF-α) inhibitors, and gold. Oral lichen planus is correlated with contact metal allergens (mercury, copper, gold) used in dental restorations. Removal of the sensitizing metal leads to clearance of oral lesions.

Clinical Features

Lichen planus can display a variety of lesion types. Patient usually presents with purple, polygon-shaped, pruritic papules of few mm wide and covered in fine white lines known as Wickham striae. Lesions are firm on palpation. Hypertrophic lichen planus is usually present on shins and ankles, and is red, red-brown, or yellow-grey papules and plaques that coalesce with a thickened or verrucous surface. Ulcerative lichen planus is seen on foot and between toes. Bullous lichen planus is seen on legs.

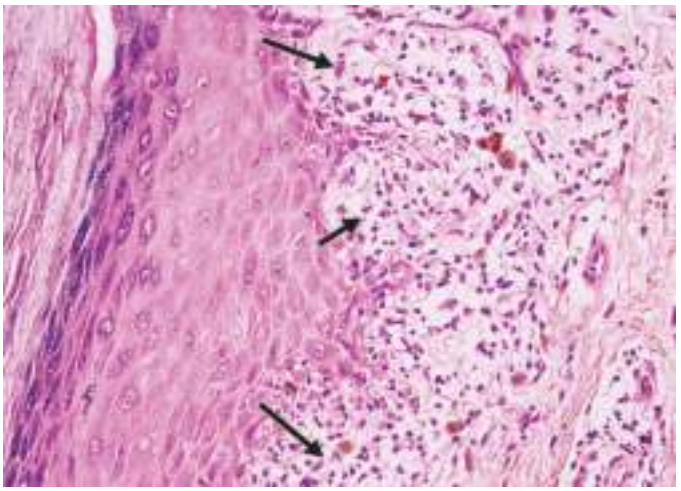


Fig. 29.40: Histology of lichen planus. Hematoxylin and eosin-stained section shows lymphoid aggregates along epidermis–dermis junction destroying basal layer of epidermis (arrows) (400X).

Surgical Pathology: Lichen Planus

Light Microscopy

- Histologic examination of lichen planus lesion on skin or oral and genital mucosa shows thickening of stratum corneum without nuclei, hyperkeratosis without parakeratosis, alteration or loss of rete ridges resulting in a sawtooth appearance, dense band-like lymphoid infiltrates along the epidermal–dermal junction, destroying the basal layer of the epithelium.
- Apoptotic keratinocytes are often demonstrated near the basal layer and are termed colloid or civatte bodies. Histology of lichen planus is shown in Fig. 29.40.

Immunofluorescence Microscopy

Direct staining by immunofluorescence microscopy may display colloid bodies with irregular deposits of IgA, IgM, IgG, or C3.

Treatment

Cutaneous lichen planus typically clears within 1–2 years. Treatment is focused on reducing pruritus by topical steroids for 2–4 weeks. Intralesional steroid injection and oral steroids are administered in patients with inadequate response to topical steroids tapered over 2–6 weeks.

PEMPHIGUS VULGARIS

Pemphigus vulgaris, an autosomal acantholytic disease, is associated with IgG antibodies against desmosomal glycoprotein desmoglein-3 and less commonly desmoglein-1, which is part of cadherin family of cell–cell adhesion molecules present in desmosomes.

- Antigen–antibody complex results in acantholysis (loss of cell–cell adhesion between keratinocytes) and formation of severe intraepidermal bullae as a result of loss of cell-to-cell adhesion of epithelial cells in lower epidermis or mucosa. It is augmented by the release of plasminogen activator (plasmin), that causes proteolysis of intercellular substance and dyshesion. Disease most often occurs in persons 30–60 years of age.
- Desmoglein-3 is a calcium-binding transmembrane glycoprotein component of desmosomes in epithelial cells in lower epidermis and mucosa, primarily responsible for maintaining intercellular adhesion in stratified squamous epithelia, such as the skin and oral mucosa.
- Patient initially develops lesions in the oral mucosa followed by extensive involvement of skin, which often rupture, leaving large denuded surfaces subject to secondary infection.

Pathology Pearls: Pathophysiology of Pemphigus Vulgaris

Pemphigus can be classified into three major forms: pemphigus vulgaris, pemphigus foliaceus and paraneoplastic pemphigus. Pemphigus vulgaris and pemphigus foliaceus are the originally characterized as classic forms of pemphigus. On histologic examination, pemphigus vulgaris blisters develop deep in the epidermis or oral epithelium above the basal layer, whereas pemphigus foliaceus blisters occur in the superficial layers of the epidermis, mostly in the granular layer.

- **Pemphigus vulgaris:** In pemphigus vulgaris, the lesions are present as mucosal dominant, mucocutaneous, and cutaneous.
  - Mucosal lesions are characterized by formation of blisters in the deep layers of the oral mucosa owing to anti-desmoglein-3 IgG autoantibodies.
  - Cutaneous lesions are characterized by formation of blisters in the deep layers of the epidermis owing to anti-desmoglein-1 and pathogenically weak anti-desmoglein-3 IgG autoantibodies. Clinical photograph of pemphigus vulgaris is shown in Fig. 29.41.
- **Pemphigus foliaceus:** Pemphigus foliaceus is caused by humoral autoimmune response; no apparent mucosal involvement blisters in the superficial layers of epidermis owing to anti-desmoglein-1 IgG autoantibodies.
- **Paraneoplastic pemphigus:** Paraneoplastic pemphigus is distinguished by the presence of a known-associated or occult-associated neoplasm, usually of lymphoid tissue and caused by humoral and cellular responses.
  - Paraneoplastic pemphigus is characterized by formation of blisters in the deep epithelial layers of mucosal and cutaneous regions owing to anti-desmoglein-3 and anti-desmoglein-1 IgG autoantibodies in combination interface dermatitis (vacuolization of basal cells, apoptosis of keratinocytes and abnormal keratinization, and inflammatory infiltrate owing to self-reacting T cells).
  - The patients also develop IgG autoantibodies against other several proteins (protease inhibitor and cytoplasmic proteins of the plakin family).

Surgical Pathology: Pemphigus Vulgaris

Light Microscopy

In pemphigus vulgaris, distinctive rounded keratinocytes (termed acantholytic cells) are shed into the vesicle during the process of dyshesion. Skin lesion shows prominent intra-epidermal acantholysis with intact basal layer as a floor and the remaining epidermis as a roof. The skin blister contains lymphocytes, macrophages, eosinophils, and neutrophils. Histology of pemphigus vulgaris is shown in Fig. 29.42.

Immunofluorescence Microscopy

Immunofluorescence microscopy shows deposition of IgG and C3 around cell membranes highlighting intercellular pattern of staining exhibiting honeycomb appearance.

BULLOUS PEMPHIGOID

Bullous pemphigoid is the most common autoimmune subepidermal vesicular (blisters) dermatitis in elderly persons of 60–80 years. It represents 80% of subepidermal blister forming disorders. Complement-fixing IgG antibodies are directed against basement membrane proteins, BPAG1 and BPAG2.

- Bullous pemphigoid resembles pemphigus vulgaris, but of less severity. Acantholysis is absent in bullous pemphigoid but present in pemphigus vulgaris.
- Before skin blisters develop, pruritus, and urticarial lesions may be present. The blisters tend to develop



Fig. 29.41: Clinical photograph of pemphigus vulgaris. Raw, erythematous and crusted lesions of pemphigus vulgaris are seen.

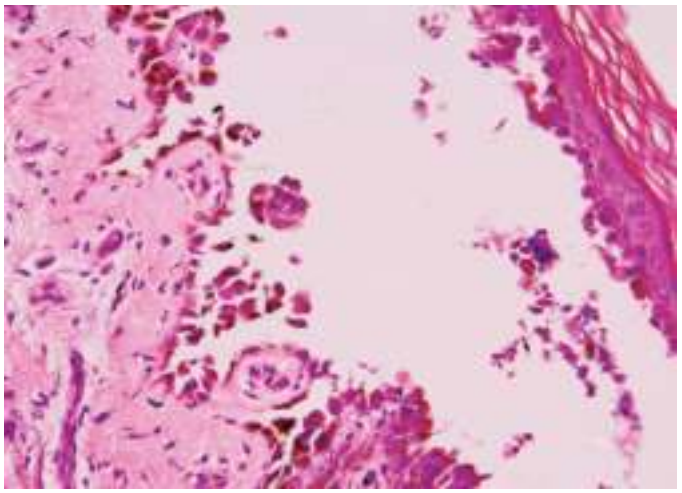


Fig. 29.42: Histology of pemphigus vulgaris. Hematoxylin and eosin stained section shows prominent intraepidermal acantholysis with intact basal layer as a floor and the remaining epidermis as a roof. The blister contains lymphocytes, macrophages, eosinophils, and neutrophils (400X).

in these regions. Since the entire epidermis forms the blister roof, hence the blisters become very stable and tense, due to fluid level, and can reach 10 cm in diameter. Oral mucosal involvement occurs in 20%; rarely presenting sign.

- Patient presents with tense bullae and intense generalized pruritus. Skin biopsy for hematoxylin and eosin staining demonstrates a subepidermal split with eosinophils.
- Direct immunofluorescence microscopy shows the autoantibodies against the basement membrane zone. Enzyme-linked immunosorbent assay (ELISA) testing is also useful in diagnosing bullous pemphigoid. Treatment depends on the severity of the disease.

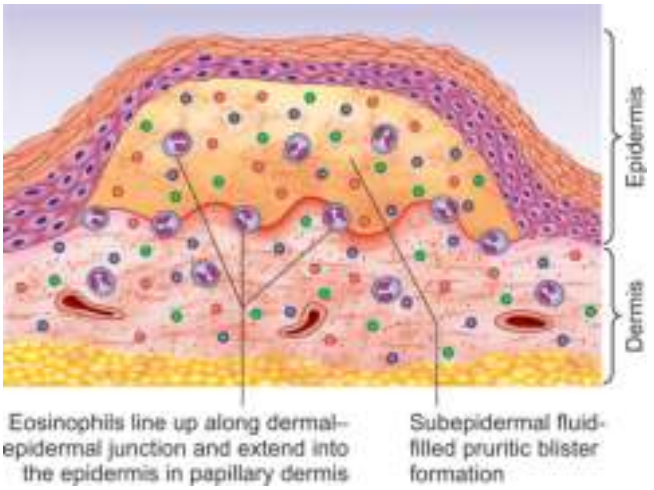


Fig. 29.44A: Schematic representation of bullous pemphigoid. This autoimmune disorder occurs due to autoantibodies against hemidesmosomal antigen, bullous antigens 1 and 2 with subepidermal fluid-filled pruritic blister formation and eosinophilic spongiosis.

- Standard treatment involves topical or systemic immunosuppressive agents. Prognosis is variable hence long-term monitoring is essential. Clinical photograph of bullous pemphigoid is shown in Fig. 29.43. Schematic representation of bullous pemphigoid is shown in Fig. 29.44A.

Surgical Pathology: Bullous Pemphigoid
Light Microscopy
Skin biopsy in bullous pemphigoid shows subepidermal bullae, with a characteristic inflammatory infiltrate of eosinophils in the surrounding dermis. Histology of bullous pemphigoid is shown in Fig. 29.44B.
Immunofluorescence Microscopy
In contrast to pemphigus vulgaris, immunofluorescence microscopy demonstrates deposition of C3 and IgG along the epidermal basement membrane in bullous pemphigoid.



Fig. 29.43: Clinical photograph of bullous pemphigoid. Tense blisters are present on an erythematous base in this case.

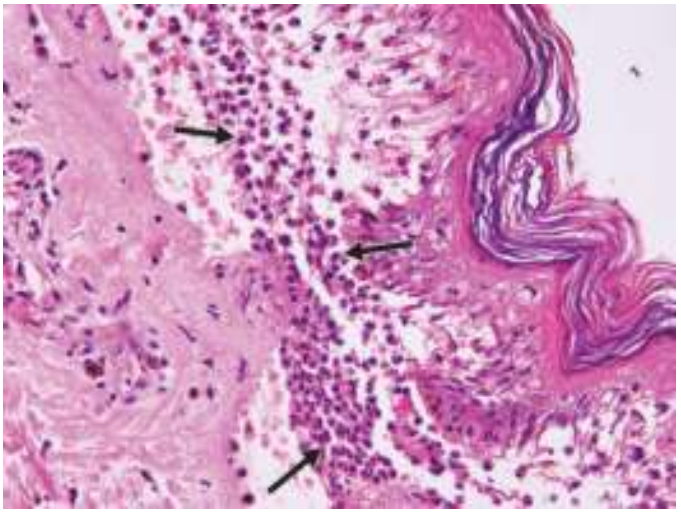


Fig. 29.44B: Histology of bullous pemphigoid. Hematoxylin and eosin-stained section shows subepidermal bullae, with a characteristic inflammatory infiltrate of eosinophils in the surrounding dermis (arrows) (400X).

NON-NEOPLASTIC MELANOCYTIC DISORDERS AND MELANOCYTIC NEOPLASMS

NON-NEOPLASTIC MELANOCYTIC DISORDERS

Biology of melanoblasts, melanocytes and melanosomes are discussed below.

- **Melanoblasts:** These cells are derived from neural crest. Melanoblasts migration/differentiation/survival in the epidermis is influenced by numerous signaling molecules synthesized by neighboring cells, which include Wnt, endothelin, bone morphogenic proteins, stem cell factor, c-KIT factor and hepatocyte growth factors. The growth factors bind to cognate cell surface receptors and induce intracellular signaling to influence gene transcription and protein synthesis. Endothelin mutation results in Hirschsprung disease and Waardenburg syndrome.
- **Melanocytes:** These cells populate the epidermis, hair follicle, eye, meninges, cochlea. Melanocytes synthesize melanin, an indole derivative of 1-3,4 dihydroxyphenylalanine (DOPA). Melanocyte stem cells are present in the hair follicle bulge. Melanocytes stain positive for TRP-2, an early marker of commitment to the melanocytic lineage, but negative for Ki-67 proliferative marker. Melanocytic lineage characterizes melanocytes migrating down to the dermal papilla during the anagen phase of cell cycle.
- **Melanosomes:** These cells are organized into supranuclear 'caps' within keratinocytes and demonstrated by Fontana-Masson stain. These cells display four maturation stages. Melanosomes contain structural matrix proteins, pH maintaining proteins, melanogenic enzymes, and free-radical scavengers.
  - Melanosomes are transported to epidermal keratinocytes and melanocyte dendritic tips.
  - Melanosomes absorb ultraviolet radiation in the skin against photodamage.
  - Melanosomes synthesize two types of melanins: (a) eumelanin dark brown-black and insoluble; and (b) phaeomelanin-light red-yellow sulfur-containing and soluble.
  - Melanin biosynthesis begins with the amino acid tyrosine that is converted to DOPA (3,4-dihydroxyphenylalanine) in the rate-limiting step of melanin biosynthesis. Melanin is stored in melanosomes.

ALBINISM

Albinism is usually inherited recessive trait disorder. It occurs due to failure of pigment synthesis by otherwise intact melanocytes as a result of failure of conversion of tyrosine to dihydroxyphenylalanine (DOPA). Clinical features of albinism include lightly pigmented or nonpigmented skin and silver-white or light color hair. Ocular nystagmus and reduced visual acuity are important features of albinism. Albinism occurs in two ocular and oculocutaneous variants.

- Ocular albinism is a melanin dysfunction that is limited to the eyes.
- Oculocutaneous albinism is an autosomal recessive disorder characterized by melanin synthetic defect that involves the eyes, skin, and hair. Risk of development of actinic keratosis, basal cell carcinoma, squamous cell carcinoma, and malignant melanoma is increased due to sensitivity of skin to sunlight.
  - **Oculocutaneous albinism type 1:** It occurs due to loss of function of the melanocyte enzyme tyrosinase resulting from mutation of the TYR gene on chromosome 11q21.
  - **Oculocutaneous albinism type 2:** It occurs due to OCA2 (P) gene mutation mapped on chromosome 15q.
  - **Oculocutaneous albinism type 3:** It occurs due to OCA3 (OMIM) gene mutation located on chromosome 9p23.
  - **Oculocutaneous albinism type 4:** It occurs due to MATP gene mutation mapped on chromosome 5p.

VITILIGO

Vitiligo is the most frequent depigmented acquired disorder involving multiple genes and environmental factors. It is characterized by progressive autoimmune mediated destruction of epidermal melanocytes in discrete areas of skin. It is sometimes associated with autoimmune disorders, such as Graves' disease and Addison disease. Antimelanocyte antibodies are sometimes demonstrated. Toxic substances may cause destruction of melanocytes resulting in vitiligo.

Clinical Features

Patient presents with acquired milk-white macules with fairly homogenous depigmentation and well-defined borders.

- **Vitiligo vulgaris:** It is characterized by multiple scattered lesions distributed in a more or less symmetrical pattern.
- **Acrofacial vitiligo:** It affects the distal end of fingers and facial orifices in a circumferential pattern.
- **Mixed vitiligo:** It is a combination of acrofacial and vulgaris, or segmental and acrofacial types.
- **Vitiligo universalis:** It is the severe form of vitiligo and characterized by complete or nearly complete depigmentation of the whole body.

- **Mucosal vitiligo:** It is confined to mucous membrane.
- **Focal vitiligo:** It is characterized by the presence of one/few macules in one area.

Clinical Course

Clinical course of any type of vitiligo is unpredictable and gradually progressive. It is difficult to control the disease with therapy.

Treatment

First-line treatment for vitiligo includes topical corticosteroids, calcineurin and ultraviolet B, systemic psoralen and ultraviolet A (PUVA). Second-line treatment includes topical calcineurin, topical PUVA and excimer laser.

FRECKLE (EPHELIS)

Freckle is produced by an increase in number of intraepidermal melanocytes laden with melanin pigment within basal keratinocytes. Freckle occurs on the sun-exposed skin areas like the cheeks, nose, and forehead in children and older adults. Newly developed lasers produce excellent results with minimal complications.

LENTIGO SIMPLEX

Lentigo simplex is a benign hyperpigmented macule located anywhere on the body in early life. Lesion results from a mild hyperplasia of melanocytes in the epidermis producing increased amounts of melanin not associated with sun exposure. Lesion occasionally forms in cutaneous scars and may be associated with psoralen/ultraviolet light therapy.

Clinical Features

Patient presents with asymptomatic, well-circumscribed, symmetric, homogeneous, light brown to black single or multiple macules measuring <5 mm in size on the trunk, extremities, genitalia, and mucous membranes. Lesion on mucous membranes can appear irregular with increased size, irregular borders, and heterogeneous pigmentation. Lentigo simplex may evolve into junctional nevi, which does not progress melanoma. Multiple lesions in lentigo simplex are associated with rare genetic disorders given in Table 29.17.

Table 29.17 Multiple lesions in lentigo simplex are associated with rare genetic disorders

Genetic Disorder	Clinical Features
Carney complex	Lentigines, atrial myxoma, mucocutaneous myxoma, and nevi
Peutz-Jeghers syndrome	Lentigines (perioral and oral), multiple gastrointestinal polyps, and visceral tumors (pancreas, ovary, testes)
Xeroderma pigmentosum	Lentigines on sun-exposed skin and multiple skin cancers
Cronkhite-Canada syndrome	Lentigines (buccal mucosa, face, palmoplantar), alopecia, nail dystrophy, and intestinal polyps

MELANOCYTIC NEOPLASMS

CONGENITAL MELANOCYTIC NEVI

Congenital melanocytic nevi are observed at birth represent a developmental abnormality of normal melanocytic development. These lesions occur presumably due to N-RAS gene mutation that occur in the progenitor cell, that result in the abnormal extensive accumulation of cutaneous melanocytic cells along migration pathways during normal development of melanoblasts in 1% of newborns. Congenital melanocytic nevi are formed between 5 and 24 weeks of gestation and present at birth or become apparent within the first year of life.

Clinical Features

The appearance of skin lesions varies considerably on the basis of morphology, texture, location, and size. Initially newborns present with nevi light in color, flat, or hairless; that can become round or oval with smooth, well-defined borders, more pigmented, raised papule or rugose, or verrucous and acquire long, coarse hairs measuring 2–20 cm in diameter.

- Congenital melanocytic nevi can appear anywhere on the body. The most common anatomic location for a giant congenital melanocytic nevus is the posterior trunk, followed by the legs, arms, head, and neck.
- The lifetime risk for malignant transformation of congenital melanocytic nevi depends largely on size of the skin lesion.

Treatment

Congenital melanocytic nevi are treated by observation of lesions, dermabrasion, chemical peel, laser ablation, and complete excision. The primary aim of management is to remove the risk for malignant transformation while minimizing functional deformity and improving cosmesis. Approach to the patient with congenital melanocytic nevi is given in Table 29.18.

MELANOCYTIC NEVUS (COMMON MOLE)

The benign melanocytic nevus (common mole) is the most common tumor, which rarely transforms to melanoma. Ultraviolet radiation (UVR) is involved in the etiology of a subset of nevi.

Table 29.18 Approach to the patient with congenital melanocytic nevi

Skin Lesion	Approach
Pigmented macular, popular or extension at birth	Small biopsy indicated in case of differential diagnosis of café' au lait macule (CALM), dermal melanocytosis (Mongolian spot) or epidermal nevus
Cranial or midline large lesions with increased risk of neurocutaneous/leptomeningeal involvement	MRI performed
One must analyze risk of melanoma	Excision of the lesion
One must analyze change in color of nonuniform lesion	Excision of the lesion

- Melanocytes are special dendritic cells derived from neural crest that have the ability to spread over the entire surface of the body.
- Nevus cells derived from melanocytes and ordinarily occur in clusters or nests.
- Depending on the involvement of skin layer melanocytic nevi are of three types: (a) junctional nevus—nevus cells are confined to the epidermal–dermal junction, (b) compound nevus—nevus cells are confined both at the epidermal–dermal junction and in the dermis; (c) intradermal nevus—nevus cells are nonpigmented and confined within the dermis.
- Patient presents with pigmented, and tan to brown, uniformly pigmented, small, 1 cm areas of elevated skin (papules, or moles) with well defined, rounded borders. Histology of intradermal nevus is shown in Fig. 29.45.

Surgical Pathology: Melanocytic Nevus

Light Microscopy

Melanocytic nevus is composed of round to oval melanocytes forming either round aggregates arranged in nests or sheets of cells in the epidermis in junctional nevus, dermis in dermal nevus or both in compound nevus.

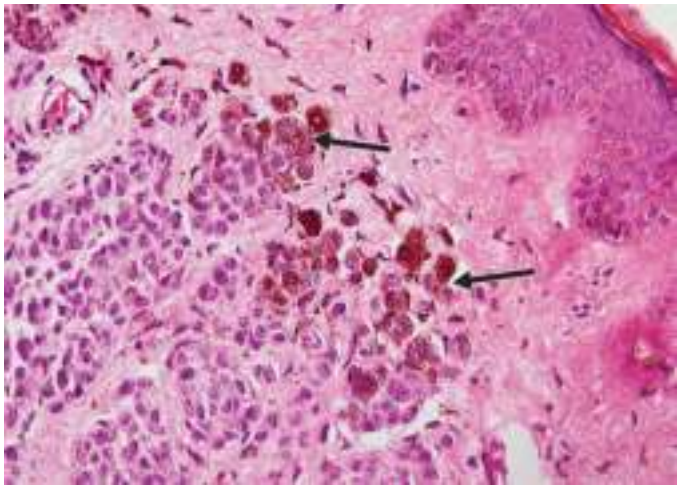


Fig. 29.45: Histology of intradermal nevus. Hematoxylin and eosin-stained section shows aggregates of melanocytes (arrows) (400X).

Dysplastic Melanocytic Nevus

Dysplastic melanocytic nevus is an atypical and irregular pigmented lesion with disorderly proliferation of melanocytes, dermal fibrosis, and lymphocytic infiltration. A clinical diagnosis of dysplastic melanocytic nevi is based on the presence of following features: skin lesion (fried egg appearance) <5 mm with ill-defined borders and color variation. Most of the atypical nevi are biopsied as these are concerning for atypical moles or melanoma.

- Dysplastic melanocytic nevus may transform into malignant melanoma. Etiology of dysplastic melanocytic nevus is not clear. However, phenotype expression is related to genetic and environmental factors. Predisposing risk factors include ultraviolet (UV) sunlight exposure especially in children, freckles, family history of dysplastic nevus syndrome, fair skin tone, light hair and light color eyes.
- Unlike patients with familial melanoma who often demonstrate a CDKN2A gene mutation, dysplastic melanocytic nevus does not commonly display CDKN2A gene mutation. Recent studies revealed that dysplastic melanocytic nevi are clonal like common nevi and some have BRAF, p16, or TP53 genetic alterations in expression.

Surgical Pathology: Dysplastic Melanocytic Nevus

Light Microscopy

- Histologic examination of dysplastic melanocytic nevus shows four characteristic features: intraepidermal lentiginous hyperplasia of melanocytes, cytologic atypia of melanocytes, lamellar and concentric fibroplasia (stromal response), and architectural atypia.
- Compound dysplastic nevus usually has 'shoulder sign' in which component extends beyond the 'shoulders' the dermal component.
- The dysplastic cells contain vesicular nuclei and prominent nuclei. The cytologic atypia is random and graded as mild, moderate or severe.

Lentigo Maligna

Lentigo maligna was first described by Hutchinson in 1890 and referred to as 'Hutchinson's melanotic

freckle'. It is characterized by atypical melanocytes at the epidermal–dermal junction. It is a precursor to lentigo maligna melanoma.

- Patient presents as an irregular brown macule on chronically ultraviolet radiation-damaged skin, particularly the head and neck, in the elderly persons.
- Ultraviolet radiation exposure causes oxidative damage and produce signature gene mutations, which in turn alter genes involved in the PI3K and MAPK pathways including BRAF, N-RAS, and KIT.
- Secondary gene mutations in CCND1, CDKN2A, and/or TP53 then result in transformation into malignant tumor.

Blue Nevus

Blue nevus can present clinically as blue, gray, brown, or black highly pigmented solitary nodules or plaques on the skin since birth. Histologically, blue nevus represents dermal pigmented dendritic melanocytes derived from embryonal precursors of melanocytes. Genetically, blue nevus harbors mutations in G protein-coupled receptor subunits, GNAQ and GNA11. Progression of blue nevus to malignant blue nevus is associated with additional gene mutations and partial gains and losses of chromosomal material.

Spitz Nevus

Spitz nevus is most often characterized by spindled and/or epithelioid nevomelanocytes, which most often affects children. Younger age is associated with more likely benign behaving Spitz nevus. Comparison of classic Spitz nevus and atypical Spitz nevus is given in Table 29.19.

MELANOMA

Melanoma is produced by the malignant transformation of melanocytes present in the basal layer of the skin derived from the neural crest, which can arise in other locations where neural crest cells migrate to brain and gastrointestinal tract. It is most often associated with excessive exposure to ultraviolet radiation.

- Clinically, melanomas are classified according to their depth: thin (≤1 mm), moderate (1–4 mm) and thick (>4 mm).
- Melanoma has two growth patterns: radial and vertical. During radiation growth, neoplastic cells grow in a radial fashion in the epidermis.
- With time, most melanomas progress to the vertical growth in which neoplastic cells invade underlying dermis and develop the ability to metastasize via lymphatic route to regional lymph nodes and via homogenous routine to distant organs (lung, liver, bone, brain). The five-year survival rate for patients with stage 0 melanoma is 97%, when compared with about 10% for patients with stage IV disease.

Table 29.19 Comparison of classic Spitz nevus and atypical Spitz nevus

Clinical Features	Classic Spitz Nevus	Atypical Spitz Nevus
Age group	<10 years	10–20 years
Location	Extremities, face, neck	Back
Size of lesion	<5–6 mm	>1 cm
Shape of lesion	Symmetrical or dome-shaped	Asymmetrical
Border	Well-defined	Irregular
Surface	Smooth	Irregular
Color	Pink, red	Irregular
Family history	No	Yes

Predisposing Factors

Melanoma is caused by combination of environmental and genetic factors. Risk factors for melanoma include ultraviolet radiation exposure, dysplastic melanocytic nevi, immunosuppressive states (hematological malignancies, transplantation patients) and positive family history, mutation in p16, BRAF, or MICR, blue eyes, fair complexion.

- Melanoma may develop in *de novo* (without evidence of precursor lesion) or precursor lesions (acquired nevus, dysplastic nevus, congenital nevus, cellular nevus). Solar irradiation predisposes to melanoma, basal cell carcinoma and squamous cell carcinoma. Melanoma may occur on unexposed areas of the skin, including the palms, soles, and perineum.
- UVB radiation spectrum (290–320 nm) induces two major DNA lesions at dipyrimidine sites: cyclobutene pyrimidine dimers and pyrimidine and pyrimidone photoproducts. UVB radiation induced DNA damage adversely affects signaling pathways resulting in impairment of cell cycle arrest, DNA repair and apoptosis. UV radiation causes TP53 and PTCH gene mutations, classical UVB signature mutations C→T (CC-TT) and dipyrimidine sites, UVA signature and oxidative mutations; and TNF-α released by exposed skin diminishes immune response. TNF-α induces dimer formation between neighboring thymine pairs in DNA.
- Depending on time and dose rate exposure to ultraviolet radiation leaves characteristic gene mutations that participate by molecular and cellular alterations in skin melanoma. Ultraviolet radiation-induced molecular changes are prevented by DNA repair, apoptosis and signaling pathways. Skin melanoma risk is increased due to genetic polymorphisms in genes for DNA repair, melanin and oxygen-derived free radical scavenging.

Table 29.20 Environmental agents associated with the development of human skin cancers

Environmental Agent	Persons at Risk	Route of Exposure to Agent	Associated Sporadic Skin Cancer
General exposure to environmental agents			
Ultraviolet radiation (UVB:290–320 nm), p53 gene mutation and induces dimer formation between neighboring thymine pairs in DNA	General population	Topical exposure	▪ Melanoma ▪ Squamous cell carcinoma ▪ Basal cell carcinoma ▪ Bowen disease ▪ Skin cancers in xeroderma pigmentosum
Therapeutic agents administered in patients			
Psoralen (PUVA)	Psoriasis patient	Topical exposure or systemic system	▪ Melanoma ▪ Squamous cell carcinoma ▪ Basal cell carcinoma

- Educational and behavioral approaches are keys to minimize skin melanoma rates. Inflammation, immunosuppression and oxidative stress enhance UV-induced carcinogenesis by causing DNA mutations.
- Clonal expansion of mutant cells increases opportunities to receive additional gene mutations for development and progression of skin melanoma. Environmental agents associated with the development of human skin cancers are given in Table 29.20.

Molecular Genetic Alterations: Melanoma

Most cases of melanoma are sporadic. It means that acquired somatic mutations in the melanocytes during a person's lifetime give rise to melanoma. Oncogenes and tumor suppressor genes in cutaneous melanoma and other human cancers are given in Table 29.21.

- Somatic germline mutations in the BRAF and CDKN2A genes are demonstrated in some cases and most common in sporadic melanoma. In about 10% of cases, melanoma occurs in multiple members of the same family. Unlike sporadic melanoma, familial melanomas are caused by inherited germline mutations in CDKN2A and MCIR genes.
- The CDKN2A gene plays important role in regulating cell senescence. MCIR gene influences skin pigmentation. In people with germline CDKN2A and MCIR gene mutations, changes in other genes, together with environmental and lifestyle factors contribute to possible development of melanoma.
  - **CDKN2A-CDK4-TP53 pathway disruption:** Germline mutations in cyclin-dependent kinase inhibitor 2A (CDKN2A) tumor suppressor gene account for 40% of hereditary melanoma and pancreatic carcinoma. CDKN2A gene is a regulator of cell division. Mutations in CDKN2A gene are the most common cause of inherited melanoma. The risk of melanoma in CDKN2A mutation carriers is approximately 14% by age 50 years, 24% by age 70 years and 28% by age 80 years.
  - **RAS signaling pathway disruption:** Somatic mutation in the BRAF gene is demonstrated in 66% of melanomas occurring on skin subject to intermittent ultraviolet radiation.
  - **Microphthalmia associated transcription pathway disruption:** Recently somatic amplifications of the microphthalmia (MITF) gene has been detected in a subset of melanomas. Overexpression of MITF in conjunction with BRAF can result in melanocytic transformation.
  - **KIT tyrosine kinase receptor mutation:** KIT tyrosine kinase receptor has been demonstrated to be mutated or amplified in a subset of melanomas predominantly in acral and mucosal sites. Tyrosine kinase receptor inhibitor 'imatinib' may be useful in the treatment of c-KIT mutated melanomas.
  - **Melanocortin 1 receptor mutation:** Recently mutations in the melanocortin 1 receptor (MCIR) increase the risk of melanoma.
  - **Xeroderma pigmentosum gene mutation:** Xeroderma pigmentosum is an autosomal recessive disorder. Mutations of XP genes increase the risk of melanoma.
  - **Breast cancer susceptibility gene mutation:** Carriers of the breast cancer susceptibility gene (BRCA) increase risk for melanoma.

Melanoma: Clinical Variants

Clinical variants of melanomas include superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma and acral lentiginous melanoma. Clinical variants of melanomas are shown in Fig. 29.46A to D and clinical variants of melanomas are given in Table 29.22.

- **Superficial spreading melanoma:** It accounts for 70% of melanomas and occurs on trunk and extremities. Patient initially develops a flat growth in a radial fashion with irregular border and variegated dark brown pigmentation resulting in about 2 cm growth with variegated colors, as well as peripheral notches, indentations, or both.
- **Nodular melanoma:** It accounts for 15–30% of melanoma diagnoses. Patient presents with blue-black tumor (sometimes lacking pigmentation) on head, neck and trunk, that grows in vertical fashion. This variant has the poorest prognosis of the clinical variants.

Table 29.21 Oncogenes and tumor suppressor genes in cutaneous melanoma and other human cancers

Gene	Locus	Function	Tumor Type	
Oncogenes				
H-RAS	11p15.5	Cell cycle, survival	▪ Melanoma	▪ Squamous cell carcinoma
K-RAS	1p13.2	Cell cycle, survival	▪ Melanoma	▪ Squamous cell carcinoma
BRAF	7q34	Cell cycle, survival	▪ Melanoma ▪ Leukemia	▪ Colon carcinoma
CDK4	q13–15	Cell cycle during G1/S transition by inhibiting RB1 protein	▪ Melanoma ▪ Glioblastoma multiforme	▪ Sarcoma
FGFR3'INT2	Unknown	Regulation of cell growth, division, angiogenesis and embryo development	Melanoma	
Tumor suppressor genes				
p16 (INK4A)	9p21	Cyclin-dependent kinase inhibitor	▪ Melanoma ▪ Squamous cell carcinoma	▪ Mesotheliomas ▪ Astrocytoma
APC	5p21	APC inhibits Wnt signaling activator pathway, thus prevents nuclear transcription. It degrades catenin	▪ Melanoma ▪ Familial polyposis coli with malignant transformation ▪ Colon carcinoma	▪ Gastric carcinoma ▪ Pancreatic carcinoma ▪ Thyroid carcinoma
PTEN	10q23.3	Phosphatase	▪ Melanoma	▪ Trichoepithelioma

Unknown gene mutation on Xq24-q27 may cause basal cell carcinoma. Unknown gene mutation on 9q31 can cause keratoacanthoma.



Fig. 29.46: Melanoma variants: (A) Superficial spreading melanoma *in situ*, (B) nodular malignant melanoma, (C) lentigo maligna presents as hyperpigmented lesion with irregular borders located on the head, (D) acral variant of melanoma.

Table 29.22 Clinical variants of melanomas

Clinical Variant	Frequency	Locations	Median Age	Features
Superficial spreading melanoma	70%	Trunk and extremities	47 years	Initially flat pigmented growth in a radial fashion attaining 2 cm
Nodular melanoma	4–10%	Head, neck and trunk	50 years	Tumor with vertical growth pattern associated with poorest diagnosis
Lentigo maligna melanoma	2–8% (Whites) 35–60% (dark-skinned)	Nose and cheek	70 years	Large pigmented 3 cm macule with marked notching of the borders
Acral lentiginous melanoma	2–8%	Palms, soles, and subungual regions	61 years	Flat, tan, or brown stained growth with irregular borders

- **Lentigo maligna melanoma:** It represents 4–10% of melanomas. It is also known as Hutchinson melanotic freckle. Initially, patient presents with large pigmented 3 cm macule with marked notching of the borders on nose and cheek.
- **Acral subungual and mucosal lentiginous melanoma:** Acral lentiginous melanoma accounts for 2–8% in Whites and 35–60% in dark-skinned people. Patient presents with flat, tan, or brown stained growth with irregular borders on palms, soles, and subungual regions. Subungual lentiginous melanoma can be brown or black, with ulcerations in later stages. A similar tumor occurs on the mucous membranes known as mucosal lentiginous melanoma.

Surgical Pathology: Melanoma

Gross Morphology

Gross morphology of malignant melanoma in skin with lymph node metastasis is shown in Fig. 29.47A and B.

- **Superficial spreading melanoma:** Tumor occurs on trunk and extremities. Initially, tumor is flat growth in a radial fashion with irregular border and variegated dark brown pigmentation resulting in about 2 cm growth with variegated colors, as well as peripheral notches, indentations, or both.
- **Nodular melanoma:** Tumor is blue-black tumor (sometimes lacking pigmentation) on head, neck and trunk, that grows in vertical fashion. This variant has the poorest prognosis of the clinical variants.
- **Lentigo maligna melanoma:** Tumor is large pigmented 3 cm macule with marked notching of the borders on nose and cheek.
- **Acral subungual and mucosal lentiginous melanoma:** Acral lentiginous melanoma is flat, tan, or brown stained growth with irregular borders on palms, soles, and subungual regions. Subungual lesions can be brown or black, with ulcerations in later stages. A similar tumor occurs on the mucous membranes known as mucosal lentiginous melanoma.

Light Microscopy

- **Dermoepidermal melanoma:** Melanoma cells are usually considerably larger than nevus cells. Tumor cells contain large nuclei with irregular contours with eosinophilic nuclei. Chromatin is clumped at the periphery of the nuclear membrane.

All melanomas start as *in situ* lesion involving only the epidermis. Nests of melanoma cells are present at the dermoepidermal junction. Histology of malignant melanoma is shown in Fig. 29.48. Cytology of malignant melanoma metastasizing to lymph node is shown in Fig. 29.49.

- **Pagetoid spread melanoma:** The lesion progresses to involve all layers of the epidermis known as epidermal tropism or Pagetoid spread.
- **Radial growth melanoma:** As melanoma cells expand laterally (so-called radial growth phase), the area of involved skin enlarges. Tumor grows in all directions predominantly lateral within epidermis and papillary zone of the dermis. There is presence of numerous lymphocytes. This variant does not metastasize; hence prognosis is good.
- **Vertical growth melanoma:** Eventually, melanoma cells invade the dermis and the so-called vertical growth phase begins (which indicates the progression from *in situ* to invasive melanoma), which extends into the reticular dermis or beyond. Prognosis depends on depth of the lesion. It may spread by lymphatic and hematogenous routes.

Electron Microscopy

Sometimes a melanoma will not be well-differentiated enough to show the typical melanin pigmentation. Electron microscopy shows oval premelanosome structures.

Immunohistochemistry Technique

Panel of markers is applied on histologic sections in melanoma.

Melanoma *in situ*	
Melan-A (A103) or also known as melanoma antigen recognized by T cells 1 (MART-1)	Positive (80–100%)
Human melanoma black 45 (HMB-45)	Positive (60–90%)
S-100 protein (calcium-binding protein)	Positive
Microphthalmia transcription factor (MITF)	Positive

Melanoma (epithelioid variant)	
Melan-A (A103) or also known as melanoma antigen recognized by T cells 1 (MART-1)	Positive (80–100%)
Human melanoma black 45 (HMB-45)	Positive (60–90%)
Ki-67 proliferative marker	Positive

▪ S-100 (calcium-binding protein)	▪ Positive
▪ Microphthalmia transcription factor (MITF)	▪ Positive
▪ SOX 100	▪ Positive
▪ PNL2	▪ Positive
▪ MUM1	▪ Positive
Melanoma (desmoplastic variant)	
▪ S-100 protein (calcium-binding protein)	▪ Positive
▪ Melan-A (A103) or also known as melanoma antigen recognized by T cells 1 (MART-1)	▪ Negative
▪ Human melanoma black 45 (HMB-45)	▪ Negative
▪ Microphthalmia transcription factor (MITF)	▪ Negative
▪ SOX 100	▪ Negative
▪ PNL2	▪ Negative
▪ MUM1	▪ Negative

Prognostic Factors

The most important prognostic indicator is tumor thickness. Superficial variant of melanoma has better prognosis than vertical variant. Ulceration, high mitotic rate, absence of significant lymphocytic response are poor prognostic indicators.

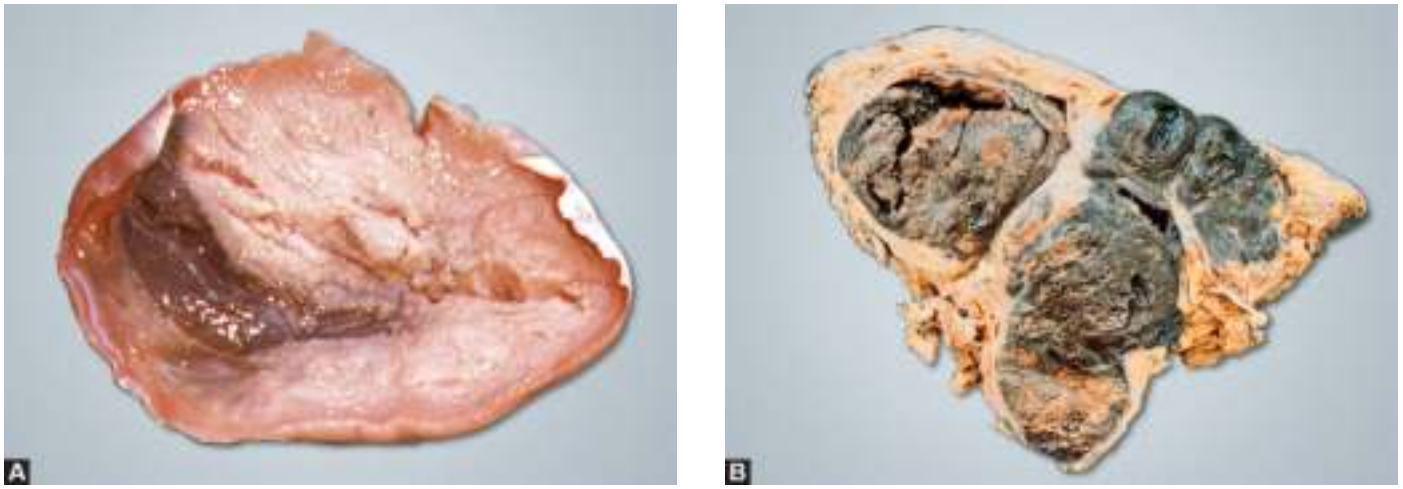


Fig. 29.47: Malignant melanoma. (A) Tumor arises from skin. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka) and (B) melanoma metastases in lymph node. (*Courtesy*: Dr. Sujata Kanetkar, Professor and Head of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra.)

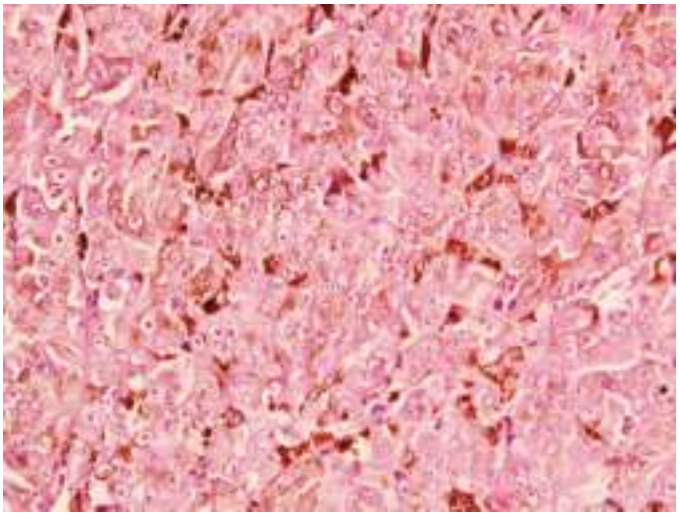


Fig. 29.48: Histology of malignant melanoma. Melanoma cells are usually considerably larger than nevus cells. Tumor cells contain large nuclei with irregular contours. Chromatin is clumped at the periphery of the nuclear membrane. All melanomas start as *in situ* lesion involving only the epidermis. Nests of melanoma cells are present at the dermoepidermal junction (400X).

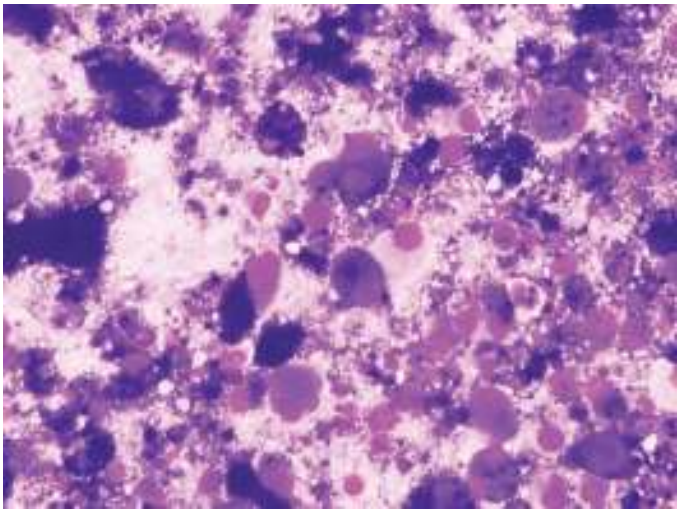


Fig. 29.49: Fine needle aspiration cytology of malignant melanoma metastasizing to lymph node. Malignant melanoma is produced by the malignant transformation of melanocytes derived from neural crest. Positive family history, exposure to UVA and UVB radiation, and atypical mole syndrome are linked to malignant melanoma. Tumor can metastasize via lymphatic and hematogenous routes (400X).

NONMELANOCYTIC CUTANEOUS NEOPLASMS

Skin cancer is the most common type of malignancy worldwide. Possible risk factors include exposure to sunlight or UV radiation, human papillomavirus (HPV), fair skin type, presence of large numbers or certain types of nevi (moles), exposure to arsenic, immunosuppression, certain genetic conditions (e.g. xeroderma pigmentosum), and family history of skin cancer.

- Epithelial neoplasms of skin include basal cell carcinoma, basal cell nevus syndrome, Bowen disease (squamous cell carcinoma *in situ*), squamous cell carcinoma and keratoacanthoma. Melanocytic neoplasms include nevi and melanoma discussed under disorders of pigmentation and melanocytes.
- Tumors of the cutaneous appendages arising from hair follicle, eccrine glands, sebaceous glands include chondroid syringoma, nodular hidradenoma and pilomatrixoma. Merkel cell carcinoma arises from neuroendocrine cell. Fibrohistiocytic tumors include dermatofibroma and dermatofibrosarcoma protuberans. Vascular tumors include capillary hemangioma, spindle cell hemangioma, kaposiform hemangioendothelioma, Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma, Kaposi sarcoma and angiosarcoma.

CARCINOGENESIS OF SKIN CANCERS

Carcinogenesis is a multistep process of formation of malignant tumor growth; whereby normal cells are transformed to malignant cells. Carcinogenesis process is characterized at the cellular, genomic and epigenetic levels and abnormal cell division.

GENOMIC INSTABILITY

Integrity of the genome is constantly threatened by exogenous agents (ultraviolet radiation, ionization radiation, tobacco smoke, chemical agents) or endogenous agents (reactive oxygen species, DNA-damaging agents).

- DNA damage can cause changes in DNA sequence due to alterations in the structure of nucleotides, DNA double-strand breaks, DNA cross-links, incorrect nucleotide base pairing, and DNA adducts.
- The ability of cells with DNA damage to repair and maintain the genomic stability is important in prevention of malignant transformation. Different agents induce different types of DNA damage, which in turn require different cellular responses and repair pathways.
- Many hereditary disorders are characterized by genomic stability due to defects in genes involved in cellular DNA repair or DNA repair defects. Inherited or acquired genomic instability is associated with an increased risk for development of cancer. Oncogenes and tumor suppressor genes in human cutaneous cancers are given in Table 29.23.

CHEMICAL CARCINOGENESIS

Chimney sweep has been linked with environmental exposure and cutaneous scrotal carcinoma. Chemical agents, such as arsenic and hydrocarbons, have been implicated in human skin cancers. Chemical

Table 29.23 Oncogenes and tumor suppressor genes in human cutaneous cancers

Gene	Locus	Function	Tumor Type
Oncogenes			
H-RAS	11p15.5	Cell cycle, survival	▪ Squamous cell carcinoma ▪ Melanoma
K-RAS	1p13.2	Cell cycle, survival	▪ Squamous cell carcinoma ▪ Melanoma
SMO	7q31–q32	Hedgehog signaling pathway receptor	Basal cell carcinoma
BRAF	7q34	Cell cycle, survival	▪ Melanoma ▪ Leukemia ▪ Colon carcinoma
CDK4	q13–15	Cell cycle during G1/S transition by inhibiting RB1 protein	▪ Melanoma ▪ Glioblastoma multiforme ▪ Sarcoma
CTNNB1	3p22–p21.3	Cell-cell adhesion, transcription	Pilomatricoma (pilomatrixoma)
LEF1	4q23–q25	Transcription	Sebaceous tumors

Contd...

Table 29.23 Oncogenes and tumor suppressor genes in human cutaneous cancers (*Contd...*)

Gene	Locus	Function	Tumor Type
FGFR3/INT2	Unknown	Regulation of cell growth, division, angiogenesis and embryo development	Melanoma
Tumor suppressor genes			
TP53	17p13.1	DNA repair, cell cycle regulation, apoptosis	▪ Squamous cell carcinoma ▪ Basal cell carcinoma
p16 (INK4A)	9p21	Cyclin-dependent kinase inhibitor	Squamous cell carcinoma
Melanoma			
APC	5p21	APC inhibits Wnt signaling activator pathway, thus prevents nuclear transcription. It degrades β-catenin	▪ Melanoma ▪ Familial polyposis coli with malignant transformation ▪ Colon carcinoma ▪ Gastric carcinoma ▪ Pancreatic carcinoma ▪ Thyroid carcinoma
PTCH1	9p22.3	Hedgehog signaling pathway receptor	▪ Basal cell carcinoma ▪ Trichoepithelioma
PTEN	10q23.3	Phosphatase	▪ Melanoma ▪ Trichoepithelioma
CYLD	16q12–q13	NF-κβ inhibitor	Cylindroma
▪ MSH2 ▪ MLH1	▪ 2p22–p21 ▪ 3p21.3	Mismatch repair	Sebaceous gland carcinoma
FLCN	17p11.2	Ribosomal protein S6 modulator	Fibrofolliculoma

Unknown gene mutation on Xq24-q27 may cause basal cell carcinoma. Unknown gene mutation on 9q31 can cause keratoacanthoma.

carcinogenic agents linked to human cancers are classified as tumor initiators, promoters, or 'complete carcinogens'. Incomplete chemical carcinogens act as promoters in the carcinogenesis.

- Tumor initiation, associated with gene mutations permanently alter the cellular biological responsiveness, is irreversible change. Tumor promotion process does not produce gene mutations, that provides a selective growth at early stages; genotoxic carcinogenic agents that facilitate malignant progression.
- Most chemical carcinogens must undergo metabolic activation, which involves cellular enzymes involved in xenobiotic metabolism, including cytochrome p-450 enzymes and glutathione S-transferase. Presence of additional risk factors such as UV exposure including polymorphisms influence the susceptibility to develop human cancers.

ULTRAVIOLET RADIATION CARCINOGENESIS

Ultraviolet (UV) radiation from the sun is most important carcinogen in human beings. There is continuous increase in the incidence of development of all types of skin cancers (squamous cell carcinoma, basal cell carcinoma, melanoma and skin cancers in xeroderma pigmentosum; Merkel cell carcinoma is also induced by polyomavirus). Exposure of the skin to UV radiation has multiple cellular and clinical effects including development of skin cancers.

- UVB radiation spectrum (290–320 nm) induces two major DNA lesions at dipyrimidine sites: cyclobutene pyrimidine dimers and pyrimidine and pyrimidone photoproducts. UVB radiation-induced DNA damage adversely affects signaling pathways resulting in impairment of cell cycle arrest, DNA repair and apoptosis. UV radiation causes p53 and PTCH gene mutations, classical UVB signature mutations C→T (CC-TT) and dipyrimidine sites, UVA signature and oxidative mutations; and TNF-α released by exposed skin diminishes immune response. UVB radiation induces dimer formation between neighboring thymine pairs in DNA.
- Depending on time and dose rate exposure to ultraviolet radiation leaves characteristic mutations that participate by molecular and cellular alterations in skin cancers. Ultraviolet radiation-induced molecular changes are prevented by DNA repair, apoptosis and signaling pathways. Skin cancer risk is increased due to genetic polymorphisms in genes for DNA repair, melanin and oxygen-derived free radical scavenging.
- Educational and behavioral approaches are keys to minimize skin cancer rates. Inflammation,

immunosuppression and oxidative stress enhance UV-induced carcinogenesis by causing DNA mutations.

- Clonal expansion of mutant cells increases opportunities to receive additional mutations for development and progression of skin cancers. Environmental agents associated with the development of human skin cancers are given in Table 29.24. Panel of immunohistochemical markers applied in assessment of selected skin tumors is given in Table 29.25.

Table 29.24 Environmental agents associated with the development of human skin cancers

Environmental Agent	Persons at Risk	Route of Exposure to Agent	Associated Sporadic Skin Cancer
General exposure to environmental agents			
Ultraviolet radiation (UVB:290–320 nm) causes p53 gene mutation and induces dimer formation between neighboring thymine pairs in DNA	General population	Topical	▪ Squamous cell carcinoma ▪ Basal cell carcinoma ▪ Melanoma ▪ Bowen disease ▪ Skin cancers in xeroderma pigmentosum
Tobacco smoke	Tobacco smokers	Topical, systemic	Squamous cell carcinoma
Soot	Chimney sweep	Topical	Squamous cell carcinoma
Occupational exposure			
Coat tar, pitch	Coker of coal, steel worker	Topical	Squamous cell carcinoma
Petroleum oils	Machinist, textile worker	Topical	Squamous cell carcinoma
Arsenic	Agriculture worker, soil contamination, living in areas of high groundwater	Topical, systemic	▪ Squamous cell carcinoma ▪ Basal cell carcinoma ▪ Bowen disease
4,4'-Bipyridyl	Pesticide manufacturer	Topical	▪ Squamous cell carcinoma ▪ Bowen disease
Polychlorinated biphenyl	Petrochemical worker	Topical, systemic	Melanoma
Dry cleaning agents	Dry cleaner	Topical, systemic	Basal cell carcinoma
Fiberglass	Insulator	Topical	Basal cell carcinoma
Therapeutic agents administered in patients			
Psoralen (PUVA)	Psoriasis patient	Topical, systemic	▪ Squamous cell carcinoma ▪ Basal cell carcinoma ▪ Melanoma
Nitrogen mustard	Cutaneous T cell lymphoma	Topical	Squamous cell carcinoma
Immunosuppressants	Transplant recipients	Systemic	▪ Squamous cell carcinoma ▪ Basal cell carcinoma
Ionizing radiation	Cancer therapy patient	Topical	▪ Squamous cell carcinoma ▪ Basal cell carcinoma

Table 29.25 Panel of immunohistochemical markers applied in assessment of selected skin tumors

Immunohistochemical Marker	Expression
Melanoma *in situ*	
Melan-A (A103) or also known as melanoma antigen recognized by T cells 1 (MART-1)	Positive (80–100%)
Human melanoma black 45 (HMB-45)	Positive (60–90%)
S-100 (calcium-binding protein)	Positive
Microphthalmia transcription factor (MITF)	Positive
Melanoma (epithelioid variant)	
Melan-A (A103) or also known as melanoma antigen recognized by T cells 1 (MART-1)	Positive (80–100%)
Human melanoma black 45 (HMB-45)	Positive (60–90%)
S-100 (calcium-binding protein)	Positive
Microphthalmia transcription factor (MITF)	Positive
SOX 100	Positive
PNL2	Positive
MUM1	Positive
Melanoma (desmoplastic variant)	
S-100 (calcium-binding protein)	Positive

Contd...

Table 29.25 Panel of immunohistochemical markers applied in assessment of selected skin tumors (*Contd...*)

Immunohistochemical Marker	Expression
Melan-A (A103) or also known as melanoma antigen recognized by T cells 1 (MART-1)	Negative
HMB-45 (human melanoma black 45)	Negative
Microphthalmia transcription factor (MITF)	Negative
SOX 100	Negative
PNL2	Negative
MUM1	Negative
Mycosis fungoides (MF)	
CD45 (T cell marker)	Positive
CD3 (T cell marker)	Positive
CD20 (T cell marker)	Negative
CD2 (T cell marker)	Negative
CD5 (T cell marker)	Negative
CD7 (T cell marker)	Negative
Squamous cell carcinoma *in situ*	
CK903 (HMWCK)	Positive
Squamous cell carcinoma (spindle cell variant)	
Cytokeratins	Positive
p63	Positive
Actin	Negative/positive
Desmin	Negative/positive
Sebaceous carcinoma	
BerEP4	Positive
Epithelial membrane antigen (EMA)	Positive
Cytokeratin 7 (CK7)	Positive
Merkel cell carcinoma	
CK20	Positive (dot-like perinuclear staining pattern)
Neurofilament	Positive (100%)
Synaptophysin (neuroendocrine marker)	Positive (cytoplasmic staining)
Chromogranin A (neuroendocrine marker)	Positive (cytoplasmic staining)
Neuron-specific enolase (NSE) neuroendocrine marker	Positive (cytoplasmic staining)
CD56 (neuronal cell adhesion molecule—NCAM) neuroendocrine marker	Positive (membranous staining)
Cytokeratins (AE1/AE3, CAM 5.2)	Positive
Merkel cell polyomavirus	Positive
Metastatic small cell lung carcinoma	
Thyroid transcription factor 1 (TTF1)	Positive
Cytokeratins (AE1/AE3, CAM 5.2)	Positive
Cytokeratin 20 (CK20)	Negative
Neurofilament	Negative
Synaptophysin (neuroendocrine marker)	Positive
Chromogranin A (neuroendocrine marker)	Positive
Neuron-specific enolase (NSE) neuroendocrine marker	Positive
CD56 (neuronal cell adhesion molecule—NCAM) neuroendocrine marker	Positive
Cytokeratins (AE1/AE3, CAM 5.2)	Positive
Dermatofibroma	
Factor XIIIa	Positive
D2–40	Positive
CD34	Negative
Dermatofibrosarcoma protuberans (DFSP)	
Factor XIIIa	Negative
D2–40	Negative
CD34	Positive
Atypical fibroxanthoma	
CD10	Positive
Primary cutaneous marginal zone lymphoma	
CD20	Positive
CD79a	Positive
BCL-2	Positive
IgM	Positive/negative
CD43	Positive/negative
CD5	Negative
CD23	Negative
Primary cutaneous follicular cell lymphoma	
CD20	Positive
CD79a	Positive
BCL-2	Negative/positive (staining weak)
Primary cutaneous diffuse large B cell lymphoma	
CD20	Positive
CD79a	Positive
BCL-2	Positive
BCL-6	Positive/negative
MUM1	Positive
Monotypic κ or λ	Positive
IgM	Positive (cytoplasmic staining)

Contd...

EPIDERMIS TUMORS

Skin is composed of squamous cells, basal cells, and melanocytes. Cancers arise from these skin cells are called squamous cell carcinoma, basal cell carcinoma and melanoma respectively. Squamous cell carcinoma and basal cell carcinoma are the most common types of skin cancer.

EPITHELIAL PRECANCEROUS LESIONS

Epithelial precancerous lesions for skin epidermis carcinomas include actinic keratosis, arsenical keratosis and viral-associated lesions (Bowenoid papulosis, epidermodysplasia verruciformis).

Actinic Keratosis

Actinic keratosis is a precancerous lesion on a spectrum from ultraviolet radiation-induced skin damage to squamous cell carcinoma. With time, actinic keratoses may evolve into squamous cell carcinoma *in situ*, and finally, resulting in invasive squamous cell carcinoma.

- However, actinic keratosis lesion may remain stable or regress. It is strongest predictor of subsequent development of skin melanoma.
- Risk factors for development of actinic keratoses include individual susceptibility (e.g. elderly male gender, fair skin, light-colored eyes), cumulative ultraviolet radiation exposure, immunosuppression, prior history of actinic keratoses, squamous cell carcinomas, basal cell carcinoma, and melanoma; genetic syndromes (xeroderma pigmentosum, Bloom syndrome, Rothmund-Thompson syndrome).

Clinical Features

Actinic keratoses can affect elderly and young persons in the significant sun-exposed regions of body such as head, neck, forearms and dorsal hands. Patient presents with pruritus, burning or stinging pain, bleeding in the scaly erythematous papules of 2–6 mm size or nodules. Clinical photograph of actinic keratoses is shown in Fig. 29.50.

Molecular Genetic Alterations

Ultraviolet radiation-induced TP53 gene mutation provides selective growth advantage to neighboring cells, which leads to clonal expansion in the epithelium in 50% of cases. Actinic keratoses also demonstrate overexpression of cyclin D1 and RAS genes and loss of heterozygosity on chromosomes 9p, 9q, 13q, 17p and 17q. Human papillomavirus (HPV) has been detected in 40% of cases.



Fig. 29.50: Actinic keratosis. Actinic keratosis (AK) is a lesion that arises as a result of excessive exposure to solar radiation and appearing predominantly on Fitzpatrick phototype I and II skin. Lesion of actinic keratosis is present on the lip (arrow).

Light Microscopy

Histologic examination of actinic keratoses demonstrates atypical cells along the basal layer with sparing of adnexal epithelium, shows patchy parakeratosis and agranulosis. Dyskeratotic cells are sometimes present. Dysplasia does not involve full thickness of the epidermis. Solar elastosis is most often seen.

Prognosis

The prognosis of actinic keratoses includes persistence, regression or malignant transformation into invasive squamous cell carcinoma in 1–20% of cases. The relative risk for progression to squamous cell carcinoma depends on period of exposure to ultraviolet radiation and immune status.

Treatment

Treatment modalities for actinic keratoses include liquid nitrogen cryotherapy, curettage with or without electrosurgery, shave excision, topical agents (5-fluorouracil cream and solution, imiquimod cream, 3% diclofenac cream), photodynamic therapy, cryopeeling, dermabrasion, medium-depth chemical peel and laser resurfacing.

Arsenical Keratosis

Arsenical keratosis is precancerous lesion associated with chronic arsenical exposure due to occupational, medicinal and environmental exposure. Chronic arsenicism is associated with Bowen disease, squamous cell carcinoma, basal cell carcinoma and internal malignancies. Bowen disease tends to persist for many years and progression to invasive squamous cell carcinoma. Most lesions are followed clinically or treated

symptomatically. Treatment modalities for arsenical keratoses include surgical excision, cryosurgery, curettage with or without topical electrosurgery, CO_2 laser treatment and topical chemotherapy.

Viral-associated Epithelial Precancerous Lesions

Viral-associated epithelial precancerous lesions are Bowenoid papulosis caused by HPV-16, HPV-18; and epidermodysplasia verruciformis caused by HPV-5, HPV-8. Lesions caused by HPV-16 include vulvar intraepithelial neoplasia (VIN), anal intraepithelial neoplasia (AIN), perianal intraepithelial neoplasia (PaIN), penile intraepithelial neoplasia (PIN) and digital or periungual Bowen disease.

- **Bowenoid papulosis of the genitalia:** It is caused by human papillomavirus (HPV), especially types 18 and 33. The lesion may regress spontaneously, persist or rarely progresses to Bowen disease or squamous cell carcinoma. Sexual partners of the patients must be examined and followed for the development of penile carcinoma, cervical carcinoma and valvular carcinoma. Patients with Bowenoid papulosis are treated by topical imiquimod, curettage, excision and laser vaporization.
- **Epidermodysplasia verruciformis (EV):** It is a rare lesion associated with mutations in EVER1 and EVER2 genes. Affected persons are prone to acquired HPV infections (HPV-5, HPV-8). Patient presents with widespread flat, wart-like papules in childhood. The patients are prone to develop squamous cell carcinoma. These patients are advised to avoid sun exposure, use sun-protective measures, regular follow-up and screening of family members.

BOWEN DISEASE (SQUAMOUS CELL CARCINOMA *IN SITU*)

Bowen disease is a squamous cell carcinoma *in situ* with the potential to progress to invasive squamous cell carcinoma. Predisposing factors for Bowen disease include UV radiation exposure, ionizing radiation exposure, chronic arsenicism, immunosuppression, HPV infection and previous psoralen therapy. Clinical variants of Bowen disease include pigmented intertriginous, periungual and subungual Bowen disease. The lesions of Bowen disease progress to invasive carcinomas in 3–5% of cases. Extragenital Bowen disease harbors HPV DNA in 30% of cases.

Clinical Features

Patient presents with discrete, slow-growing pink to erythematous macule or thin plaque with well-demarcated, irregular borders overlying scale or crust on skin exposed regions on face and legs especially in fair skinned person.

Molecular Genetic Alterations

TP53 gene mutation has been observed in majority of cases. Allelic deletion of one or more chromosome has been detected in occasional cases. Hyperkeratotic and verrucous surface changes may be demonstrated. Pigmented Bowen disease has been reported in 2% of cases.

Surgical Pathology: Bowen Disease

Light Microscopy

- In Bowen disease, epidermis exhibits full-thickness (stratum corneum to basal layer) cytologic atypia in the intraepidermal portions of adnexal structures.
- Basement membrane of epidermis remains intact. Epidermis shows presence of parakeratosis, hyperkeratosis, acanthosis, dyskeratosis, mitoses and loss of maturation.
- Numerous pleomorphic keratinocytes with hyperchromatic nuclei and eosinophilic cytoplasm are present throughout full-thickness of the epidermis.
- The upper dermis is infiltrated by numerous lymphocytes, plasma cells and histiocytes.

Bowen Disease: Histologic Variants

- **Psoriasiform Bowen disease:** It shows parakeratoses and marked acanthosis with broad, sometimes, fused rete ridges of epidermis.
- **Atrophic Bowen disease:** It shows thinned epidermis with cellular atypia in full-thickness of epidermis with lack of cellular maturation as well as adnexal involvement.
- **Acantholytic Bowen disease:** It shows marked acantholysis in the epidermis.
- **Epidermolytic Bowen disease:** It shows epidermolytic hyperkeratosis.
- **Pagetoid Bowen disease:** It shows nests of atypical cells within the epidermis.

Treatment

Patient with Bowen disease is treated by excision and Mohs micrographic surgery, curettage with or without electrosurgery, liquid nitrogen cryosurgery, topical therapies, laser ablation, radiotherapy, and photodynamic therapy. Histopathologic evaluation of biopsy is essential to rule out invasive carcinoma. Curettage treatment may miss appendageal involvement.

SQUAMOUS CELL CARCINOMA OF SKIN

Squamous cell carcinoma of skin is a common malignant locally invasive neoplasm derived from suprabasal dermal keratinocytes. Most common sites are lower part of face and back of the hands. Predisposing factors for squamous cell carcinoma of skin are ultraviolet radiation exposure actinic keratosis, Bowen disease, scars/chronic scarring, burns or long-term heat exposure, exposure to

environmental carcinogens. inflammatory dermatoses, human papillomavirus (HPV) infection, albinism, xeroderma pigmentosum, epidermolysis bullosa and porokeratosis.

- UVB radiation spectrum (290–320 nm) induces two major DNA lesions at dipyrimidine sites: cyclobutene pyrimidine dimers and pyrimidine and pyrimidone photoproducts. UVB radiation-induced DNA damage adversely affects signaling pathways resulting in impairment of cell cycle arrest, DNA repair and apoptosis.
- UVB radiation causes TP53 and PTCH gene mutations, classical UVB signature mutations C→T (CC-TT) and dipyrimidine sites, UVA signature and oxidative mutations; and TNF-α released by exposed skin diminishes immune response. It induces dimer formation between neighboring thymine pairs in DNA. Treatment options are excision, Mohs micrographic surgery and radiation. Predisposing factors for squamous cell carcinoma of skin are given in Table 29.26. Environmental agents associated with the development of squmous cell carcinoma of skin are given in Table 29.27.

Table 29.26 Predisposing factors for squamous cell carcinoma of skin

Precursor lesions (actinic keratosis, Bowen disease)
Ultraviolet radiation exposure
Scars and underlying diseases (chronic osteomyelitis sinuses, granuloma inguinale, lupus vulgaris, discoid lupus erythematosus)
Burns or long-term heat exposure
Exposure to environmental carcinogens (arsenic, aromatic hydrocarbons, insecticides, herbicides)
Immunosuppressive state
Inflammatory dermatoses
Human papillomavirus (HPV) infection
Albinism
Xeroderma pigmentosum
Epidermolysis bullosa
Porokeratosis
Third degree burns
Sinuses in chronic osteomyelitis

Table 29.27 Environmental agents associated with the development of squamous cell carcinoma of skin

Environmental Agent	Persons at Risk	Route of Exposure to Agent	Associated Sporadic Skin Cancer
General exposure to environmental agents			
Ultraviolet radiation (UVB:290–320 nm) causes p53 gene mutation and induces dimer formation between neighboring thymine pairs in DNA	General population	Topical	Squamous cell carcinoma
Tobacco smoke	Tobacco smokers	Topical, systemic	Squamous cell carcinoma
Soot	Chimney sweep	Topical	Squamous cell carcinoma
Occupational exposure			
Coal tar, pitch	Coker of coal, steel worker	Topical	Squamous cell carcinoma
Petroleum oils	Machinist, textile worker	Topical	Squamous cell carcinoma
Arsenic	Agriculture worker, soil contamination, living in areas of high groundwater	Topical, systemic	Squamous cell carcinoma
4,4'-Bipyridyl	Pesticide manufacturer	Topical	Squamous cell carcinoma
Therapeutic agents administered in patients			
Psoralen (PUVA)	Psoriasis patient	Topical, systemic	Squamous cell carcinoma
Nitrogen mustard	Cutaneous T cell lymphoma	Topical	Squamous cell carcinoma
Immunosuppressants	Transplant recipients	Systemic	Squamous cell carcinoma
Ionizing radiation	Cancer therapy patient	Topical	Squamous cell carcinoma

Clinical Features

Patient initially presents with firm or erythematous nodules, which later become shallow ulcers with firm elevated or indurated surroundings, sometimes with crust or scale especially in fair-skin persons as well as immunocompromised adults. High-risk squamous cell carcinoma of skin is given in Table 29.28.

Molecular Genetic Alterations

Development of squamous cell carcinoma of skin from normal keratinocytes begins with mutations in the cellular DNA and genetic instability. Molecular alterations in gene expression results in loss of growth controls, penetration of the basement membrane, and ultimately invasion into surrounding tissue. Along the pathway to squamous cell carcinoma, keratinocytes become resistant to apoptosis and immune attack.

- Genetic changes associated with development of squamous cell carcinoma of skin occurs by multistage evolution. In the early lesions, mutations of TP53, K-RAS, H-RAS occur in the normal keratinocyte induced by ultraviolet light rays. Later changes are associated with genomic instability due to loss of heterozygosity (17p, 17q, 9p, 9q, 3p, 13q), INK4A-ARF mutation (9pq), TP53 mutation (17p21), upregulation of telomerase and amplification of EGFR in the development of squamous cell carcinoma *in situ*, then invasive squamous cell carcinoma.
- Mutations of DNA repair genes allow cells with lethal damage to proliferate, which increases the risk of cancer. Oncogenes and tumor suppressor genes in cutaneous squamous cell carcinoma, basal cell carcinoma and melanoma are given in Table 29.29.

Table 29.28 High-risk squamous cell carcinoma of skin

Diameter of tumor >2 cm
Depth of tumor >4 mm, and Clark levels IV and V (based on depth and invasion)
Tumor involving muscle, nerve and bone
Location of tumor on ear and lip
Tumor arising in scar tissue
Broders grade 3 and 4
Immunosuppressive state
Absence of inflammatory infiltrate in tumor

With respect to depth and invasion, tumors <4 mm is rated as Clark levels I–III.

Surgical Pathology: Squamous Cell Carcinoma of Skin

Gross Morphology

- Diagnosis of squamous cell carcinoma of skin is always made by skin biopsy, that should include sufficient depth in invasive tumor. Superficial shaving technique is applied in flat or minimally elevated lesion <1 mm. Punch biopsy is taken from elevated tumor.
- The margins of tumor may be indistinct. Large tumor is firm in consistency. Progressive invasion of the tumor results in fixation of tumor to underlying soft tissue.
- Presence of enlarged lymph node indicates tumor metastases. Gross morphology of squamous cell carcinoma of skin is shown in Fig. 29.51.
- Squamous cell carcinoma of skin is a firm, flesh colored or erythematous, keratotic papule or plaque. Tumor may be pigmented or ulcerated or smooth nodule or a thick cutaneous horn or verrucous. Tumor may be present as an abscess in periungual location.

Light Microscopy

- Invasive squamous cell carcinoma of skin shows variable degree of differentiation. Highly atypical cells are demonstrated at all levels of the epidermis beneath basement membrane.
- Tumor is composed of polygonal cells arranged in orderly lobules and exhibiting numerous large zones of keratinization forming squamous or keratin pearls to highly anaplastic, rounded cells with foci of necrosis and only abortive, single-cell keratinization (dyskeratosis). Histology of cutaneous squamous cell carcinoma is shown in Fig. 29.52.

Table 29.29 Oncogenes and tumor suppressor genes in cutaneous squamous cell carcinoma, basal cell carcinoma and melanoma

Gene	Locus	Function	Tumor Type
Oncogenes			
H-RAS	11p15.5	Cell cycle, survival	▪ Squamous cell carcinoma ▪ Melanoma
K-RAS	1p13.2	Cell cycle, survival	▪ Squamous cell carcinoma ▪ Melanoma
Tumor suppressor genes			
TP53	17p13.1	DNA repair, cell cycle regulation, apoptosis	▪ Squamous cell carcinoma ▪ Basal cell carcinoma
p16 (INK4A)	9p21	Cyclin-dependent kinase inhibitor	▪ Squamous cell carcinoma ▪ Melanoma

Unknown gene mutation on Xq24–q27 may cause basal cell carcinoma. Unknown gene mutation on 9q31 can cause keratoacanthoma.

Squamous Cell Carcinoma of Skin: Histologic Variants

- Histologic variants of squamous cell carcinoma of skin include: SCC (NOS), keratoacanthoma (low-grade variant), verrucous variant, acantholytic variant, adenosquamous variant, spindle cell variant, basaloid variant, clear cell variant, signet-ring variant, pseudoglandular variant and other rare variants (lymphoepithelial-like SCC, pseudovascular SCC, and SCC with sarcomatoid differentiation).
- Keratoacanthoma exhibits as symmetric keratin-filled crater, with the epidermis on each side extending over to form a distinct lip.
- In verrucous squamous cell carcinoma of skin, superficial component resembles verruca vulgaris with prominent acanthosis and papillomatosis; and the deeper component extends downward, displacing collagen bundles.
- Spindle cell variant of squamous cell carcinoma of skin reveals spindle-shaped atypical cells.
- Adenoid (pseudoglandular) variant of squamous cell carcinoma of skin shows glandular microscopic pattern and keratinocyte acantholysis.
- In clear cell variant of squamous cell carcinoma of skin keratinocytes exhibit hydropic cytoplasmic swelling and accumulation of lipid vacuoles.
- Signet-ring variant of squamous cell carcinoma of skin is rare and characterized by concentric rings composed of keratin and large vacuoles corresponding to markedly dilated endoplasmic reticulum.
- Basaloid variant of squamous cell carcinoma of skin is another histologic subtype.

Immunohistochemistry Technique

Panel of markers used for squamous cell carcinoma (spindle cell variant) of skin is mentioned below.

Markers	Expression
▪ Cytokeratins	▪ Positive
▪ p63	▪ Positive
▪ Actin	▪ Negative/positive
▪ Desmin	▪ Negative/positive

Treatment and Clinical Course

Squamous cell carcinoma of skin causes local tissue destruction. Metastasis tends to occur in patient with large tumors that invade deep structures or cutaneous nerves in <5% of cases.

- Large tumors are discovered early and cured by nonexcisional ablative therapy for *in situ* carcinoma, Mohs micrographic surgery, conventional surgical excision, topical therapy for *in situ* carcinoma and radiation therapy.
- Indications of Mohs micrographic surgery are infiltrative growth with poorly defined clinical margins on lip, ear, nail bed, nasal tip, eyelid and genitalia; history of radiation at site, immunosuppression state, recurrence of large tumor, verrucous carcinoma and tumor arising from chronic scarring conditions.



Fig. 29.51: Squamous cell carcinoma of skin. Gross examination shows cauliflower-like growth. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

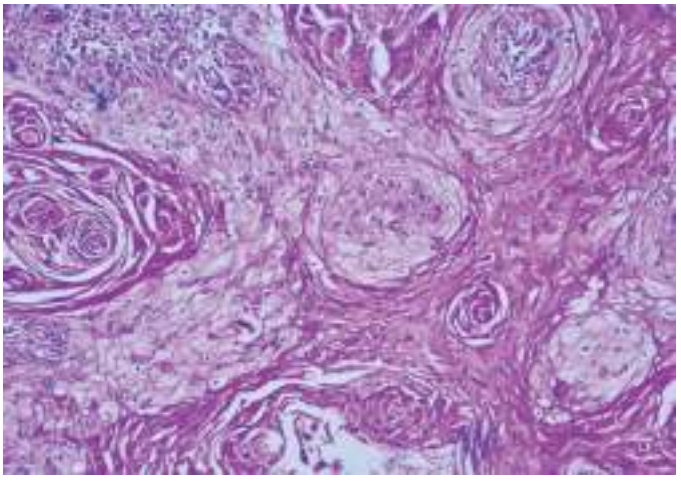


Fig. 29.52: Histology of squamous cell carcinoma of skin. Tumor is composed of solid nests of polygonal-shaped cells with distinct borders, intercellular bridges, pink keratinized cytoplasm and keratin formation (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

KERATOACANTHOMA OF SKIN

Keratoacanthoma is a low-grade squamous cell carcinoma of skin characterized by initial rapid growth with central keratin plug followed by a period of variable tumor stability and spontaneous regression. Tumor originates from pilosebaceous unit and shares histopathologic features with squamous cell carcinoma (SCC) requiring treatment.

- Cytologic pleomorphism is less common in keratoacanthoma, which has glassy pale eosinophilic cytoplasm. There is an abrupt transition between the tumor and adjacent epidermis.
- Keratoacanthoma has predilection for cheeks, nose, ears, and dorsa of hands on sun-exposed areas. The peak incidence of solitary keratoacanthoma is

between 50 and 69 years of age. They occur more frequently in men with a male to female ratio of 2:1.

Molecular Pathogenesis

Predisposing factors for keratoacanthoma include ultraviolet (UV) radiation, exposure to chemical carcinogens, immunosuppression, human papillomavirus (HPV), recent trauma or surgery to the location, mutations of TP53 or H-RAS; Hedgehog pathway inhibitors for basal cell carcinoma and use of BRAF inhibitors in melanoma. Keratoacanthoma can be associated with autosomal dominant disorders such as Muir-Torre syndrome, Ferguson-Smith syndrome or multiple keratoacanthoma of Witten-Zak; autosomal recessive xeroderma pigmentosum; and X-linked dominant incontinentia pigmenti.

Clinical Features

Most common sites of keratoacanthoma are hair-bearing areas and sun-exposed hair-bearing areas with the face, head, neck, and dorsum of extremities; within the mouth, lip, gingiva, hard palate, and other mucosal surfaces. Keratoacanthoma has three stages: (a) proliferation stage—rapid growth of flesh-colored, dome-shaped nodule of 1–2 cm with a central crater filled with keratinaceous material with a central crater filled with keratinaceous material within 6–8 weeks, (b) maturation stage—lasts for several weeks to months where the keratoacanthoma maintains its crateriform appearance, and (c) involution stage—heals spontaneously after several weeks leaving atrophic scar without treatment.

Surgical Pathology: Keratoacanthoma

Gross Morphology

Keratoacanthoma is solitary nodule with central keratin plug. The lesion is solitary nodule with central keratin plug.

Light Microscopy

- Exoendophytic keratoacanthoma is composed of central, keratin-filled crater, surrounded by keratinizing well differentiated squamous epithelium. There is marked acanthosis and hyperkeratosis.
- Cells in the center of lesion have glassy eosinophilic appearance of cytoplasm, which extend upward over the sides of the crater and downward into the dermis as irregular tongues or nests of squamous cells. Prominent inflammatory cells are present around the lesion. Microabscesses are present at the edge of tumor.
- Tumor does not extend beyond sweat glands. Perineural and vascular invasion may be present. Histology of keratoacanthoma is shown in Fig. 29.53.

Keratoacanthoma: Histologic Variants

Histologic subtypes of keratoacanthoma include solitary keratoacanthoma, subungual keratoacanthoma, mucosal keratoacanthoma, giant keratoacanthoma, keratoacanthoma centrifugum marginatum, generalized eruptive keratoacanthoma of Grzybowski, and multiple keratoacanthomas in Ferguson-Smith syndrome.

Treatment and Prognosis

While keratoacanthoma is a low-grade squamous cell carcinoma of skin, hence treatment is recommended due to the relation to squamous cell carcinoma. Tumor should be excised with 4 mm margins. Tumor <2 cm on extremities is treated by electrodesiccation and curettage. Aggressive tumor >2 cm in cosmetically sensitive areas require tissue sparing and should consider Mohs micrographic surgery. Mohs micrographic surgery is the treatment of choice for tumor with perineural invasion. Keratoacanthoma has an excellent prognosis after surgical excision.

BASAL CELL CARCINOMA OF SKIN

Basal cell carcinoma of skin is the most common slow growing locally destructive malignant tumor in persons with fair skin that rarely metastasizes. It is caused by exposure to ultraviolet light radiation; associated with PTCH gene mutation.

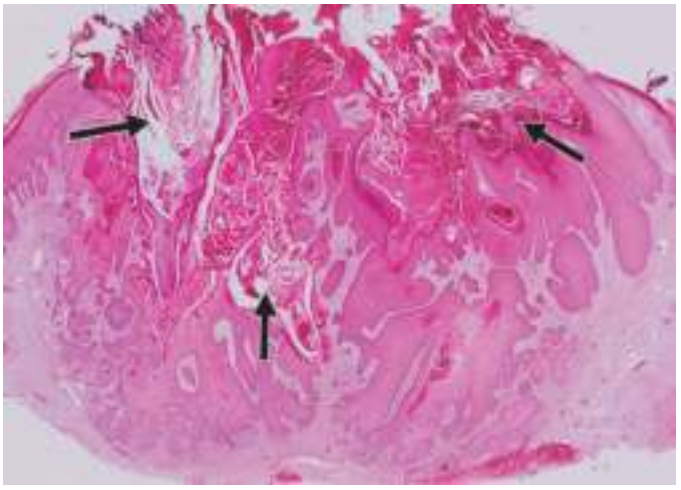


Fig. 29.53: Histology of keratoacanthoma. Tumor is composed of central, keratin-filled crater, surrounded by keratinizing well differentiated squamous epithelium. There is marked acanthosis and hyperkeratosis. Cells in the center of lesion have glassy eosinophilic appearance of cytoplasm. These cells extend upward over the sides of the crater and downward into the dermis as irregular tongues or nests of squamous cells. Prominent inflammatory cells are present around the lesion. Microabscesses are present at the edge of lesion. Tumor does not extend beyond sweat glands. Perineural and vascular invasion may be present (arrows) (400X).

- There is a direct correlation between total exposure to sunlight on head and neck region resulting in basal cell carcinoma, squamous cell carcinoma and melanoma of skin. In contrast to squamous cell carcinoma, it tends to involve the upper part of the face. Incidence is increasing in younger populations.
- The great danger of basal cell carcinoma of skin results from local invasion rather than metastases. The doubling time is estimated to be between six months to one year. Metastases of basal cell carcinoma occur rarely to regional lymph nodes and lungs.
- Metastases of basal cell carcinoma of skin to the bone marrow have been reported. Lethal extension to the central nervous system from aggressive basal cell carcinoma on scalp has been reported.
- Basal cell carcinoma of skin is treated by surgical excision, electrodesiccation and curettage, Mohs micrographic surgery, and occasionally radiation.

Pathogenesis

Solar ultraviolet radiation causes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), which often appear on sun-exposed regions of skin. Ultraviolet B-radiation dose influences the induction of apoptosis and TP53 gene in human keratinocytes. Two main DNA lesions in keratinocytes formed by exposure to UVB radiation are cyclobutene pyrimidine dimers (CPD) and 6–4 pyrimidine pyrimidone photoproducts (6–4PPs), and their Dewar isomers at dipyrimidine sites.

- Cutaneous absorption of ultraviolet B (UVB) radiation (290–320 nm) induces mutations in TP53 gene, PTCH gene and UV signature genes, which directly interferes with the bonding between the nucleotide base pairs in the DNA, resulting in impairment of cell cycle arrest, DNA repair and apoptosis.
- The cellular response to ultraviolet radiation is dependent upon the induced expression of many genes including TP53 tumor suppressor gene. Increased expression of p53 protein is cell-type specific and activation is due to an alteration in transcription, translation or conformation. Once expression of TP53 tumor suppressor gene is activated, p53 protein can bind directly to single-stranded DNA, interact with DNA replication machinery and regulate the transcription of genes involved in both cell cycle arrest DNA repair and apoptosis.
- Environmental agents associated with the development of basal cell carcinoma of skin and other cancers are given in Table 29.30. Heritable disorders predisposing to multiple basal cell carcinomas of skin are given in Table 29.31.

Molecular Genetic Alterations

Mutations of PTCH1 gene on chromosome 9q22.3 and SMD gene on chromosome 7q31–32 involved in Hedgehog signaling pathway have been demonstrated in sporadic basal cell carcinoma. Oncogenes and tumor suppressor genes in basal cell carcinoma of skin are given in Table 29.32.

Table 29.30 Environmental agents associated with the development of basal cell carcinoma of skin and other cancers

Environmental Agent	Persons at Risk	Route of Exposure to Agent	Associated Sporadic Skin Cancer
General exposure to environmental agents			
Ultraviolet radiation (UVB:290–320 nm) uses p53 gene mutation and induces dimer formation between neighboring thymine pairs in DNA	General population	Topical	▪ Basal cell carcinoma ▪ Squamous cell carcinoma ▪ Melanoma ▪ Bowen disease ▪ Skin cancers in xeroderma pigmentosum
Occupational exposure			
Arsenic	Agriculture worker, soil contamination, living in areas of high groundwater	Topical, systemic	▪ Basal cell carcinoma ▪ Squamous cell carcinoma ▪ Bowen disease
Dry cleaning agents	Dry cleaner	Topical, systemic	Basal cell carcinoma
Fiberglass	Insulator	Topical	Basal cell carcinoma
Therapeutic agents administered in patients			
Psoralen (PUVA)	Psoriasis patient	Topical, systemic	▪ Basal cell carcinoma ▪ Squamous cell carcinoma ▪ Melanoma
Immunosuppressants	Transplant recipients	Systemic	▪ Basal cell carcinoma ▪ Squamous cell carcinoma
Ionizing radiation	Cancer therapy patient	Topical	▪ Basal cell carcinoma ▪ Squamous cell carcinoma

Table 29.31 Heritable disorders predisposing to multiple basal cell carcinomas of skin

Heritable Disorder	Comments
Nevoid basal cell carcinoma syndrome (NBCCS)	PTCH gene mutation, associated with multiple basal cell carcinomas >100 broad nasal root, borderline intelligence jaw cysts, palmar pits, multiple skeletal abnormalities
Bazex syndrome	X-linked disorder, associated with multiple basal cell carcinomas, follicular atrophoderma, dilated follicular ostia, with ice scars, hypotrichosis, hypohidrosis
Rombo syndrome	Autosomal dominant disorder associated with multiple basal cell carcinomas, atrophoderma, milia, hypertrichosis, trichoepitheliomas, peripheral vasodilation

Table 29.32 Oncogenes and tumor suppressor genes in basal cell carcinoma of skin

Gene	Locus	Function	Tumor Type
Oncogenes			
SMO	7q31–q32	Hedgehog signaling pathway receptor	Basal cell carcinoma
Tumor suppressor genes			
TP53	17p13.1	DNA repair, cell cycle regulation, apoptosis	▪ Basal cell carcinoma ▪ Squamous cell carcinoma
PTCH1	9p22.3	Hedgehog signaling pathway receptor	▪ Basal cell carcinoma ▪ Trichoepithelioma

Clinical Features

Patient presents with friable, nonhealing translucent lesion, pearly papules with areas of ulceration, telangiectasias (subepidermal dilated blood vessels) and presence of rolling border on the sun-exposed areas of head and neck. It is locally aggressive tumor extending in facial tissues, including underlying bone.

- Clinical features may vary in different clinical subtypes, which may include nodular, pigmented (similar to nevi or melanomas), superficial, morpheaform (sclerosing) and fibroepithelioma of Pinkus (FEP). The anatomical location of basal cell carcinoma may favor the occurrence of a particular clinical subtype.
- Basal cell carcinoma of skin almost never metastasizes. Tumor is usually curable by surgical resection. Clinical photograph of basal cell carcinoma of skin is shown in Fig. 29.54.

Surgical Pathology: Basal Cell Carcinoma of Skin

Gross Morphology

Gross morphology of basal cell carcinoma of skin depends on the clinical subtypes. Tumor is pearly papule or nodule, often with central ulceration, and overlying telangiectatic vessels.

- **Nodular basal cell carcinoma:** It is most common clinical subtype, which occurs on unexposed regions of head and neck as translucent papule or nodule depending on duration of tumor. Large tumor undergoing central necrosis is called 'rodent ulcer'.
- **Pigmented basal cell carcinoma:** It is also clinical subtype of nodular subtype and presents as hyperpigmented translucent papule undergoing erosion.
- **Superficial basal cell carcinoma:** It usually occurs on trunk as erythematous patch that resembles eczema.
- **Morpheaform basal cell carcinoma:** It presents as ivory-white small scar-like or small lesion of morphea in the absence of pervious surgical procedure or trauma. Fibroepithelioma of Pinkus basal cell carcinoma presents as papule on the lower back; and expresses androgen receptors.

Light Microscopy

Tumor is composed of infiltrative deeply basophilic epithelial (basaloid) cells with narrow rims of cytoplasm arranged in clusters. Tumor cell clusters are attached to the epidermis and protruding into the subjacent papillary dermis. Nuclei in the periphery of the clustered cells are arranged in palisading manner. Mitotic figures are absent. Frequently, slit-like retraction of stroma from tumor islands is present creating lacunae. Histology of basal cell carcinoma of skin is shown in Fig. 29.55. Histology of basal cell carcinoma with squamous differentiation of skin is shown in Fig. 29.56.

- **Nodular basal cell carcinoma:** Tumor is composed of discrete nests of basaloid cells in either the papillary or reticular dermis.
- **Pigmented basal cell carcinoma:** Tumor contains melanocytes in 75% of cases. In 25% cases, tumor contains large amounts of melanin.
- **Superficial basal cell carcinoma:** Tumor contains buds of malignant cells extending into the dermis from basal layer of the epidermis. Tumor cells show palisading pattern in the peripheral region of tumor. There is minimal epidermal atrophy and dermal invasion. Upper dermis shows chronic inflammatory infiltrate.

- **Morpheaform basal cell carcinoma:** Tumor is composed of cells arranged in packed columns. Recurrent basal cell carcinoma may show tumor cells arranged in nests embedded within the dense fibrous stroma of scar.
- **Fibroepithelioma of Pinkus:** Tumor is composed of long strands of interwoven basaloid cells embedded in fibrous stroma with abundant collagen fibers.
- **Basosquamous carcinoma:** Tumor is a form of aggressive basal cell carcinoma composed of cells arranged in infiltrating jagged tongues admixed with squamous intercellular bridges and cytoplasmic keratinization.

Treatment

Basal cell carcinoma of skin is treated by surgical excision, radiotherapy, cryotherapy, curettage, electrodesiccation and Mohs micrographic surgery. Recurrence rate for non-Mohs modalities is nearby four times higher than Mohs micrographic surgery.

- Nonaggressive basal cell carcinoma on trunk and extremities are treated by surgical excision. Aggressive basal cell carcinoma on trunk and extremities are treated by surgical excision or Mohs micrographic surgery.
- Recurrent tumor or tumor located in canthus, nasolabial fold, periorbital or postocular region are treated by Mohs micrographic surgery. The 5-year recurrence rate for Mohs micrographic surgery is approximately 6%.



Fig. 29.54: Basal cell carcinoma of skin. A case of pigmented basal cell carcinoma (BCC) on the face in a farmer (arrow).

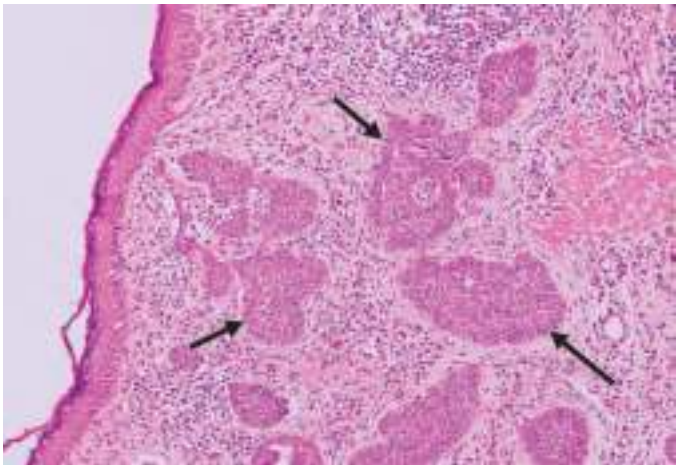


Fig. 29.55: Histology of basal cell carcinoma of skin. Tumor is composed of infiltrative deeply basophilic epithelial (basaloid) cells with narrow rims of cytoplasm arranged in clusters, which are attached to the epidermis and protruding into the subjacent papillary dermis. Nuclei in the periphery of the clustered cells are arranged in palisading manner. Mitotic figures are absent (arrows) (400X).

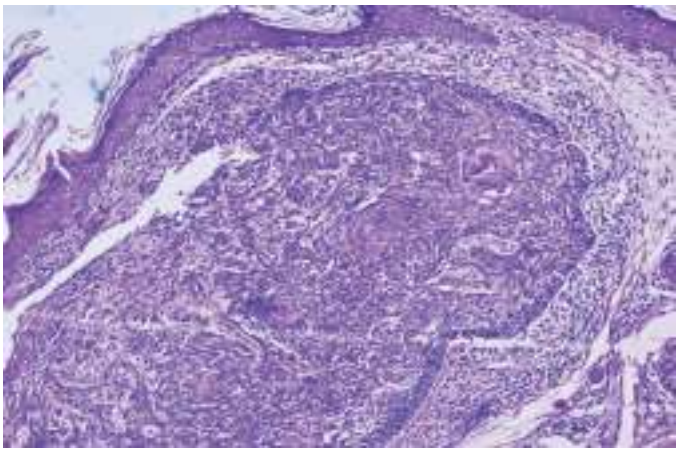


Fig. 29.56: Histology of basal cell carcinoma with squamous differentiation of skin. Tumor is composed of infiltrative deeply basophilic epithelial (basaloid) cells with narrow rims of cytoplasm arranged in clusters, which are attached to the epidermis and protruding into the subjacent papillary dermis. Nuclei in the periphery of the clustered cells are arranged in palisading manner. Mitotic figures are absent (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

BASAL CELL NEVUS SYNDROME

Basal cell nevus syndrome (BCNS) is an autosomal dominant disorder with complete penetrance but variable expression of traits. It is characterized by developmental anomalies and postnatal tumors especially multiple basal cell carcinomas in young persons, and medulloblastoma, pits of the palms and soles; and odontogenic cysts of the jaw. Other related findings include enlarged body habitus, calcifications of the falx cerebri, and skeletal abnormalities of the ribs. Histologic features of basal cell carcinomas and medulloblastoma are identical to those seen in tumors.

Molecular Genetic Alterations

Basal cell nevus syndrome carries tumor suppressor gene mutations of PTCH1 on long arm of chromosome 9. PTCH1 plays a central role in Hedgehog signaling pathway, that is essential for the development of normal body. Basal cell nevus syndrome patients

inherit one defective PTCH1 gene allele and tumors demonstrate somatic mutations. Basal cell nevus syndrome may also occur by two-hit gene mutations.

SEBORRHEIC KERATOSIS

Seborrheic keratosis is the most common benign epidermal tumor. It usually begins as well-circumscribed, dull, flat, tan or brown scaly patches with pseudohorn cysts and rapidly progressing to symptomatic or atypical lesions over years with velvety to granular surface on the head, neck, trunk, and extremities of middle-aged to older individuals.

- Rapidly growing growth should be biopsied to rule out basal cell carcinoma, squamous cell carcinoma or melanoma of skin.
- Clinical and histologic variants of seborrheic keratoses include common seborrheic keratosis, clonal seborrheic keratosis, stucco keratosis, reticulated keratosis, irritated keratosis, seborrheic keratosis with squamous atypia, melanoacanthoma and dermatosis papulosa nigra. The sudden appearance of numerous seborrheic keratosis has been associated with internal malignancies (sign of Leser-Trelat), especially gastric adenocarcinoma.

Surgical Pathology: Seborrheic Keratosis

Gross Morphology

- Seborrheic keratosis tumor measures few millimeters to several centimeters in size. It may be single or multiple.
- Tumor is soft, tan-black with greasy surface, sharply demarcated pigmented lesion that protrudes above surface of skin. It appears stuck to skin. Gross morphology of seborrheic keratitis is shown in Fig. 29.57.

Light Microscopy

- Hematoxylin and eosin-stained section in a case of seborrheic keratosis shows broad anastomosing cords of mature stratified squamous epithelium with abrupt area of acanthotic proliferations of small cuboidal keratinocytes without cytologic atypia, hyperkeratosis, papillomatosis and small 'horn pseudocysts' (round intralesional cysts of loose keratin).
- Clonal seborrheic keratosis is characterized by intraepidermal proliferation of keratinocytes (basaloid to epithelioid cells) without horn pseudocysts. String sign refers to lesion extends to a uniform depth and one could draw a horizontal line parallel to the epidermal surface underlying the lesion. Histology of seborrheic keratosis is shown in Fig. 29.58.



Fig. 29.57: Gross morphology of seborrheic keratosis. Gross morphology of tumor is soft, tan-black with greasy surface, sharply demarcated pigmented lesion that protrudes above surface of skin. It appears stuck to skin (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

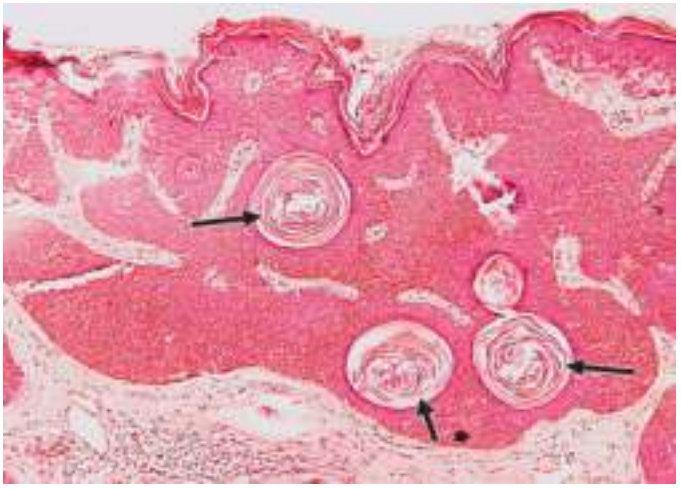


Fig. 29.58: Histology of seborrheic keratosis. Hematoxylin and eosin-stained sections from skin biopsy of seborrheic keratosis shows broad anastomosing cords of mature stratified squamous epithelium with abrupt area of acanthosis, hyperkeratosis, papillomatosis and small pseudohorn keratin cysts (arrows) (400X).

NEUROENDOCRINE TUMORS

Neuroendocrine tumors (NETs) are neoplasms that rise from cells of nervous system and endocrine system. These tumors may contain higher-than-normal quantity of hormones into the blood in response to a signal from the nervous system which can cause various clinical manifestations.

- Neuroendocrine tumors may arise at almost any anatomical site, including paraganglia and are distributed throughout the body in organs including soft tissues.
- Some examples of neuroendocrine tumors are prostatic neuroendocrine tumor, carcinoid tumors in intestine, pancreatic islet cell tumors, medullary thyroid carcinoma, pheochromocytoma, neuroendocrine carcinoma of the skin (Merkel cell carcinoma), small cell lung carcinoma and large cell lung carcinoma.

- Three grading parameters of prognostic relevance in neuroendocrine tumors are the mitotic index, Ki-67 labeling index and presence or absence of necrosis defined by morphological criteria.
- Immunohistochemical stains for neuroendocrine differentiation include neuron-specific enolase, chromogranin A, chromogranin B, synaptophysin and CD56 as well as site specific markers such as hormones and transcription factor.

MERKEL CELL CARCINOMA

Merkel cell carcinoma (MCC) is an uncommon primary cutaneous aggressive neuroendocrine cancer associated with a high-risk of local recurrence, lymph nodal metastasis, and distant metastases. Tumor most commonly occurs on ultraviolet (UV) radiation exposed regions (head, neck) of white elderly patients >65 years of age especially in immunosuppressive state and Merkel cell polyomavirus infection (80%).

- Five-year survival is 51% for localized disease and as low as 14% for distant disease. Sentinel lymph node, surgery and radiation are indicated in many cases.
- On clinical examination, tumor appears as a red or violaceous nodule or plaque.
- Advances in molecular genetics and immunology are helpful to understand pathophysiology and treatment of Merkel cell carcinoma. Deletion of chromosome 1p36 and trisomy 6 are most often demonstrated.

Clinical Features

Patient presents with nontender, firm, red, purple, or skin colored papule or nodule; on the ultraviolet radiation exposed regions such as head and neck of <2 cm at the time of diagnosis in immunocompromised elderly persons. Growth may expand rapidly within three months.

Surgical Pathology: Merkel Cell Carcinoma

Gross Morphology

Tumor is fleshy tan brown nodule or ill-defined lesion showing variable ulceration.

Light Microscopy

- Markel cell carcinoma is composed of dermal proliferation of small, blue cells with indistinct borders arranged in sheets, nests or a trabecular array.
- Tumor cells contain scant cytoplasm and vesicular round nuclei containing finely granular chromatin and inconspicuous nucleoli.
- Numerous mitotic figures are present. Tumor may demonstrate vascular invasion, perineural invasion, or cellular necrosis. Tumor cells invade dermis and cutis. Histology of Merkel cell carcinoma is shown in Fig. 29.59.

Immunohistochemistry Technique

- Immunohistochemistry technique differentiates Merkel cell carcinoma (MCC) from other dermal, blue cell tumors such as lymphoma, Ewing sarcoma, neuroblastoma, melanoma, and small cell lung carcinoma.
- In addition to immunostaining for neuroendocrine markers (synaptophysin, chromogranin), perinuclear dot-like staining with cytokeratin 20 is characteristic.

Markers	Expression
Cytokeratin 20	Positive (highly sensitive)
Neuron-specific enolase (NSE)	Positive
Chromogranin	Positive
Synaptophysin	Positive
Epithelial membrane antigen (EMA)	Positive
Cytokeratin CAM 5.2	Positive
S-100 protein	Negative
Thyroid transcription factor 1 (TTF-1)	Negative

Staging and Prognosis

According to the new American Joint Committee on cancer, there are four stages for Merkel cell carcinoma. Survival after a diagnosis of Merkel cell carcinoma is highly dependent on the stage of presentation. Survival decreases markedly with lymph node involvement and metastatic disease. Unlike melanoma, Merkel cell carcinoma recurs rapidly.

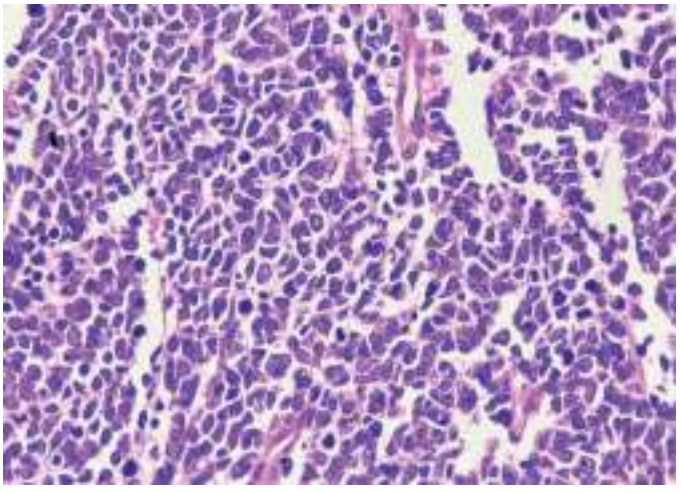


Fig. 29.59: Histology of Merkel cell carcinoma. Hematoxylin and eosin-stained section reveals a dermal proliferation of small, blue cells with indistinct borders arranged in sheets, nests or a trabecular array. Tumor cells contain scant cytoplasm and vesicular round nuclei containing finely granular chromatin and inconspicuous nucleoli. Numerous atypical mitotic figures are present. Tumor may demonstrate vascular invasion, perineural invasion, or cellular necrosis. Tumor cells invade dermis and cutis (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

SKIN APPENDAGEAL TUMORS AND HAMARTOMATOUS OF THE SKIN

Skin appendageal tumors comprise a wide spectrum of benign and malignant tumors that exhibit morphological differentiation from cutaneous adnexal structures found in normal skin. Majority of the tumors are benign and clinically asymptomatic. Classification of skin appendageal tumors is given in Table 29.33.

- Skin appendages arise from pluripotent stem cells that are undifferentiated and present within the epidermis or the appendageal structures.
- Patients can present with papules and nodules. The most important tool for diagnosing and classifying skin appendageal tumors is histopathology. Immunohistochemical analysis has been of little value in diagnosing skin appendageal tumors.
- Skin appendages are represented by three histologically distinct structures: (a) the pilosebaceous unit (hair follicle and sebaceous glands); (b) the eccrine sweat glands; and (c) the apocrine glands.
  - **Pilosebaceous unit:** The sebaceous glands are present in the opening shaft of hair. Palms of the hands or the soles of the feet lack sebaceous glands. These glands secrete an antibacterial moisture known as sebum fluid, that also softens the hands. The secretion activity is related to hormonal release. Blockage of sebaceous ducts results in acne.
  - **Eccrine sweat glands:** The eccrine sweat glands arise from epidermal epithelial germs protruding into the dermis similar to those of pilosebaceous units, but contain less mesenchymal condensation. These are distributed all over the body except nipples and outer genital organs. Although the nipples do have the mammary glands, these are known as modified sweat glands.
  - **Apocrine glands:** The apocrine glands are present mainly in the axillae, groin, pubic and perineal regions. In contrast with eccrine glands, apocrine glands develop from an upper bulge in hair follicles that are in the early bulbous peg stage.

NODULAR HIDRADENOMA

Nodular hidradenoma (synonym: eccrine acrospiroma) is usually a solitary, well-circumscribed and solid tumor measuring 0.5–≥2.0 cm with few of them exhibiting cystic changes on the scalp and axilla. Tumor arises from sweat gland distal excretory duct.

- Long-standing nodular hidradenoma has been reported to undergo malignant transformation. No local recurrence or metastasis has been observed within one year.

Table 29.33 Classification of skin appendageal tumors

Origin	Hamartomas, Hyperplasias and Cysts	Benign Skin Appendageal Neoplasms	Malignant Skin Appendageal Neoplasms
Hair and hair follicle	▪ Hair follicle nevus ▪ Basaloid follicular hamartoma	▪ Trichofolliculoma ▪ Pilar sheath acanthoma ▪ Dilated pore of Winer ▪ Fibrofolliculoma ▪ Trichodiscoma ▪ Fibrous papule ▪ Trichoblastoma ▪ Trichoepithelioma ▪ Desmoplastic trichoepithelioma ▪ Trichoadenoma ▪ Pilomatricoma ▪ Trichilemmoma ▪ Tumor of the follicular infundibulum ▪ Proliferating trichilemmal cyst	▪ Pilomatrical carcinoma ▪ Malignant proliferative trichilemmal tumor ▪ Trichilemmal carcinoma ▪ Trichoblastic carcinoma
Sebaceous glands	▪ Nevus sebaceous ▪ Sebaceous hyperplasia	▪ Sebaceous adenoma ▪ Sebaceoma/sebaceous epithelioma	▪ Sebaceous carcinoma ▪ Basal cell; carcinoma with sebaceous differentiation
Apocrine	▪ Apocrine hidrocystoma ▪ Apocrine nevus	▪ Apocrine fibroadenoma ▪ Erosive adenomatosis of the nipple ▪ Hidradenoma papilliferum ▪ Syringocystadenoma	▪ Apocrine adenocarcinoma ▪ Hidradenocarcinoma ▪ Syringocystadenocarcinoma papilliferum ▪ Cylindrocarcinoma

- The tumor is composed of tubules of various sizes along with papillary projections that are lined by an outer layer of myoepithelial cells and inner layer of cuboidal cells. Solid portions of the tumor show nodules composed of clear and polygonal cells. Tumors have no predilection.

Clinical Features

Patient presents with slow-growing, solitary, firm, flesh-colored or red to blue, mobile, solid or cystic nodule measuring 1–2 cm in diameter on scalp, face and trunk.

Surgical Pathology: Nodular Hidradenoma

Gross Morphology

Majority of tumors are solitary, circumscribed, and non-encapsulated, solid and cystic, dermal, symmetrical, lobulated tumors. Cut surface shows pinkish or bluish or red-blue or brownish nodules with cystic areas.

Light microscopy

- Nodular hidradenoma tumor is composed of round, fusiform or polygonal cells with clear to eosinophilic cytoplasm and a regular oval grooved nucleus and a small inconspicuous nucleolus. Tumor cells arranged in sheet-like and papillary architecture.
- Squamous differentiation is an important feature, especially of an infundibular type of keratinization. Sebaceous differentiation is also common. The stroma varies from fine to dense fibrous tissue to dense hyalinized collagen fibers to myxoid. Cystic spaces may be due to degeneration of tumor cells. Histology of nodular hidradenoma is shown in Fig. 29.60.

Immunohistochemistry Technique

Panel of markers is used in nodular hidradenoma.

Markers	Expression
▪ Cytokeratin	▪ Positive
▪ EMA	▪ Positive
▪ CEA	▪ Positive
▪ S-100 protein	▪ Positive
▪ Vimentin	▪ Positive

Treatment and Clinical Course

Nodular hidradenoma is treated by surgical excision with clear margins. Incomplete surgical excision, nodular hidradenoma has recurrence rate. Mohs micrographic surgery is employed for margin control in irregular tumor.

ECCRINE SPIRADENOMA

Eccrine spiradenoma is an extremely painful slowly growing solitary pink gray benign tumor with eccrine differentiation that may occur anywhere in the body on trunk, scalp and female genital area (vulva, clitoris, labia majora). It may be derived from the secretory coil. It may rarely undergo high-grade malignant transformation.

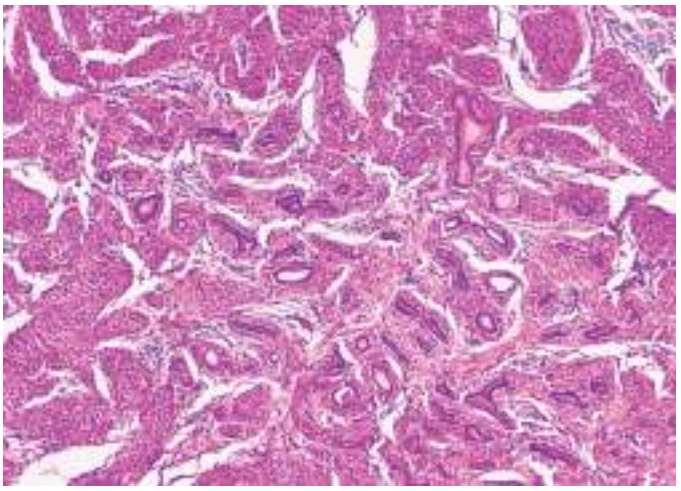


Fig. 29.60: Nodular hidradenoma. Tumor cells are round, fusiform or polygonal exhibiting squamous appearance (400X).

- Tumor is composed of round to oval cells in dermis or subcutaneous tissue with small basaloid cells on the outside and larger polygonal cells with vesicular nuclei and prominent nucleoli towards the center.
- Prognosis is excellent unless malignant transformation with features of rapid growth, distortion and ulceration.

Surgical Pathology: Eccrine Spiradenoma

Gross Morphology

Tumor is usually solitary measuring >1 cm, with pink to gray or tan nodule.

Light Microscopy

- Tumor has delicate fibrovascular capsule. Tumor is composed of round to oval cells in dermis or subcutaneous tissue.
- Small basaloid cells on the outside and larger polygonal cells with vesicular nuclei and prominent nucleoli towards the center.
- Lymphocytes are common throughout the lesion. Histology of eccrine spiradenoma is shown in Fig. 29.61.

CHONDROID SYRINGOMA

Chondroid syringoma, or mixed tumor of the skin is a benign slow-growing tumor of sweat glands with a mucoid stroma showing cartilaginous metaplasia in dermis or subcutaneous region. Though tumor is usually benign, malignant counterpart (malignant chondroid syringoma) does exist and should be ruled out on morphologic features on histologic examination.

- Morphologically, it is identical to pleomorphic adenoma. Tumor is composed of both epithelial cells and mesenchymal cells. Epithelial cells are arranged in solid nests, small clusters and ducts in the myxoid, chondroid or fibrous stroma. It most often seen in middle aged men; mean age at presentation is 48 years.

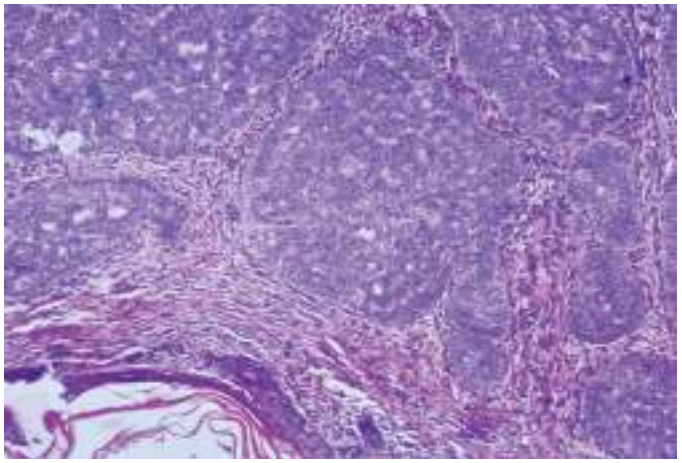


Fig. 29.61: Histology of eccrine spiradenoma. Tumor is composed of round to oval cells in dermis or subcutaneous tissue. Small basaloid cells on the outside and larger polygonal cells with vesicular nuclei and prominent nucleoli towards the center (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

- Most common sites of chondroid syringoma in decreasing frequency are nose, cheek, upper lip, face, head and neck extremities, trunk and back. Chondroid syringoma can easily confused with epidermal cyst and pilomatricoma.

Molecular Genetic Alterations

Chondroid syringoma is positive for gene rearrangement of PLAG1 in largest study to date, but lacks the characteristic translocations of PLAG1 seen in pleomorphic adenoma of salivary gland.

Surgical Pathology: Chondroid Syringoma

Gross Morphology

Chondroid syringoma is well circumscribed but unencapsulated, multilobulated tumor in deep dermis or subcutaneous fat with a prominent chondroid or myxoid stroma.

Light Microscopy

- Major diagnostic criteria of chondroid syringoma include chondroid matrix, tubuloalveolar structures lined by a bilayered epithelium, ductal structures lined by a single epithelium, nests of polygonal cells and the presence of keratinous cysts.
- Tumor is biphasic with both epithelial and stromal components. Epithelial cells are elongated branching tubular structures with two cell layers. Outer layer is cuboidal or columnar with mild nuclear pleomorphism; inner layer is columnar with eosinophilic cytoplasm.
- Lobules are separated by fibrous septae of varying thickness. Features suggestive of malignancy include severe cytologic atypia, markedly increased cellularity, numerous mitoses, destruction of surrounding tissue and tumor necrosis. Histology of chondroid syringoma is shown in Fig. 29.62.

Immunohistochemistry Technique

Panel of markers are used to demonstrate inner epithelial cells and outer myoepithelial cells in benign chondroid syringoma.

Markers	Expression
Inner epithelial cells	
▪ EMA	▪ Positive
▪ CEA	▪ Positive
▪ GCDFP	▪ Positive
▪ Actin	▪ Positive
▪ Cytokeratin 15	▪ Positive
Outer myoepithelial cells	
▪ Vimentin	▪ Positive
▪ GFAP	▪ Positive
▪ SMA	▪ Positive
▪ Calponin	▪ Positive
▪ p63	▪ Positive

p16 is negative in benign syringoma in contrast to strong nuclear. p16 is positive in malignant chondroid syringoma.

PILOMATRIXOMA

Pilomatrixoma is also known as calcifying epithelioma of Malherbe, which arises from pilosebaceous unit. The most sites are head (cheek, periorbital, scalp) and neck region, followed by the upper extremities, trunk and the lower extremities in descending order. Although pilomatrixoma can occur at any age two peaks of presentation, the first and the sixth decades. It is associated with activating mutations in β-catenin, a mediator in the Wnt signaling pathway.

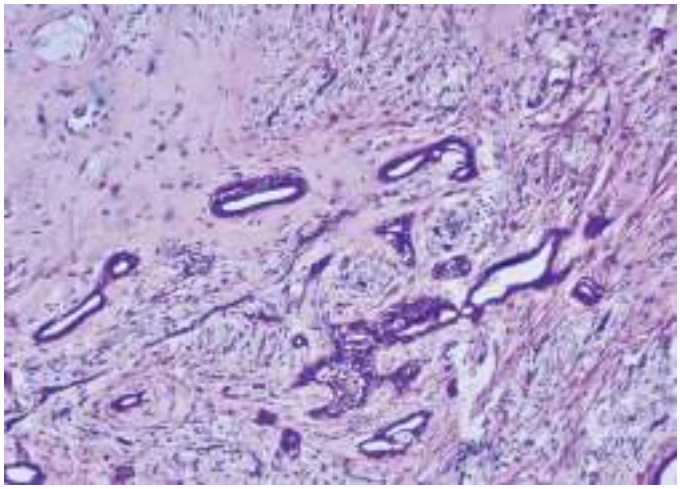


Fig. 29.62: Histology of chondroid syringoma. Tumor consists of chondroid matrix, tubuloalveolar structures lined by a bilayered epithelium, ductal structures lined by a single epithelium, nests of polygonal cells and the presence of keratinous cysts. Tumor is biphasic with both epithelial and stromal components. Lobules are separated by fibrous septae of varying thickness (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

Clinical Features

Patient presents with firm, nontender, solitary cutaneous skin-colored or bluish, firm nodule, cystic to firm in consistency measuring 1–2 cm with adherence to the overlying normal or hyperemic skin but not to the underlying structure on the head and neck, and upper extremities especially during childhood. Overlying epidermal atrophy is common.

- Giant pilomatrixoma has been associated with hypercalcemia and elevated parathyroid hormone-related protein (PTH-rp).
- Multiple pilomatrixomas are demonstrated in Gardner's syndrome, Turner syndrome, myotonic dystrophy, Rubinstein-Taybi syndrome.

Surgical Pathology: Pilomatrixoma
Light Microscopy
▪ Pilomatrixoma is sharply demarcated in the lower dermis most often the subcutis. It is composed of two types of cells: basaloid and shadow (ghost) cells. ▪ The ghost cells have well-defined cell borders, abundant pale eosinophilic cytoplasm, distinct cell borders and no nuclear staining with a central portion of the tumor and may later calcify to give a bony hard consistency to the lesion. ▪ Basaloid cells resemble the cells of basal cell carcinoma of skin. The basaloid cells are deep blue stained, round-to-ovoid cells with ill-defined cell borders having minimal cytoplasm. ▪ Shadow cells have eosinophilic cytoplasm, distinct cell borders and no nuclear staining. Calcification can be present. Histology of pilomatrixoma is shown in Fig. 29.63.

Treatment

Pilomatrixoma is treated by surgical excision. It rarely recurs after surgery. Rare malignant transformation to pilomatrical carcinoma has been reported on head, neck, upper extremities and buttocks. Metastases to regional lymph nodes and distant sites are seen in 10% of patients. Malignant tumor rarely arises *de novo*.

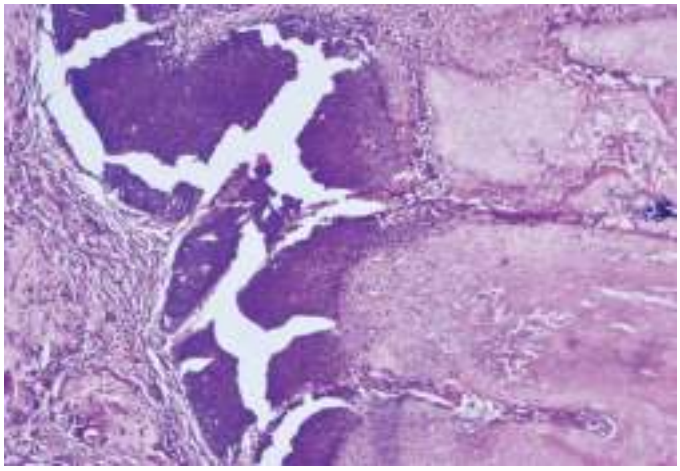


Fig. 29.63: Histology of pilomatrixoma. Tumor is composed of small epithelial cells arranged in solid nests, small clusters and ducts in the mesenchymal components (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

DERMIS AND SUBCUTANEOUS FAT: TUMORS

DERMATOFIBROMA

Skin lesions in dermatofibroma are tan to brown, firm dermal papules. Clinical photograph of dermatofibroma is shown in Fig. 29.64.

Surgical Pathology: Dermatofibroma
Gross Morphology
Tumor is well circumscribed. Cut surface is gray white. Gross morphology of dermatofibroma is shown in Fig. 29.65.
Light Microscopy
On histologic examination, tumor is composed of homogenous spindle-shaped fibroblasts, histiocytes and blood vessels. At the periphery, collagen bundles are surrounded by fibroblasts. The overlying epidermis shows acanthosis, basal keratinocyte hyperpigmentation and broad flattened rete ridges or basaloid proliferation, which may be mistaken for basal cell carcinoma. Histology of dermatofibroma is shown in Fig. 29.66.
Immunohistochemistry Technique
Tumor cells show positivity for factor XIIIa, but negative with CD34.

DERMATOFIBROSARCOMA PROTUBERANS

Dermatofibrosarcoma protuberans (DGSP) is slow growing, solitary or multiple polypoid infiltrative, locally aggressive and rarely metastasizing neoplasm on the trunk, proximal upper extremity and lower extremity of 20–50 years of age group.



Fig. 29.64: Clinical photograph of dermatofibroma. Child shows dermatofibroma on the abdominal wall (arrow).



Fig. 29.65: Gross morphology of dermatofibroma. Tumor is well circumscribed. Cut surface is gray white. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Benguluru, Karnataka.)

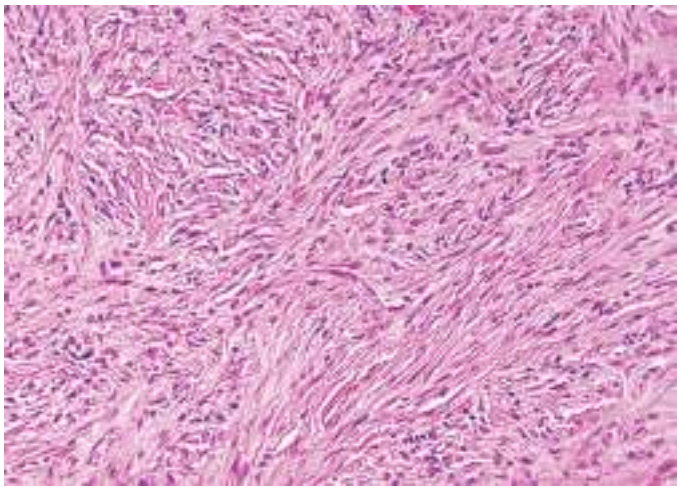


Fig. 29.66: Histology of dermatofibroma. Tumor is composed of homogenous spindle-shaped fibroblasts, histiocytes and blood vessels. At the periphery, collagen bundles are surrounded by fibroblasts (400X).

Clinical Features

Patient presents with plaque-like thickening of skin and steadily progressing to form subcutaneous nodular mass over the course of several years. However, wide surgical excision with generous tumor margins is most often curative.

Surgical Pathology: Dermatofibrosarcoma Protuberans

Gross Morphology

Dermatofibrosarcoma protuberans is a solitary multinodular mass measuring several centimeters with average 5 cm. Although the neoplasm is highly infiltrative, yet it may appear as circumscribed mass. Gross morphology of dermatofibrosarcoma protuberans is shown in Fig. 29.67.

Light Microscopy

- Dermatofibrosarcoma protuberans is composed of bland appearing spindled fibroblasts arranged in an irregularly whorled or storiform pattern or cartwheel pattern; which infiltrate between adnexa, extending into the subcutis with trapping of fat resulting in thinned out epidermis.
- Overlying epidermis may be normal, atrophic or ulcerated. Neoplastic cells show mild atypia and occasional mitosis.
- Occasional tumor can have a more aggressive component, where neoplastic spindle cells are arranged in fascicular pattern. This variant of neoplasm is known as fibrosarcomatous variant of dermatofibrosarcoma protuberans. Histology of dermatofibrosarcoma protuberans is shown in Fig. 29.68.

Differential Diagnosis

Dermatofibrosarcoma protuberans should be differentiated from fibrous histiocytoma (dermatofibroma). Unlike dermatofibrosarcoma protuberans, fibrous histiocytoma tends to limited to the dermis. Analysis of COL1A1-PDGFB fusion protein by fluorescence *in situ* hybridization can be helpful in diagnosing neoplasm in difficult cases. It is worth mentioning that storiform pattern may be demonstrated in dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, liposarcoma, peripheral nerve sheath tumor and nodular fasciitis.

Immunohistochemistry Technique

Dermatofibrosarcoma protuberans shows positivity for CD34.

Markers	Expression
▪ CD34	▪ Positive
▪ Factor XIIIa	▪ Negative



Fig. 29.67: Gross morphology of dermatofibrosarcoma protuberans is a solitary multinodular mass measuring several centimeters with average 5 cm. Although the neoplasm is highly infiltrative, yet it may appear as circumscribed mass. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

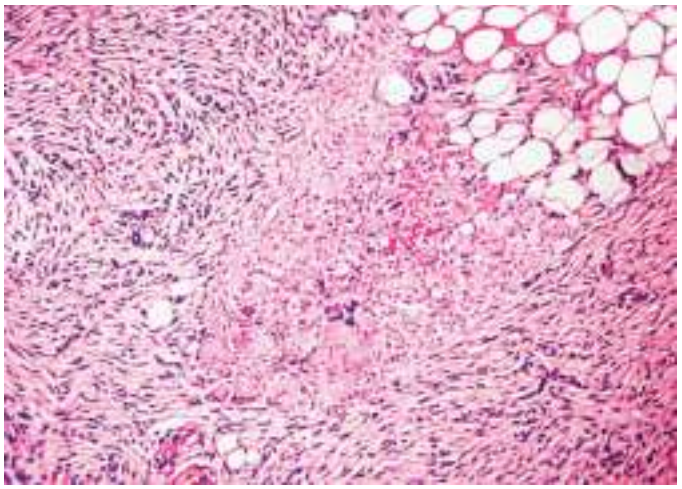


Fig. 29.68: Histology of dermatofibrosarcoma protuberans (DFSP). Tumor is composed of bland-appearing spindled fibroblasts arranged in an irregularly whorled or storiform pattern; which infiltrate into the periphery of tumor entrapping fat resulting in thinned out epidermis. Overlying epidermis may be normal, atrophic or ulcerated. Neoplastic cells show mild atypia and occasional mitosis. Occasional tumor can have a more aggressive component, where neoplastic spindle cells are arranged in fascicular pattern. This variant of neoplasm is known as fibrosarcomatous variant of dermatofibrosarcoma protuberans (400X).

Molecular Genetic Alterations

Dermatofibrosarcoma protuberans exhibits t(17;22)(q22;q13) chromosomal translocation resulting in formation of COL1A1-PDGFB fusion protein analyzed by fluorescence *in situ* hybridization. Supernumerary ring chromosome in dermatofibrosarcoma protuberans may contain centromere of 22 and comprise sequences from chromosomes 17 and 22.

Prognosis and Treatment

Prognosis is excellent after surgical excision of dermatofibrosarcoma protuberans. Recurrence is demonstrated in 11–33% of cases. Tumor may metastasize to lymph node in 1% and distant organs in 5% of cases.

VASCULAR TUMORS OF SKIN

CAPILLARY HEMANGIOMA

Capillary hemangioma is a regressing benign neoplasm of vascular endothelial cells. It is the most common tumor of soft tissue either present at birth or during infancy in 15–20% of cases and children. Initially, it grows rapidly followed by stabilization and involution. Familial capillary hemangioma may show abnormality of chromosome 5.

Age, Sex Predilection and Locations

Capillary hemangioma affects infants and children without sex predominance. Most common sites of hemangioma are skin and mucosal surface followed by viscera. Large hemangioma in infants on the head and neck region occurs as a part of PHACE syndrome (posterior fossa refers to possible abnormal structure in the brain especially cerebellum, hemangiomas of the cervicofacial region, abnormalities of arteries, cardiac and ocular region). Lumbosacral hemangioma occurs in infants with occult spinal malformations.

Clinical Features

Patient with small superficial hemangioma presents with red macules, usually level with the surface of the skin or slightly elevated. Large capillary hemangioma appears as firm, blue-violet torturous mass. Some capillary hemangiomas are associated with Sturge-Weber syndrome and von Hippel-Lindau syndrome. Clinical photograph of capillary hemangioma is shown in Fig. 29.69.

- **Port-wine stain:** It is flat, purple-red birthmark on the face or neck on face of infants. It is composed of large vascular channels frequently interspersed with small capillaries.
- **Cherry hemangioma:** It is small, dome-shaped red papule.
- **Sturge-Weber syndrome:** It is characterized by port-wine stain of face, ipsilateral glaucoma, vascular lesions of choroidal tissue and extensive hemangiomatous involvement of meninges. Patient presents with convulsions, mental retardation and retinal detachment.



Fig. 29.69: It is most common benign vascular tumor in head and neck region of children and infants (arrow).

- **von Hippel-Lindau syndrome:** It is characterized by multiple vascular tumors, other tumors and cysts widely scattered in many organ systems.

Surgical Pathology: Capillary Hemangioma

Gross Morphology

Capillary hemangioma is well circumscribed but lacking capsule. Gross morphology of capillary hemangioma varies depending on its size and location. Small sized capillary hemangioma in the superficial region appears as red macules. Capillary hemangioma located in deep tissues appears as firm and blue violet mass.

Light Microscopy

- Capillary hemangioma is composed of closely packed aggregates of thin-walled capillaries usually filled with blood lined by single layer of bland endothelial cells. Each capillary is surrounded by scant connective tissue stroma. The lumen of capillaries may be partially or completely thrombosed and organized. Histology of capillary hemangioma is shown in Fig. 29.70.
- Juvenile capillary hemangioma in young children may be highly cellular and appearing solid with lumen formation due to protrusion of plump endothelial cells into the lumen and admixed with large number of pericytic cells. Mitoses may be demonstrated.
- Capillary hemangioma in adults, lumen of capillaries is more obvious due to flattening of endothelial cells. Each capillary is surrounded by scant connective tissue stroma. Old lesions are usually fibrotic.

Differential Diagnosis

Capillary hemangioma should be differentiated from kaposiform hemangioendothelioma (KHE) and pediatric round cell tumors.

Immunohistochemistry Technique

Capillary hemangioma shows positivity for CD31, CD34, FLI-1, vWF and GLUT-1 (glucose transporter protein 1).

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ FLI-1	▪ Positive
▪ vWF	▪ Positive
▪ GLUT-1 (glucose transporter protein 1)	▪ Positive

Prognosis and Treatment

Treatment of capillary hemangioma depends on the age of patient, size of lesion and location. Large capillary hemangioma is treated by administration of interferon-α or glucocorticoids or both. Unresectable capillary hemangioma is treated by anti-angiogenesis agents.

KAPOSI SARCOMA

Kaposi sarcoma is a locally aggressive vascular neoplasm, which occurs in several distinct populations with a variety of presentations and courses. It usually occurs in patients with immunosuppression, such as those with acquired immunodeficiency syndrome (AIDS) or those undergoing immunosuppression due to an organ transplant.

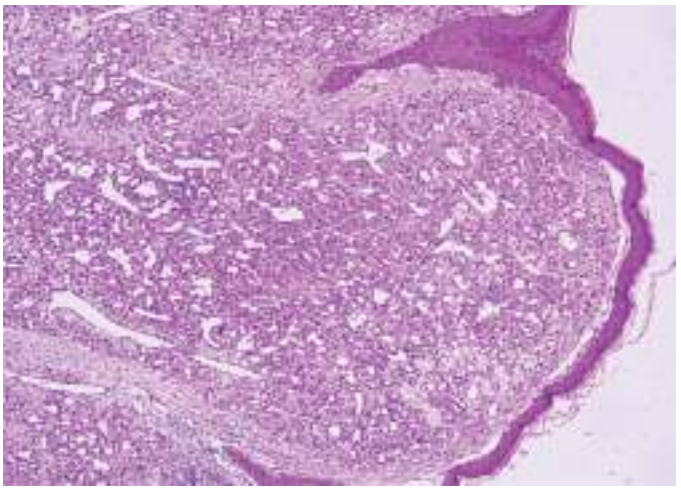


Fig. 29.70: Histology of capillary hemangioma. Capillary hemangioma is composed of closely packed aggregates of thin-walled capillaries usually filled with blood lined by single layer of bland endothelial cells. Each capillary is surrounded by scant connective tissue stroma. The lumen of capillaries may be partially or completely thrombosed and organized (400X).

- In AIDS-related Kaposi sarcoma, most common sites are mucosal surfaces, skin, face, lower extremities, visceral and cutaneous lesions in HIV-infected homosexual or bisexual persons. Patient with organ transplantation develops cutaneous or visceral lesions of the disease with unpredictable clinical course. Lesions resolve on withdrawal of immunosuppressive therapy.
- In classic Kaposi sarcoma, East European and Mediterranean elderly men present with tumor in the lower extremities in the subcutaneous region, that has indolent course.
- In endemic (African) Kaposi sarcoma, young male patient presents with bulky lymphadenopathy and soft tissue tumor. Disease has indolent clinical course in adults; and aggressive clinical course in African children with bulky lymphadenopathy. Clinicopathologic types of Kaposi sarcoma are given in Table 29.34.

Clinical Features

Patient presents with cutaneous violaceous patch, plaque, papule, macule or tumor mass usually on lower extremities. Lymphedema is demonstrated in an occasional patient. Involvement of viscera and lymph nodes presents with hemorrhagic mass. Clinical photograph of Kaposi sarcoma is shown in Fig. 29.71.

Table 29.34 Clinicopathologic types of Kaposi sarcoma

Kaposi Sarcoma Type	Clinical Groups	Location of Lesion	Clinical Course
Classic Kaposi sarcoma	Elderly males of East European and Mediterranean descent	Skin of lower extremities in the subcutaneous region	Indolent course
Endemic (African) Kaposi sarcoma	Children and middle-aged males from equatorial Africa	Skin of lower extremities; and possibly viscera (bulky lymph nodes in children) and soft tissue tumor	Indolent clinical course in adults; and aggressive clinical course in African children with bulky lymphadenopathy
Iatrogenic Kaposi sarcoma	Immunocompromised persons (e.g. post-renal transplantation patients)	Skin of lower legs; and possibly viscera	Unpredictable clinical course; lesions resolve on withdrawal of immunosuppressive therapy
Epidemic (AIDS-related) Kaposi sarcoma	Homosexual or bisexual males with HIV infection	Widespread involvement of skin, face, lower extremities, genitalia, and widespread involvement of viscera (e.g. lungs, lymph nodes, gastrointestinal tract, oral mucosa)	Aggressive clinical course



Fig. 29.71: Kaposi sarcoma of the lower extremity. Papule and ulcerated nodules are seen on foot.

Surgical Pathology: Kaposi Sarcoma

Gross Morphology

- Cutaneous lesions in Kaposi sarcoma are variable, i.e. violaceous patch, plaque, papule, macule or tumor mass.
- Visceral and lymph nodes involved in Kaposi sarcoma are large hemorrhagic mass.

Light Microscopy

Histologic features of Kaposi sarcoma are always identical regardless of clinical settings.

- **Patch stage in Kaposi sarcoma:** It is composed of small infiltrative blood vessels lined by endothelial cells surrounding large ectatic blood vessels; dissecting collagen bundles in the upper reticular dermis and infiltrated by lymphocytes and plasma cells.
- **Plaque stage in Kaposi sarcoma:** It is composed of proliferation of relatively bland, vaguely myxoid spindled cells producing slit-like vascular channels showing jagged outlines. There is presence of extravasated red blood cells and extracellular hyaline globule.
- **Papule (nodule) stage in Kaposi sarcoma:** It is well circumscribed cellular nodular polypoidal mass simulating pyogenic granuloma. It is composed of intersecting fascicles of spindle cells with little or without cytologic atypia. Numerous slit spaces contain red blood cells.

Differential Diagnosis

Kaposi sarcoma should be differentiated from acroangiodermatitis, spindle cell hemangioma and spindle cell sarcomas.

Immunohistochemistry Technique

Kaposi sarcoma shows positivity for immunomarkers CD31, CD34, D2–40, VEGFR3, HHV8 LANA protein (most sensitive marker), factor VIII-related antigen (demonstrated by immunoperoxidase method), Ulex europaeus 1, Fli-1 and Flt-4 (vascular endothelial growth factor receptor 3—VEGFR3).

Markers	Expression
▪ CD31 (PECAM1)	▪ Positive
▪ CD34	▪ Positive
▪ D2–40	▪ Positive
▪ VEGFR3	▪ Positive
▪ HHV8 LANA protein (most sensitive marker)	▪ Positive
▪ Factor VIII-related antigen (demonstrated by immunoperoxidase method)	▪ Positive
▪ Ulex europaeus 1	▪ Positive
▪ Fli-1	▪ Positive
▪ Flt-4 (vascular endothelial growth factor receptor 3—VEGFR3)	▪ Positive

Prognosis and Treatment

Kaposi sarcoma is treated by cryotherapy and radiotherapy. Prognosis is good in immunocompetent patients with cutaneous lesions. Prognosis is poor

in immunocompromised patients without highly active antiretroviral therapy (HAART). Prognosis can be improved by administration of HAART and chemotherapy.

CUTANEOUS ANGIOSARCOMA

Angiosarcoma is highly malignant tumor showing endothelial differentiation, which affects elderly persons without sex predominance. Characteristics and clinical behavior of angiosarcoma depend on the clinical presentation.

- Primary angiosarcoma is related to prolonged sun exposure and therapeutic radiation. Elderly patient presents with single or multiple lesions in the superficial region of head and neck.
- Angiosarcoma associated long-standing lymphedema may arise in the long-standing lymphedema due to Stewart-Treves syndrome, secondary to lymph node dissection and mastectomy performed for breast cancer.
- Stewart-Treves syndrome is a rare deadly cutaneous angiosarcoma that develops in long-standing chronic lymphedema.
- Post-irradiation angiosarcoma arises from soft tissue of patient treated by radiation as adjuvant treatment for breast cancer. It may arise in the settings of use of radiocontrast media thorostat, vascular grafts and arteriovenous fistulae.

Clinical Features

Patient presents with violaceous, erythematous plaque and nodule. Angiosarcoma in deep soft tissue is a hemorrhagic lump. Mammary angiosarcoma presents with rapidly growing mass in the women.

Surgical Pathology: Angiosarcoma

Gross Morphology

Gross morphology of angiosarcoma in cutaneous region is variable ranging from erythematous or violaceous plaque or nodule. Angiosarcoma in the deep tissue is large hemorrhagic mass.

Light Microscopy

Histologic features of angiosarcoma are variable ranging from well differentiated lesion simulating hemangioma; spindled lesion resembling fibrosarcoma; or epithelioid lesion simulating carcinoma.

- **High-grade angiosarcoma:** Tumor is composed of epithelioid like endothelial cells with high nuclear grade and sieve-like to solid architectural pattern. Mitoses and necrosis are frequently demonstrated.
- **Well differentiated angiosarcoma:** Tumor is composed of cells with low nuclear grade lacking mitotic activity and dissecting the adjacent connective tissue.
- **Epithelioid angiosarcoma:** Tumor is composed of epithelioid-like endothelial cells with abundant cytoplasm and uniform high nuclear grade and arranged in sheets. Such lesion is mistaken for carcinoma.

Differential Diagnosis

Angiosarcoma should be differentiated from hemangioma, diffuse angiomatosis, atypical vascular proliferation after radiation, carcinoma, spindle cell carcinoma, melanoma and other spindle cell sarcomas.

Immunohistochemistry Technique

Angiosarcoma shows positivity for CD31, CD34, Fli-1, vWF, D2–40, VEGFR3 and cytokeratins.

Markers	Expression
CD31	Positive (100%)
CD34	Positive (100%)
Fli-1	Positive (100%)
vWF	Positive (100%)
D2–40	Positive (some cases)
VEGFR3	Positive (some cases)
Cytokeratins (low molecular weight) in epithelioid tumors	Positive (25%)

Prognosis and Treatment

Patient with angiosarcoma has poor prognosis. Taxanes chemotherapeutic agent administration may be of value in cutaneous angiosarcoma related to sun exposed skin.

NEURAL TUMORS OF SKIN

PLEXIFORM NEUROFIBROMA

Plexiform neurofibromas can occur anywhere in the body outside the brain and spinal cord. These can occur on the face, around eye, neck, arms, legs, back, chest, abdomen and internal organs.

- Large plexiform neurofibroma can cause thickening of nerve that may affect nearby structures such as skin, muscle and bone.
- Patient presents with severe pain, restricted mobility, vision loss, hearing loss, high blood pressure and other medical problems.
- Most plexiform neurofibromas are benign in nature, but some may undergo malignant transformation. These tumors may be solitary.
- Multiple plexiform neurofibromas affect children as a part of genetic disorder of von Recklinghausen's disease (neurofibromatosis type 1), in which patients can develop multiple café au lait spots (six or more pale brown pigmented macules each >1.5 cm in size).

Surgical Pathology: Plexiform Neurofibroma

Gross Morphology

On gross examination, plexiform neurofibroma may be seen as a fusiform tumor originating from a nerve. Gross morphology of plexiform neurofibroma is shown in Fig. 29.72.

Light Microscopy

- Tumor shows well defined but non-encapsulated masses.
- Tumor is composed of wavy spindle cells with a lot of intervening collagen. Histology of plexiform neurofibroma is shown in Fig. 29.73.

TRAUMATIC NEUROMA

Traumatic neuroma is a firm small painful nodule present in the subcutis and deep soft tissue as a result of injury. The lesion develops most commonly in the soft tissue of the mental foramen area, lower lip and tongue.



Fig. 29.72: Gross morphology of plexiform neurofibroma. Plexiform neurofibroma (WHO grade I) is pathognomonic of neurofibromatosis type 1 (NF-1) involving single or multiple nerve fascicles of major nerve branches. (*Courtesy*: Department of Pathology, Sapthagiri Institute of Medical Sciences, Bengaluru, Karnataka.)

Surgical Pathology: Traumatic Neuroma

Light Microscopy

- Traumatic neuroma is composed of irregularly arranged nerve fascicles embedded in fibrous scar tissue.
- Each fascicle is surrounded by fibrous tissue and perineural cells. There is presence of scattered mast cells.

CUTANEOUS METASTASES

In adults, cutaneous metastases are seen in cancers of bronchus, large bowel, melanoma, oral cavity, breast, and rhabdomyosarcoma. Cutaneous metastases occur in children with neuroblastoma. Panel of immunohistochemical markers is employed on histologic sections to find out primary cancers metastasizing to skin.

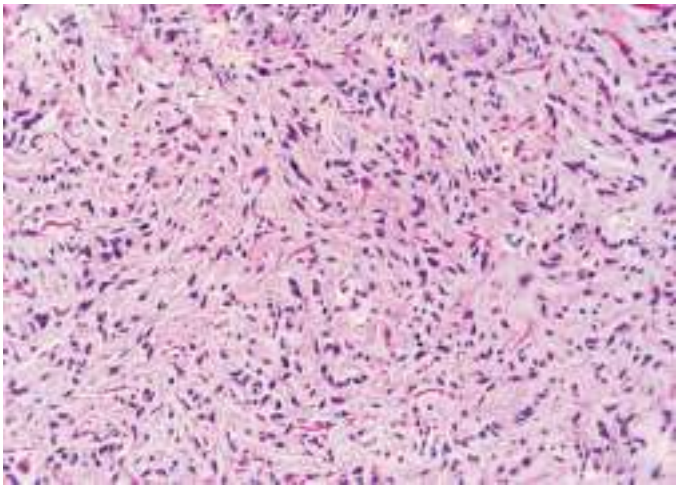


Fig. 29.73: Histology of plexiform neurofibroma. Tumor is composed of elongated wavy cells with small dark oblong nuclei. This lesion is benign. However, persons with neurofibromatosis have a long-term risk for development of malignant neoplasms: neurofibrosarcomas, malignant schwannomas, gliomas.

MISCELLANEOUS SKIN DISORDERS

ACNE VULGARIS

Acne vulgaris is an inflammatory disorder of the pilosebaceous unit, that runs a self-limited clinical course.

- During puberty, under the influence of dehydroepiandrosterone (DHEA), sebaceous glands synthesize excessive sebum. The sebum stimulates excessive proliferation of follicles resulting in retention of sebum. Distended follicles rupture and release proinflammatory chemical mediators in the dermis and induce inflammation. Schematic representation demonstrates stages of open comedone (blackhead) and closed comedone is shown in Figs 29.74 and 29.75.
- Disease is triggered by *Cutibacterium acnes*, *Staphylococcus epidermidis*, and *Malassezia furfur*, which induce inflammation and excessive proliferation of follicles. Acne vulgaris is aggravated by excessive ultraviolet radiation, drugs (steroids, anticonvulsants, lithium), use of occlusive wear, endocrine disorder (polycystic ovary), pregnancy and genetic factors affecting percentage of branched fatty acids in the sebum.
- Acne vulgaris is most often seen in adolescents and young adults involves hair follicles in areas of face,

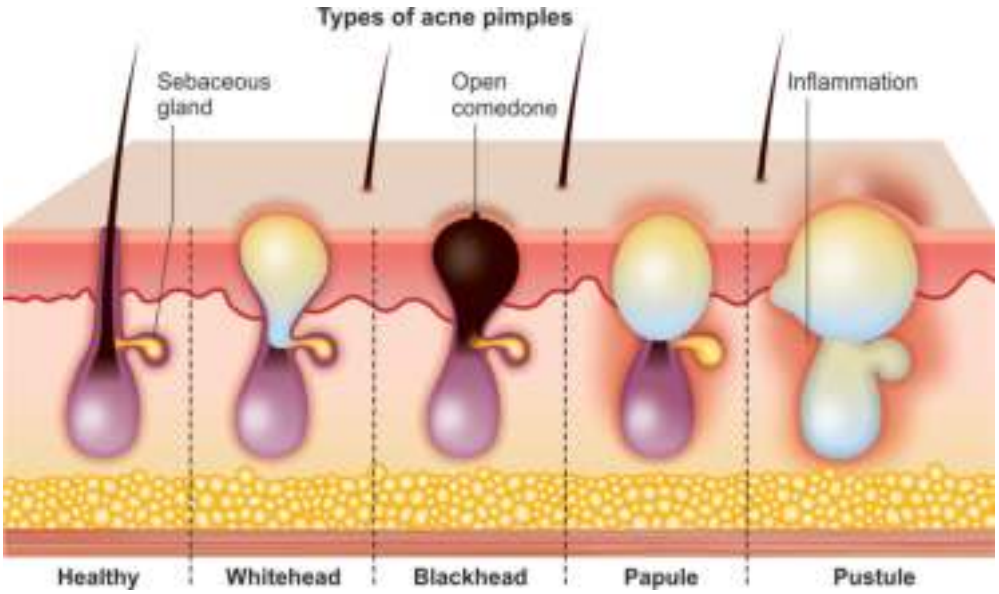


Fig. 29.74: Schematic representation demonstrates stages of open comedone (blackhead) and closed comedone (whitehead) transforming to papule and pustule in acne.

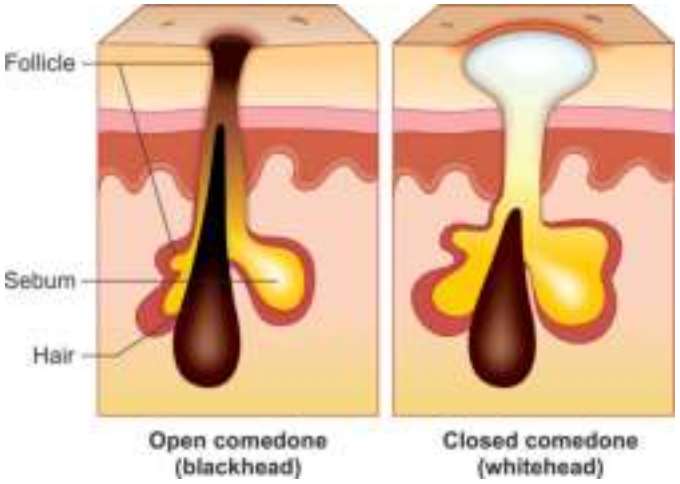


Fig. 29.75: Schematic representation demonstrates open and closed comedones in acne.



Fig. 29.76: Clinical photograph of open comedones in acne. Multiple blackheads or open comedones on the face (lower side), the skin lesions called milia are grouped together (arrows).

upper extremities, chest, and upper back. Lesions may be open or closed comedones.

- Open comedone consists of a distended hair follicle, that contains a central black keratin plug due to oxidation of melanin pigment (not dirt).
- Closed comedone is plugged follicles without a visible central opening, that most often ruptures into the dermis resulting in inflammation. Inflammatory acne is characterized by erythematous papules, nodules, and pustules. The most common lesion is the comedo that involves hair follicles.
- Clinical photograph of open comedones in acne is shown in Fig. 29.76. Clinical photograph of closed comedones in acne is shown in Fig. 29.77.



Fig. 29.77: Clinical photograph of closed comedones in acne. Closed comedone multiple whiteheads are observed on the forehead.



Fig. 29.78: Clinical photograph of fibroepithelial polyp. Fibroepithelial polyp, known as skin tag or acrochordon or papilloma, is benign tumor associated with obesity (arrows).

FIBROEPITHELIAL POLYP

Fibroepithelial polyp, known as skin tag or acrochordon or papilloma, is benign tumor associated with obesity, which most often occurs on the face near eyelids, neck, axilla, trunk and intertriginous regions. Patient presents with itching pain, soft, fleshy-colored and bag-like lesion attached to the skin by a small tender stalk.

- There may be a familial predisposition for developing the lesion, as well as the established association with obesity and insulin resistance. Skin tag is strongly associated with obesity and insulin resistance.
- On histologic examination, fibroepithelial polyp consists of papillary dermis composed of loosely arranged collagen fibers and dilated capillaries and lymphatic vessels, without appendageal structures (hair follicles, sweat glands).

Clinical photograph of fibroepithelial polyp is shown in Fig. 29.78.

EPIDERMAL KERATINOUS CYST

Epidermal keratinous cysts also called epidermal inclusion cysts are the most common cutaneous cysts in men than women during 20–40 years of age. Epidermal keratinous cysts can be primary or secondary occurring anywhere on the body especially on face, scalp, neck, back, and scrotum. Primary epidermal keratinous cysts arise directly from the infundibulum of the hair follicle.

- Plugging of the follicular orifice allows for cyst formation. The cyst often communicates with the surface of the skin through a small orifice or visible central punctum. The size of these cysts can range from a few millimeters to several centimeters in diameter.
- Lesions may remain stable or progressively enlarge over time. The center of epidermoid cysts almost always contains keratin and not sebum.
- Patient presents with well circumscribed, firm, often freely movable, fluctuant nodule with a visible central punctum. Large epidermal keratinous cyst often becomes painful upon traumatic rupture resulting in release of soft gray-white material.

Surgical Pathology: Epidermal Keratinous Cyst

Light Microscopy

- Histologic examination reveals an epidermal kerationus cyst lined by stratified squamous epithelium and is filled with laminated strands of keratinous material.
- A clinically similar lesion seen on the scalp is called a pilar cyst. The pilar cyst lacks a granular layer and is filled with more amorphous material.
- Rupture of epidermal keratinous cyst induce intense acute and chronic inflammatory reaction including formation of giant cells. Histology of unruptured epidermal keratinous cyst is shown in Fig. 29.79. Histology of ruptured epidermal keratinous cyst is shown in Fig. 29.80.

ACANTHOSIS NIGRICANS

Acanthosis nigricans is localized hyperpigmentation of the skin with poorly defined borders. It most common involves intertriginous areas such as skin folds of neck, forehead, armpits (axillae), antecubital and popliteal fossae, groin region and umbilicus in the settings of obesity and/or hyperinsulinemia, other

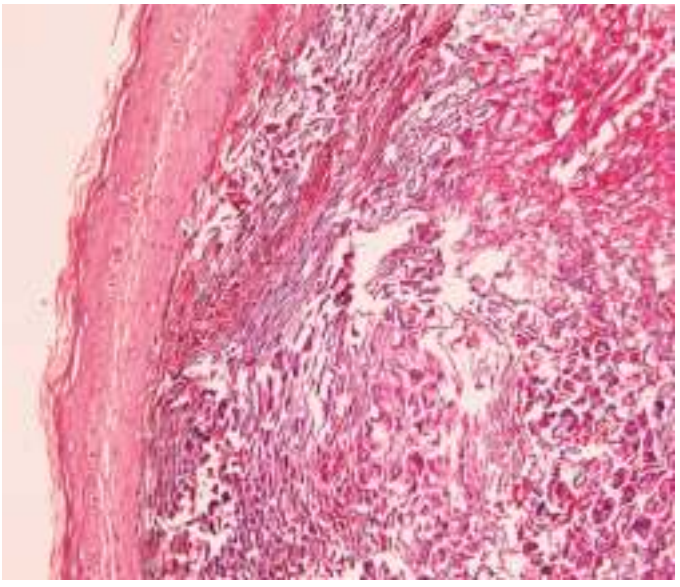


Fig. 29.79: Histology of unruptured epidermal keratinous cyst. The inclusion cyst lined by stratified squamous epithelium and is filled with laminated strands of keratinous material (400X).

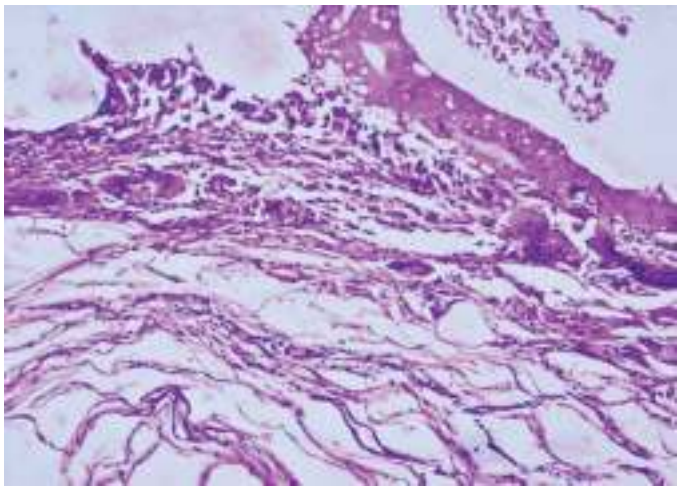


Fig. 29.80: Histology of ruptured epidermal keratinous cyst. The ruptured epidermal keratinous cyst is lined by stratified squamous epithelium and is filled with laminated strands of keratinous material. There is intense acute and chronic inflammatory reaction including formation of giant cells (400X). (*Courtesy*: Dr. Geeta Pachori, Senior Professor and Head of Pathology, Jawahar Lal Nehru Medical College, Ajmer, Rajasthan.)

endocrinopathies, administration of glucocorticoids and oral contraceptives; and visceral malignancy (stomach, colon, prostate, breast, ovary, uterus, lung). Etiology and features of acanthosis nigricans are given in Table 29.35.

- **Pathogenesis:** Multiple factors are involved in the pathogenesis of acanthosis nigricans. Increased concentration of insulin activates IGF-1 and keratinocyte insulin-like growth factor (ILGF) receptors. At higher concentration, insulin may displace IGF-1 from IGF-binding protein. Elevated circulating IGF causes proliferation of keratinocyte and dermal fibroblasts. Hereditary acanthosis nigricans variant is linked to malignancy due to synthesis of aberrant fibroblast growth factor (FGF), that acts via the epidermal growth factor receptor.
- **Clinical features:** Patient presents with pruritic, velvety, poorly defined hyperpigmented macules and patches and progress to palpable plaques in skin folds of neck, forehead, armpits (axillae), antecubital and popliteal fossae, groin region and umbilicus. In approximately one-third of cases, malignant acanthosis nigricans presents with skin changes before any signs of cancer.

Surgical Pathology: Acanthosis Nigricans

Light Microscopy

- Histologic examination of the skin lesion in acanthosis nigricans shows papillomatosis, hyperkeratosis with minimal hyperpigmentation.
- The dermal papillae usually have an upward projection with thinning of the epidermis. There is usually no dermal inflammatory infiltrate.

Table 29.35 Etiology and features of acanthosis nigricans

Acanthosis Nigricans	Comments
Familial acanthosis nigricans	Autosomal dominant trait presents at birth or during childhood due to mutation of fibroblast growth factor receptor 3 (FGFR3)
Obesity associated acanthosis nigricans	Associated with obesity and resistance to insulin
Medications associated acanthosis nigricans	Administration of nicotinic acid, systemic glucocorticoids, diethylstilbestrol, combined oral contraceptive pill, growth hormone therapy, estrogen, protease inhibitors, niacin, injected insulin
Acanthosis nigricans associated with endocrine dysfunction	▪ **Insulin resistance syndromes type A:** Hyperandrogenemia, insulin resistance and acanthosis nigricans ▪ **Insulin resistance syndromes type B in females:** Uncontrolled diabetes mellitus, ovarian hyperandrogenism, systemic lupus erythematosus, scleroderma, Sjögren's syndrome and acanthosis nigricans
Malignant acanthosis nigricans syndromes	▪ Most common cancers include gastrointestinal tract, adenocarcinoma, prostatic adenocarcinoma, breast carcinoma, ovarian carcinoma ▪ Rare cancers include lung carcinoma and lymphoma
Autoimmune disorders associated acanthosis nigricans	Systemic lupus erythematosus, scleroderma, Sjögren's syndrome and Hashimoto's thyroiditis
Unilateral acanthosis nigricans	Autosomal dominant disorder associated with unilateral lesions in infancy, childhood and adulthood

XANTHOMAS

Xanthomas are lesions characterized by accumulation of lipid-laden macrophages. These lesions can develop in the settings of altered systemic lipid metabolism or as a result of local cell dysfunction, e.g. hyperlipidemia, diabetes mellitus, hypothyroidism, primary biliary cirrhosis, cholestasis, nephrotic syndrome, monoclonal gammopathy, metabolic lipid disorders and drugs (prednisone, cyclosporine, tamoxifen).

- **Clinical features:** Patient presents with well circumscribed most often multiple plain or nodules in cutaneous regions, eyelids (xanthelasma), over tendon sheath or synovium, elbows, knees, feet and buttock regions associated with familial dysbetalipoproteinemia. Well controlled diabetes mellitus and cholesterol normal levels are less likely to cause xanthoma. Xanthomas resolve by treating underlying cause of hyperlipidemia.
- **Treatment:** Surgical excision of xanthoma is indicated in case of compromised joint function. Hyperlipidemia should be treated. Xanthomas are treated by surgical excision, laser surgery, or chemical treatment with trichloroacetic acid. Recurrence of xanthomas may occur unless hyperlipidemia is treated.

Surgical Pathology: Xanthoma
Gross Morphology
Xanthoma is well circumscribed white or yellow to brown masses.
Light Microscopy
Histologic examination of xanthoma shows focal dermal collections of lipid-laden histiocytes. Histology of xanthoma is shown in Fig. 29.81.

GRANULOMA PYOGENICUM

Granuloma pyogenicum is also known as lobular capillary hemangioma in the skin or mucous membranes and occasionally it can be found subcutaneously or intravascularly. It most often occurs following trauma in all age groups. Lesion is composed of numerous capillaries, venules with plump endothelial cells and fibromyxoid stroma. Hands, lower lips and gingiva are most frequently affected.

- **Pathogenesis:** Risk factors for granuloma pyogenicum include trauma, BRAF mutations, herpesvirus or human papillomavirus 2 (HPV-2); and certain medications such as oral contraceptives, retinoids, gefitinib, cabecitabine, and afatinib. Development of granuloma pyogenicum occurs three phases: (a) cellular phase, (b) vascular phase or capillary phase, and (c) involutionary phase.
- **Clinical features:** Patient presents with small or large, smooth or lobulated, reddish exophytic vascular nodule on hands, lower lips and gingiva; that can grow rapidly. Larger lesion becomes lobulated and sometimes develop into mushroom-like, pedunculated tumor. Profuse bleeding is the most common symptom. Depending on the area, size and patient wishes, granuloma pyogenicum is treated by surgical excision, curettage, electrocautery, radiosurgery, cryosurgery, sclerotherapy, or laser surgery. Clinical photograph of granuloma pyogenicum is shown in Fig. 29.82.

KELOID

Keloid is an exaggerated response to injury to skin, that extends outside the original wound due to ear piercing

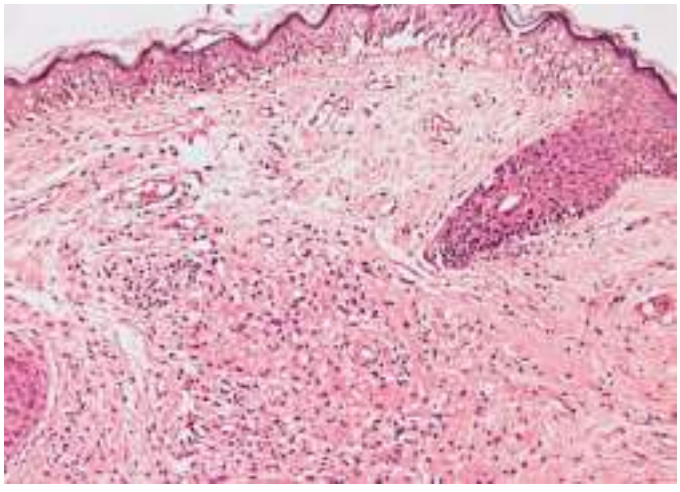


Fig. 29.81: Histology of xanthoma. Xanthoma is composed of focal dermal collections of lipid-laden histiocytes (100X).

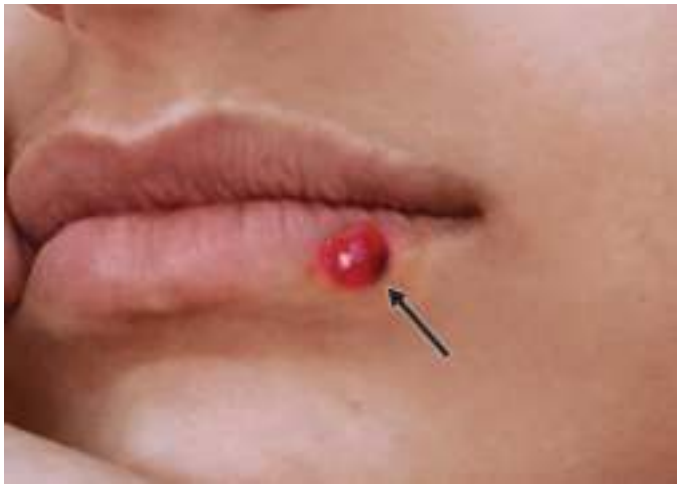


Fig. 29.82: Clinical photograph of granuloma pyogenicum (arrow).

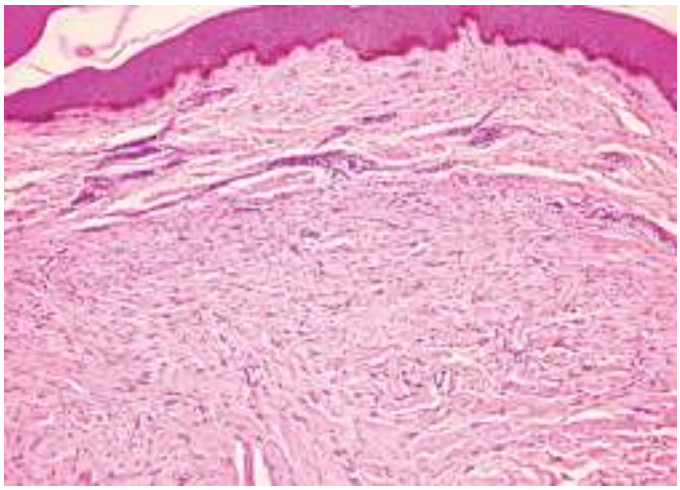


Fig. 29.83: Histology of keloid. Skin consists of thin top layer of epidermis and deeper layer of skin dermis. The pathology of the keloid resides in the dermis, where there is an excessive amount of tightly packed haphazard fascicles of dense and hyalinized collagen fibers randomly oriented in multiple directions, keloid tissue fibroblasts and lymphocytic infiltrate. Overlying epidermis is most often thinned out (400X).

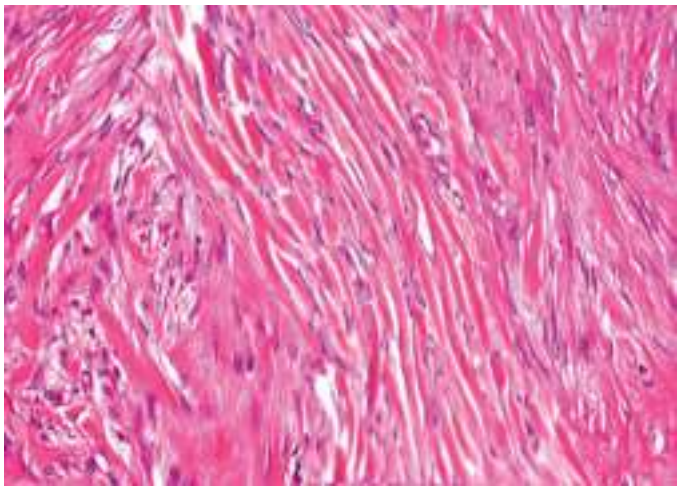


Fig. 29.84: Histology of keloid. Keloid scar is composed of homogenous, broad, eosinophilic fibrillary collagen bundles arranged in haphazardly array. Increased number of fibroblasts are found arrayed with an orientation that parallel the accompanying collagen bundles. Vascularity is diminished within the lesion. It is worth mentioning that overlying epidermis is most often thinned out.

or surgical wounds especially in dark persons. It is characterized by exuberant proliferation of fibroblasts and synthesis of abundant collagen bundles which caused by overexpression of cytokines and growth factors resulting in formation of large nodular scar. Keloid may recur after surgical excision.

- Most common sites of keloid are hands, ear lobe, neck, sternum, chest and shoulder. It most often in females on the earlobe secondary to ear piercing during 10–30 years of age.
- Patient presents with large nodular scar on the upper back, shoulder, presternal region and earlobe. Lesion is most often asymptomatic. Sometimes, lesion may be tender, pruritic or painful. On examination, lesion is elevated and protruding surrounding skin.

Surgical Pathology: Keloid

Gross Morphology

Keloid is polypoidal lesion and covered by thin and hairless skin.

Light Microscopy

- Hematoxylin and eosin-stained section examination of keloid reveals nonencapsulated haphazard fascicles of dense, hyalinized collagen that appears edematous due to dermal mucosubstances in dermis, scattered fibroblasts and myofibroblasts are present.
- Keloid blends with adjacent tissue, overlying epithelium is thin and lacks adnexal structures.
- There is presence of widely scattered dilated blood vessels. Histology of keloid is shown in Figs 29.83 and 29.84.

CHAPTER 30

Nervous System

Vinay Kamal, Anubhav and Vigyat

LEARNING OBJECTIVES

CENTRAL NERVOUS SYSTEM

- Anatomy of nervous system
- Histology of nervous system
- Intracranial compartments
- Intraventricular space

CENTRAL NERVOUS SYSTEM INFECTIONS

- Meningitis
  - Pyogenic meningitis
  - Tuberculous meningitis
  - Viral meningitis
  - Fungal meningitis
  - Iatrogenic meningitis
  - Metastatic cancer-induced meningitis
- Encephalitis
  - Parasitic encephalitis
  - Viral encephalitis
- Cerebral abscess
- Tabes dorsalis in tertiary syphilis

CENTRAL NERVOUS SYSTEM TUMORS

- Nervous system tumors: overview
  - Primary nervous system tumors
  - Metastases to nervous system
- Astrocytic tumors
  - Diffuse astrocytoma, IDH-mutant
  - Diffuse midline glioma, histone H3K27M-mutant
  - Anaplastic astrocytoma, IDH-mutant
  - Primary glioblastoma multiforme, IDH-wildtype
  - Secondary glioblastoma multiforme, IDH-mutant
  - Pilocytic astrocytoma
  - Epithelioid glioblastoma
  - Glioblastoma multiforme with a primitive neuroectodermal tumor component
- Oligodendroglial tumors
- Ependymal tumors
  - Ependymoma
  - Ependymoma with RELA fusion positive
- Choroid plexus tumors
  - Choroid plexus papilloma
- Embryonal tumors
  - Medulloblastoma
  - Embryonal tumor with multilayered rosettes
- Meningeal tumors
  - Meningioma
  - Diffuse leptomeningeal glioneuronal tumor
- Tumors of the cranial and paraspinal nerves
  - Schwannoma (neurilemmoma)
  - Neurofibroma, neurofibromatosis type 1 and malignant change in neurofibroma
  - Malignant peripheral nerve sheath tumor
- Tumors of the sellar region
- Mesenchymal nonmeningeal tumors
- Primary CNS lymphoma
- Germ cell tumors of nervous system
- Primitive neuroectodermal tumors

HYPOXIA-INDUCED BRAIN INJURY

- Cerebral hypoxia
  - Clinical features
  - Pathophysiology
  - Laboratory diagnosis

CEREBROVASCULAR DISORDERS

- Cerebral infarction
- Cerebral hemorrhage
- Subarachnoid hemorrhage
  - Berry aneurysm
  - Mycotic aneurysm
  - Arteriovenous fistula
  - Cavernous hemangiomas
- Transient ischemic attacks
- Fat embolism syndrome

HEAD INJURY

- Epidural hematoma
- Subdural hematoma
- Intracerebral hemorrhage

DEMYELINATING DISORDERS OF NERVOUS SYSTEM

- Multiple sclerosis
- Acute disseminated encephalomyelitis
- Guillain-Barré syndrome
- Hydrocephalus
- Arnold-Chiari malformation

CONGENITAL DISORDERS OF NERVOUS SYSTEM

- Neural tube defects
- Agenesis of corpus callosum
- TORCH complex
- Fetal alcohol syndrome
- Tuberous sclerosis syndrome

CENTRAL NERVOUS SYSTEM

ANATOMY OF NERVOUS SYSTEM

Central nervous system consists of cerebrum, cerebellum, brainstem, spinal cord, 12 cranial nerves and blood vessels supplying these structures.

- Brain and spinal cord are covered by meninges. Meninges are composed of outer dense fibrous dura mater and delicate leptomeninges (pia and arachnoid mater).
- Falx cerebri divides cerebrum into left and right hemispheres. Supratentorial compartment consists of cerebral cortex (frontal, temporal, parietal and occipital lobes), white matter and deep gray nuclei such as basal ganglion, thalamus, and hypothalamus.

- Neuroaxis term denotes brain and spinal cord. Tumors that involve brain parenchyma are said to be 'intra-axial' (e.g. astrocytoma, central neurocytoma or ependymoma).
- Tumors located outside the parenchyma are referred to as 'extra-axial' (meningiomas, hemangiopericytoma, schwannoma). Similar terms used for spinal lesions are intramedullary or extramedullary.

HISTOLOGY OF NERVOUS SYSTEM

Central nervous system (CNS) is composed of gray and white matter that differ quantitatively on gross and histopathologic examination. Nervous system is composed of neurons, glial supporting cells (e.g. astrocytes, oligodendrocytes, ependymal cells), microglial cells, connective tissue in meninges and blood vessels.

NEURONS

Central nervous system consists of 100 billion polarized neurons. Neurons cannot divide and hence have limited regeneration. Neurons respond to physical and chemical stimuli; conduct electrical impulses and release chemical regulators.

- A neuron is a microscopic structure composed of three major parts, namely neuronal cell body (perikaryon), dendrites and axons.
- Neuronal cell bodies and dendrites reside in the gray matter (cortex and deep gray matter). Axons create the framework of the white matter. Neuroglial cells (astrocytes, oligodendrocytes and microglia) are present in different proportions in these tissues.
- Oligodendrocytes are present in abundance in the white matter; and their processes form myelin sheath that insulin CNS axons. Neuropil is a fibrillary material between neuronal cell bodies and formed by the processes of neurons (axons and dendrites) and glial cells.

NEUROGLIAL TISSUE

Neuroglial Cells in CNS

- **Astrocytes:** Astrocytes are present in white matter of cerebral cortex, which participate in metabolism of neurotransmitters. Astrocytes form blood–brain barrier by providing a link between neuron and blood vessels.
- **Microglia cells:** Microglia cells participate in phagocytosis of microbes and cellular debris.
- **Ependymal cells:** Ependymal cells form links between the ventricles of the brain and the central canal of spinal cord. These cells participate in synthesis of cerebrospinal fluid in choroid plexus and its circulation.
- **Oligodendrocytes:** Oligodendrocytes form a supporting network by forming myelin sheath around neurons in CNS.

Neuroglial Cells in Peripheral Nervous System

- **Neurilemmocytes or Schwann cells:** Each Schwann cell produces part of myelin sheath around a single axon of peripheral nervous system neuron. Schwann cell is a type of glial cell wrapping axon forming insulating myelin sheath, which does not insulate node of Ranvier.
- **Satellite neuroglial cells:** These cells support neurons in ganglia of peripheral nervous system.

MENINGES

The meninges are the connective tissue coverings of the brain and spinal cord. The outermost layer is the dura mater, a dense and tough tissue, that forms the periosteum of the inner skull. Beneath the dura is the arachnoid, a network of loose connective tissue that lacks blood vessels.

BLOOD VESSELS OF BRAIN

The brain receives the blood through two internal carotid arteries and two vertebral arteries, which fuse to form the basilar artery. The basilar artery bifurcates into two posterior cerebral arteries, which interconnect with branches of the carotid arteries to form the 'circle of Willis' at the base of the brain. The blood vessels are non-fenestrated and tight junctions with adjacent cells.

Clinical Pearls: Blood–Brain Barrier

- Blood vessels are critical to deliver oxygen and nutrients to all of the tissues and organs throughout the body.
- The blood vessels that vascularize the CNS possess unique properties, termed the blood–brain barrier, which allow these vessels to tightly regulate the movement of ions, molecules, and cells between the blood and the brain.
- Blood–brain barrier protects the neural tissue from toxins and pathogens.
- The physiologic barrier is coordinated by a series of physical and metabolic properties possessed by the vascular endothelial cells (ECs) that form the walls of the blood vessels.
- These properties are regulated by interactions with different vascular, immune, and neural cells.
- Understanding how these different cell populations interact to regulate the blood–brain barrier, which is essential for understanding how the brain functions during health and disease (Fig. 30.1).

INTRACRANIAL COMPARTMENTS

Intracranial lesions or processes can be described according to which intracranial compartment they occur within. The simplest division is into three intracranial

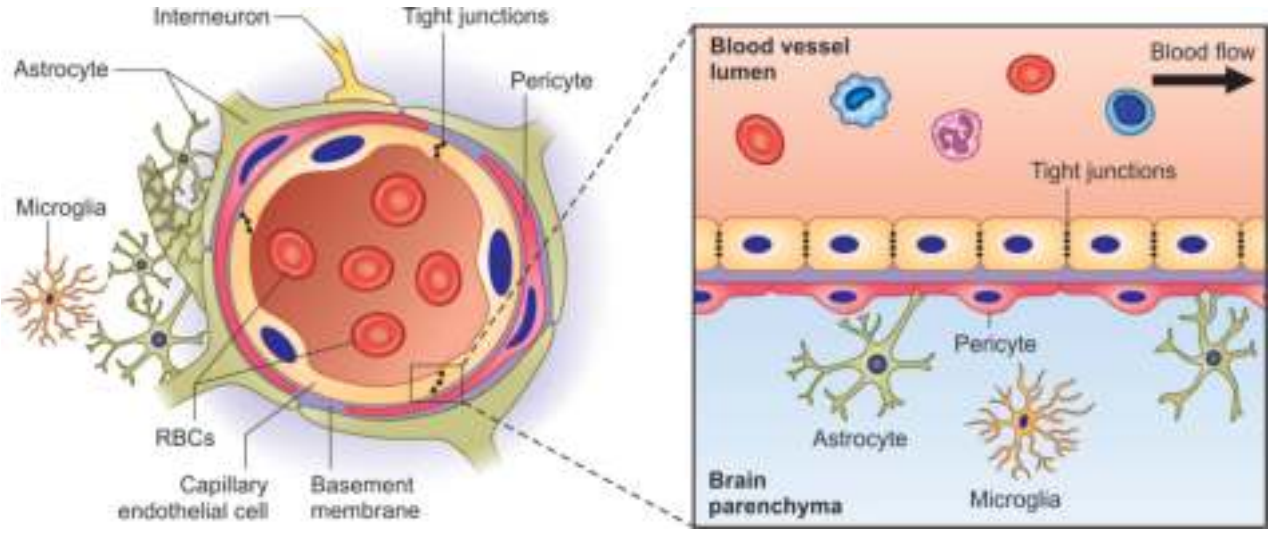


Fig. 30.1: Blood–brain barrier. Astrocytes form blood–brain barrier by providing a link between neuron and blood vessels.

compartments: extra-axial is external to the brain parenchyma; intra-axial is present within the brain parenchyma.

- **Epidural space:** This is a virtual space between the inner aspect of the cranial bones and the dura mater. The epidural space contains the middle meningeal arteries and three main branches, which are 'grooved' into the inner surface of the temporal–parietal bones.
- **Subdural space:** Subdural space is located between the arachnoid and pia mater and is filled with cerebrospinal fluid (CSF). It is contiguous with the perivascular 'Virchow-Robin spaces' surrounding the blood vessels penetrating from the meninges into the brain.
- **Cerebral parenchymal compartment:** Cerebral parenchymal compartment is occupied by the neural, glial, and vascular cells.

INTRAVENTRICULAR SPACE

Intraventricular space includes the lateral ventricles, the third and fourth ventricles, and the '**aqueduct of Sylvius**'. This compartment is filled with cerebrospinal fluid (CSF). CSF circulation in ventricles in central nervous system are shown in Figs 30.2 and 30.3.

- **Cerebrospinal fluid synthesis:** Most of the CSF is produced by choroid plexuses in the lateral cerebral ventricles at a rate of approximately 500 ml per day. The choroid plexus stretches along the roof of the third ventricle and then angles posteriorly to span the lateral ventricles.
- **Cerebrospinal fluid flow/circulation:** Cerebrospinal fluid flows from its intraventricular origin to sites of reabsorption, principally through the arachnoid villi,

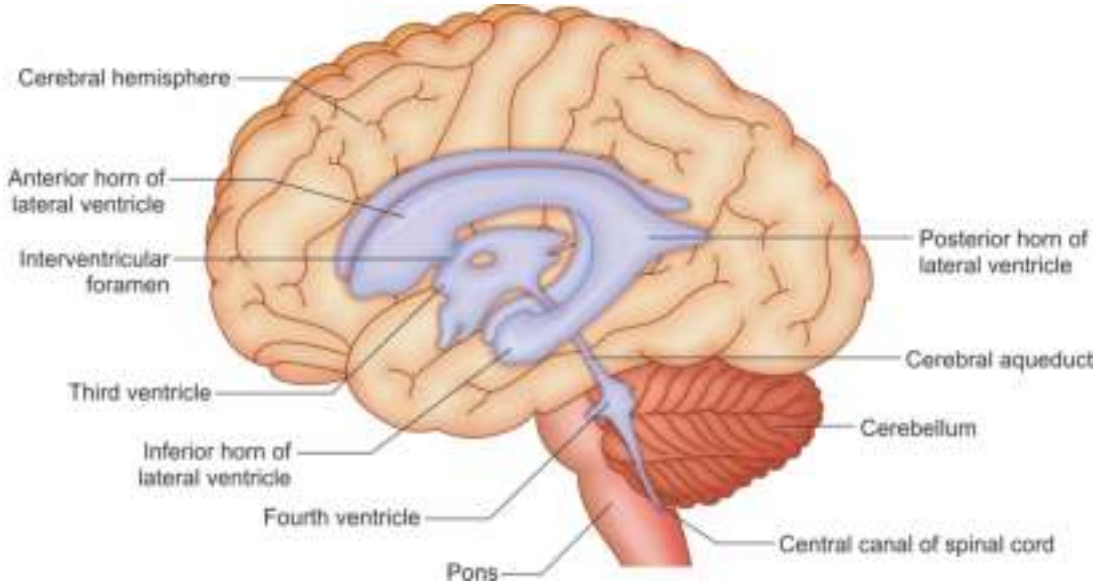


Fig. 30.2: Cerebrospinal fluid (CSF) circulation in ventricles in central nervous system.

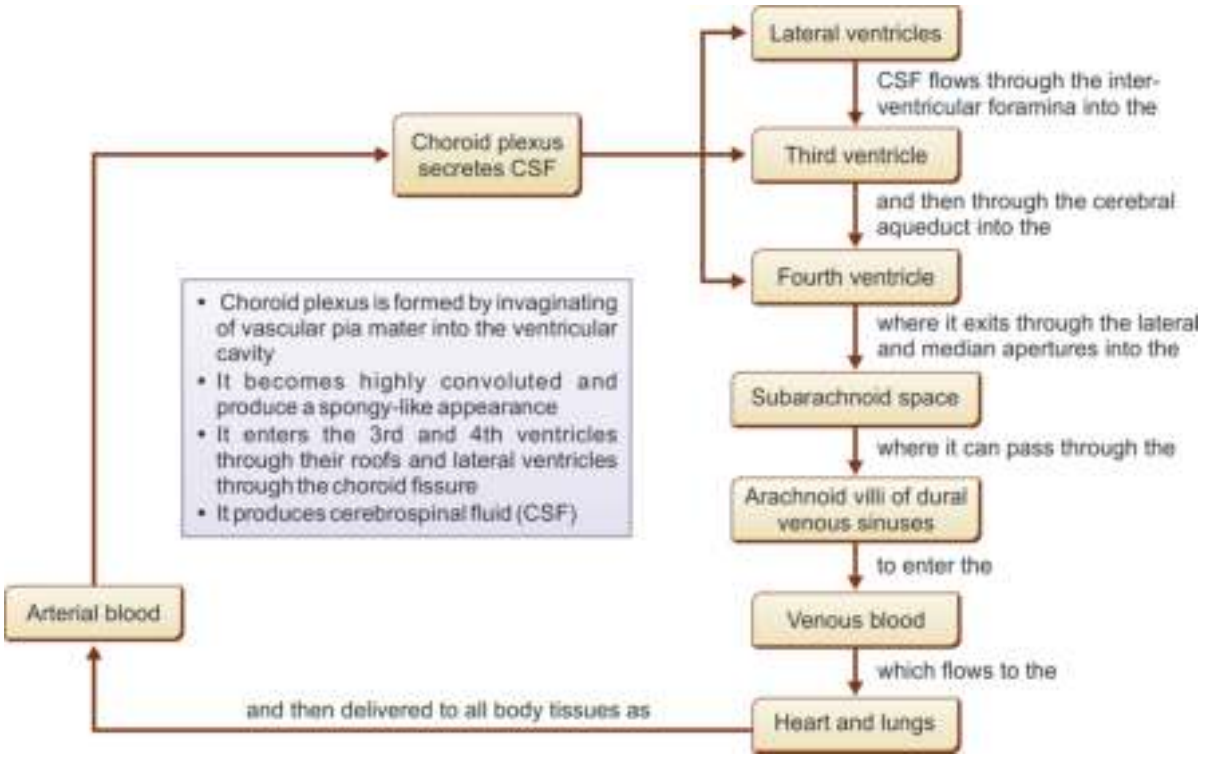


Fig. 30.3: Cerebrospinal fluid (CSF) circulation in central nervous system.

into the dural sinuses. From the ventricles, the CSF flows via the cerebral aqueduct and enters the space through the foramina of Luschka and Magendie. CSF is absorbed by the arachnoid villi, which provide the 'outlet' for CSF to flow into the major dural spaces. Normal values of CSF are given in Table 30.1.

- **Cerebrospinal fluid functions:** Cerebrospinal fluid supports and protects the brain and spinal cord. CSF acts as a cushion and shock absorber and reservoir to regulate the contents of the cranium. CSF keeps the brain and spinal cord moist. Cerebrospinal fluid may act as a medium for the interchange of metabolic substances between nerve cells. Cerebrospinal fluid clears metabolic waste of central nervous system. CSF is poor in colloids. Bilirubin is absent in CSF.

Table 30.1 CSF normal values

CSF Parameters	Normal Values
Volume	90–150 ml
Pressure	80–180 cm of water (20 drops/minute)
Specific gravity	1.006–1.008
pH	7.30–7.40
Colloids	Poor
Total solids	0.85–1.70 g/dl
Bilirubin	Absent
Proteins	▪ Total proteins (20–40 mg%) ▪ Albumin (50–70%) ▪ α_1-Globulin (3–9%) ▪ α_2-Globulin (4–10%) ▪ β-Globulin (4–10%) ▪ γ-Globulin (3–9%) ▪ Fibrinogen: Nil

CSF Parameters	Normal Values
Sugar	40–80 mg%
Chloride	118–132 mEq/L
Sodium	144–154 mEq/L
Potassium	2.0–3.5 mEq/L
Thyroxine	0.1–0.2 mg/dl
Iron	1.0–2.0 mg/dl
Glutamine	6–16 mg/dl
Urea	6–16 mg/dl
Uric acid	0.5–4.5 mg/dl
Creatinine	0.5–1.2 mg/dl
Cholesterol	0.2–0.5 mg/dl
Light microscopy	0–4 lymphocytes/mm^3
Culture and sensitivity	Sterile

Contd...

CENTRAL NERVOUS SYSTEM INFECTIONS

Infections of central nervous system may be classified into meningitis, encephalitis, myelitis and brain abscess. Meningitis refers to infection of meninges.

- Encephalitis is characterized by infection of brain parenchyma.
- Myelitis refers to infection of spinal cord.
- Encephalomyelitis is characterized by infection of brain parenchyma and spinal cord.
- CNS infections are caused by potentially life-threatening bacteria and viruses. Rarely fungi or *Entamoeba histolytica* and therapeutic drugs procaine or methotrexate in neurosurgical may cause meningitis. Prompt diagnosis and treatment of CNS infection is essential for patient survival, these infections to prevent morbidity and mortality.

MENINGITIS

Meningitis is inflammation of the meninges that surround the brain and spinal cord, which involves pia mater, the arachnoid and the CSF-filled subarachnoid space. Inflammation spreads rapidly because of CSF circulation occurs around the brain and spinal cord.

- Patchy meningitis denotes an inflammatory process restricted to dura mater.
- Leptomeningitis denotes an inflammatory process localized to arachnoid and pia mater. The infection may be limited to subarachnoid space or may spread to the brain (meningoencephalitis).
- Meningitis is most often caused by bacteria and viruses. Rarely, fungi or *Entamoeba histolytica* or therapeutic chemical may also cause meningitis.
  - **Bacteria:** *Escherichia coli*, Salmonella, *Haemophilus influenzae*, Neisseria, streptococci, pneumococci-3 and Mycobacterium bacilli cause bacterial meningitis.
  - **Viruses:** Viral meningitis is caused by viruses, which include enteroviruses (echoviruses 3, 7, 9, 11, 21, 30), Epstein-Barr virus (EB virus), coxsackie-viruses (B1-5 and A-9), herpes II virus and mumps virus. Viruses are most often transmitted by fecal–oral route. Viral meningitis is less severe than bacterial meningitis.
  - **Fungi:** *Cryptococcus neoformans* causes meningitis in immunocompromised persons, which is demonstrated by India ink preparation of CSF in a black background. Capsule of *Cryptococcus neoformans* is also demonstrated by mucicarmine stain (magenta colored) and silver stain (black color).
  - **Iatrogenic agents:** Therapeutic administration of procaine or methotrexate in neurosurgical procedures may produce sterile chemical meningitis.
  - **Metastatic cancers:** Lymphoma, breast cancer, melanoma, and lung cancer (especially small cell lung cancer) are associated with the greatest predilection to metastasize to the subarachnoid space resulting in carcinomatous meningitis.
- Most CNS infections occur in children less than 5 years of age, young adults and elderly persons. Otitis media is most important risk factor for meningitis in children. Such infection can be acute or chronic. The pia mater is the layer most commonly affected. CNS infection increases the permeability of blood–brain barrier, which leads to cerebral edema and influx of toxic waste products resulting in increased intracranial pressure and damage to cranial nerves (especially VIII cranial nerve). Patient develops hydrocephalus that limits the normal circulation of cerebrospinal fluid within brain and spinal cord.
- The presence of neutrophils in the CSF is the most definitive index of bacterial meningitis.
- Lymphocytes are the hallmark of tuberculosis and the viral meningitis, as well as some chronic infections.
- In untreated cases, delirium gives way to coma and death. CSF findings in normal persons are given in Table 30.2. Color changes in CSF suggestive of disease states are given in Table 30.3. Differentiation between blood CSF caused by subarachnoid hemorrhage versus traumatic lumbar puncture is given in Table 30.4. Major cells demonstrated on microscopic examination of cerebrospinal fluid are given in Table 30.5. Laboratory analysis of cerebrospinal fluid (CSF) for infectious meningitis is given in Table 30.6.

Pathology Pearls: Lumbar Puncture—Clinical Alert

- Before lumbar puncture, fundus examination with ophthalmoscope should be performed for evidence of papilledema (swelling of optic disc generally due to an increased intracranial pressure).
- Because presence of papilledema may signal potential problems or complications of lumbar. However, if increased CSF pressure is found while performing lumbar puncture, it should not be essential to stop the procedure unless neurologic signs are present.
- Blood levels for specific substances should always be measured simultaneously with CSF determinations for meaningful interpretations of the results.

PYOGENIC MENINGITIS

Most cases of pyogenic meningitis are caused by *Streptococcus pneumoniae* (pneumococcus 3) or *Neisseria meningitidis* (meningococcus) in adults. *Escherichia*

Table 30.2 CSF findings in normal persons

Features	Normal CSF Findings
Causative organisms	No organisms
CSF pressure	60–150 cm of H_2O (20 drops/minute)
Color	Clear, colorless
CSF proteins	Normal 15–40 mg%
CSF sugar	50–80 mg% (CSF sugar : blood sugar ratio is 0.6)
CSF white blood cells	0–4 lymphocytes /mm^3
Gram staining	Organisms absent, hence insignificant
CSF culture	Sterile

coli, Salmonella, *Haemophilus influenzae* may also cause meningitis. Neonates develop meningitis by *Escherichia coli*, Salmonella and group B streptococci. Risk factors associated with contracting pyogenic meningitis include head trauma with basilar skull fractures, otitis media, sinusitis or mastoiditis, neurosurgery, dermal sinus tracts, systemic sepsis, or immunocompromised persons.

Pyogenic Meningitis and Age Group Predilection

In pyogenic meningitis, majority of the organisms originate in nasopharynx. Causative organisms and age group predilection for pyogenic meningitis are given in Table 30.7.

Table 30.3 Color changes in CSF suggestive of disease states

Appearance of CSF	Cause	Clinical Significance
Clear CSF	Normal persons	Normal findings
Hazy, cloudy, opalescent, purulent slight yellow to frank pus	▪ WBCs ▪ Microorganisms ▪ Protein	▪ Pyogenic meningitis ▪ Blood–brain barrier disorders ▪ IgG production in the central nervous system
Slightly yellow with delicate clot (cobweb turbid)	Fibrinogen converted to fibrin	Tubercular meningitis
Viscous appearance	▪ Neoplastic cells ▪ Meningococci	▪ Metastatic colon carcinoma ▪ Severe meningococcal infection
Bloody	Red blood cells	▪ Traumatic lumbar puncture tap ▪ Subarachnoid hemorrhage ▪ Primary amebic meningoencephalitis
Xanthochromia	▪ Hemoglobin ▪ Microorganism ▪ Bilirubin ▪ Protein ▪ Carotene	▪ Old hemorrhage ▪ *Toxoplasma gondii* ▪ Elevated serum bilirubin ▪ Blood–brain barrier disorders ▪ Elevated carotene levels
Generally clear or slightly xanthochromia	Neoplastic cells	Tumors of brain and spinal cord
Pellicle	Clotting factors	Refrigerated tuberculous meningitis by CSF specimens

Table 30.4 Differentiation between blood CSF caused by subarachnoid hemorrhage versus traumatic lumbar puncture

CSF Findings	Subarachnoid Hemorrhage	Traumatic Lumbar Puncture
CSF pressure	CSF pressure most often increased	CSF pressure normal
Blood in test tubes for collecting CSF	Mixture of CSF with blood is uniform in all test tubes	Test tubes 1 and 2 more bloody than test tubes 3 and 4
CSF clotting	CSF does not clot	CSF often clots
Xanthochromia	Present if >8–12 hours since cerebral hemorrhage	Absent unless patient is jaundiced
Immediate repeat of lumbar puncture at higher level of intervertebral space	CSF same as initial procedure	CSF clear (if traumatic)

CSF with red blood cells >6000 per mm^2 appears grossly bloody.

Table 30.5 Major cells demonstrated on microscopic examination of cerebrospinal fluid

Cell Types	Disorders	Light Microscopy Findings
Blast forms	Acute myelogenous or lymphoblastic leukemia	Myeloblasts or lymphoblasts
Malignant cells	Metastatic carcinomas	Clusters of neoplastic cells with fused cell borders and nuclei
Neutrophils	▪ Bacterial meningitis ▪ Viral meningitis (early case) ▪ Fungal meningitis (early case)	Neutrophil containing granules
Lymphocytes	▪ Normal CSF ▪ Viral meningitis ▪ Fungal meningitis ▪ Multiple sclerosis	All stages of development of lymphocytes possible
Macrophages	▪ Viral meningitis ▪ Tubercular meningitis ▪ Red blood cells in CSF ▪ Contrast media administration	Macrophages may contain phagocytized red blood cells (appearing as empty vacuoles or ghost cells) and hemosiderin granules
Monocytes	▪ Chronic bacterial meningitis ▪ Viral meningitis ▪ Tubercular meningitis ▪ Fungal meningitis ▪ Multiple sclerosis	Monocytes are mixed with lymphocytes and neutrophils
Plasma cells	▪ Multiple sclerosis ▪ Tubercular meningitis ▪ Sarcoidosis	Classic plasma cells
Ependymal and choroid cells	Traumatic lumbar puncture	Clusters with distinctive cellular outlines and nuclei
Pia arachnoid mesothelial cells	Normal mixed reactions including neutrophils, lymphocytes, monocytes and plasma cells	Pia arachnoid mesothelial cells resemble young monocytes with a round, not indented, nucleus

Table 30.6 Laboratory analysis of cerebrospinal fluid (CSF) for infectious meningitis

Features	Pyogenic Meningitis	Tubercular Meningitis	Viral Meningitis	Normal Range
Causative organisms	*E. coli*, Salmonella, *Haemophilus influenzae. Neisseria meningitidis,* streptococci or pneumococci	Mycobacterium tubercle bacillus	Enteroviruses (echoviruses 3, 7, 9, 11, 21, 30), including coxsackievirus (B1–5 and A-9), poliovirus, and echovirus. Epstein-Barr virus, mumps virus, HSV, and West Nile virus	No pathogens in normal CSF
CSF pressure	Increased	Increased	Increased	60–150 cm H_2O (20 drops/minute)
Color	Opaque and turbid to frank pus	Straw colored	Clear or colorless	Clear, colorless
CSF proteins	Increased	Increased rich in fibrinogen. When allowed to stand forms 'cobweb turbidity' (fibrinogen → fibrin)	Increased	15–40 mg%
CSF sugar	▪ CSF sugar low or absent due to consumption by pyogenic bacteria ▪ CSF glucose: Serum glucose = <0.4; lactate >35 mg/dl	CSF sugar low and it starts rising on ATT therapy; lactate >25 mg/dl	CSF sugar normal except in mumps and herpes; lactate <25 mg/dl	50–80 mg%
CSF WBC count	Elevated WBCs and neutrophils	Elevated WBCs, lymphocytes and monocytes	Elevated WBCs and lymphocytes	0–4 lymphocytes/mm^3
Staining technique	Gram staining positivity sensitivity (75–80%)	ZN staining for AFB	Not stained by Gram stain or ZN stain	Insignificant
CSF culture	Positive in 70–80%	Detection of AFB antigens by CSF chromatography, ELISA and PCR	▪ Amplification of viral DNA or RNA from CSF by PCR amplification ▪ CSF IgG/serum IgG antibody titer >1.5	Insignificant

Table 30.7 Causative organisms and age group predilection for pyogenic meningitis

Age Group	Organisms
Neonates	*Escherichia coli*, Salmonella, Group B streptococci, *Listeria monocytogenes*
Infants and young children	*Streptococcus pneumoniae*, *Neisseria meningitidis*
Adults <30 years	*Haemophilus influenzae*, *Neisseria meningitidis*
Adults >30 years	*Streptococcus pneumoniae*, Mycobacterium tubercle bacilli
Epidemics in young adults in overcrowded living conditions (e.g. college dormitories and military barracks)	*Neisseria meningitidis*

Clinical alert: CSF should be cultured for bacteria, tuberculosis and fungi. In children, Haemophilus influenzae type B is the most cause of bacterial meningitis. In adults, meningococci and pneumococci are most common pathogens for meningitis. CSF with any degree of cloudiness should be treated with extreme care because it could be an indication of contagious disease.

- **Neonates:** *Escherichia coli* is the prime cause of meningitis in the newborn, whose resistance to gram-negative bacteria has not fully developed. The transplacental transfer of maternal IgG imparts protection to the newborn against many bacteria. Salmonella also causes meningitis in neonates.
- **Infants and young children:** *Streptococcus pneumoniae* or *Neisseria meningitidis* causes pyogenic meningitis in this age group. Pyogenic meningitis caused by *Neisseria meningitidis* may develop 'Waterhouse-Friderichsen syndrome', which is characterized by hemorrhagic destruction of the adrenal cortex, acute hypocorticism with circulatory collapse, and disseminated intravascular coagulation.
- **Adults <30 years:** *Haemophilus influenzae* and *Neisseria meningitidis* cause pyogenic meningitis at a later age.
- **Adults >30 years:** Adults more than 30 years of age develop pyogenic meningitis caused by *Streptococcus pneumoniae*. Mycobacterium tubercle bacilli may affect any age group.

Portal of Entry of Pyogenic Bacteria in Nervous System

Bacteria enter central nervous system via hematogenous route, direct inoculation route due to trauma, local spread from regional infective focus, ascending infection in surgical procedures and iatrogenic route.

- **Hematogenous spread:** Hematogenous spread is most common portal of entry of organisms to brain and meninges. Venous blood may serve as a carrier of infectious agents from the infected periocular and perinasal tissues (e.g. bacterial endocarditis and bronchiectasis) to brain and meninges. It is worth mentioning that CNS has no lymphatic channels.
- **Trauma:** Bacteria may be inoculated directly into the brain by bullets and sharp objects, such as a knife, or by direct entry of bacteria through an open or surgical wound or skull fracture.
- **Local spread:** Pyogenic meningitis occurs from spread of infection from paranasal sinuses, middle ear (drum perforation) and dental infections.
- **Ascent of infection:** Infection may ascend by various procedures like cystoscopy to brain and meninges.
- **Iatrogenic infection:** *Staphylococcus epidermidis* is most common organism to cause pyogenic meningitis.

Pathology

- **Organism's entry:** In the pathophysiologic process of bacterial meningitis, the bacteria replicate and undergo lysis in the cerebrospinal fluid, releasing endotoxins or cell wall fragments.
- **Release of chemical mediators:** Pyogenic bacteria products lead to release of inflammatory chemical mediators resulting in movement of glucose and neutrophils across the capillary wall into CSF.
- **Purulent exudate in the subarachnoid space:** Entry of pathogens into the subarachnoid space leads to formation of cloudy purulent exudate. Purulent exudate in the subarachnoid space is characteristic. CSF findings of diagnostic significance include numerous neutrophils, decreased glucose (<2/3 of the serum glucose concentration) and increased protein.
- **Leptomeningeal venulitis:** Thrombophlebitis occurs in dural sinuses and bridging leptomeningeal veins. Obliteration of arterioles by inflammation leads to vascular congestion, hemorrhagic infarction and cerebral abscess in the surrounding tissues. Net result is thickening of the meninges and formation of adhesions in patient with pyogenic meningitis.
- **Reactive fibroblastic arachnoiditis with scarring:** Formation of adhesions may impinge on the cranial nerves resulting in cranial nerve palsy. Adhesions may impair the outflow of cerebrospinal fluid resulting in hydrocephalus.

Surgical Pathology: Pyogenic Meningitis

Gross Morphology

- In pyogenic meningitis, the brain is edematous and congested. The subarachnoid space is filled with purulent exudates.
- The pus is most obvious in the sulci and subarachnoid cisterns at the base of the brain (Fig. 30.4).

Light Microscopy

The exudate in meninges consists predominantly of live and dead neutrophils (pus).

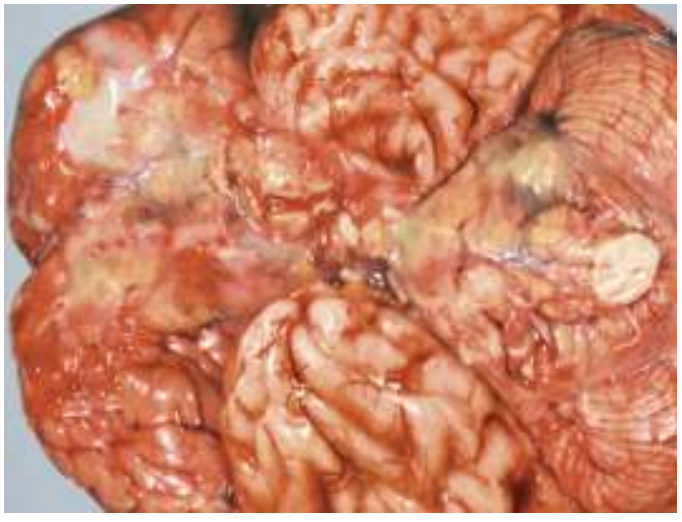


Fig. 30.4: Pyogenic meningitis. One cause for acute swelling is infection. The yellow-tan exudate of acute bacterial meningitis seen here obscures the sulci.

Clinical Features

The most common symptoms in patient with pyogenic meningitis are fever (>40°C) and chills; severe headache localized to the forehead; stiff neck; back, abdominal, mental confusion, somnolence, photophobia, seizures, neurologic symptoms and extremity pains; nausea and vomiting. Patient experiences knee pain on flexion of hip joint is called Kernig sign. Upon flexion of the neck, there is spontaneous similar movement of the hips and knees are known as Brudzinski's sign. Clinical manifestations of pyogenic meningitis are shown in Fig. 30.5. Complications of pyogenic meningitis are given in Table 30.8.

Laboratory Diagnosis

Diagnosis of pyogenic meningitis is based on the history and physical examination, along with laboratory data. Lumbar puncture findings are necessary for accurate diagnosis. These include a cloudy and purulent CSF under increased pressure. CSF findings in pyogenic meningitis are given in Table 30.9.

- CSF typically contains large numbers of polymorphonuclear neutrophils (≤90,000/mm^3), increased protein content, and reduced sugar content. Bacteria can be seen on smears and can easily be cultured with appropriate media.
- Occasionally, previous antibiotic use limits culture sensitivities, in which case latex agglutination can be used, or polymerase chain reaction (PCR) testing for *Neisseria meningitidis*, *Haemophilus influenzae*, and *Listeria* species.

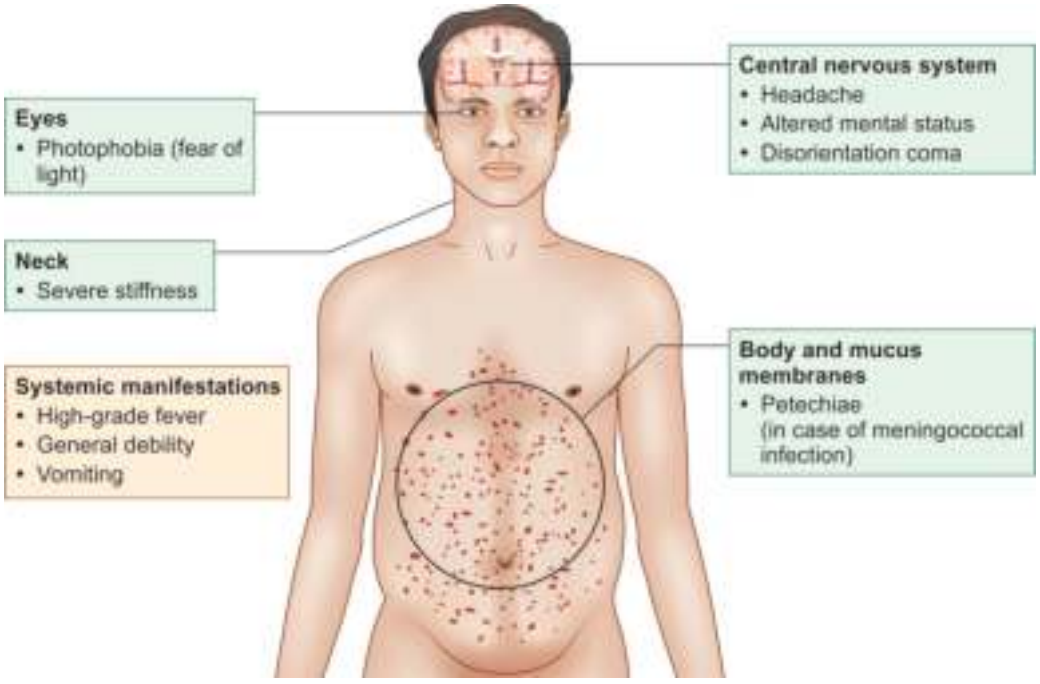


Fig. 30.5: Clinical manifestations of pyogenic meningitis.

Table 30.8 Complications of pyogenic meningitis

Cerebral infarction
Obstructive hydrocephalus
Cerebral abscess
Subdural empyema
Epilepsy (seizures) focal neurologic deficits
Cranial nerve palsies
Sensorineural hearing loss
Communicating and non-communicating hydrocephalus

Vaccination

Effective polysaccharide vaccines are available to protect against meningococcal groups A, C, Y, and W-135. These vaccines are now recommended for military recruits and college students, who are at increased risk for invasive meningococcal disease.

TUBERCULOUS MENINGITIS

Tuberculous meningitis has insidious onset and may last for weeks or months, which is secondary to tuberculous infection occurring elsewhere in the body. It has a predilection for the base of the brain. Basal cisterns and the lateral sulci contain gelatinous whitish gray material. Inadequately treated tuberculous meningitis results in meningeal fibrosis, communicating hydrocephalus and vasculitis. Inflammation of striate arteries results in cerebral infarcts.

Pathology

Tuberculous meningitis develops in two steps: (a) *Mycobacterium tuberculosis* bacilli, that enter the host by droplet inhalation and escalate within the lungs, and then disseminate to the regional lymph nodes and via hematogenous route to meninges or brain parenchyma resulting in the formation of small subpial or subependymal foci of disseminated caseous necrosis lesion, termed **Rich foci**. (b) Second step in the development of tuberculous meningitis is an increase in the size of Rich focus until it ruptures in the subarachnoid space of brain and spinal cord. Expansion of the tubercle 'Rich focus' determines the type of central nervous system involvement.

Clinical Pearls: Diagnostic Approach of Tuberculoma of the Brain

- Tuberculoma of the brain is an important clinical entity. It is main challenge in the management to diagnose it.
- CT scan of brain demonstrates solitary or multiple ring-enhancing lesion with moderate perilesional edema, but these findings are not specific for brain tuberculoma.
- Neurocysticercosis (NCC), coccidioidomycosis, toxoplasmosis, metastasis and few other diseases have identical appearance on CT scan of brain.
- Cerebrospinal fluid examination is most often normal.
- Magnetic resonance imaging (MRI) of brain with magnetic resonance spectroscopy (MRS) have advantage over CT scan in diagnosis of brain tuberculoma.
- Magnetic resonance spectroscopy (MRS) demonstrates a specific lipid peak in cases of brain tuberculoma, which is not seen in any other differential diagnosis of brain tuberculoma.
- Histologic examination of biopsy and tissue culture from the lesion can establish the diagnosis of tuberculoma.
- The tuberculoma consists of epithelioid cell granulomas, caseous necrosis and Langhans' giant cells (Fig. 30.6).

Clinical Features

Patient with tuberculous meningitis presents with headache, fever and stiff neck. Although the meningeal signs may be absent in the early stage of disease. The duration of symptoms before clinical presentation persist from several days to several weeks.

Laboratory Diagnosis

Tuberculous meningitis is difficult to diagnose. It is essential to enquire patient's history, including recent

Table 30.9 CSF findings in pyogenic meningitis

Features	Pyogenic Meningitis	Normal CSF
Causative organisms	*Escherichia coli*, Salmonella, *Haemophilus influenzae*, *Neisseria meningitidis*, streptococci or pneumococci	No organisms
CSF pressure	Increased	60–150 cm of H_2O (20 drops/minute)
Color	Opaque and turbid to frank pus	Clear, colorless
CSF proteins	Due to increased vascular permeability	Normal 15–40 mg%
CSF sugar	▪ ↓ or absent due to consumption by pyogenic bacteria ▪ CSF sugar:blood sugar ratio is <0.4	▪ 50–80 mg% ▪ CSF sugar:blood sugar ratio is 0.6
CSF white blood cells	Polymorphonuclear cells ≤90,000/mm^3	0–4 lymphocytes /mm^3
CSF culture	Positive in 70–80% of cases	Sterile
Gram staining	Sensitivity in 75–80% of cases	Organisms absent, hence insignificant

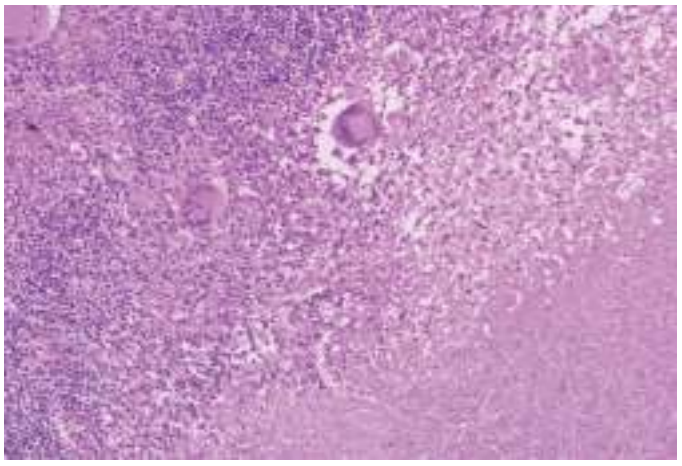


Fig. 30.6: Tuberculosis of brain. It shows epithelioid granulomas, Langhans giant cells and caseous necrosis (100X).

contact with patient suffering from tuberculosis. Lumbar puncture reveals increased cerebrospinal pressure yielding straw-colored cerebrospinal fluid (CSF). Proteins content is markedly increased. Light microscopy of CSF shows numerous lymphocytes. Acid-fast bacteria may be grown on culture media. CSF findings in tuberculous meningitis are given in Table 30.10.

VIRAL MENINGITIS

Viral meningitis may be caused by many different viruses, most often enteroviruses (echoviruses 3, 7, 9, 11, 21, 30), including coxsackieviruses (B1-5 and A-9), poliovirus, and echovirus. Others include Epstein-Barr virus, mumps virus, HSV, and West Nile virus.

- Viruses are most often transmitted by fecal–oral route. It is worth mentioning that herpesviruses especially HHV6 residing in the trigeminal ganglion of infected persons can also infect brain. Rabies virus ascends into brain along with the axons of peripheral nerves.
- The clinical manifestations of viral infections of CNS parenchyma are heterogeneous, owing to viral tropism to specific regions of the brain.
- The classic hallmark of most CNS viral infections is the presence of perivascular lymphocytes around arteries and arterioles.
- Viral meningitis is less severe than bacterial meningitis. Viral infections are limited to the meninges. It seldom involves the brain or spinal cord.
- The acute viral meningitis is self-limited and usually require only symptomatic treatment, except for herpes simplex virus (HSV) type 2, which responds to intravenous acyclovir.

Laboratory Diagnosis

Cerebrospinal fluid examination demonstrates moderately increase in CSF pressure and proteins with normal sugar. Light microscopy shows increase in lymphocytes in the cerebrospinal fluid rather than polymorphonuclear cells. Although often the virus cannot be identified, newer assays are emerging that allow in some circumstances for rapid identification of viral ribonucleic acid (RNA) in CSF. Cerebrospinal fluid findings in viral meningitis are given in Table 30.11.

FUNGAL MENINGITIS

Meningitis caused by fungi has insidious onset and may last for weeks or months. *Cryptococcus neoformans*, *Coccidioides immitis*, Aspergillus, or *Histoplasma capsulatum* involve the brain substance or the meninges in immunocompromised persons.

- *Cryptococcus neoformans* is the most common virulent fungi causing fungal meningitis in immunocompromised persons especially in AIDS patients. *Cryptococcus neoformans* is demonstrated by mixing a drop of infected CSF with India ink. Histopathologic examination shows a clear halo about the encapsulated organism.
- Histologic identification of *Cryptococcus neoformans* can be performed by methenamine silver stain for yeast forms and mucicarmine stain for yeast and capsule forms and Fontana-Masson stain for the melanin in the yeast.

Table 30.10 Cerebrospinal fluid (CSF) findings in tuberculous meningitis

Features	Tubercular Meningitis	Normal CSF
Causative organism	Mycobacterium tubercle bacillus	No organisms
CSF pressure	Increased	60–150 cm of H_2O (20 drops/minute)
Color	Straw colored	Clear and colorless
CSF proteins	Increased due to increased vascular permeability	Normal 15–40 mg%
CSF sugar	Within normal range	50–80 mg%
CSF white blood cells	Lymphocytes increased	0–4 lymphocytes/mm^3
ZN staining	Acid-fast bacteria demonstration	Organisms absent, hence insignificant
CSF culture	Bacterial growth on LJ medium	Sterile

Table 30.11 Cerebrospinal fluid (CSF) findings in viral meningitis

Features	Viral Meningitis	Normal CSF
Causative organism	Enteroviruses (echoviruses 3, 7, 9, 11, 21, 30), including coxsackieviruses (B1–5 and A-9), poliovirus, and echovirus. Others include Epstein-Barr virus, mumps virus, HSV, and West Nile virus	No organisms
CSF pressure	Increased	80–180 cm of H_2O (20 drops/min)
Color	Clear colorless	Clear, colorless
CSF proteins	↑ due to increased vascular permeability	Normal 15–40 mg%
CSF sugar	Normal range	50–80 mg%
CSF white blood cells	Lymphocytes increased	0–4 lymphocytes/mm^3

IATROGENIC MENINGITIS

Iatrogenic meningitis is a rare, but potentially fatal complication of diagnostic and therapeutic lumbar puncture (LP).

- Therapeutic administration of procaine or methotrexate in neurosurgical procedures may produce sterile chemical meningitis, iatrogenic bacterial meningitis (IBM) is a rare but serious complication of neuraxial procedures such as spinal and epidural anesthesia or lumbar puncture.
- The cerebrospinal fluid (CSF) culture is positive for *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

METASTATIC CANCER-INDUCED MENINGITIS

Metastatic cancer-induced meningitis results from metastatic spread of cancer to the leptomeninges of central nervous system.

- Lymphoma, breast carcinoma, melanoma, and lung carcinoma (especially small cell lung carcinoma) are associated with the greatest predilection to metastasize to the subarachnoid space. Patient has overall median survival of 2–4 months.
- There are many new approaches to the diagnosis and treatment of metastatic cancer-induced meningitis that promise to extend life and prevent disability. Leptomeningeal metastatic cancer-induced meningitis is diagnosed by molecular techniques.
- Patients are treated by drugs available for intrathecal administration, using systemic chemotherapy; and applying strategies such as gene therapy and immunotoxins to the management of leptomeningeal metastases.
- Leptomeningeal metastases consist of atypical tumor cells growing either attached to the pia mater of brain and spinal cord or floating in the cerebrospinal fluid (CSF) in the subarachnoid space, that provides hospitable environment for the growth of metastatic tumor cells.
- Rich vascular supply of meninges provides hematogenous metastatic tumor cells access to the subarachnoid space.
- Cerebrospinal fluid has high content of glucose and oxygen to support metastatic tumor cells with high metabolic activity.
- Leptomeningeal metastases can therefore, escape from the need for angiogenesis, which limits the growth of parenchymal metastases.

ENCEPHALITIS

PARASITIC ENCEPHALITIS

Parasitic encephalitis occurs due to neurocysticercosis, cerebral malaria, amoebic encephalitis and toxoplasmosis.

Neurocysticercosis

Neurocysticercosis is a common CNS parasitic infection and seizures worldwide caused by *Taenia solium*. Death of the parasite triggers granulomatous inflammatory response. Inflammation subsides over months to years and cyst becomes small fibrotic calcified nodule.

Cerebral Malaria

Cerebral malaria is caused by lodging of parasitized RBCs within the microvasculature of the brain and accompanied by petechial hemorrhages in the white matter around necrotic blood vessels. Prompt treatment with corticosteroids and antimalarial drugs reduces mortality.

Amoebic Encephalitis

Amoebic encephalitis is caused by highly lethal CNS infection caused by free-living amoebae found in

rivers such as *Naegleria fowleri*, *Balamuthia mandrillaris* and *Acanthamoeba* species. *Naegleria fowleri* enters CNS through cribriform plates during swimming. Patient develops acute meningitis progressing to coma and death. Organisms are demonstrated in subarachnoid space. It is worth mentioning that *Entamoeba histolytica* causes brain abscess.

Toxoplasma Gondii Encephalitis

Toxoplasma gondii is a parasite causing brain infection. It is one of the TORCH infections. It is transmitted via transplacental route in neonates, and ingestion of contaminated food due to urine or feces of household cats and pets. Newborn manifests with hydrocephalus, mental retardation, neurologic abnormalities of cerebral cortex, basal ganglia, retinae, and involvement of heart, lungs, and liver. Calcification in periventricular is demonstrated on radiograph. Immunocompromised adults present with lymphadenitis and CNS involvement.

VIRAL ENCEPHALITIS

Inflammation of the brain parenchyma is most often caused by viruses. It may be diffuse/localized inflammation of brain. The nervous system is subject to invasion by many viruses such as arbovirus, poliovirus, and rabies virus.

- Person contracts arbovirus infection from reservoirs such as horses, birds and mosquitoes.
- Herpes simplex encephalitis is most common in teenagers and young adults. Herpes simplex targets the temporal lobes by binding on the plasma membranes of CNS cells. The virus has ability to remain latent or selective replication in distinct intracellular microenvironments.

Mode of Transmission

The mode of transmission of arbovirus may be due to bite of mosquito, rabid animal such as dog, fox and bat (rabies virus in saliva), or ingestion (poliovirus). Common causes of encephalitis in the United States are HSV and West Nile virus. Patient develops severe encephalitis with increased excitability of the CNS.

Clinical Features

Patient suffering from rabies presents with hydrophobia (fear from water) characterized by violent muscle contractions and convulsions. It is usually fatal once clinical signs develop.

Surgical Pathology: Viral Encephalitis

Light Microscopy

- Light microscopy of affected brain shows eosinophilic intracytoplasmic inclusions (Negri bodies) in the hippocampus and Purkinje cells of the cerebellum.
- Brainstem and spinal cord show neuronal degeneration, perivascular accumulations of mononuclear cells.

CEREBRAL ABSCESS

Cerebral abscess is a focal area necrosis with surrounding membrane within the brain parenchyma usually resulting from infectious process by bacteria, *Entamoeba histolytica* and fungi.

- Cerebral abscess can result from penetrating skull injuries, which may be solitary or multiple.
- Cerebral abscess can originate directly from otitis media, mastoiditis secondarily cause inferior temporal lobe and cerebellar abscesses, paranasal sinuses infections, dental infection and facial trauma. Metal fragments or other foreign bodies left in the brain parenchyma can also serve as a nidus for infection.
- Bacteria may gain entry via hematogenous route from bronchopulmonary infections (bronchiectasis, lung abscess, emphysema), pulmonary arteriovenous malformation, and bronchopleural fistula, cyanotic congenital heart diseases, bacterial endocarditis, ventricular aneurysms, skin infection, pelvi infection and intra-abdominal infection.
- Cerebral abscesses associated with bacteremia usually cause multiple abscesses mostly in the distribution of the middle cerebral artery and usually at the gray and white matter junction. Most important bacteria isolated from cerebral abscess are *Staphylococcus aureus* and *Streptococcus viridans*.

Pathophysiology

Damaged brain tissue and immunosuppression contribute to cerebral abscess in some cases. Cerebral abscess matures over two weeks' period. Initially vascular endothelial swelling occurs within 1–2 days followed by neutrophilic infiltration over 3–4 days. Chronic inflammatory cells composed of macrophages, lymphocytes and plasma cells are present over 5–7 days. Granulation tissue is formed over 8–14 days, which is followed by fibrosis (gliosis).

Clinical Features

The triad of fever, headache, and the focal neurologic deficit is observed in 50% of cerebral abscess patients.

Patient presents with headache, lethargy, focal neurological deficits, seizures (frontal lobe abscess), nausea, vomiting, nuchal rigidity (occipital lobe abscess), third and sixth cranial deficits. Rupture of cerebral abscess presents with sudden worsening headache and followed by emerging signs of meningismus.

Laboratory Diagnosis

Routine tests carried out include total and differential leukocyte count, erythrocyte sedimentation rate, platelet count, C-reactive protein, blood culture at least twice preferably before antibiotic therapy. CT scan and MRI scan are done to rule out increased intracranial pressure. Lumbar puncture is rarely required. Stereotactic CT-guided surgical aspiration is performed to obtain a sample for culture, Gram stain, serology, histopathology and polymerase chain reaction.

Treatment

Cerebral abscess is treated by medical and surgical approaches. Patient is treated with specific medicines targeting the etiological pathogens. Steroids can be considered in selected patients specially to reduce the brain abscess mass effect and improve antibiotic penetration and cerebral edema. The surgical approach has important role in the management of brain abscess. CT-guided needle aspiration is performed via stereotactic procedure, burr hole and craniotomy for loculated multiple abscess. Intravenous or intrathecal agents are administered against specific microorganism along with surgical procedure.

TABES DORSALIS IN TERTIARY SYPHILIS

Tabes dorsalis is a manifestation of tertiary syphilis involving the lumbar spinal cord. Patient develops chronic fibrosing syphilitic meningitis, and compression of the posterior nerve roots (carrying sensory nerves) associated with lancinating pain in extremities. It also damages the transmission of proprioceptive impulses, causing gait disturbances (ataxia). Joint deformities are common and known as Charcot joints.

Surgical Pathology: Tabes Dorsalis
Gross Morphology
Focal irregular thickening is seen around spinal cord but may be seen over the convexity of the brain or over the cerebellum.
Light Microscopy
▪ Tertiary syphilis may also present in the form of granulomatous inflammation (gumma syphilis). ▪ Meninges contain infiltrates of lymphocytes and plasma cells, typically arranged around the meningeal blood vessels.

CENTRAL NERVOUS SYSTEM TUMORS

Primary brain tumors account for 2% of all cancer deaths. Brain tumors can arise from neuroepithelial tissue, non-neuroepithelial tissue and metastases. Intracranial brain tumors are more frequent than spinal cord neoplasms. All brain tumors are malignant except meningioma and schwannoma.

- Primary malignant of CNS tumors rarely metastasize. Most often, metastatic brain tumors occur secondary to breast carcinoma, lung carcinoma, colorectal carcinoma, renal cell carcinoma, and malignant melanoma.
- Astrocytoma, glioblastoma multiforme, ependymoma and oligodendroglioma arising from neuroglial tissue occur in the temporoparietal regions. Astrocytoma is the most common tumor in adults. Neuron-specific enolase and synaptophysin are markers for neurons.
- World Health Organization (WHO) system updated in 2021 is the most widely used classification system for CNS tumors based on histopathologic characteristics with addition of genetic basis of tumorigenesis and molecular markers. This classification provides prognostic or predictive data analysis within diagnostic categories established by conventional histology which help effective targeted therapy.
- Revised 2021 WHO classification of tumors of central nervous system is given in Table 30.12. Anatomic predilection of primary central nervous system tumors is given in Table 30.13 and Figs 30.7 to 30.9.

NERVOUS SYSTEM TUMORS: OVERVIEW

PRIMARY NERVOUS SYSTEM TUMORS

Most primary brain tumors are derived from neuroglial cells. These tumors are characterized by an extensive infiltration of growth into the surrounding brain parenchyma and confined to the central nervous system, and the tumor metastasis to other organs is rare. Because leptomeningeal tissue is barrier against tumor invasion. However, other tumors of non-neural origin may frequently metastasize to the central nervous system.

- In adults, the majority of intracranial tumors (70%) originate in the supratentorial region, which include glioblastoma multiforme, meningioma, ependymoma and acoustic neuroma in order of frequency.

Table 30.12 Revised 2021 WHO classification of tumors of central nervous system

Astrocytic Tumors	
▪ Adult-type diffuse gliomas	▪ Diffuse pediatric-type high-grade glioma, H3-wildtype and IHD-wildtype
▪ Astrocytoma, IHD-mutant (grade II)	▪ Infant-type hemispheric glioma
▪ Oligodendroglioma, IHD-mutant and 1p/19q-codeleted	▪ Circumscribed astrocytic glioma
▪ Glioblastoma, IHD-wildtype (grade IV)	▪ Pilocytic astrocytoma
▪ Pediatric-type diffuse low-grade gliomas	▪ High-grade astrocytoma with piloid features
▪ Diffuse astrocytoma, MYB- or MYBL1-altered	▪ Pleomorphic xanthoastrocytoma
▪ Angiocentric glioma	▪ Subependymal giant cell astrocytoma
▪ Polymorphous low-grade neuroepithelial tumor of the young	▪ Choroid glioma
▪ Diffuse low-grade glioma, MAPK pathway-altered	▪ Astroblastoma, MN1-altered
▪ Pediatric-type diffuse high-grade gliomas	▪ Glioneuronal and neuronal tumors
▪ Diffuse midline glioma, H3K27M-altered (grade IV)	
▪ Diffuse hemispheric glioma, H3G34-mutant	
Glioneuronal and Neuronal Tumors	
▪ Ganglioglioma	▪ Myxoid glioneuronal tumor
▪ Desmoplastic infantile ganglioglioma/desmoplastic infantile astrocytoma	▪ Diffuse leptomeningeal glioneuronal tumor
▪ Dysembryoplastic neuroepithelial tumor	▪ Gangliocytoma
▪ Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters	▪ Multinodular and vacuolating neuronal tumor
▪ Papillary glioneuronal tumor	▪ Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)
▪ Rosette-forming glioneuronal tumor	▪ Central neurocytoma
	▪ Extraventricular liponeurocytoma
Ependymal Tumors	
▪ Supratentorial ependymoma	▪ Spinal ependymoma
▪ Supratentorial ependymoma, ZFTA fusion-positive	▪ Spinal ependymoma, MYCN-amplified
▪ Supratentorial ependymoma, YAP1 fusion-positive	▪ Myxopapillary ependymoma
▪ Posterior fossa ependymoma, group PFA	▪ Subependymoma
▪ Posterior fossa ependymoma, group PFB	
Choroid Plexus Tumors	
▪ Choroid plexus papilloma	▪ Choroid plexus carcinoma (grade III)
▪ Atypical choroid plexus papilloma	
Tumors of the Pineal Region	
▪ Pineocytoma	▪ Papillary tumor of the pineal region
▪ Pineal parenchymal tumor of intermediate differentiation	▪ Desmoplastic myxoid tumor of the pineal region, SMARCB1-mutant
▪ Pineoblastoma (grade IV)	
Embryonal Tumors	
▪ Medulloblastoma	▪ Medulloblastoma, SHH-activated and TP53-mutant
▪ Medulloblastoma, molecularly defined	▪ Medulloblastoma, non-Wnt/non-SSH
▪ Medulloblastoma, Wnt-activated	▪ Medulloblastoma, histologically defined (grade IV)
▪ Medulloblastoma, SHH-activated and TP53-wildtype	
Other Embryonal Tumors	
▪ Schwannoma	▪ CNS neuroblastoma, FOXR2-activated
▪ Atypical teratoid/rhabdoid tumor	▪ CNS tumor with BCOR internal tandem duplication
▪ Cribriform neuroepithelial tumor	▪ CNS embryonal tumor
▪ Embryonal tumor with multilayered rosettes	

Contd...

Table 30.12 Revised 2021 WHO classification of tumors of central nervous system (*Contd...*)

Cranial and Paraspinal Tumors	
▪ Schwannoma ▪ Neurofibroma ▪ Perineuroma ▪ Hybrid nerve sheath tumor	▪ Malignant melanocytic nerve sheath tumor ▪ Paraganglioma ▪ Meningiomas
Mesenchymal and Non-meningoepithelial Tumors	

▪ Soft tissue tumors
  • Fibroblastic and myoblastic tumors
  • Solitary fibrous tumor
▪ Vascular tumors
  • Hemangiomas and vascular malformations
  • Hemangioblastoma
▪ Skeletal muscle tumors
  • Rhabdomyosarcoma
	▪ Tumors of uncertain differentiation
	  • Intracranial mesenchymal tumor, FET-CREB fusion-positive
	  • Sarcoma with CIC-rearrangement
	  • Primary intracranial sarcoma, DICER1-mutant
	  • Ewing sarcoma
	▪ Chondro-osseous tumors
	  • Chondrogenic tumors
	  • Mesenchymal chondrosarcoma

Notochordal Tumors	
▪ Chordoma	▪ Poorly differentiated chordoma
Melanocytic Tumors	
▪ Diffuse meningeal melanocytic neoplasms ▪ Meningeal melanocytosis and meningeal melanomatosis	▪ Circumscribed meningeal melanocytic neoplasms ▪ Meningeal melanocytoma and meningeal melanoma
Hematolymphoid Tumors	
▪ CNS lymphoma ▪ Primary diffuse large B cell lymphoma of the CNS ▪ Immunodeficiency-associated CNS lymphomas ▪ Lymphomatoid granulomatosis ▪ Intravascular large B cell lymphoma	▪ Miscellaneous rare lymphomas in the CNS ▪ MALT lymphoma of the dura ▪ Low-grade B-cell lymphoma of CNS ▪ Anaplastic large cell lymphoma (ALK+/ALK–) ▪ T cell and NK/T cell (natural killer/T cell) lymphomas
Histiocytic Tumors	
▪ Erdheim-Chester disease ▪ Rosai-Dorfman disease ▪ Juvenile xanthogranuloma	▪ Langerhans cell histiocytosis ▪ Histiocytic sarcoma
Germ Cell Tumors	

▪ Germinoma
▪ Embryonal carcinoma
▪ Yolk sac tumor (endodermal sinus tumor)
▪ Choriocarcinoma
	▪ Teratoma
	  • Mature teratoma
	  • Immature teratoma
	  • Teratoma with somatic type malignancy
	▪ Mixed germ cell tumor

Tumors of the Sellar Region	
▪ Adamantinomatous craniopharyngioma ▪ Papillary craniopharyngioma ▪ Pituicytoma, granular cell tumor of the sellar region, and the spinal cell oncocytoma	▪ Pituitary adenoma/pituitary neuroendocrine tumor (PitNET) ▪ Pituitary blastoma
Metastatic Tumors	
▪ Metastases to the brain and spinal cord parenchyma	▪ Metastases to meninges

Adapted from 2021 WHO classification of tumors of the central nervous system (5th edition)
SSH (sonic Hedgehog)
Histologic variants of meningiomas under cranial and spinal tumors include: meningoepithelial, fibrous, transitional, psammomatous, angiomatous, microcytic, secretory, lymphoplasmacytic-rich, metaplastic, choroid, clear cell, atypical, papillary, rhabdoid anaplastic types.

Table 30.13 Anatomic predilection of primary central nervous system tumors

Anatomic Predilection	Primary Central Nervous System Tumors	
Cerebral hemispheres	▪ Glioblastoma multiforme ▪ Astrocytoma	▪ Oligodendroglioma
Meninges	Meningioma	
Ventricles	▪ Ependymoma (children)	▪ Choroid plexus papilloma
Cerebellum	▪ Astrocytoma (children) ▪ Hemangioblastoma	▪ Medulloblastoma
Medulla and spinal cord	▪ Glioblastoma multiforme ▪ Astrocytoma (adults)	▪ Ependymoma of cauda equina (adults)
Cranial nerve	Schwannoma	

All brain tumors are malignant except for meningioma and schwannoma.

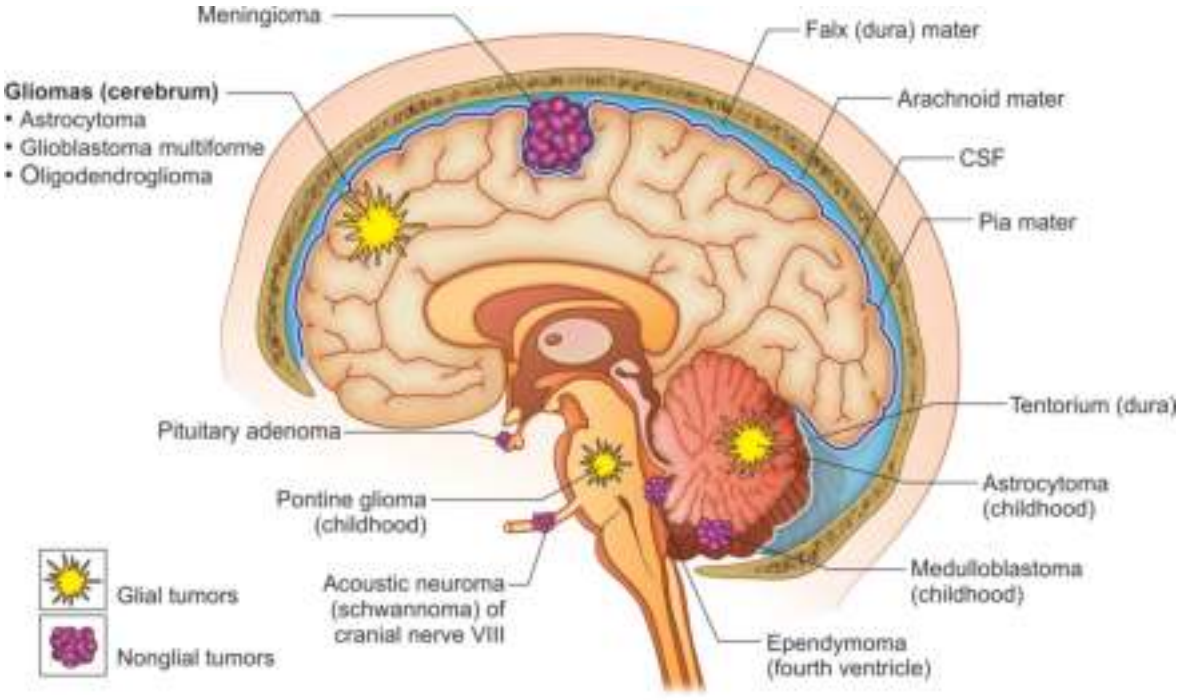


Fig. 30.7: Most common intracranial primary central nervous system tumors.

- In children, the majority of intracranial tumors originate in the infratentorial region, which include cerebellar astrocytoma, medulloblastoma, glioma in brainstem, and ependymoma in the fourth ventricle in order of frequency.

Predisposing Factors

Exposure to environmental (tobacco smoke) and occupational agents (vinyl chloride and EB virus); immunosuppression (organ transplant and HIV) and familial tumor syndromes and related chromosomal abnormalities increase the risk for primary brain tumors (Tables 30.14 and 30.15).

Molecular Genetic Alterations

Several molecular genetic alterations have emerged in recent years as powerful prognostic factors in diffuse glioma (astrocytoma, oligodendroglioma, mixed glioma, and glioblastoma). These molecular genetic alterations may guide patient management.

- Specific molecular genetic alterations include: (a) DNA methylation of the 6-methylguanine-DNA methyltransferase (MGMT) gene promoter, (b) mutation of isocitrate dehydrogenase (IDH), i.e. IDH1 or IDH2 genes, and (c) codeletion of chromosomes 1p and 19q.

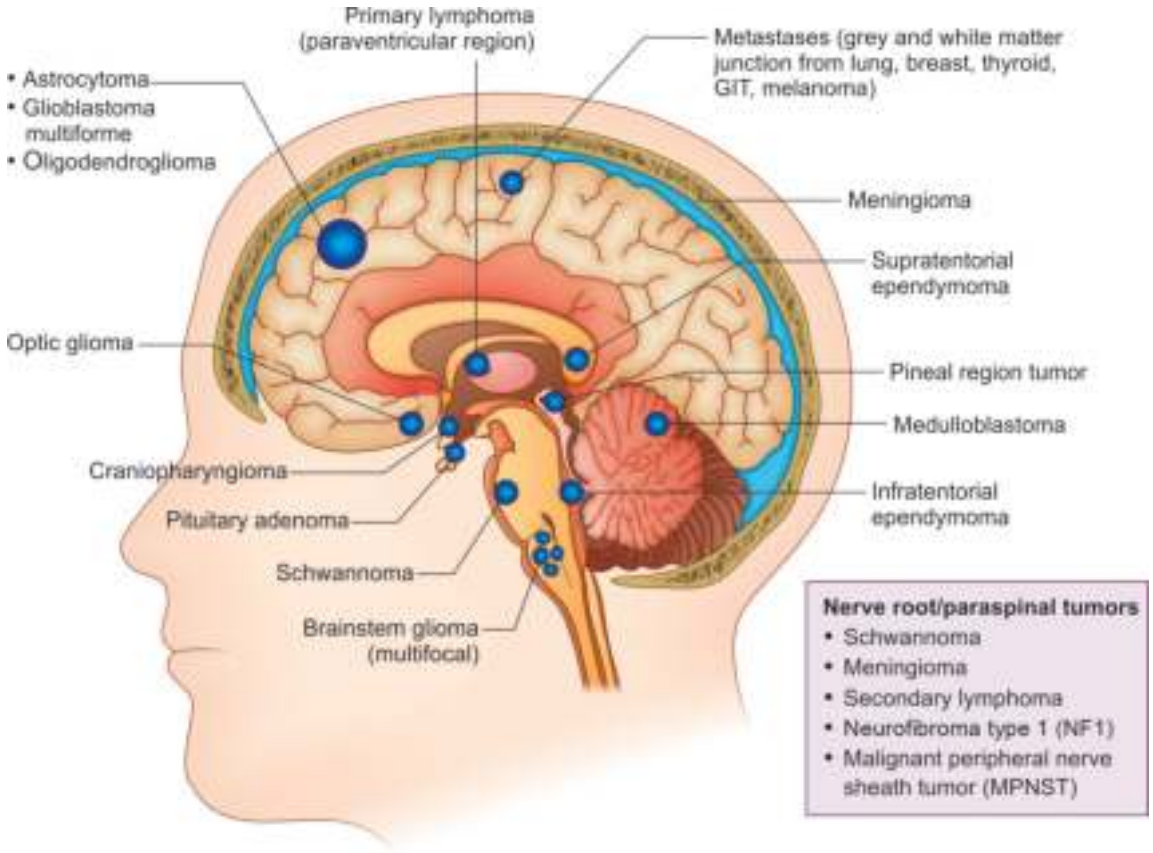


Fig. 30.8: Common primary CNS tumors in adults.

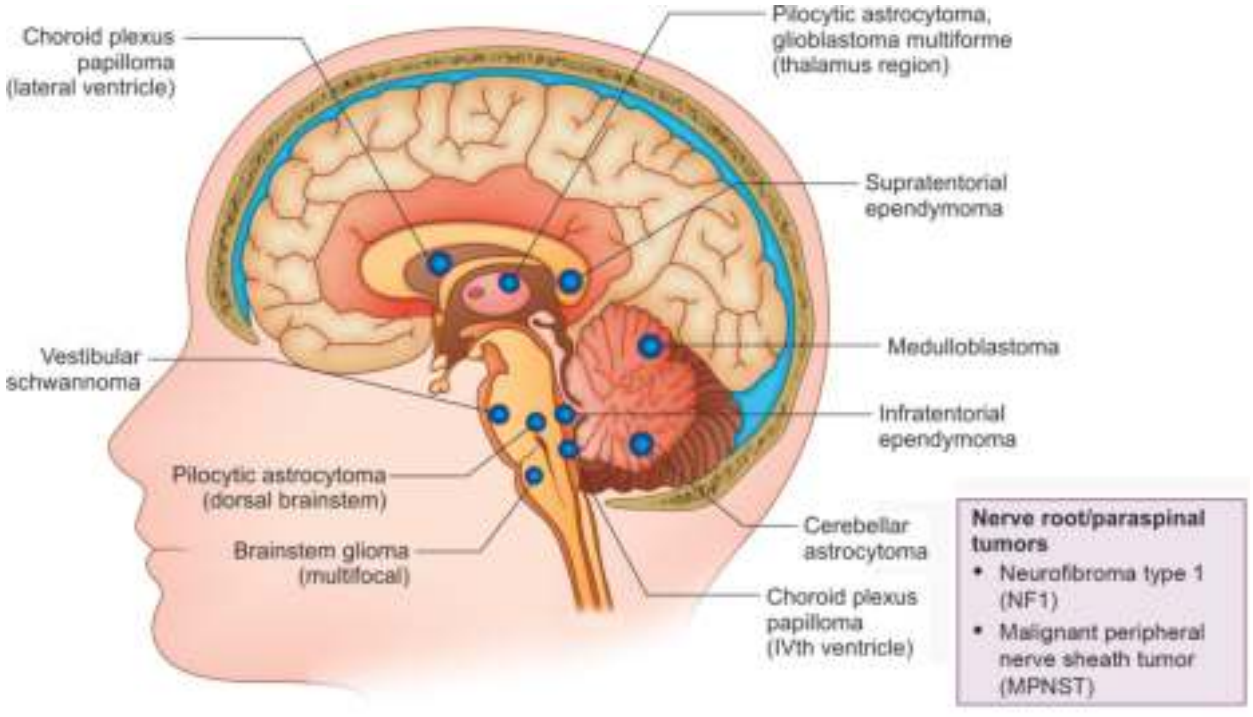


Fig. 30.9: Common primary CNS tumors in children.

Table 30.14 Environmental and occupational predisposing factors for primary CNS tumors

Predisposing Risk Factors	Primary CNS Tumors
Tobacco smoking	Brain tumors
Exposure to vinylchloride	Glioma
Epstein-Barr virus infection	CNS lymphoma
Transplant recipients and patients with AIDS	CNS lymphoma

- Main molecular markers used in diagnosis of gliomas include isocitrate dehydrogenase (IDH), i.e. IDH1 and IDH2, 1p19q codeletion, MGMT, TERT, ATRX and TP53 genes, which are of diagnostic significance. Molecular genetic markers for diagnosis of brain tumors are given in Table 30.16. Oncogenes and tumor suppressor genes (inactivated/biallelic loss) associated with primary CNS tumors are given in Tables 30.17 and 30.18.

Table 30.15 The familial tumor syndromes and related chromosomal abnormalities that are associated with CNS neoplasms

Syndrome	Chromosome
Neurofibromatosis type 1	17q11
Neurofibromatosis type 2	22q12
von Hippel-Lindau disease	3p25–26
Tuberous sclerosis	9q34, 16p13
Li-Fraumeni syndrome	17p13
Turcot syndrome type 1	3p21, 7p22
Turcot syndrome type 2	5q21
Nevoid basal cell carcinoma syndrome	9q22.3

Table 30.16 Molecular genetic markers for diagnosis of brain tumors

Tumor	Molecular Genetic Markers
Astrocytoma	IDH1, IDH2, TP53, ATRX
Oligodendroglioma	IDH1, TERT, 1p/19q codeletion
Glioblastoma	IDH1, TERT, MGMT methylation
Diffuse midline glioma, H3K27M-mutant	H3K27M-mutant, TP53, ATRX

IDH: Isocitrate dehydrogenase, TP53: Tumor protein 53, ATRX: Alpha-thalassemia/mental retardation syndrome X-linked, MGMT: O-6-methylguanine-DNA methyltransferase.

Table 30.17 Oncogenes associated with primary CNS tumors

Category	Proto-oncogene	Mode of Activation	Associated Malignancies
Growth factors			
PDGFB chain	PDGFB (*sis*-Simian sarcoma)	Overexpression	▪ Astrocytoma ▪ Osteosarcoma
TGFA	TGFA	Overexpression	Astrocytoma
Growth factor receptors			
PDGF receptor	PDGFRB	▪ Overexpression ▪ Translocation	▪ Glioma ▪ Leukemia
Cyclin-dependent kinases (cell cycle regulators)			
Cyclin-dependent kinase	CDK4	Amplification or point mutation	▪ Glioblastoma multiforme ▪ Melanoma ▪ Sarcoma

Table 30.18 Tumor suppressor genes (inactivated/biallelic loss) associated with primary CNS tumors

Gene	Protein	Function	Familial Tumors	Sporadic Cancers
Mitogenic signaling pathway inhibitors				
NF1	▪ Neurofibromin 1 (locus 17p11) ▪ Mutation occurs by deletion	NF1 inhibits RAS/MAP-kinase signaling activator pathway	Neurofibromatosis type 1 (neurofibroma and malignant peripheral nerve tumors)	▪ Schwannoma ▪ Neuroblastoma ▪ Juvenile myeloid leukemia

Contd...

Table 30.18 Tumor suppressor genes (inactivated/biallelic loss) associated with primary CNS tumors (*Contd...*)

Gene	Protein	Function	Familial Tumors	Sporadic Cancers
NF2	▪ Merlin (locus 22q) ▪ NF2 stabilizes cytoskeleton membrane linkage	Mutation by deletion or nonsense mutation	Neurofibromatosis type 2 (acoustic schwannoma and meningioma)	▪ Schwannoma ▪ Meningioma
PTCH	Patched	Inhibits Hedgehog signaling pathway	Gorlin's syndrome (basal cell carcinoma, medulloblastoma, other benign tumors)	▪ Medulloblastoma ▪ Basal cell carcinoma
Cell cycle progression inhibitors				
RB gene	▪ Retinoblastoma protein (locus 13p14) ▪ Mutation by deletion and nonsense	RB gene codes for pRB protein, master brake on cell cycle. It inhibits G1 to S transition during cell cycle. It inhibits nuclear transcription factor	▪ Retinoblastoma ▪ Osteosarcoma	▪ Retinoblastoma ▪ Osteosarcoma ▪ Breast carcinoma ▪ Colon carcinoma ▪ Prostatic carcinoma ▪ Urinary bladder carcinoma ▪ Lung carcinoma

Molecular Genetic Alterations in Primary Brain Tumors

- **IDH mutations:** Both IDH1 and IDH2 are enzymes in Krebs' cycle which catalyzes the conversion of isocitrate to α-ketoglutarate.
  - IDH1 involves an amino acid substitution (glycine to arginine) in the active site of the enzyme in codon 132 (R132H) resulting in the abnormal production of 2-hydroxyglutarate, which causes histone and DNA methylation, hence promoting tumorigenesis.
  - IDH2 mutation involves codon 172 results in seizures, weak muscle tone (hypotonia), and progressive damage to the cerebrum. IDH is most important diagnostic marker, that can differentiate glioma from gliosis.
  - IDH is positive in astrocytoma, oligodendroglioma, and secondary glioblastoma (10%).
  - IDH1 or IDH2 mutation can be demonstrated by immunohistochemistry using gene mutation-specific antibody against R132H mutant IDH1. If immunohistochemical analysis is negative, then it should be followed by IDH1/IDH2, DNA genotyping.
  - Mutation in both IDH1 and IDH2 entity is known as IDH mutant and if both are negative then it is known as IDH wildtype. If IDH testing is not available or cannot be fully performed or is inconclusive, then it is labeled as IDH glioma, NOS.
- **1p/19q codeletion:** In 1p/19q codeletion, there is complete deletion of both the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q).
  - 1p/19q codeletion can be demonstrated by fluorescent *in situ* hybridization (FISH), polymerase chain reaction (PCR), chromogenic in situ hybridization (CISH), or molecular genetic testing for diagnosis of grade II and grade III (anaplastic) oligodendroglioma.
  - 1p/19q codeletion is a strong prognostic factor associated with improved survival and also a predictive factor for response to chemotherapy as well as radiotherapy.
- **O-6-Methylguanine-DNA methyltransferase (MGMT) methylation mutations:** O-6-Methylguanine-DNA methyltransferase (MGMT) methylation gene encodes a DNA repair enzyme that can nullify the effects of alkylating chemotherapy such as temozolomide. Alkylating chemotherapeutic drugs cause damage to DNA by adding methyl group. Therefore, a brain tumor with a high degree of MGMT activity will become resistant to chemotherapies which target DNA at this location.
  - Addition of methyl group to the promoter region of MGMT results in silencing of the gene. Removal of methyl group to the promoter region of MGMT gene results in activation of the gene.
  - If the MGMT gene is active, the DNA is rapidly repaired. Methylation of the MGMT gene promoter is a favorable prognostic and predictive factor in glioblastoma patients, but it is not a diagnostic marker for the same.
- **Telomerase reverse transcriptase (TERT) promoter mutations:** TERT mutations involve C228T and C250T mutations of the promoter region. TERT promoter mutations and long telomere length predict radiotherapy resistance in gliomas and poor survival. TERT gene mutations most often occur in glioblastoma and oligodendroglioma.
- **α-Thalassemia/mental retardation syndrome X-linked (ATRX):** ATRX is a chromatin remodeling protein essential in DNA replication, gene transcription, chromosome congression (assembly), and cohesion between cell division.
  - ATRX mutation leads to lengthening of telomerase, that helps in chromatic maintenance and remodeling. Immunohistochemical analysis demonstrates positive staining of cells with normal ATRX.
  - If ATRX mutation is present, then there will be a loss of staining in the cells.
  - ATRX mutations are almost always accompanied by other mutations such as IDH, TP53, and H33K27M in the histone regulation.

- Loss of ATRX expression is demonstrated in anaplastic astrocytomas (45%), anaplastic oligoastrocytomas (27%), and anaplastic oligodendrogliomas (10%) and also in pediatric and adult high-grade astrocytoma.

- **TP53 mutation:** The TP53 is a tumor suppressor gene located on the short arm of chromosome 17. Loss of TP53 results in DNA damage, hypoxia, oxidative damage, oncogene activation, microtubule disruption, which in turn contributes to the tumorigenesis of CNS tumors mainly medulloblastoma, glioblastoma, and in IDH-mutant astrocytomas (56–58%). Astrocytomas demonstrate mutations of both TP53 and IHD1.

Clinical Features

The clinical presentation of various primary brain tumors is best appreciated by considering the relationship of signs and symptoms to anatomy.

- General signs and symptoms include headache, seizures, visual disturbances, anorexia, nausea, vomiting and changes in personality, mood, mental capacity, and concentration.
- Seizures are the presenting symptom in about 20% of patients with supratentorial slow-growing brain tumors in the supratentorial region over months to years before the clinical diagnosis. All patients with either primary brain tumors or metastatic brain tumors can present with seizures.

Imaging Techniques

Contrast-enhanced computed tomography (CT) scan and magnetic resonance imaging (MRI) have complementary roles in the diagnosis of CNS neoplasms. CT scan is superior to detect calcifications, skull lesion and bleeding of <24 hours, and thus helps diagnosis and immediate management.

- High-quality MRI is the diagnostic tool of choice in the evaluation of intramedullary and extramedullary spinal cord lesions.
- When a glioma spreads across the corpus callosum into both hemispheres of the brain, the tumor often has the appearance of a butterfly on a CT scan or MRI scan. Hence, it is given the general term 'butterfly glioma'.

Surgical Pathology: Primary Brain Tumors

Gross Morphology

- Fast growing primary brain tumors may have apparent pseudocapsule.
- Primary large size brain tumors are poorly delineated. When a glioma spreads across the corpus callosum into both hemispheres of the brain, the tumor often has the appearance of a butterfly, hence it is known as butterfly glioma.
- Peritumoral edema is most commonly demonstrated.
- Cut section of brain tumor gives variegated appearance, i.e. yellowish necrotic central area, grayish peripheral rim, recent and old hemorrhage, cysts due to liquefaction of the necrotic tissue.

Light Microscopy

Salient features of selected primary central nervous system tumors including metastatic tumors are given in Table 30.19.

Immunohistochemistry Technique

Immunohistochemistry (IHC) is a powerful microscopy-based diagnostic technique, which involves the process of selectively identification of target antigen (protein) in the cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues (Tables 30.20 to 30.22).

Table 30.19 Salient features of selected primary central nervous system tumors including metastatic tumors

Histologic Variants	Age Predilection	Most Frequent Site	Characteristics
Glioblastoma multiforme	Older persons (40–70 years)	Cerebral hemispheres frontal lobe, crossing corpus callosum involving opposite cerebral hemisphere	▪ Most common highly malignant intracranial tumor ▪ Butterfly shaped tumor on MRI ▪ Increased intracranial pressure and seizures ▪ Dual loss of 1p,19q associated with good prognosis ▪ Mutations of EGFR, MDM2, PTEN ▪ Seeding CSF ▪ Surgical resection impossible, but treated by radiotherapy and chemotherapy
Astrocytoma	Children	Cerebellum, optic nerve, brainstem	▪ Low-grade tumor associated with good prognosis ▪ Immunohistochemistry (GFAP positive)
Pilocytic astrocytoma	Any age, common in children	Any location, common in cerebellum and third ventricle	▪ Hair-like processes ▪ Rosenthal fibers
Oligodendroglioma	4th–5th decades	Cerebral hemispheres frontal lobe white matter	▪ Prone to spontaneous hemorrhage owing to delicate vasculature ▪ Round cells with perinuclear halos; satellitosis

Contd...

Table 30.19 Salient features of selected primary central nervous system tumors including metastatic tumors (*Contd...*)

Histologic Variants	Age Predilection	Most Frequent Site	Characteristics
			▪ Calcification seen on radiograph ▪ IDH1/IDH2, mutation in FUBP1 due to 1p/19q codeletion ▪ Chemosensitive tumor
Medulloblastoma	Most often during 15–35 years, may occur in children (<15 years), peak at 7 years	Cerebellum (close to 4th ventricle) in adults, cerebellar vermis in children	▪ Highly malignant intracranial tumor ▪ Small round tumor cells ▪ Homer Wright rosettes ▪ Chromosome 17 inversion ▪ Seeding CSF ▪ Resection difficult, highly radiosensitive associated with poor prognosis ▪ Immunohistochemistry (synaptophysin or nestin, neuron-specific enolase and GFAP positive)
Ependymoma	Most common during first two decades	4th ventricle of brain, spinal cord	▪ Histologic variants (well differentiated, myxopapillary and anaplastic) ▪ Immunohistochemistry (GFAP, S-100, vimentin positive) ▪ Increased intracranial pressure, hydrocephalus
Choroid plexus	Children	Lateral ventricle, 4th ventricle of brain	▪ Uncommon neuroectodermal tumor but seeding CSF ▪ Hydrocephalus
Meningioma	20–40 years (more common in women)	Convexity of cerebral hemispheres, falx cerebri, olfactory groove beneath frontal lobe, lesser wing of sphenoid, sella turcica, foramen magnum, thoracic region of spinal cord	▪ Second most common primary intracranial neoplasm (WHO grade I) ▪ Aggressive meningiomas (clear cell and chondroid variant—WHO grade II, papillary and rhabdoid variant—WHO grade III) ▪ Benign tumor presents external to the brain hence easy resection ▪ Associated with type 2 neurofibromatosis
Schwannoma (neurilemmoma)	Middle to later life	8th cranial nerve (when schwannoma is intracranial)	Acoustic schwannoma, common intracranial tumor, ranking third after glioblastoma multiforme and meningioma, most often benign tumor is usually respectable
Retinoblastoma	Young children (<3 years)	Retina, bilateral and multifocal in familial form; unilateral and unifocal in sporadic forms	Most common eye tumor of young children; linked to Rb gene deletion or inactivation
Metastatic tumors	Variable age	Metastases from lung, breast, kidney, colon, thyroid, skin melanoma	Metastatic tumors are more common than primary intracranial tumors

Table 30.20 Panel of immunohistochemical markers applied in central nervous system tumors showing neuroglial differentiation

Immunohistochemical Marker	Expression in Tumors
CNS tumor with neuroendocrine (NE) differentiation	
▪ Synaptophysin	▪ Pheochromocytoma, carcinoid tumor, medulloblastoma, PNET/Ewing sarcoma, paraganglioma, thyroid medullary carcinoma
▪ Chromogranin A	▪ Pheochromocytoma, carcinoid tumor, medulloblastoma, PNET/Ewing sarcoma, paraganglioma, thyroid medullary carcinoma
▪ NSE (neuron-specific enolase)	▪ Pheochromocytoma, carcinoid tumor, medulloblastoma, PNET/Ewing sarcoma, paraganglioma, thyroid medullary carcinoma
▪ CD56 (neuronal cell adhesion molecule—NCAM)	▪ Pheochromocytoma, carcinoid tumor, medulloblastoma, PNET/Ewing sarcoma, paraganglioma, thyroid medullary carcinoma

Contd...

Table 30.20 Panel of immunohistochemical markers applied in central nervous system tumors showing neuroglial differentiation (*Contd...*)

Immunohistochemical Marker	Expression in Tumors
CNS tumor with glial differentiation	
GFAP (glial fibrillary acidic protein)	Astrocytes, astrocytoma, oligodendrocytes, oligodendroglioma, ependymal cells, ependymoma, GFAP may be used to differentiate a glioma from non-glial neoplasm (lymphoma, carcinoma, melanoma, and multiple sclerosis), GFAP also reacts variably with Schwann cells
CNS tumor with neuronal/ganglion differentiation	
▪ Sm311 (pan-NF)	▪ Sm31 (axon) may be used to highlight axon
▪ Sm32 (cell body)	▪ Sm32 (cell body) may be used to highlight normal axons and identify permeation of normal brain parenchyma by a glioma or meningioma
▪ NeuN (neuronal nuclei)	▪ NeuN (neuronal nuclei) to highlight neuronal nucleoli
▪ Synaptophysin	▪ Gangliocytoma, neuroblastoma, medulloblastoma
▪ Chromogranin A	▪ Gangliocytoma, neuroblastoma, medulloblastoma
▪ NSE (neuron-specific enolase)	▪ Gangliocytoma, neuroblastoma, medulloblastoma
CNS tumor with Schwann cells	
▪ S-100 protein	▪ Schwannoma, neurofibroma, glioma, melanoma, ependymoma, melanocytes, myoepithelial cells, histiocytes
▪ CD57 (Leu7) lymphoid marker	▪ CD57 cross-reacts with myelin-associated protein and second-tier marker for Schwann cells

All glial/neuronal/ nerve sheath are CAM 5.2 negative and AE1/AE3. However, AE1/AE3 staining is observed in normal and neoplastic brain and reactive astrocytes.

Table 30.21 Panel of immunohistochemical markers applied in central nervous system tumors with neuroglial differentiation

Immunohistochemical Marker	Expression
Astrocytoma	GFAP (glial fibrillary acidic protein) (++), p53 (+30%), (1p/19q deletion absent)
Oligodendroglioma	GFAP (glial fibrillary acidic protein variable staining), p53 (–), (1p/19q deletion present), reticulin (–)
Ependymoma	GFAP (glial fibrillary acidic protein) (+), vimentin (+), EMA (epithelial membrane antigen) (+)
Meningioma (grade I variants include angiomatous, fibroblastic, lymphocyte-rich, meningothelial, metaplastic, psammomatous, secretory, transitional types; grade II variants include atypical, choroid and clear cell types, and grade III variants include anaplastic, papillary and rhabdoid types)	Vimentin (+++), EMA (epithelial membrane antigen) (+), PR (50%), S-100 protein (80%); Note: secretory meningioma shows positivity for cytokeratin (CK), CEA (carcinoembryonic antigen), PAS (periodic acid–Schiff); and atypical meningioma shows positivity for Ki-67 proliferative marker
Primitive neuroectodermal neoplasms (medulloblastoma)	Synaptophysin (+), chromogranin A (+), nestin (+), NSE (neuron-specific enolase) (+)
Primitive neuroendocrine tumor (PNET)	Synaptophysin (+), GFAP (glial fibrillary acidic protein) (+), CD99 (–)
Retinoblastoma	GFAP (glial fibrillary acidic protein) (+), S-100 protein (+), synaptophysin (+), NSE (neuron-specific enolase) (+), MBP (myelin basic protein) (+)
Hemangioblastoma	CD31 (+), CD34 (+), CD56, S-100 protein (+), vimentin (+), NSE (neuron-specific enolase) (+), inhibin A (+)
Atypical teratoid/rhabdoid tumor (AT/RT)	EMA (epithelial membrane antigen) (+), GFAP (glial fibrillary acidic protein) (+), cytokeratin (CK) (+), vimentin (+), SMA (smooth muscle actin) (+), IN11 (HSNF/BAF47) loss of expression due to 22q11 deletion, synaptophysin (variable staining), chromogranin A (variable staining)
Dysembryoplastic neuroepithelial tumor (DNT)	S-100 protein (+), NeuN (+), synaptophysin (–)
Pituitary neoplasms	Synaptophysin (+), chromogranin A (+), growth hormone (+), ACTH (+), prolactin
Choroid plexus tumors	Cytokeratin (CK) (+), transthyretin (+), S-100 protein (+), GFAP (glial fibrillary acidic protein) (+), EMA (epithelial membrane antigen) (+)

Table 30.22 Panel of immunohistochemical markers applied in nerve and nerve sheath tumors

Immunohistochemical Marker	Expression
Traumatic neuroma	S-100 protein (stains Schwann cell), neurofilament (stains axons), EMA (epithelial membrane antigen stains perineural cells), collagen IV
Schwannoma (cellular variant)	S-100 protein (strong uniform staining), GFAP (glial fibrillary acidic protein variable staining), CD57 (Leu7) lymphoid marker (variable staining), laminin (pericellular staining), collagen IV (pericellular staining)
Schwannoma (ancient variant)	S-100 protein (strong uniform staining), GFAP (glial fibrillary acidic protein positive in minority of cases), CD57 (Leu7) lymphoid marker (positive in minority of cases)
Ganglioneuroma	Synaptophysin (+), neurofilament (+), tubulin (+), NSE (neuron-specific enolase) (+), S-100 protein (+), GFAP (glial fibrillary acidic protein) (+); all markers are positive for ganglion cells
Neurothekeoma	NKIC3 (+), S-100 protein (+), PGP9.5 (+), MITF (microphthalmia transcription factor) (+), SMA (smooth muscle actin) (+)
Cellular schwannoma	NKIC3 (+), S-100 protein (+), GFAP (glial fibrillary acidic protein variable staining), CD57 (Leu7) lymphoid marker (variable staining), PGP9.5 (–)
Neurofibroma	S-100 protein (+), CD57 (Leu7) lymphoid marker (variable staining), CD34 (variable staining), factor XIIIa (variable staining), EMA (epithelial membrane antigen variable staining)
Plexiform neurofibroma	S-100 protein (+), CD57 (Leu7) lymphoid marker (variable staining), CD34 (variable staining), factor XIIIa (variable staining), EMA (epithelial membrane antigen) (–)
Perineuroma	EMA (epithelial membrane antigen) (+), S-100 protein (+), GLUT1 (glucose transporter 1 protein isoform) (+), claudin (+), laminin (outlines the basal lamina around individual cells or small group of cells), type 4 collagen (outlines the basal lamina around individual cells or small group of cells)
Malignant peripheral nerve sheath tumor with spindle cell variant	HMB-45 (human melanoma black 45) (+), S-100 protein (+), CD57 (Leu7) lymphoid marker (+)
Malignant peripheral nerve sheath tumor with epithelioid variant	HMB-45 (human melanoma black 45) (+), S-100 protein (+), CD57 (Leu7) lymphoid marker (+)
Malignant peripheral nerve sheath tumor with rhabdoblastic differentiation	Desmin (+), myogenin (+), MyoD1 (+), HMB-45 (human melanoma black 45) (+), CD57 (Leu7) lymphoid marker (+)
Malignant-Triton tumor	S-100 protein (++++ in 50%), CD57 (Leu7) lymphoid marker (+ in 30–40%), cytokeratin (CK) (+), desmin (+)
Granular cell tumor (benign tumor)	CD68 (++++), SMA (smooth muscle actin), inhibin (+), NSE (neuron-specific enolase) (+), laminin (+), myelin P0 (+), myelin P2 (+)
Granular cell tumor (malignant tumor)	S-100 protein (+), NSE (neuron-specific enolase) (+), vimentin (+), PAS-D CD68 (–)
Neuroglial heterotopia	S-100 protein (stains glial tissue), GFAP (glial fibrillary acidic protein stains glial tissue)
Neuroblastoma	Synaptophysin (+), chromogranin A (+), NSE (neuron-specific enolase) (+), neurofilament (+), N-myc amplification (+ demonstrated by FISH), deletion of chromosome 1p (demonstrated by cytogenetic analysis), CD99 (–)
Esthesioneuroblastoma	Synaptophysin (+), chromogranin A (+), CD56 (+), neurofilament (+), NSE (neuron-specific enolase) (+), S-100 (+ around periphery of tumor), cytokeratin (CK) (–), desmin (–), myogenin (–)
Paraganglioma	NSE (neuron-specific enolase) (+), synaptophysin (+), chromogranin (+), neurofilament (+), peptide hormones, e.g. calcitonin, somatostatin (+), S-100 protein (+), GFAP (glial fibrillary acidic protein) (+)
Melanocytic neuroectodermal tumor of infancy	Cytokeratin (CK) (+), HMB-45 (human melanoma black 45) (+), NSE (neuron-specific enolase) (+), synaptophysin (+)

Prognostic Factors

Prognostic factors for primary brain tumors are age of the patient >40 years, progressive disease attaining >5 cm, tumor crossing the midline, contrast enhancement of tumor on MRI, WHO performance status 1, neurological symptoms and less than a gross total resection. Poor prognostic factors for primary brain tumors are given in Table 30.23.

Table 30.23 Poor prognostic factors for primary brain tumors

Age of the patient >40 years
Progressive disease
Tumor size >5 cm
Tumor crossing the midline
Contrast enhancement of tumor on MRI
World Health Organization performance status (≥1)
Neurological symptoms
Less than a gross total resection

METASTASES TO NERVOUS SYSTEM

Metastatic brain tumors, also called secondary brain tumors, are caused by malignant tumors metastasizing to the brain from a different part of the body. These tumors are more common than primary intracranial tumors of the CNS in adults (30%) and children (6–10%).

- Common sources of metastases in adults include lung carcinoma, breast carcinoma, skin melanoma, renal cell carcinoma, colorectal carcinoma, thyroid carcinoma and NHL.
- In children, sources of metastases include acute lymphoblastic lymphoma, osteosarcoma, Ewing sarcoma and rhabdomyosarcoma.
- The cancer stem cells break away from the primary tumor and travel through the bloodstream, break down blood–brain barrier and then invades cerebral hemisphere or cerebellum.
- Metastatic brain tumors can grow rapidly, crowding or destroying nearby brain tissue, inflammation, and edema. Sometimes, a patient may have multiple metastatic tumors in several different areas of the brain.
- Distribution throughout the central nervous system is more common in areas of high blood flow; however, different histologic subtypes tend to have different distributions of location within the brain.

Locations of Metastases in Nervous System

Most metastatic tumors seed to the gray-white junction, reflecting the rich capillary bed in this region. The borders of a metastasis deposit tend to be more discrete than those of a primary glioma. These metastatic tumors involve subarachnoid space and cause meningitis (Figs 30.10 and 30.11).

Basic Laboratory Assessment of Metastases to Brain

Basic laboratory assessment including complete blood count, metabolic panel, and liver function test should be performed.

Imaging Techniques

- A head CT allows for a quick examination, although fine-slice MRI of the brain with contrast is the gold standard for neuroimaging in cases of suspected brain metastases.
- MRI allows for a determination of the number and anatomical location of tumors and degree of associated edema. Metastasis in brain appears as discrete, globoid shape lesion, and prominent halo of edema in contrast to primary glioma or medulloblastoma.
- Spinal cord involvement is uncommon, but if present, then metastases deposits are seen in extradural space.

Surgical Pathology: Metastases to Brain

Gross Morphology

Metastatic tumor deposits are usually multiple and well circumscribed in nervous system. Pigmented secondaries indicate secondary melanoma (Fig. 30.12).

Light Microscopy

- A biopsy is required to differentiate metastatic tumors. A solitary brain mass in an adult can be primary or metastatic.
- In some cases, the metastases become clinically apparent before primary site is observed (Fig. 30.13).

Immunohistochemistry Technique

Immunohistochemistry is useful to confirm source of primary cancers metastasizing to central nervous system in adults and children (Tables 30.24 and 30.25).

Management

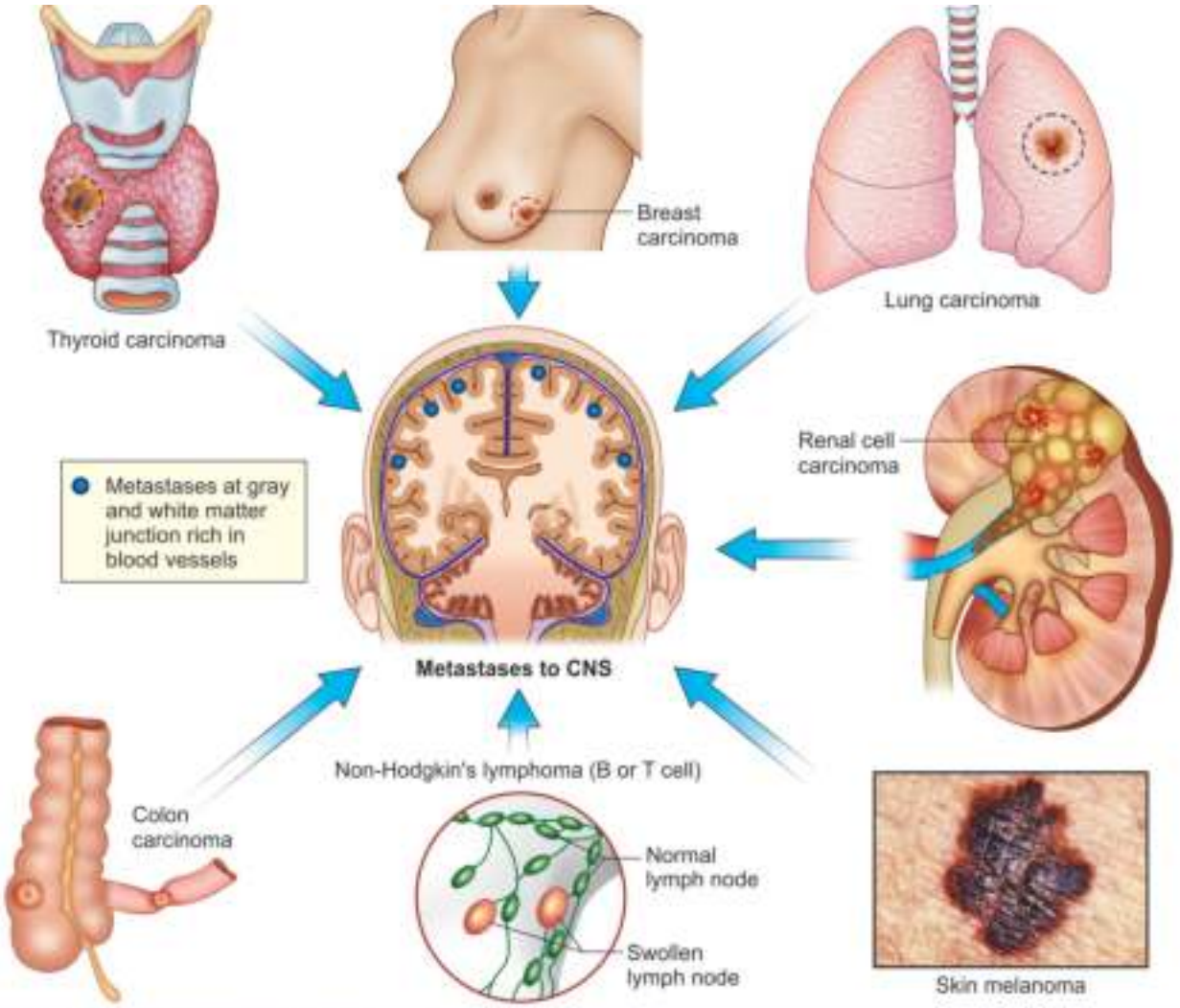
The first step in the management of newly diagnosed brain metastases is the treatment of intracranial edema.

- A loading dose of dexamethasone (10 mg) should be administered by oral or intravenous route and followed by 4 mg IV every six hours is one dosing regimen.
- Following initiation of steroids, definitive management may be initiated.
- Treatment options include surgical resection (for limited brain metastases in patients with good performance status and surgically accessible lesions), whole brain radiotherapy, and stereotactic radiosurgery.

ASTROCYTIC TUMORS

Astrocytic tumors are neuroepithelial tumors arising from neuroglial cells.

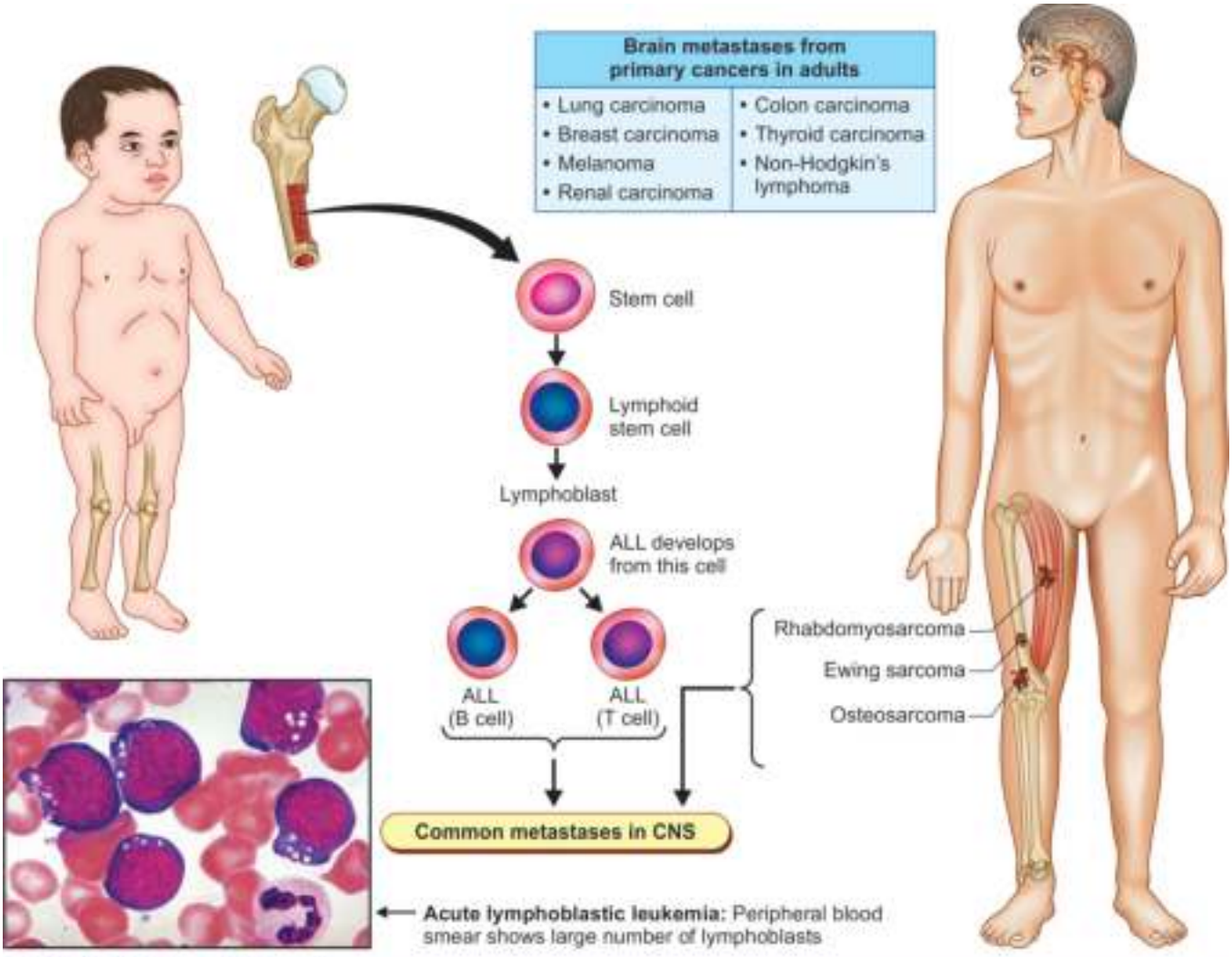
- Astrocytomas account for 20% of primary brain tumors, which originate from astrocytes in the cerebral hemisphere (most common), cerebellum, optic nerve, brainstem and spinal cord.



Primary cancer metastases to central nervous system	Immunohistochemistry
• Thyroid carcinoma	Thyroglobulin, CA 125 cell surface glycoprotein demonstrated by monoclonal antibody OC-125 and TTF1
• Breast carcinoma	ER, PR, HER2/neu, CK7, GCDFP-15 and mammoglobulin
• Lung carcinoma (squamous cell carcinoma)	EMA, CK7, TTF1, p63
• Renal cell carcinoma	Cytokeratin, vimentin
• Skin melanoma	HMB-45, melan-A (MART-1), CAM 5.2, S-100
• NHL (B cell type)	CD10, CD19, CD20
• NHL (T cell type)	CD3
• Colon carcinoma	CK20, CDX2 and β-catenin

Fig. 30.10: Common brain metastases in adults from thyroid, breast, lung, kidney, colon, skin melanoma and NHL.

- Cystic astrocytomas occur in cerebellar region in children.
- Peak occurrence of astrocytomas is in the late middle age group. Most patients die within 5–7 years.
- Diffuse astrocytoma usually occurs in the cerebral hemispheres (most common), cerebellum, optic nerve, brainstem and spinal cord.
- Histologic classification and grading: Astrocytic tumors in the cerebral hemispheres can be further categorized according to their histologic differentiation and clinical course.
  - Histologic grading of astrocytic tumors is based rapid progression and increasing pathologic anaplasia of tumors such as nuclear atypia, mitotic activity, endothelial cells or microvascular proliferation and necrosis in tumor.
  - These astocytic tumors are termed: (a) grade I: low-pilocytic astrocytomas, (b) grade II: diffuse



Primary cancer metastases to central nervous system	Immunohistochemistry
• Acute lymphoblastic leukemia	B cell lineage: cCD79a/cCD22 T cell lineage: cCD3, CD2, CD3, CD4, CD5, CD7 and TdT
• Osteosarcoma	Vimentin
• Ewing sarcoma	CD99 (MIC2 specific marker) Vimentin, neuron-specific enolase (NSE) and neurofilament Cytokeratin: Sometimes tumor cells are reactive to low molecular weight keratins
• Rhabdomyosarcoma	Desmin, vimentin, myoglobin and MyoD1

Fig. 30.11: Common brain metastases in children and young adults from acute lymphoblastic leukemia, Ewing sarcoma, osteosarcoma and rhabdomyosarcoma.

astrocytomas, (c) grade III: anaplastic astrocytomas, and (d) grade IV: astrocytomas, glioblastoma multiforme.

- Astrocytomas may be classified based on their invasion into surrounding brain parenchyma.
  - Noninvasive astrocytomas include pilocytic astrocytomas, pleomorphic xanthoastrocytoma, and subependymal giant cell astrocytomas. Many low-grade astrocytomas eventually transform to anaplastic (grade III) and highly malignant glioblastoma multiforme (grade IV).
  - Invasive (diffuse) astrocytomas include low-grade fibrillary astrocytoma, anaplastic astrocytomas and glioblastoma multiforme (grade IV). Fibrillary astrocytomas account for 80% of adult primary brain tumors. These are most common in middle age. Anaplastic astrocytoma has a peak incidence in the sixth decade. Although they usually are found in the cerebral hemispheres, they also can occur in the **cerebellum, brainstem**, or spinal cord. Clinically, infiltrating astrocytic tumors present with symptoms of increased intracranial pressure

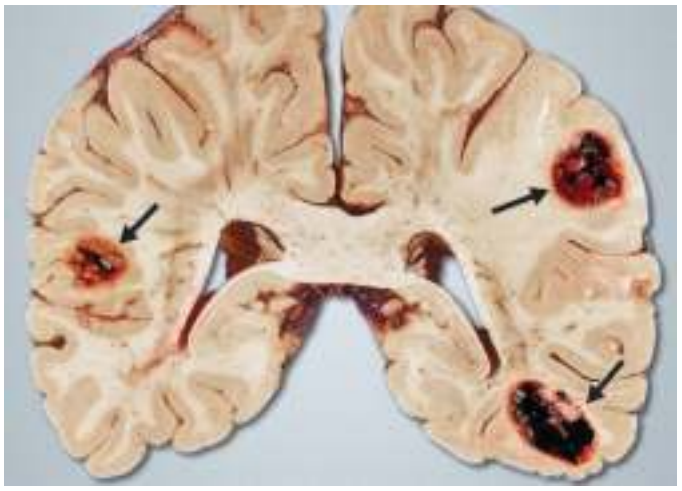


Fig. 30.12: Secondary tumor in the brain. Secondary tumor deposits are usually multiple and well circumscribed. Pigmented secondaries indicate secondary melanoma (arrows).

Table 30.24 Primary tumors metastasizing to central nervous system in adults

Primary cancers	Immunohistochemistry
Lung carcinoma	CK7+, CAM 5.2+, TTF-1+, EMA+, CK20–
Breast carcinoma	CK7+, CAM 5.2+, CK20–, TTF-1–
Melanoma	HMB-45, melan-A (MART-1), CAM 5.2, S-100
Renal cell carcinoma	CK+, CAM 5.2+, vimentin+
Colon carcinoma	CK20+, CK7–, TTF-1–
Thyroid carcinoma	TTF-1+
Non-Hodgkin's lymphoma	CD10, CD19 and CD20

Variable expression of ER, PR, HER2/neu is demonstrated in different histologic variants of breast carcinoma.

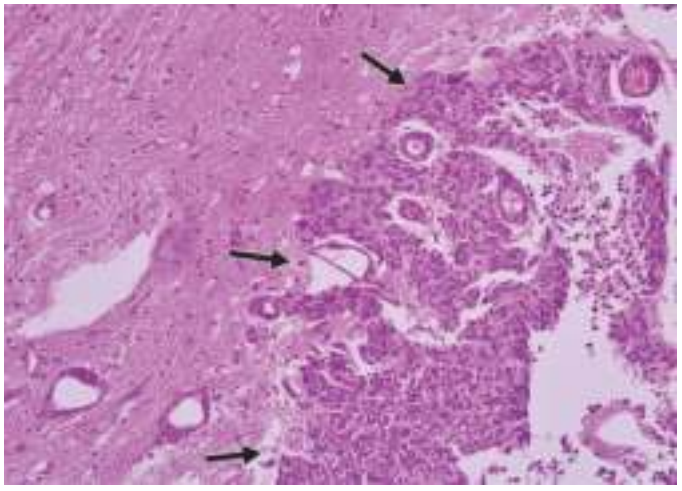


Fig. 30.13: Metastases to brain from carcinoma (arrows) (100X).

Table 30.25 Primary tumors metastasizing to central nervous system in children

Primary Cancers	Immunohistochemical Markers Expression
Acute lymphoblastic leukemia	▪ B cell lineage: cCD79a/cCD22+ ▪ T cell lineage: cCD3+, CD2+, CD3, CD4+, CD5+, CD7+ and TdT+
Osteosarcoma	Vimentin
Ewing sarcoma	MIC2+ (specific marker), vimentin+, neuron-specific enolase+ (NSE), CD99+, neurofilament+, cytokeratin+ (LMW sometimes)
Rhabdomyosarcoma	Desmin+, vimentin+, myoglobin+, MyoD1+

(e.g. headache) or focal abnormalities related to their position (e.g. seizures).

- Low-grade astrocytomas arising from cerebellum, optic nerve and brainstem in children have good prognosis. Glioblastoma multiforme arising in cerebrum is highly malignant tumor in adults associated with fatal outcome, which has ill-defined margins and grow irregularly into the surrounding brain tissue and therefore it is difficult to eradicate the tumor. These increase intracranial pressure.

DIFFUSE ASTROCYTOMA, IDH-MUTANT

Diffuse astrocytoma with a mutation in either IDH1 or IDH2 gene corresponds histologically to WHO grade II neoplasm.

- Tumor is composed of moderately pleomorphic cells and characterized by a high degree of cellular differentiation and slow growth. The diagnosis is supported by the presence of ARTX and TP53 gene mutations.
- The tumor most often affects young adults with peak in mid-30s and occurs throughout the central nervous system especially in the frontal lobes of brain.
- Diffuse astrocytoma can progress to IDH-mutant anaplastic astrocytoma and eventually to IDH-mutant glioblastoma. The median ages of patients with IDH-mutant diffuse astrocytoma are in the mid-30s.
  - Tumor can be located in any region of central nervous system, but most common site is frontal lobe in the supratentorial region.
  - It is worth mentioning that astrocytoma and oligodendroglioma with codeletion of 1p/19q arise in the supratentorial region. It supports the hypothesis that these gliomas arise from a distinct population of precursor cells.

- Gemistocytic astrocytoma, IDH-mutant is a variant of IDH-mutant diffuse astrocytoma characterized by the presence of variable proportion of gemistocytic neoplastic astrocytes in adults. Diffuse astrocytoma, NOS has morphologic features without IDH mutations.

Genetic Susceptibility

Diffuse astrocytoma especially anaplastic astrocytoma and glioblastoma multiforme can occur in patients with inherited TP53 germline mutations/Li-Fraumeni syndrome. There is increased risk for development of low-grade astrocytoma in patients with inherited Ollier-type multiple enchondromatosis.

Clinical Features

Patient presents with seizures (most common), speech difficulty, sensation and vision disturbances. Patient with frontal lobe tumor develops behavioral or personality changes.

Radiologic Findings

CT scan demonstrates poorly defined, homogenous mass without contrast enhancement. However, calcification and cystic change may be demonstrated early in the evolution of the astrocytoma.

- MRI studies usually demonstrates T1-hypodensity and T2-hyperintensity.
- Gadolinium enhancement is uncommon in low-grade astrocytoma, but tends to demonstrate during tumor progression.

Surgical Pathology: Diffuse Astrocytoma, IDH-mutant

Gross Morphology

- Because of infiltrative nature, astrocytoma usually exhibits blurring of the gross anatomical boundaries.
- Enlargement of the tumor distorts invaded anatomical structures without causing destruction of cortex.
- Tumor is gray or white with indistinct boundaries, cysts formation and spongy, soft to firm in consistency.
- Extensive microcyst formation can cause gelatinous appearance. Focal calcification may be demonstrated.
- Extension of tumor into contralateral structures, particularly frontal lobes are rarely observed (Figs 30.14 and 30.15).

Light Microscopy

- Diffuse astrocytoma is composed of well-differentiated fibrillary astrocytes in the background of a loosely structured, often microcystic tumor matrix.
- Tumor is moderately cellular without cytologic atypia, necrosis and microvascular proliferation, when compared with normal brain.
- Presence of necrosis and/or microvascular proliferation is incompatible with the diagnosis of diffuse astrocytoma.
- The histologic patterns with respect of their size, cell processes and cytoplasmic glial filaments may vary in different regions of the tumor.
- Histological recognition of neoplastic astrocytes is studied by using hematoxylin and eosin stained sections. Tumor is composed of cells with vesicular nuclei, intermediate chromatin and distinct nucleoli.
- When compared, normal astrocytes contain oval or elongated nuclei and without stainable cytoplasm, that is distinct from the background neuropil (Fig. 30.16).

Immunohistochemistry Technique

Panel of immunohistochemical markers is applied on paraffin-embedded hematoxylin and eosin stained sections in diffuse astrocytoma with IDH mutation.

Markers	Expression
Glial fibrillary acidic protein (GFAP)	Positive
IDH1 R132H mutation	Positive
p53	Positive
Ki-67 proliferation index of <4%	Positive

Molecular Genetic Alterations

Molecular genetic alterations for diffuse astrocytoma are IDH1, IDH2, TP53 and ATRX.

DIFFUSE MIDLINE GLIOMA, HISTONE H3K27M-MUTANT

Diffuse midline glioma, histone H3K27M mutant is a new tumor entity defined by the recent 2021 WHO classification of tumors of the central nervous



Fig. 30.14: Astrocytoma. Brain slice examined from its posterior aspect. The tumor has arisen in the right hemisphere and extends through the genu of the corpus callosum into the left hemisphere. There is hemorrhage and necrosis in the tumor, indicating that it is a high-grade malignancy. It has extended into the third ventricle, obstructing the interventricular foramina and resulting in hydrocephalus, as shown by the dilatation of the anterior horns of both lateral ventricles (arrow).



Fig. 30.15: Astrocytoma in cerebellum. Cerebellum containing a large astrocytoma. The majority of the brain tumors in children occur below the tentorium cerebelli (arrow).

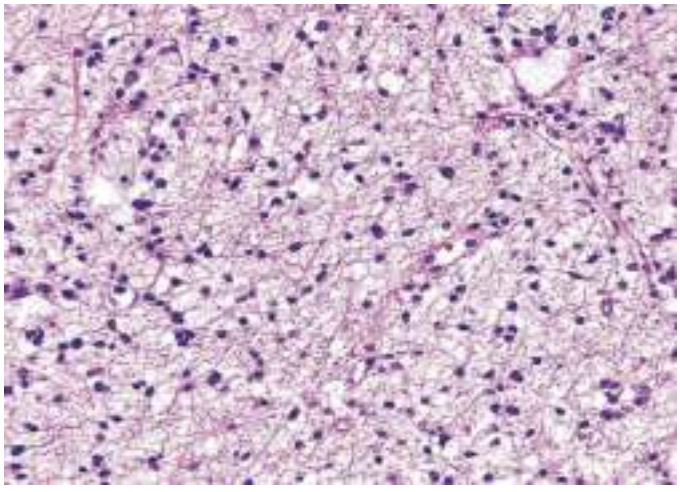


Fig. 30.16: Diffuse astrocytoma. Tumor is composed of well-differentiated fibrillary astrocytes with vesicular nuclei, intermediate chromatin and distinct nucleoli. in the background of a loosely structured, often microcystic tumor matrix. Tumor is moderately cellular without atypia, necrosis and microvascular proliferation, when compared with normal brain. There is no necrosis or microvascular proliferation. The histologic patterns with respect their size, cell processes and cytoplasmic glial filaments may vary in different region of the tumor (400X).

system corresponding to WHO grade IV. It affects adults and children in the region of brainstem, thalamus and spinal cord. It was previously known as glioma in brainstem tumor and diffuse intrinsic pontine glioma in spinal cord.

Radiologic Findings

Radiographic features of diffuse midline glioma histone H3K27M-mutant are diffuse growth with hypointense on T1 and hyperintense on contrast enhancement along with hemorrhage and necrosis. Tumor spreads by leptomeningeal dissemination associated with poor prognosis.

Surgical Pathology: Diffuse Midline Glioma, Histone H3K27M-mutant

Gross Morphology

Tumor distorts anatomic structures and demonstrates areas of hemorrhage and necrosis.

Light Microscopy

- Tumor is composed of primitive, poorly differentiated, small uniform cells with scant cytoplasm, nuclear molding, and brisk mitotic activity, exhibiting PNET-like appearance. In the hypocellular area, oligodendroglioma-like cells are demonstrated.
- Neuropil-like islands are observed in the cellular area.
- Microvascular proliferation and necrosis are also present.

Immunohistochemistry Technique

In diffuse midline glioma, histone H3K27M mutant panel of markers is applied on paraffin-embedded sections such as H3K27M, p53, neural adhesion molecule 1, S-100 protein, OLIG2, MAP2 and nuclear p53.

Markers	Expression
H3K27M	Positive (strong nuclear staining)
p53	Positive
Neural adhesion molecule 1	Positive
S-100 protein	Positive
OLIG2	Positive
MAP2	Positive
Nuclear p53	Positive
NeuN	Negative
Chromogranin A	Negative

ANAPLASTIC ASTROCYTOMA, IDH-MUTANT

Anaplastic astrocytoma, IDH-mutant is a diffuse infiltrating astrocytoma with focal or dispersed anaplasia and significant proliferative activity. The cell of origin is not known. The fact that diffuse astrocytoma grade II and astrocytoma grade III and glioblastoma IDH-mutant grade IV carry IDH mutations suggest these tumors may share a cell of origin different from other IDH-wildtype glioblastomas and pilocytic astrocytoma.

- Anaplastic astrocytoma, IDH mutant tumor can originate from low-grade diffuse astrocytoma, which corresponds histologically to WHO grade III. It is usually diagnosed without indication of low-grade diffuse astrocytoma.
  - Tumor resembles identical to oligodendroglioma in the absence of 1p/19q codeletion.
  - Anaplastic astrocytoma has tendency for malignant progression to IDH-mutant glioblastoma. It occurs in adults with mean age of 38 years. Tumor can

develop in any region of central nervous system, but cerebrum (especially in frontal lobe) is the most common site.

- Anaplastic astrocytoma, IDH wildtype corresponding to WHO grade III is a diffusely infiltrating astrocytoma with focal or dispersed anaplasia with significant proliferative activity but without mutations in the IDH genes, which can extensively involve cerebrum (three lobes involving deep gray mater), brainstem, cerebellum and spinal cord. Another histologic variant anaplastic astrocytoma, NOS corresponds to WHO grade III and has identical histopathologic features but lacks IDH mutation.

Clinical Features

Patient presents with a history of seizures and neurological deficits due to increased intracranial pressure depending on location of the tumor, degree of edema and tumor effect on the surrounding anatomical structures. Tumor is generally characterized by diffuse infiltrative growth in the brain. Occasionally, leptomeningeal spread of anaplastic astrocytoma, IDH mutant is evident.

Radiologic Findings

Anaplastic astrocytoma, IDH-mutant appears as a poorly defined mass of low density. Partial contrast enhancement is demonstrated unlike WHO grade II diffuse astrocytoma. Tumor lacks necrosis with ring enhancement. Rapidly growing tumor demonstrates peritumoral edema, that can lead to tumor mass shift and increased intracranial pressure.

Surgical Pathology: Anaplastic Astrocytoma, IDH-mutant

Gross Morphology

- Anaplastic astrocytoma, IDH-mutant tends to infiltrate the surrounding brain, with causing frank destruction.
- Invasion in surrounding brain parenchyma results in enlargement of adjacent gyri and basal ganglion.
- Cut section of tumor reveals discernible opaque tumor mass, soft in consistency with granular surface, which is distinguished from the surrounding brain structures. Macroscopic cysts are uncommon.
- It is most often difficult on gross examination to differentiate grade III anaplastic astrocytomas from grade II diffuse astrocytoma.

Light Microscopy

- Histologic features of anaplastic astrocytoma, IDH-mutant and diffuse astrocytoma are identical.
- Anaplastic astrocytoma, IDH-mutant is highly cellular and composed of cells with nuclear atypia and mitoses.
- Ki-67 labeling in the range of 5–10% may be helpful in analyzing significant proliferative activity of tumor.
- With progressive anaplasia, tumor is composed of cells with variation in size and shape of nuclei, dispersion of chromatin and prominent nucleoli.
- Multinucleated tumor giant cells and abnormal mitoses may be demonstrated (Fig. 30.17).

Immunohistochemistry Technique

Panel of immunohistochemical markers is applied on paraffin-embedded hematoxylin and eosin stained sections in anaplastic astrocytoma, IDH mutation.

Markers	Expression
GFAP	Positive
IDH1 R132H mutation	Positive
p53	Positive
Ki-67 proliferation index of 5–10%	Positive

Molecular Genetic Alterations

Molecular genetic markers for anaplastic astrocytoma, IDH mutant are IDH1, IDH2, TP53 and ATRX. There are higher frequencies of chromosome 9p and 19p losses.

Clinical Course

Patient with anaplastic astrocytoma, IDH mutant has median survival in the range of 3–5 years. The extent of surgical resection of tumor at diagnosis also has impact on outcome. EGFR amplification and genotype of 7q gain and 10q loss have been associated with fatal outcome.

PRIMARY GLIOBLASTOMA MULTIFORME, IDH-WILDTYPE

Primary glioblastoma multiforme, IDH-wildtype is a high grade glioma corresponding to WHO grade IV with predominantly astrocytic differentiation with features of

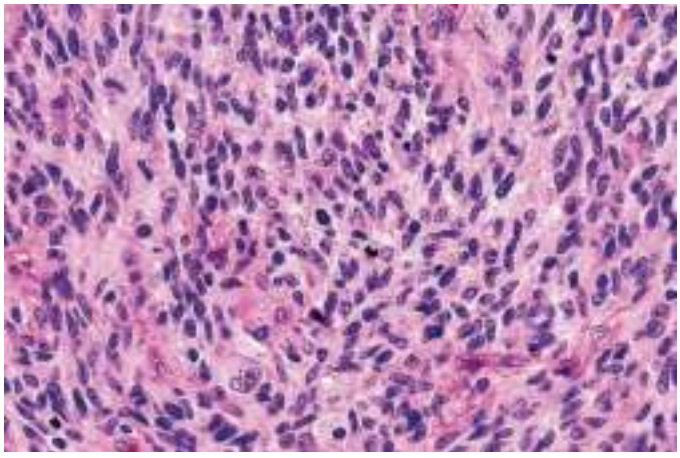


Fig. 30.17: IDH-multant. Histologic features of anaplastic astrocytoma, IHD-mutant is highly cellular and composed of pleomorphic cells with nuclear atypia, dispersion of chromatin, and prominant nucleoli. Ki-67 labeling in the range of 5–10% may be helpful in analyzing significant proliferative activity of tumor. Multinucleated tumor giant cells and atypical mitoses may be demonstrated (400X).

nuclear atypia, cellular pleomorphism, mitotic activity, diffuse growth pattern, microvascular proliferation and/or necrosis, which lacks mutations in IDH gene. It arises in *de novo* and usually affects adults of 40–60 years.

- Tumor has infiltrative growth that can affect bilateral cerebral hemispheres (temporal lobe, parietal lobe, occipital lobe). Tumor infiltration occurs along white mater tracts, but can also involve cortical and deep gray mater. Tumor extends into the adjacent cortex and through the corpus callosum into the contralateral cerebral hemisphere.
- Similarly, tumor spreads rapidly along white matter tracts of the internal capsule, fornix, and anterior commissure.
- Despite its rapid and infiltrative growth, primary glioblastoma multiforme, IDH-wildtype does not extend into the subarachnoid space or spread through cerebrospinal fluid. Although tumor spreads via cerebrospinal fluid in children. Extracranial metastasis of primary glioblastoma multiforme, IDH wildtype is uncommon.
- Tumor in basal ganglion and thalamus is common in children. It rarely occurs in brainstem, cerebellum and spinal cord.
- Tumor is invariably lethal within 8–12 months of diagnosis. It accounts for 15% of all intracranial neoplasms and 45–50% of all primary malignant brain tumors.
- Genetic susceptibility in primary glioblastoma multiforme, IDH-wildtype are given in Tables 30.26 and 30.27.

Clinical Features

Primary glioblastoma multiforme, IDH-wildtype develops rapidly. The symptoms depend on location of tumor. Patient presents with focal neurological deficits (aphasia, hemiparesis), seizures, nausea, vomiting, severe pulsating headache, behavioral changes and neurocognitive changes.

Radiologic Findings

Primary glioblastoma multiforme, IDH-wildtype tumors are irregularly shaped. These tumors demonstrate ring-shaped zone of contrast enhancement around a dark, central area of necrosis. These tumors may extend widely into the adjacent lobes of cerebral hemisphere.

Table 30.26 Genetic susceptibility in primary glioblastoma multiforme, IDH-wildtype

Syndrome	Gene	Chromosome
Li-Fraumeni syndrome	TP53	17p13.1
Turcot syndrome	MLH1, PMS2, MSH2, MSH6	3p21.3, 7p22, 2p22–p21, 2p16
Neurofibromatosis type 1	NF1	7p12
Ollier/Maffucci syndrome	PTHR1	3p21–22
L-2-hydroxyglutamic aciduria	L2HGDH	14q21.3

Table 30.27 Genetic alterations in primary glioblastoma multiforme, IDH-wildtype

Genetic Alterations	Expression (%)
IDH1 promoter	≤80%
CDKN2A/CDKN2B	≤60%
Loss of chromosome 10p	≤50%
Loss of chromosome 10q	≤70%
EGFR alterations (rearrangement, splicing or amplification)	≤55%
PTEN mutations/deletions	≤40%
TP53 mutations	≤30%
P13K mutations	≤25%
TERT	70–90%
NF1 mutation	15–18%
RB1 mutation	8–12%
MDM4 amplification	5–15%
MET amplification	4%
MGMT methylation	Gives information about response of tumor to alkylating chemotherapeutic agents

Surgical Pathology: Primary Glioblastoma Multiforme, IDH-wildtype

Gross Morphology

- Most glioblastomas, IDH-wildtype of cerebral hemisphere are large tumors either unilateral or bilateral within brain parenchyma. These are rarely found to involve leptomeninges.
- Tumor is poorly circumscribed. As the tumor invades and causes obliteration of blood vessels, the tumor features patchy (multifocal areas) yellow areas of necrosis and red zones of hemorrhage.
- Macroscopic cysts in the tumor, when present contain turbid fluid with liquefied necrotic material (Fig. 30.18).

Light Microscopy

- Glioblastoma multiforme, IDH-wildtype is a highly cellular tumor and composed of poorly differentiated, pleomorphic, round, or fusiform astroglial cells with nuclear atypia, brisk mitotic activity, multinucleated giant cells and marked proliferation of endothelial cells.
- Tumor cells are arranged in palisading pattern around prominent vessels. Areas of hemorrhage and necrosis are common.
- Pseudopalisading necrosis is prominent feature of glioblastoma multiforme, IDH-wildtype.
- The term multiforme derives from the variegated gross appearance of these tumors and the histologic pleomorphism of the tumor cells.

- Diagnosis of glioblastoma multiforme, IDH-wildtype is mainly based on the identification of the tissue pattern rather than of specific cell types (Figs 30.19 and 30.20).

Immunohistochemistry Technique

Panel of markers is applied to paraffin-embedded sections of primary glioblastoma multiforme, IDH-wildtype such as GFAP, α_1-antitrypsin, α_1-antichymotrypsin, actin, EMA, S-100 protein, OLIG2, nestin, TP53 mutation and cytokeratins with AE1/AE3.

Markers	Expression
GFAP	Positive
α_1-Antitrypsin	Positive
α_1-Antichymotrypsin	Positive
Actin	Positive
EMA	Positive
S-100 protein	Positive
OLIG2	Positive
Nestin	Positive
TP53 mutation	Positive
Cytokeratin with AE1/AE3	Positive

Treatment

Surgical resection of tumor is impossible, but radiation and chemotherapy may add months to patient's survival.

SECONDARY GLIOBLASTOMA MULTIFORME, IDH-MUTANT

Secondary glioblastoma, IDH-mutant develops through malignant progression from diffuse astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III) is almost associated with IDH mutation. It is a high-grade glioma corresponding to WHO grade IV with astrocytic differentiation; featuring cellular pleomorphism, mitotic activity, diffuse growth pattern and/or necrosis; with mutation in either IDH1 or IDH2 gene.



Fig. 30.18: Primary glioblastoma multiforme (GBM), IDH-wildtype. This is the worst possible form of glioma, a GBM. These neoplasms are quite vascular with prominent areas of necrosis and hemorrhage. Note how this one has crossed the midline to the opposite hemisphere (arrow).

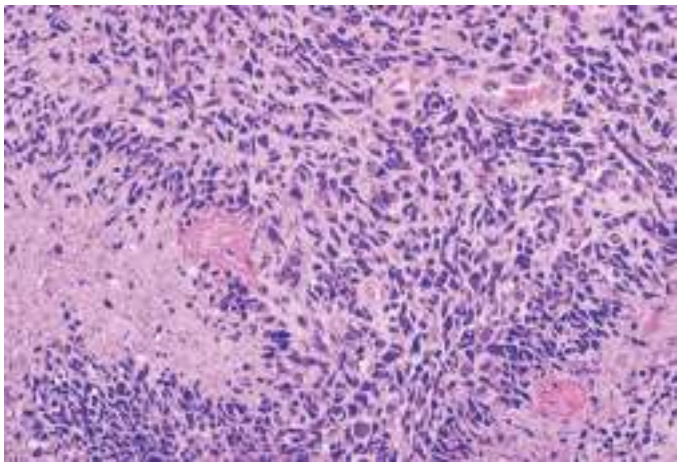


Fig. 30.19: Primary glioblastoma multiforme (GBM), IDH-wildtype. Tumor demonstrates marked cellularity with marked hyperchromatism and pleomorphism.Note the prominent vascularity as well as the area of necrosis at the left with neoplastic cells palisading around it (400X).

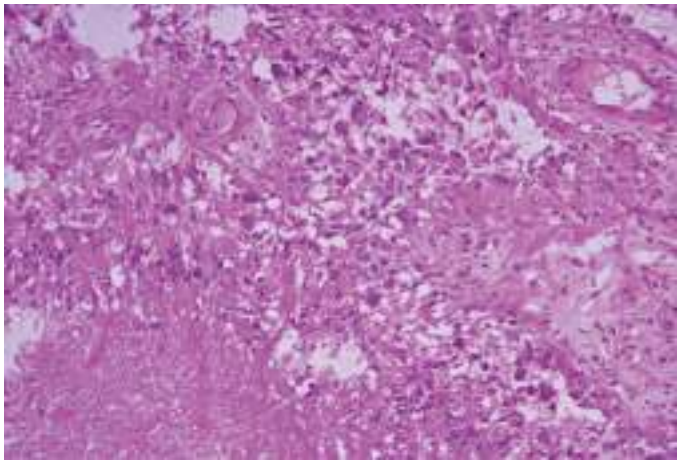


Fig. 30.20: Primary glioblastoma multiforme (GBM), IDH-wildtype. Tumor demonstrates marked cellularity with marked hyperchromatism and pleomorphism. Note the prominent vascularity as well as the area of necrosis with neoplastic cells palisading around it (400X).

- Tumor is morphologically indistinguishable from primary glioblastoma multiforme, IDH-wildtype except for a lesser extent of necrosis.
- Tumor affects young adults. It occurs in the frontal lobe of cerebral hemisphere and associated with better prognosis than primary glioblastoma multiforme, IDH-wildtype.

Molecular Genetic Alterations

Secondary glioblastoma multiforme, IDH-mutant arises from common precursor cells with IDH1/IDH2 mutation, in contrast to primary glioblastoma, IDH-wildtype that arises from glial progenitor cells. Development of secondary glioblastoma multiforme, IDH-mutant develops from common precursor cells with IDH1/IDH2 mutation through malignant progression from diffuse astrocytoma with TP53 and ATRX mutations

Table 30.28 Comparison of primary glioblastoma multiforme, IDH-wildtype, and secondary glioblastoma multiforme, IDH-mutant

Synonym	Primary Glioblastoma Multiforme, IDH-wildtype	Secondary Glioblastoma Multiforme, IDH-mutant
General features		
▪ Origin	▪ Glial progenitor cells	▪ Common precursor cells
▪ Precursor lesion	▪ Originates in *de novo* without identifiable lesion	▪ Diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III)
▪ Frequency	▪ 90%	▪ 10%
▪ Median age at diagnosis	▪ 62 years	▪ 44 years
▪ Sex predilection	▪ Males > females	▪ Males < females
▪ Location of tumor	▪ Supratentorial region	▪ Frontal lobe of cerebral hemisphere
▪ Clinical history mean duration	▪ 4 months	▪ 15 months
Gene mutations		
▪ TERT promoter mutations	▪ 72%	▪ 26%
▪ TP53 mutation	▪ 27%	▪ 81%
▪ ATRX mutation	▪ Exceptional	▪ 71%
▪ EGFR amplification	▪ 35%	▪ Exceptional
▪ PTEN mutation	▪ 24%	▪ Exceptional
Gross morphology		
Necrosis	Extensive necrosis	Limited necrosis
Median survival		
▪ Surgery and radiotherapy	▪ 10 months	▪ 24 months
▪ Surgery, radiotherapy and chemotherapy	▪ 15 months	▪ 30 months

(WHO grade II) or anaplastic astrocytoma with loss of heterozygosity LOH 19p and 10q (WHO grade III). Comparison of primary glioblastoma multiforme, IDH-wildtype and secondary glioblastoma multiforme, IDH-mutant is given in Table 30.28.

Clinical Features

Patient with secondary glioblastoma multiforme, IDH-mutant located in frontal lobe, presents with behavioral and neurocognitive changes, focal neurological deficits such as hemiparesis and aphasia. Due to its slow evolution from diffuse astrocytoma and anaplastic astrocytoma, tumor is associated with cerebral edema due to increase in intracranial pressure.

Radiologic Findings

Unlike in primary glioblastoma multiforme, IDH-wildtype, large areas of central necrosis are absent in secondary glioblastoma multiforme and IDH-mutant.

Surgical Pathology: Secondary Glioblastoma Multiforme, IDH-mutant

Gross Morphology

- Secondary glioblastoma multiforme, IDH-mutant diffusely infiltrates in the brain parenchyma.
- Tumor is not delineated.
- Cut surface shows large yellowish areas of necrosis or hemorrhage.

Light Microscopy

- Histologic features of secondary glioblastoma multiforme, IDH-mutant are identical to primary glioblastoma multiforme, IDH-wildtype. However, morphologic studies revealed two significant differences.
- Secondary glioblastoma multiforme, IDH-mutant demonstrates areas of ischemic and/or palisading necrosis in 50% of cases; while 90% in primary glioblastoma multiforme, IDH-wildtype.
- Focal oligodendroglial components are more common in secondary glioblastoma multiforme, IDH-mutant than primary glioblastoma multiforme, IDH-wildtype.

Immunohistochemistry Technique

Panel of immunohistochemical markers is applied to paraffin-embedded sections of secondary glioblastoma multiforme, IDH-mutant such as TERT promoter, p53, ATRX and GFAP.

Markers	Expression
▪ IDH1/IDH2	▪ Positive (100%)
▪ TERT promoter	▪ Positive (26%)
▪ p53	▪ Positive (81%)
▪ ATRX	▪ Positive (71%)
▪ GFAP	▪ Positive (variable)

Treatment and Prognosis

Secondary glioblastoma multiforme, IDH-mutant is treated by surgery, radiotherapy and chemotherapy. Overall survival is 24–36 months.

PILOCYTIC ASTROCYTOMA

Pilocytic astrocytoma is the most common pediatric WHO low-grade I brain tumor in children and young adults. Tumor tends to occur in cerebellum and optic chiasma, and floor and wall of third ventricle in the hypothalamic region. However, it can occasionally occur in cerebral hemispheres.

- Child with neurofibromatosis 1 can predispose to pilocytic astrocytoma primarily within the optic pathway.
- Alterations in the RAS/RAF/mitogen-activating protein kinase (MAPK) pathway are exclusively found in almost all cases, indicating that pilocytic astrocytoma represents a one-pathway disease. Dissemination of tumor is uncommon.

Clinical Features

Patient with pilocytic tumor in cerebellum presents with ataxia, cranial nerve defects, and signs of increased intracranial pressure, i.e. headache, nausea and vomiting. When tumor is present in the optic pathway, the tumor may produce loss of visual acuity or field defects. Tumor localized in the hypothalamus, may result in endocrine syndromes, such as diabetes insipidus, precocious puberty, or electrolyte imbalance. Blocking of CSF pathways may result in hydrocephalus associated with rapid deterioration.

Surgical Pathology: Pilocytic Astrocytoma

Gross Morphology

Tumor is discrete. Cut surface shows macrocystic or microcystic areas.

Light Microscopy

- Pilocytic astrocytoma tumor is composed of bipolar neoplastic cells with long hair-like cells cytoplasmic processes, that are arranged in parallel bundles.
- Tumor has biphasic pattern with varying proportions of piloid areas alternating with spongy areas.
- Piloid areas are composed of compacted strongly GFAP+ bipolar cells (with hair-like bipolar processes) associated with Rosenthal fibers (tapered corkscrew shaped, brightly eosinophilic, hyaline masses).
- Spongy areas are composed of loosely textured and formed of weakly GFAP+ multipolar cells (protoplasmic astrocytes) associated with microcysts and eosinophilic granular bodies (brightly eosinophilic PAS+ globular aggregates).
- Vascular changes are commonly demonstrated, which include glomeruloid vascular proliferation and vascular hyalinization.
- Other findings in tumor demonstrated include hyalinized blood vessels, infarct-like necrosis, degenerative nuclear atypia, calcification, hemosiderin deposits and lymphocytic infiltrate.
- Leptomeningeal extension is not a sign of malignancy. Some tumors in cerebellum show a diffuse growth pattern.

Immunohistochemistry Technique

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein expressed in variable proportions in tumor.

Treatment and Prognosis

When required, treatment of pilocytic astrocytoma consists of surgery, chemotherapy, and/or radiation, although new molecular agents targeting the RAS/RAF and related MAPK signaling pathways are promising new approaches. The 10-year survival rates are >90% in pediatric patients; however, they are poorer in adults.

- Tumors that are amenable to complete resection (i.e. cerebellum and cortex) have the best overall survival.
- The prognosis of people with pilocytic astrocytomas is influenced primarily by their location.
- The prognosis is usually better for patients with surgically respectable tumors located in the cerebellar cortex, than for patients with less accessible tumors, such as those involving the hypothalamus or brainstem.

EPITHELIOID GLIOBLASTOMA

Epithelioid glioblastoma has predilection for children and young adults. *De novo* tumor usually expresses SMARCB1. Tumor frequently has BRAF V600E mutations. Most common sites of tumor are temporal and frontal lobes of cerebral cortex and diencephalon. Tumor spreads by leptomeningeal dissemination. It is an aggressive tumor associated with fatal outcome with six months.

Radiologic Findings

Gadolinium enhanced magnetic resonance imaging demonstrates epithelioid glioblastoma as solid mass in the cerebral cortex (frontal or temporal region) or diencephalon with leptomeningeal seeding.

Surgical Pathology: Epithelioid Glioblastoma

Gross Morphology

Tumor is unifocal with prominent areas of hemorrhage and necrosis.

Light Microscopy

- Tumor is composed of monomorphic population of large epithelioid cells containing abundant eosinophilic cytoplasm, vesicular nucleus with prominent nucleoli.
- Tumor cells are reminiscent of melanoma cells.
- Rhabdoid cells are also sometimes demonstrated.

Immunohistochemistry Technique

Panel of immunomarkers is applied on paraffin-embedded sections of epithelioid glioblastoma such as vimentin, S-100 protein, GFAP, cytokeratin AE1/AE3, EMA. Tumor is negative for melan-A, desmin, myoglobin and smooth muscle actin.

Markers	Expression
▪ Vimentin	▪ Positive
▪ S-100 protein	▪ Positive
▪ GFAP	▪ Positive
▪ Cytokeratin AE1/AE3	▪ Positive
▪ EMA	▪ Positive
▪ Melan-A	▪ Negative
▪ Desmin	▪ Negative
▪ Myoglobin	▪ Negative
▪ Smooth muscle actin (SMA)	▪ Negative

GLIOBLASTOMA MULTIFORME WITH A PRIMITIVE NEUROECTODERMAL TUMOR COMPONENT

Glioblastoma multiforme with a primitive neuroectodermal tumor component (GBM-PNET) affects elderly persons. It has high rate of cerebrospinal fluid dissemination.

Surgical Pathology: Glioblastoma Multiforme with a Primitive Neuroectodermal Tumor Component

Light Microscopy

- Tumor is composed of two histologically distinct different components: astrocytic cells and fibrillary network, supports the glial nature of the tumor.
- Another component of the tumor demonstrates cellular areas of small tumor cells distributed in multifocal pattern.
- Tumor cells contain narrow cytoplasm and hyperchromatic nuclei, which can occasionally form pseudorosettes and numerous mitoses.

Immunohistochemistry Technique

GFAP is demonstrated in astrocytic cells and fibrillary network in glioblastoma with a primitive neuroectodermal tumor component.

Marker	Expression
GFAP	Positive

OLIGODENDROGLIAL TUMORS

Oligodendroglial tumors are neuroepithelial tumors arising from neuroglial cells, which include oligodendroglioma, IDH-mutant (WHO grade II) and anaplastic oligodendroglioma, IDH-mutant and 1p/19q codeleted (WHO grade III).

OLIGODENDROGLIOMA

Oligodendrogliomas account for <20% of all intracranial tumors and 25% of gliomas. Oligodendroglioma is slow growing and well circumscribed low-grade tumor (WHO grade I) affecting middle age group (30–50 years).

- Tumor arises from oligodendrocytes in the white matter of frontal lobe (most common).
- Molecular genetic analysis of oligodendroglioma demonstrates mutation in IDH1/IDH2, mutation in FUBP1 due to 1p/19q codeletion, and promoter hypermethylation of the MGMT gene.

Clinical Features

Patient presents with seizures, increased intracranial pressure, focal neurological deficits and cognitive changes.

Radiographic Findings

Radiograph most often shows intra-axial mass, occasionally well-circumscribed mass, and small foci of calcification (best seen on CT scan).

Surgical Pathology: Oligodendroglioma

Gross Morphology

- Oligodendroglioma is soft well-defined grayish-pink tumor.
- Cut surface is gritty due to calcification. Tumor has gelatinous appearance.

Light Microscopy

- Oligodendroglioma is diffusely growing highly cellular tumor and composed of cells with round uniform nuclei, distinct borders, eosinophilic to clear cytoplasm, moderate to marked nuclear atypia resembling 'fried egg' appearance.
- Acutely branched delicate capillary sized blood vessels with 'chicken wire' like appearance are present between group of neoplastic cells.
- Calcification (calcispherite) is an important feature throughout the tumor.
- Anaplastic oligodendroglioma (WHO grade III) shows brisk mitotic activity (>6 mitoses/10 high power fields), microvascular proliferation and necrosis (Fig. 30.21).

Immunohistochemistry Technique

Oligodendroglioma is positive for immunomarker GFAP.

Marker	Expression
GFAP	Positive

Molecular Genetic Alterations

A translocation between chromosomes 1p and 19q is the characteristic molecular finding in oligodendroglioma.

Prognosis

Prognosis of patient with oligodendroglioma depends on the histologic grade of the tumor, its location, and recognition of molecular genetic features that can be linked to chemosensitivity. The oligodendroglial tumors are prone to spontaneous hemorrhage owing to their delicate vasculature.

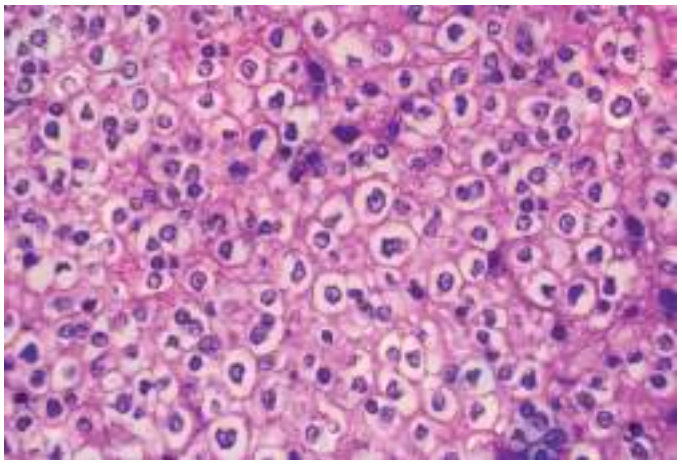


Fig. 30.21: Oligodendroglioma. The tumor cells show acute swelling of the perinuclear zones giving them a characteristic poached egg-like appearance (400X).

EPENDYMAL TUMORS

Ependymal tumors are neuroepithelial tumors arising from neuroglial cells, which include subependymoma (WHO grade I), myxopapillary ependymoma (WHO grade II), ependymoma, RELA fusion positive (WHO grade II or III) and anaplastic ependymoma (WHO grade III).

EPENDYMOMA

Ependymoma is a slow-growing malignant tumor derived from ependymal cells, which line the choroid plexus and ventricles and spinal cord. Ependymal cells participate in synthesis of cerebrospinal fluid.

- In children, tumor most often occurs in the 4th ventricle, where papillary growth produces obstruction and results in hydrocephalus.
- Whereas in middle-aged adults, tumor is most often located in the spinal cord and cauda equina. Although tumor can occur at any age, yet it most often occurs in the first two decades of life.

Clinical Features

The clinical features depend on the location of ependymal tumor. Intracranial tumor is often associated with hydrocephalus and evidence of increased intracranial pressure (ICP).

Surgical Pathology: Ependymoma

Gross Morphology

- Ependymoma is well-circumscribed, soft tan tumor with discrete margin from surrounding brain or spinal cord.
- Tumor grows exophytically into fourth ventricle and exit.
- Tumor can fill fourth ventricle and exit out through Luschka foramen or Magendie foramen.
- Gross morphology of ependymoma in right lateral ventricle is shown in Fig. 30.22.

Light Microscopy

- **Well-differentiated ependymoma:** Most ependymomas are well differentiated giving epithelial appearance similar to that of normal ependymal cells and glial differentiation. Tumor is composed of round to oval cells forming tubules with 'carrot-shaped nuclei' with abundant granular cytoplasm. A characteristic finding is the orientation of ependymal cells around vascular channels forming pseudorosettes.
- **Myxopapillary ependymoma:** A special variant, myxopapillary ependymoma may also originate from the lining of the central canal of the spinal cord and the filum terminale in lumbosacral portion region. Extensive invasion of adjacent CNS structures is uncommon. Light microscopy shows myxoid background (Fig. 30.23).
- **Anaplastic ependymoma:** Tumor is highly cellular and composed of numerous mitoses, blood vessels proliferation and necrosis with infiltrative vascularized cores lined by elongated cells forming papillary structures.

Immunohistochemistry Technique

Panel of markers is used to analyze expression in ependymoma on histopathologic sections.

Markers	Expression
GFAP	Positive
S-100 protein	Positive
Vimentin	Positive

Epithelial membrane antigen (EMA) is expressed on luminal surface of rosettes.

EPENDYMOMA WITH RELA FUSION POSITIVE

Ependymoma with RELA fusion positive tumor of central nervous system most often occurs in supratentorial region (mostly in lateral or third ventricle)

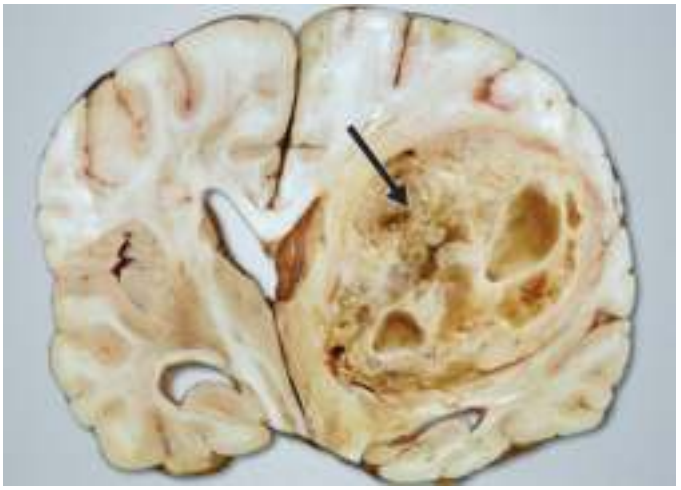


Fig. 30.22: Ependymoma in the right lateral ventricle. The tumor is well circumscribed and apparently confined to the ventricle. It has compressed the CSF pathways, causing dilatation of the third ventricle and the left lateral ventricle. The macroscopic appearance of this tumor suggests an ependymoma, but microscopic confirmation would be required (arrow).

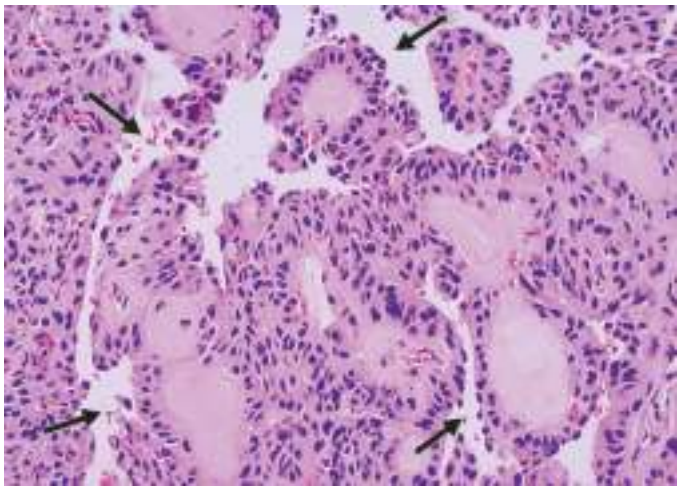


Fig. 30.23: Myxopapillary ependymoma. It is typically found arising in the filum terminale of the spinal cord. Note the cells around papillae structures that have a myxoid connective tissue core. Surgical removal is made easier if this tumor has not grown around nerve roots of the cauda equina (arrows) (400X).

in 70% of children and 30% of adults. It has not been reported in the infratentorial region. Detection of RELA fusion gene by fluorescence *in situ* hybridization (FISH) technique is diagnosis of ependymoma. It is worth mentioning that ependymoma in posterior fossa and spinal compartment do not harbor RELA fusion gene. Patient presents with headache, seizures or location-dependent focal neurological deficits. **Tumor spreads via cerebrospinal fluid** and is associated with poor prognosis.

Radiologic Features

Gadolinium enhanced magnetic resonance imaging demonstrates well circumscribed supratentorial mass (most in lateral or third ventricle but may not have obvious connection to a ventricle) with various degrees of contrast enhancement. Intratumoral hemorrhage, necrosis, calcification or cystic components may be present.

Surgical Pathology: Ependymoma with RELA Fusion Positive

Gross Morphology

- Tumor is well circumscribed arising in or near ventricular system.
- Cut surface is tan colored and spongy.
- Occasionally, gritty calcium deposit is demonstrated.

Light Microscopy

- Tumor is composed of well delineated glioma with monomorphic cells containing round to oval nuclei with speckled nuclear chromatin.
- Main features of the tumor are perivascular pseudorosettes and ependymal true rosettes, or anaplastic cells with high nucleocytoplasmic ratio with brisk mitotic activity, delicate network capillary blood vessels and focal clear cells.
- Uncommon variants of ependymoma such as tanycytic ependymoma are less likely to harbor RELA fusion.
- It should be differentiated from astrocytoma and medulloblastoma.

Immunohistochemistry Technique

- Panel of immunomarkers is applied on paraffin-embedded sections of ependymoma with RELA fusion positive such as GFAP, EMA, S-100 protein, vimentin and L1CAM with C11 or 95 rearrangement.
- Presence of RELA fusion gene can be analyzed by fluorescence *in situ* hybridization (FISH).

Markers	Expression
GFAP	Positive
EMA	Positive
S-100 protein	Positive
Vimentin	Positive
L1CAM with C11 or 95 rearrangement	Positive

CHOROID PLEXUS TUMORS

Choroid plexus tumors are neuroepithelial tumors arising from neuroglial cells, which include choroid plexus papilloma (WHO grade I), atypical choroid plexus papilloma (WHO grade II) and choroid plexus carcinoma (WHO grade III).

CHOROID PLEXUS PAPILLOMA

Choroid plexus papilloma is an uncommon tumor, which originates from neuroectodermal cells adjacent to embryonic lateral ventricles and fourth ventricle. Tumor leads to increased intracranial pressure due to obstruction to CSF flow resulting in obstructive hydrocephalus. There is little tendency of local infiltration. It spreads via CSF. It commonly affects children.

Surgical Pathology: Choroid Plexus Papilloma

Gross Morphology

- Choroid plexus papilloma is well circumscribed tumor.
- Cut surface shows cystic areas, calcification and hemorrhage.

Light Microscopy

- Tumor is composed of benign cuboidal to columnar cells arranged in tubules and acini.
- Tumor cells exhibiting pleomorphism, numerous mitoses, invasion, and lack of papillary pattern indicates malignant behavior.

EMBRYONAL TUMORS

Embryonal tumors are neuroepithelial tumors arising from neuroglial cells, which include medulloblastoma (all subtypes, WHO grade IV), embryonal tumor with multilayered rosettes, C19MC-amplification (WHO grade IV), medulloepithelioma (WHO grade IV), CNS embryonal tumor, NOS (WHO grade IV), atypical teratoid/rhabdoid tumor (WHO grade IV) and CNS embryonal tumor with rhabdoid features (WHO grade IV).

MEDULLOBLASTOMA

Medulloblastoma, WHO grade IV is second most common and highly malignant brain tumor of cerebellum. It originates from primitive neuroectodermal cells (fetal external granular layer) within cerebellar vermis (midline) in children; and within cerebral hemispheres in adults.

- Tumor accounts for 10% of primary central nervous system tumors. However, it accounts for 60% primary central nervous system tumors in children <15 years of age. It can occur in adults between 15 and 35 years.
- Molecular genetic alterations involving chromosome 17 are common in this tumor.
- Tumor originating within cerebellar vermis most often spreads down the neuroaxis and occludes the fourth ventricle resulting in hydrocephalous. Tumor may metastasize to bone and lymph nodes. Tumor suppressor genes associated with medulloblastoma are given in Table 30.29.

Surgical Pathology: Medulloblastoma

Gross Morphology

- Medulloblastoma is soft, fleshy, gray-tan tumor with variable hemorrhage and sometimes necrosis.
- Extensive stromal reticulin deposition may impart a firm consistency to desmoplastic variant of medulloblastoma.
- Medulloblastoma of spinal cord is shown in Fig. 30.24.

Light Microscopy

- Tumor is composed of poorly differentiated small cells with round-to-oval hyperchromatic nuclei and scant cytoplasm.
- Mitotic figures are often present in abundance. These cells form 'Homer-Wright' rosettes, histologic feature of neuroblasts.
- Histologic variants include classic and desmoplastic/nodular medulloblastomas (Figs 30.25 and 30.26).

Immunohistochemistry Technique

Panel of markers is used to differentiate from metastatic epithelial tumors, lymphomas, or other neuroectodermal malignancies.

Markers	Expression
Synaptophysin	Positive
Neuron-specific enolase (NSE)	Positive

Molecular Genetic Alterations

In 2021 WHO classification update, medulloblastoma is classified according to molecular characteristics and histologic features. Molecular classification is based on transcriptome, micro-RNA (miRNA), and methylome profiling for clinical treatment and histologic classification has also been retained due to its clinical utility when molecular genetic analysis is not feasible.

- **Wnt and sonic Hedgehog activation:** Wnt-activated and some Hedgehog active groups demonstrate activation of cell signaling pathways resulting in tumorigenesis in medulloblastoma.
- **C19MC alterations:** Amplification of the C19MC region on chromosome 9 is observed in embryonal tumor with multilayered rosettes (ETMR). The presence of focal amplification at chromosome region 19q13.42 is associated with an upregulation of the oncogenic miRNA cluster.

Metastasis

Since medulloblastoma occurs close to the fourth ventricle, it commonly disseminates into the subarachnoid space and via CSF seeds into the spinal cord.

Treatment

The location of the medulloblastoma tumor often makes it difficult to excise completely. Although highly radiosensitive, yet patients have poor prognosis.

EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES

Embryonal tumor with multilayered rosettes (ETMR) is new entity defined by recent 2021 WHO classification of tumors of the central nervous system.

- Tumor is WHO grade IV in infants also known as embryonal tumor with abundant neuropil and true rosettes. Tumor demonstrates combined histologic features of neuroblastoma and ependymoblastoma, which had predilection for children in first five years of life in the cerebrum, cerebellum and brainstem.
- Presence amplification of the C19MC on chromosome 19 in both central nervous system PNET and embryonal tumor with abundant neuropil and true rosettes suggesting that these are the one entity exhibiting variable growth patterns.
- Tumor is locally invasive and disseminates via leptomeningeal extraneural routes. Tumor is aggressive and associated with poor prognosis.

Clinical Features

Patient presents with symptoms due to increased intracranial pressure such as seizures, hemiparesis, cerebellar signs, nerve palsies and other neurological deficits.

Table 30.29 Tumor suppressor genes associated with medulloblastoma

Tumor Suppressor Gene	Protein	Function	Familial Tumors	Sporadic Cancers
Mitogenic signaling pathway inhibitors				
PTCH	Patch	Inhibits Hedgehog signaling pathway	Gorlin's syndrome (basal cell carcinoma, medulloblastoma, other benign tumors)	▪ Medulloblastoma ▪ Basal cell carcinoma



Fig. 30.24: Medulloblastoma. Transverse section of spinal cord shows tumor in the subarachnoid space around the cord. This mode of spread is characteristically seen in medulloblastoma (arrow).

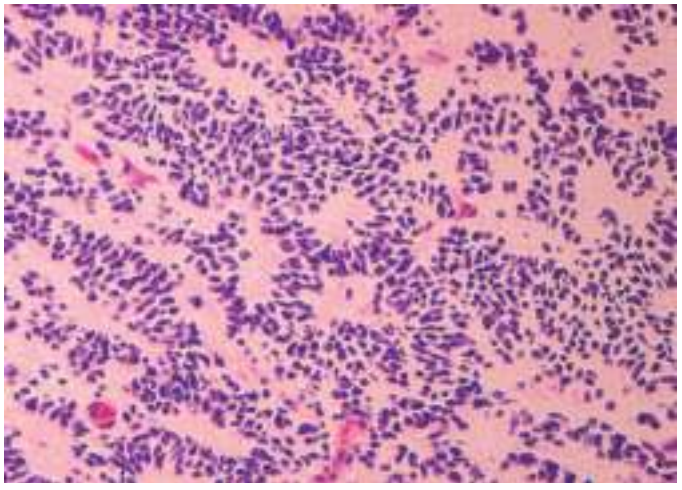


Fig. 30.25: Medulloblastoma. Tumor is composed of poorly differentiated small cells with round-to-oval hyperchromatic nuclei and scant cytoplasm. Mitotic figures are often present in abundance. These cells form 'Homer-Wright' rosettes, histologic feature of neuroblasts. Histologic variants include classic and desmoplastic/nodular medulloblastomas (400X).

Radiologic Findings

Magnetic resonance imaging (MRI) demonstrates a large, well demarcated and solid tumor with surrounding edema. There is no contrast enhancement. Macrocalcifications and cystic components may be demonstrated in some cases. Magnetic resonance spectroscopy demonstrates choline peak and high ratio of choline and aspartate suggesting hypercellularity of the tumor.

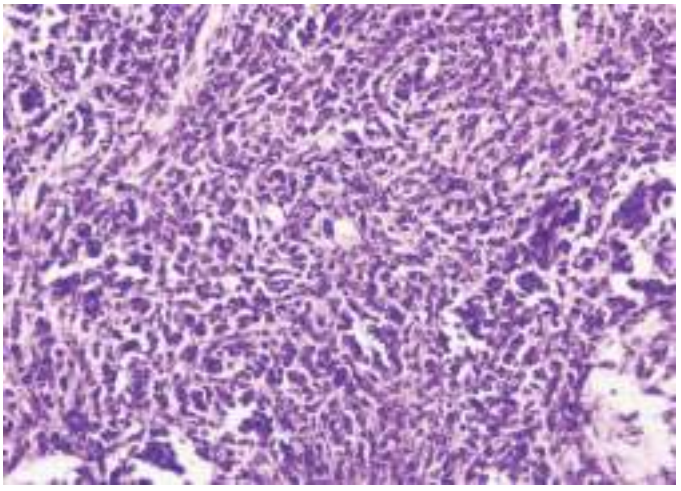


Fig. 30.26: Medulloblastoma. Tumor is composed of poorly differentiated small cells with round-to-oval hyperchromatic nuclei and scant cytoplasm. Mitotic figures are often present in abundance. These cells form 'Homer-Wright' rosettes, histologic feature of neuroblasts. Histologic variants include classic and desmoplastic/nodular medulloblastomas (400X).

Surgical Pathology: Embryonal Tumor with Multilayered Rosettes

Gross Morphology

- Large tumor involving multiple lobes can protrude to cerebellopontine cistern.
- Tumor is well circumscribed, grayish to pink with areas of hemorrhage, necrosis and calcification.

Light Microscopy

- Tumor is composed of highly cellular population of primitive cells alternating with hypocellular neuropil-rich regions containing multilayered pseudostratified neuroepithelium, true rosettes and cells with glial and neuronal differentiation.
- The lumen is either empty or filled with eosinophilic debris. Nucleus of cells is pushed away from the lumen.

Molecular Genetic Alterations Analysis

Amplification of 19q13.42 chromosomal region on fluorescence *in situ* hybridization analysis confirms the diagnosis.

Immunohistochemistry Technique

Panel of immunomarkers is applied on paraffin-embedded sections such as vimentin, EMA, cytokeratin, CD99, neuropil, synaptophysin and NFPs.

Markers	Expression
▪ Vimentin	▪ Positive
▪ EMA	▪ Positive
▪ Cytokeratin	▪ Positive
▪ CD99	▪ Positive
▪ Neuropil	▪ Positive
▪ Synaptophysin	▪ Positive
▪ NFPs	▪ Positive
▪ Glial marker	▪ Negative
▪ Neuronal marker	▪ Negative

MENINGEAL TUMORS

Meningeal tumors include meningoepithelial meningioma (WHO grade I), fibroblastic meningioma (WHO grade I), transitional meningioma (WHO grade I), psammomatous meningioma (WHO grade I), metaplastic meningioma (WHO grade II), chondroid meningioma (WHO grade II), clear cell meningioma (WHO grade II), atypical meningioma (WHO grade II), papillary meningioma (WHO grade III), rhabdoid meningioma (WHO grade III) and anaplastic meningioma (WHO grade III).

MENINGIOMA

Meningioma is the second most common primary intracranial benign neoplasm of adults, which arises from the meningoepithelial cells of the arachnoid mater. Tumor accounts for 20% of all primary intracranial neoplasms. Tumor may compress the brain or permeate the overlying bone. It most often occurs in women than men between 20 and 60 years of age with a peak incidence around 45. Spinal meningiomas are more common in females than males. Tumor is uncommon in children.

Locations

Common locations of meningiomas include convexities of the brain near venous sinuses, parasagittal region along the falx cerebri (most common site), along skull base, or tentorium, olfactory nerve sheath in the groove beneath the frontal lobe, lesser wing of sphenoid wing, sella turcica, and foramen magnum and within the spinal cord in thoracic segment. Occasionally, these tumors may arise in the ventricular system.

Clinical Features

Meningioma is slow-growing, well-circumscribed, and often highly vascular benign tumors. Complete removal is possible if the tumor does not involve vital structures especially present external to the brain.

Radiologic Findings

Meningiomas often infiltrate overlying bone and cause increased bone density (hyperostosis) seen on X-ray.

Surgical Pathology: Meningioma

Gross Morphology

- Meningiomas are popcorn shaped, firm, well circumscribed lobulated masses adherent to dura and invade dura or dural sinuses.
- Tumor may indent the surface of the brain without invading, which is common cause of new-onset focal seizures (Fig. 30.27).

Light Microscopy

Meningiomas are classified based on grade according to WHO classification. Grade I tumor has benign course. Grades II and III grow rapidly with great propensity to recur following resection. Fluorescence *in situ* hybridization (FISH) technique can detect genetic alterations and histologic grading of tumor. Histologic variants, grades, mitotic figures and survival rate in meningiomas are given in Table 30.30.

- **WHO grade I meningiomas:** Examples are meningoepithelial, fibroblastic, transitional and psammomatous meningiomas. Meningiomas exhibit many different microscopic patterns.
  - Tumor consists of swirling masses of fusiform meningothelial cells with oval nuclei containing dispersed chromatin in a composite solid and whorled pattern.
  - Small foci of calcification (psammoma bodies–calcified bodies) are common. These express estrogen receptors.
  - Meningoepithelial or transitional meningiomas should be differentiated from metastatic carcinoma and fibroblastic meningioma from schwannoma (Figs 30.28 to 30.31).
- **WHO grade II meningiomas (atypical meningiomas):** Examples are metaplastic, chondroid, clear cell and atypical meningiomas. These tumors have a higher mitotic index (≥4 mitoses/10 high power fields), increased cellularity, increased nucleocytoplasmic ratio, prominent nucleoli. Immunohistochemistry reveals increased Ki-67. These are associated with local invasion and increased risk for recurrence after resection.
- **WHO grade III meningiomas (anaplastic meningiomas):** Examples are papillary, rhabdoid and anaplastic meningiomas. These tumors must exhibit increased mitoses (≥20 mitotic figures/10 or 40 high power fields).

Immunohistochemistry Technique

Most meningiomas show weak positivity for epithelial membrane antigen (EMA) and progesterone receptors. Immunoreactivity for progesterone receptor is less in grades II and III meningiomas. S-100 protein immunoreactivity is patchy rather than diffuse in meningiomas. Atypical meningiomas exhibit increased Ki-67 (MIB-1 antibody).

Molecular Genetic Alterations

Meningioma is associated with neurofibromatosis type 2 due to mutation of NF2 gene located on long arm of chromosome 22 in person with previous history of irradiation. It is considered to be important for tumor development. Loss of the CDKN2A/p16 region on 9p/21 in anaplastic meningiomas is associated with

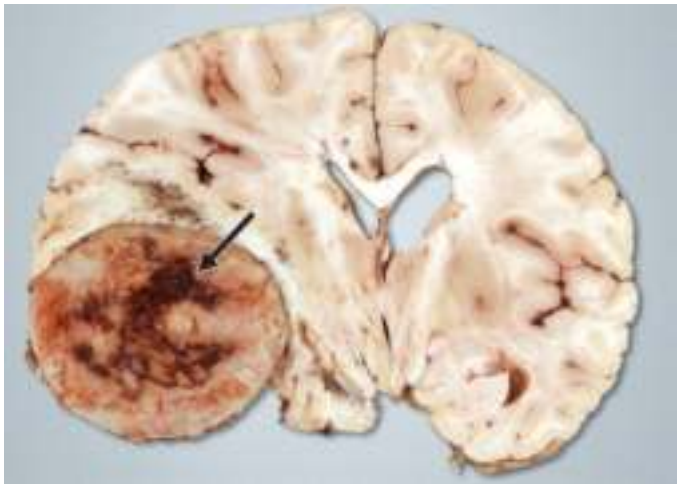


Fig. 30.27: Meningioma. Tumor arises from the dura is well encapsulated. It is cured by surgical excision (arrow).

poor prognosis. Lack of genetic 9p/21 alteration in meningiomas is associated with long survival. Tumor suppressor genes associated with meningiomas are given in Table 30.31.

DIFFUSE LEPTOMENINGEAL GLIONEURONAL TUMOR

Diffuse leptomeningeal glioneuronal tumor is a new entity defined by recent 2021 WHO classification of tumors of the central nervous system. It is also known as disseminated oligodendroglial-like leptomeningeal tumor of childhood, that presents as a plaque-like subarachnoid tumor, commonly involving the basal cisterns and interhemispheric fissure but lacking intraparenchymal tumor. It usually affects children and adolescents. Most common sites of tumor are posterior fossa, spinal region, intracranial leptomeninges.

Table 30.30 Histologic variants, grades, mitotic figures and survival rate in meningioma

Tumor and Histologic Grade	Histologic Features and Mitoses	Clinical Course and Survival
WHO grade I meningiomas		
▪ Meningothelial (syncytial) meningioma	▪ Cell borders are not easily demonstrated (syncytium-like histologic features) ▪ Mitoses nil ▪ Survival 5–20 years	▪ Slow-growing benign tumors ▪ Enlargement of tumor growth leads to clinical manifestations like headache, seizures, or focal neurologic deficits due to compression
▪ Fibroblastic (fibrous) meningioma	▪ Tumor is composed of spindle cells admixed with syncytium-like cells; and calcification in a linear fashion ▪ Mitoses nil	
▪ Transitional meningioma	▪ Tumor is composed of syncytial and fibroblastic elements; and psammoma bodies ▪ Mitoses nil	
▪ Psammomatous meningioma	▪ Tumor is composed of syncytial and fibroblastic elements; and numerous psammoma bodies ▪ Mitoses nil	
▪ Angiomatous meningioma	▪ Tumor is composed of neoplastic cells and dense vasculature ▪ Mitoses nil	
▪ Metaplastic meningioma	▪ Tumor is composed of neoplastic cells and areas of cartilage and/or bone ▪ Mitoses nil	
▪ Secretory meningioma	▪ Tumor is composed of neoplastic cells with brightly eosinophilic intracytoplasmic globular inclusions of proteinaceous secretions of varying size and shape termed 'pseudopsammomatous bodies', strongly positive for epithelial membrane antigen and cytokeratins ▪ Mitoses nil	
▪ Lymphoplasmacytic-rich meningioma	▪ Tumor is composed of bioplastic cells admired with chronic inflammatory cells, i.e. lymphocytes and plasma cells ▪ Mitoses nil	
▪ Microcystic meningioma	▪ Tumor is showing microcystic architecture throughout and often hyalinized, vasculature and angiomatous subtype ▪ Mitoses nil	
WHO grade II meningiomas		
▪ Choroid meningioma	▪ Tumor is composed of neoplastic cells, separated by myxoid matrix ▪ Mitoses ≥4 mitoses/10 high power fields	▪ Rapidly growing tumors ▪ Recurrence following excision

Contd...

Table 30.30 Histologic variants, grades, mitotic figures and survival rate in meningioma (*Contd...*)

Tumor and Histologic Grade	Histologic Features and Mitoses	Clinical Course and Survival
▪ Clear cell meningioma	▪ Tumor is composed of neoplastic cells with cytoplasmic clearing obscuring meningothelial nature; highlighted by immunostaining for vimentin or epithelial membrane antigen ▪ ≥4 mitoses/10 high power fields	▪ Survival rate of 5 years
WHO grade III meningiomas		
▪ Rhabdoid meningioma	▪ Tumor is composed of small epithelioid cells with globular eosinophilic cytoplasm and eccentric nuclei termed 'rhabdoid'; and increased mitotic activity ▪ Mitoses ≥20 mitotic figure/10 or 40 high power fields	▪ Rapidly growing tumors ▪ Recurrence following excision ▪ Survival rate of <2 years
▪ Papillary meningioma	▪ Tumor is composed of perivascular pseudorosettes giving papillary appearance; and increased mitotic activity ▪ Mitoses ≥20 mitotic figure/10 or 40 high power fields	

Genetic alterations: Grade I meningiomas demonstrate NF gene mutation (locus 22q); and grade III meningiomas exhibit loss of the CDKN2A/p16 region on 9p/21.

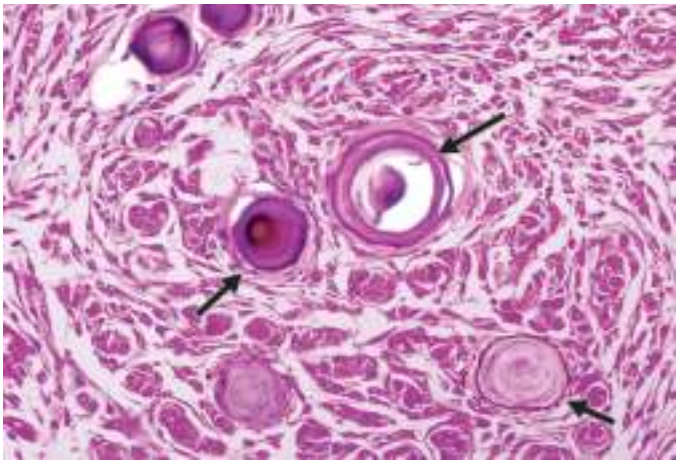


Fig. 30.28: Meningioma. Tumor consists of swirling masses of fusiform meningoepithelial cells with oval nuclei containing dispersed chromatin in a composite solid and whorled pattern. Small foci of calcification (psammoma bodies–calcified bodies) are common (arrows) (400X).

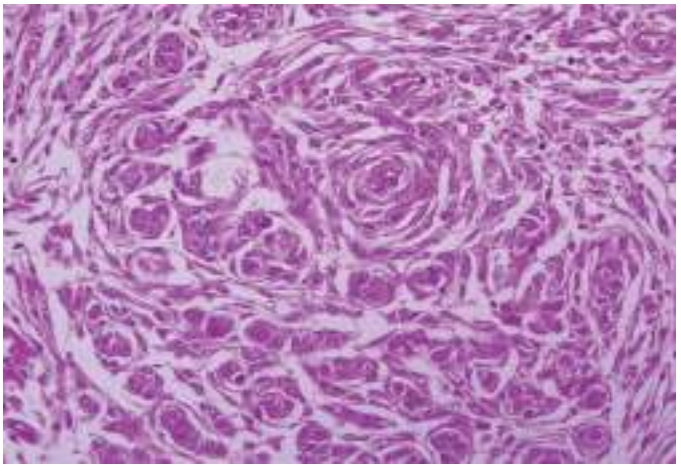


Fig. 30.30: Meningothelial meningioma. Tumor is composed of sheets, whorls, or syncytia of neoplastic cells with round to oval centrally located smooth nuclei with dispersed chromatin, small indistinct nucleoli, and absence of mitoses (400X).

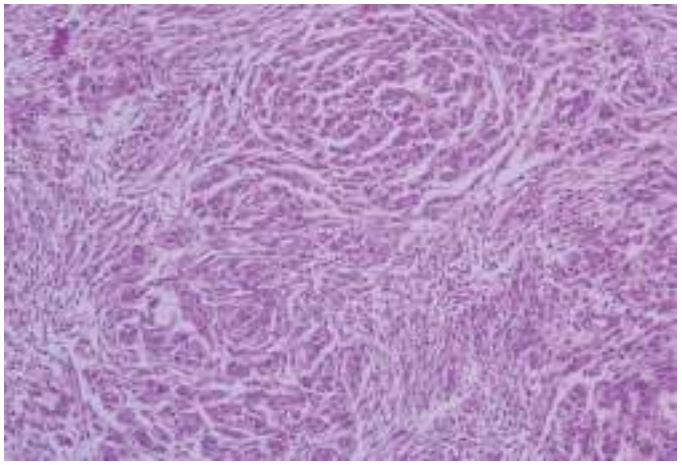


Fig. 30.29: Meningothelial (syncytial) meningioma. Cell borders are not easily demonstrated (syncytium-like histologic features) and absence of mitoses (400X).

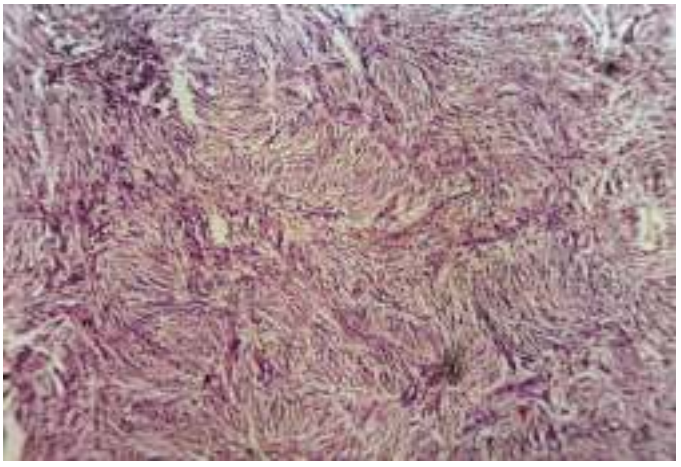


Fig. 30.31: Fibroblastic meningioma. Tumor composed of spindle cells admixed with syncytium-like cells; and calcification in a linear fashion. Mitoses are absent (400X).

Table 30.31 Tumor suppressor genes associated with meningiomas

Gene	Protein	Function	Familial Tumors	Sporadic Cancers
Mitogenic signaling pathway inhibitors				
NF2	▪ Merlin (locus 22q) ▪ Mutation by deletion or nonsense mutation	NF2 stabilizes cytoskeleton membrane linkage	Neurofibromatosis type 2 (acoustic schwannoma and meningioma)	▪ Meningiomas ▪ Schwannomas

Clinical Features

Patient presents with headache, nausea and vomiting due to increased intracranial pressure as a result of obstructive hydrocephalus; opisthotonos (spasm of skeletal muscles causing backward arching of the head and neck), and signs of meningeal or cranial nerve damage. Prognosis is moderate.

Radiologic Findings

Magnetic resonance imaging (MRI) demonstrates meningeal enhancement with thickening and hyperintense T2.

Mode of Spread

Tumor spreads via cerebrospinal fluid. Cerebrospinal fluid examination shows high protein and negative cytology.

Surgical Pathology: Diffuse Leptomeningeal Glioneuronal Tumor

Gross Morphology

- Intraparenchymal tumor is well circumscribed and solid/cystic nodules.
- Multifocal extension of the tumor occurs along Virchow-Robin space.

Light Microscopy

- Tumor demonstrates low-to-moderate cellularity. Tumor is composed of sheets of monotonous rounded cells with perinuclear halos identical to oligodendroglial cells.
- Tumor cells contain round-to-oval nuclei with finely granular dispersed chromatin and inconspicuous nucleoli and low mitotic index (<1%).
- Intense desmoplastic fibrous response is seen.

Immunohistochemistry Technique

Panel of immunomarkers is applied on paraffin-embedded sections such as BRAF fusion, synaptophysin, OLIG2, S-100 protein, glial fibrillary acidic protein (GFAP).

Markers	Expression
▪ BRAF fusion	▪ Positive (most common)
▪ Synaptophysin	▪ Positive (diffuse staining)
▪ OLIG2	▪ Positive (diffuse staining)
▪ S-100 protein	▪ Positive (patchy staining)
▪ GFAP	▪ Positive (patchy staining)
▪ Neurofilament	▪ Negative
▪ EMA	▪ Negative
▪ IDH mutation absent	▪ Negative

TUMORS OF THE CRANIAL AND PARASPINAL NERVES

Tumors of the cranial and paraspinal nerves include schwannoma (WHO grade I), neurofibroma (WHO grade II), perineuroma (WHO grade I) and malignant peripheral nerve sheath tumor (WHO grade III).

SCHWANNOMA (NEURILEMMOMA)

Schwannoma (also known as neurilemmoma) is a slowly growing benign encapsulated tumor (WHO grade I).

- The tumor originates from differentiated Schwann cell intimately surrounding axons of cranial nerves (e.g. vestibulocochlear cranial nerve), posterior spinal nerve roots or peripheral nerves. Tumor is eccentric to nerve.
- Essential features of schwannoma include: (a) biphasic tumor with highly ordered cellular component (Antoni A) that palisades (Verocay bodies) plus myxoid hypocellular component (Antoni B); (b) tumor usually associated with an identifiable nerve; and (c) strong immunoreactivity for S-100 calcium-binding protein.
- The tumor usually affects adults of 20–50 years of age with equal sex predilection. About 90% are sporadic and 3% associated with neurofibromatosis type 2.

Locations

Schwannomas are most often intracranial in cerebellopontine angle with involvement of the vestibulocochlear cranial nerve (acoustic neuroma). Expansion of the tumor results in tinnitus (most common) and sensorineural deafness. These tumors also originate frequently in posterior spinal nerve roots and peripheral nerves (upper limbs, head and neck, salivary glands, posterior mediastinum and retroperitoneum).

Pathophysiology

Schwannoma can occur sporadic in 90% and 3% with neurofibromatosis type 2 (NF2), schwannoma or Carney complex, which occurs due to direct genetic alteration involving the NF2 gene on chromosome 22 or secondarily to merlin inactivation. NF2 gene mutation can cause other neoplasms including meningioma, mesothelioma, glioma multiforme and carcinomas of breast, colorectum, kidney (clear cell type), liver, prostate and skin.

Table 30.32 Comparison of neurofibromatosis type 1 and neurofibromatosis type 2

Parameters	Neurofibromatosis Type 1 (NF1)	Neurofibromatosis Type 2 (NF2)
Mitogenic signaling pathway inhibitors		
Germline NF1 and NF2 gene mutation	NF1 gene mutation on chromosome 17p11 by deletion	NF2 gene mutation on chromosome 22q12.2 by deletion or nonsense mutation
Encoded defective protein by NF1 and NF2	Neurofibromin 1 inactivation	Merlin inactivation
Encoded protein function by NF1 and NF2 genes	Neurofibromin 1 homologous to GAP protein, that influences cell cycle by downregulating p21 ras gene (RAS/MAP-kinase signaling pathway)	Merlin stabilizes skeleton membrane linkage
Diagnostic criteria	Patient presents with two or more neurofibromas or one plexiform neurofibroma, six or more café au lait macules >5 mm (pubertal) or >15 mm (postpubertal), ocular iris hamartomas, sphenoid bone dysplasia and first degree relative with neurofibromatosis type 1	Diagnostic criteria of neurofibromatosis type 2 include bilateral vestibular schwannomas or family history of neurofibromatosis type 2 in first degree relative and unilateral vestibular schwannoma in below 30 years of age and any two of the lesions, i.e. meningioma, schwannoma, glioma, posterior subcapsular lenticular opacities/juvenile cortical cataract

Pathology Pearls: Neurofibromatosis Type 2

- Diagnostic criteria of neurofibromatosis type 2 include bilateral vestibular schwannomas or family history of neurofibromatosis type 2 in first degree relative and unilateral vestibular schwannoma in below 30 years of age and any two of the lesions, i.e. meningioma, schwannoma, glioma, posterior subcapsular lenticular opacities/juvenile cortical cataract.
- Comparison of neurofibromatosis type 1 and neurofibromatosis type 2 is given in Table 30.32.

Radiologic Findings

Schwannoma is well circumscribed mass eccentric to nerve, which displaces adjacent structures without direct invasion. Cystic degeneration or hemorrhage can be seen as areas of heterogeneity in large tumor. Hemorrhage and calcification are less frequent.

Surgical Pathology: Schwannoma (Neurilemmoma)

Gross Morphology

Classic schwannoma is encapsulated tan-yellow mass measuring 2–10 cm in diameter. Sinonasal and intracranial schwannomas are not encapsulated.

Light Microscopy

Classic schwannoma is encapsulated and composed of alternating Antoni A cellular component with Verocay bodies (cellular areas surrounded by nuclear palisading); and Antoni B hypocellular component with myxoid architecture (Fig. 30.32).

- **Antoni A cellular component of schwannoma:** It is cellular component composed of monomorphic spindle-shaped Schwann cells containing poorly defined cytoplasm, pointed basophilic nuclei set in a variably collagenous stroma showing nuclear palisading surrounding pink areas known as 'Verocay bodies'.
- **Antoni B cellular component of schwannoma:** It is composed of Schwann cells containing inconspicuous cytoplasm and nuclei suspended in abundant myxoid microcystic matrix (the pattern resembles the stripes of a zebra).

Differential Diagnosis

Schwannoma should be differentiated from neurofibroma, nevomelanocytic lesions, leiomyosarcoma, gastrointestinal stromal tumor and malignant peripheral nerve sheath tumor (MPNST).

Immunohistochemistry Technique

Schwannoma shows strong positivity for S-100 protein.

Marker	Expression
S-100 protein	Positive

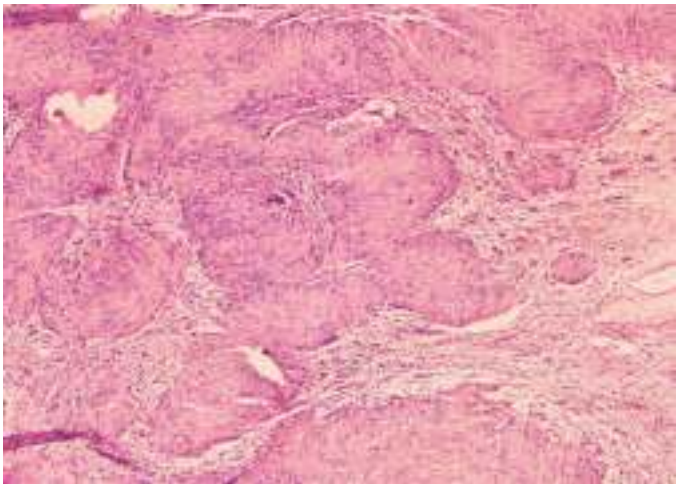


Fig. 30.32: Schwannoma (neurilemmoma). Tumor is composed of cells with palisading nuclei arranged in compact whorls and fascicles (Antoni type A), interspersed with less cellular loosely structured myxomatous appearing areas designated Antoni type B areas (100X).

Prognosis and Treatment

Schwannoma is a slow-growing tumor that very rarely undergoes malignant transformation. It is treated by surgical excision. Sudden progressive increase in size of tumor is a concerning feature. Schwannoma may transform to malignant peripheral nerve sheath tumor (MPNST), angiosarcoma and epithelioid malignant change. Neurofibromatosis type 2 caused due to germline NF2 gene mutation is difficult to treat.

NEUROFIBROMA, NEUROFIBROMATOSIS TYPE 1 AND MALIGNANT CHANGE IN NEUROFIBROMA

Neurofibroma is a benign tumor, which arises from within a peripheral nerve. Solitary neurofibroma is relatively common, which can be associated with neurofibromatosis type 1. Neurofibromatosis type 1 is characterized by multiple neurofibromas, café au lait spots, optic iris hamartoma, sphenoid bone dysplasia, macrocephaly, short stature and mental retardation.

Pathology Pearls: Diagnostic Criteria of Neurofibromatosis Type 1

- Patient presents with two or more neurofibromas or one plexiform neurofibroma, six or more café au lait macules >5 mm (pubertal) or >15 mm (postpubertal), ocular iris hamartomas, sphenoid bone dysplasia and first degree relative with neurofibromatosis type 1.
- Out of these clinical manifestations, patient must have at least two or more features.

Locations and Age Distribution

Solitary neurofibroma is relatively a common tumor. Plexiform neurofibroma associated with neurofibromatosis type 1 is relatively uncommon. Both tumors originate from peripheral nerve especially in the scalp region and lower extremities. Neurofibroma usually affects persons 20–40 years of age. Neurofibroma associated with neurofibromatosis type 1 affects children and adolescents with male predominance.

Clinical Features

Patient with neurofibromatosis type 1 presents with solitary or multiple neurofibromas, café au lait spots, optic iris hamartoma, sphenoid bone dysplasia, macrocephaly, short stature and mental retardation. Clinical photograph of patient with multiple neuromas in neurofibromatosis type 1 is shown in Fig. 30.33.

Surgical Pathology: Neurofibroma, Neurofibromatosis Type 1 and Malignant Change in Neurofibroma

Gross Morphology

- **Solitary neurofibroma:** Tumor is well delineated and fusiform-shaped mass measuring 1–2 cm in diameter.
- **Diffuse neurofibroma:** Tumor is gray plaque-like thickening of dermis with soft in consistency.
- **Plexiform neurofibroma:** Tumor is large soft tissue mass giving 'bag of worms' appearance (Fig. 30.34).

Light Microscopy

- **Neurofibromatosis type 1:** Cellular and stromal milieu in neurofibromatosis type 1 include Schwann cells, intraneural fibroblasts, collagen bundles, myxoid change and increased cellularity in the center of neurofibroma, when compared with edematous appearance in the peripheral region of neurofibroma.
- **Solitary neurofibroma:** Tumor is composed of spindle cells arranged in storiform pattern with abundant intervening collagen fibers. Intraneural growth is extending into the surrounding tissue (Fig. 30.35).
- **Diffuse neurofibroma:** Tumor is composed of small round to spindle cells associated with fibrillar and myxoid background. It infiltrates subcutaneous fat in a honeycomb pattern. Presence of 'Wagner-Meissner bodies' is the hallmark of diffuse neurofibroma.
- **Plexiform neurofibroma:** Tumor is tortuous distension of peripheral nerve showing plexiform architecture demonstrated on imaging technique, gross morphology and histologic features with multiple intraneural nodules of tumor separated by epineurium and stroma. It can involve the subcutis or skeletal structures or both. Clinicopathologic diagnosis is established due to its 'bag of worms' appearance.
- **Low-grade malignant peripheral nerve sheath tumor (low-grade MPNST):** Neurofibroma may undergo early malignant transformation. Low-grade malignant peripheral nerve sheath tumor resembles neurofibroma but with increased cellularity with pleomorphism and arranged in fascicular architecture and scattered mitotic activity in the deep area of tumor.

Differential Diagnosis

Neurofibroma should be differentiated from schwannoma, malignant peripheral nerve sheath tumor (MPNST), benign fibrous histiocytoma and desmoplastic malignant melanoma.

Immunohistochemistry Technique

Neurofibroma and neurofibromatosis type 1 may undergo early malignant transformation show positivity for immunomarkers S-100 protein (Schwann cells), epithelial membrane antigen (EMA), CD34 (intraneural fibroblasts), neurofilament protein (NFP) and glial fibrillary acidic protein (GFAP).

Markers	Expression
S-100 protein	Positive
EMA	Positive
CD34	Positive
NFP	Positive
GFAP	Positive (occasional)

Molecular Genetic Alterations

Neurofibromatosis type 1 occurs due to germline mutation of NF1 tumor suppressor gene located on chromosome 17.



Fig. 30.33: Multiple neurofibromas in neurofibromatosis type 1.



Fig. 30.34: Plexiform neurofibroma. Plexiform neurofibroma (WHO grade I) is pathognomonic of neurofibromatosis type 1 (NF1) involving single or multiple nerve fascicles of major nerve branches.

- NF1 gene encodes aberrant protein product known as 'neurofibromin', homologous that affects cell cycle by disrupting p21 and RAS gene.
- Malignant transformation of neurofibroma is common in these patients.
- Neurofibromatosis type 1 is characterized by multiple neurofibromas, café au lait spots, optic iris hamartoma, sphenoid bone dysplasia, macrocephaly, short stature and mental retardation.

Prognosis and Treatment

Solitary neurofibroma, diffuse neurofibroma and plexiform neurofibroma may be associated with neurofibromatosis type 1. These are treated by surgical excision. Recurrence is common in diffuse neurofibroma after surgical excision. Early malignant peripheral nerve sheath tumor in a case of neurofibromatosis type 1 is treated similar to low-grade sarcoma. High-grade malignant transformation in neurofibroma is treated similar to high-grade sarcoma.

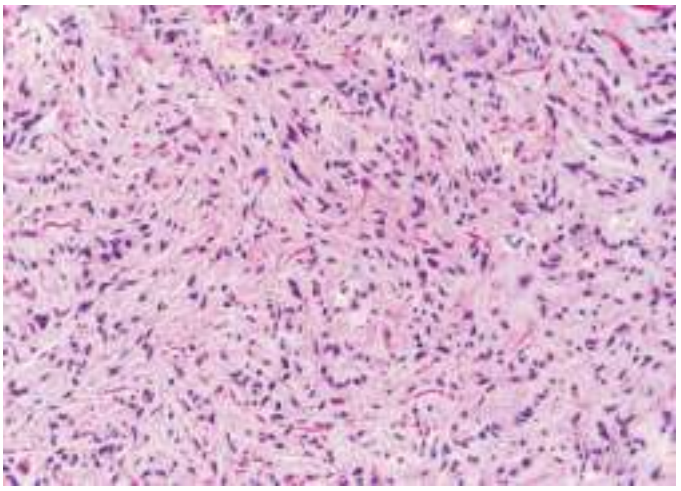


Fig. 30.35: Neurofibroma. Tumor is composed of elongated wavy cells with small dark oblong nuclei. This tumor is benign. However, persons with neurofibromatosis type 1 have a long-term risk for development of malignant neoplasms such as neurofibrosarcomas, malignant schwannomas, gliomas (400X).

MALIGNANT PERIPHERAL NERVE SHEATH TUMOR

Malignant peripheral nerve sheath tumor (MPNST) can originate in *de novo* or in the setting of neurofibroma and neurofibromatosis type 1.

- Most common sites of MPNST buttock, thigh, brachial plexus, upper arm and paraspinal regions. Sporadic malignant nerve sheath tumor affects adults during second to fifth decades of life.
- On the other hand, malignant nerve sheath tumor originating in the setting of neurofibromatosis type 1 affects during third to sixth decades of life. Patient presents with signs and symptoms depending on location of malignant nerve sheath tumor.

Radiologic Findings

Malignant peripheral nerve sheath tumor (MPNST) poses diagnostic difficulty to differentiate from benign nerve sheath tumor on radiologic techniques. Tumor size >5 cm with irregular borders and presence of necrosis are suggestive of MPNST.

Surgical Pathology: Malignant Peripheral Nerve Sheath Tumor
Gross Morphology
▪ Malignant peripheral nerve sheath tumor is creamy gray and fusiform shaped measuring >5 cm in diameter. ▪ Cut surface shows areas of hemorrhage and necrosis.

Light Microscopy

- Malignant peripheral nerve sheath tumor exhibits diverse histologic patterns resembling marble-like appearance with alternating zones of hypercellularity and hypocellularity.
- Tumor is composed of neoplastic cells with waxy or buckled hyperchromatic nuclei and moderate amount of lightly eosinophilic cytoplasm.
- Neoplastic cells are arranged in long fascicles. In occasional area of the tumor, neoplastic cells may be arranged in storiform pattern.
- Hemangiopericytoma-like vascular pattern may also be demonstrated.
- Heterologous differentiation, e.g. skeletal muscle, cartilage, bone, blood vessels and glands, is demonstrated in few cases of neurofibromatosis type 1 related MPNST.

Differential Diagnosis

Malignant peripheral nerve sheath tumor should be differentiated from synovial sarcoma, fibrosarcoma, malignant solitary fibrous tumor and malignant melanoma.

Electron Microscopy

Malignant peripheral nerve sheath tumor shows melanosomes combined with schwannian features of well-developed basement membranes and complex processes.

Immunohistochemistry Technique

- Spindled malignant peripheral nerve sheath tumor accounts for 95% and shows positivity for HMB-45 and S-100 protein (weak and focal staining).
- Epithelioid malignant peripheral nerve sheath tumor shows positivity for HMB-45 and S-100 protein (strong staining).
- Malignant peripheral nerve sheath tumor with rhabdoblastic differentiation shows positivity for immunomarkers desmin, myogenin, MyoD1 and HMB-45.

Markers	Expression
Spindled malignant peripheral nerve sheath tumor	
▪ HMB-45 ▪ S-100 protein	▪ Positive ▪ Positive (weak and focal staining)
Epithelioid malignant peripheral nerve sheath tumor	
▪ HMB-45 ▪ S-100 protein	▪ Positive (strong staining) ▪ Positive
Malignant peripheral nerve sheath tumor with rhabdoblastic differentiation	
▪ Desmin ▪ Myogenin ▪ MyoD1 ▪ HMB-45	▪ Positive ▪ Positive ▪ Positive ▪ Positive

Prognosis and Treatment

Malignant peripheral nerve sheath tumor arising *de novo* and in the setting of neurofibromatosis type 1 has five-year survival rate in 85% of cases. Malignant nerve sheath tumor of head and neck region is a low-grade tumor with best prognosis.

TUMORS OF THE SELLAR REGION

Tumors of the sellar region include craniopharyngioma (WHO grade I), granular cell tumor of the neurohypophysis (WHO grade I), pituicytoma (WHO grade I), and spindle cell oncocytoma of the adenophysis (WHO grade I). Craniopharyngioma has been discussed in Chapter 26 (Endocrine System).

MESENCHYMAL NONMENINGEAL TUMORS

Mesenchymal nonmeningeal tumors include solitary fibrous tumor/hemangiopericytoma (WHO grade I, II or III) and hemangioblastoma (WHO grade I).

HEMANGIOBLASTOMA

Hemangioblastoma WHO grade I tumor occurs most frequently in the cerebellum, brainstem and spinal cord, which has cellular and reticular variants. Tumor sometimes produces erythropoietin, leading to secondary polycythemia. It is a slow-growing highly vascular tumor favoring cerebellum in adults.

Molecular Genetic Alterations

Hemangioblastoma occurs due to mutations of von Hippel-Lindau (vHL) disease gene located on 3p25–26. Mutation of vHL gene is associated with similar lesions in the retina, pheochromocytoma and renal cell carcinoma (clear cell variant) as part of vHL disease.

Radiologic Findings

Classic hemangioblastoma is well circumscribed tumor demonstrated by imaging techniques.

Surgical Pathology: Hemangioblastoma

Gross Morphology

- Hemangioblastoma is well circumscribed tumor.
- Cut surface shows cystic areas filled with fluid.

Light Microscopy

Tumor is composed of thin-walled vascular channels surrounded by large polygonal tumor cells with pale cytoplasm. There may be foci of hematopoietic tissue and mast cell infiltration (Fig. 30.36).

Immunohistochemistry Technique

Panel of immunomarkers is employed on histologic sections to highlight endothelial cells.

Markers	Expression
▪ CD31	▪ Positive
▪ CD34	▪ Positive
▪ CD56	▪ Positive
▪ S-100 protein	▪ Positive
▪ Vimentin	▪ Positive
▪ Neuron-specific enolase (NSE)	▪ Positive
▪ Inhibin-α	▪ Positive

PRIMARY CNS LYMPHOMA

Primary lymphoma occurs first in central nervous system (CNS) without evidence of system lymphoma, which usually occurs in cerebrum but also elsewhere. It accounts for <2% of brain tumors especially in AIDS, immunocompromised persons and even in immunocompetent elderly persons.

- Most primary CNS lymphomas are high-grade diffuse large B cell lymphoma. High incidence of T cell subtype of lymphoma is seen in Korea.
  - Patient presents with focal neurological deficits (50– 80%), neuropsychiatric symptoms (20–30%), increased intracranial pressure (10–30%), seizures (5–20%) and ocular symptoms (5–20%).
  - CSF cytology is helpful in diagnosing lymphoma of dura, leptomeningeal space or nerve roots.
  - CT and MRI scans demonstrate bilateral symmetric, subependymal, high signal foci are suggestive of primary CNS lymphoma on CT/MRI studies.
- In immunocompetent patients treated with radiotherapy and chemotherapy, 2-year overall survival is 40–75% and 5-year overall survival is 25–45%. AIDS patients have median survival of 2–6 months, increasing to 13 months with multimodal therapy.
- Poor prognostic factors for primary CNS lymphoma include multiple lesions, with periventricular or meningeal involvement, immunocompromised state, elderly person over 60 years, preoperative and Karnofsky performance scale index score (≤70).
- Karnofsky performance scale index is used to compare effectiveness of different therapies and to assess the prognosis in individual patient. In most serious illnesses, the lower the Karnofsky score, the worse the likelihood of survival. Expression of TP53, c-Myc or BCL-6 in immunocompetent patients is associated with poor prognosis in one study.

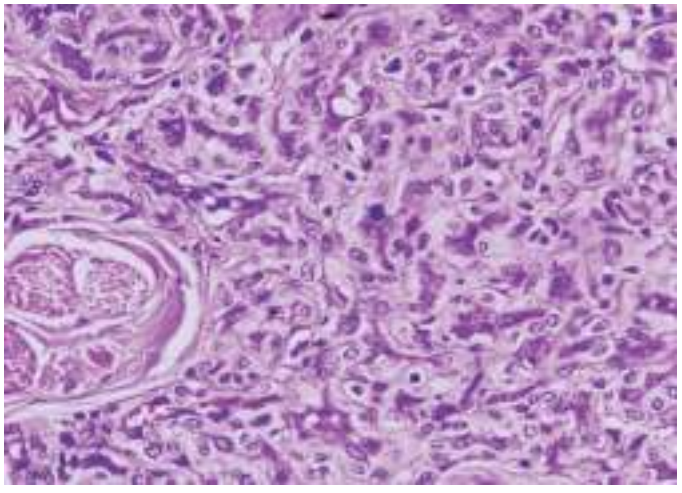


Fig. 30.36: Hemangioblastoma. Tumor is composed of thin-walled vascular channels surrounded by large polygonal tumor cells with pale cytoplasm (400X).

GERM CELL TUMORS OF NERVOUS SYSTEM

Germ cell tumors of central nervous system include germinoma, embryonal carcinoma, endodermal sinus tumor (yolk sac tumor) teratoma, and choriocarcinoma. These tumors usually occur in children, but may occur in adults especially in males than females.

- Patient with germ cell tumor located in pineal region presents with increased intracranial pressure. Tumor located in suprasellar region is associated with diabetes insipidus and hypopituitarism. Less common sites are basal ganglia and thalamus.
- Germ cell tumors exhibit heterogenous enhancement on MRI scan. Calcification is seen in teratomas. Hemorrhage is strongly suggestive of choriocarcinoma. Histologically, these tumors are homologous to their gonadal and mediastinal counterparts. Histologic examination and immunohistochemistry are diagnostic tools.
- Pure germinomas are radiosensitive tumors associated with excellent prognosis. Germinoma with syncytiotrophoblastic element has tendency to recur with less favorable prognosis. Mature teratomas are treated by surgical resection. Yolk sac tumor, embryonal carcinoma and choriocarcinoma have less favorable prognosis.

PRIMITIVE NEUROECTODERMAL TUMORS

Primitive neuroectodermal tumors (PNETs) are small blue tumors of the nervous system, which include medulloblastoma, neuroblastoma arising from autonomic ganglia in the abdomen, cerebral neuroblastoma, retinoblastoma, pineoblastoma and esthesioblastoma arising from nasal mucosa. These PNETs show similar morphology. Tumor cells possess high nucleocytoplasmic ratio and fibrillary processes demonstrated by silver staining technique. Nuclei are

small, oval and darkly stained. Mitoses are common. Many tumors show evidence of neuronal, glial or ependymal differentiation.

RETINOBLASTOMA

Retinoblastoma is a malignant retinal tumor of childhood. The tumor is sporadic (60%) and familial (40%). Sporadic retinoblastomas are unilateral and monocentric in origin. Familial retinoblastomas are more often bilateral and multicentric in origin. Some inherited cases also develop pinealoblastoma (so-called trilateral retinoblastoma). Retinoblastoma arises from the retina in children under 3 years. Around 40% of cases are inherited and the tumor may be bilateral. Remaining tumors arise sporadically and are usually unilateral.

Molecular Genetic Alterations

Retinoblastoma gene (RB gene) is a tumor suppressor gene situated on the short arm of chromosome 13(13q14), which encodes pRB. The normal RB protein prevents cell mitosis. The tumor is the prototype of the two-hit hypothesis of Knudson.

- In first hit, germline deletion inherited mutation of RB1 gene occurs in allele 1.
- In second hit, somatic deletion of RB gene occurs in remaining normal allele. Both deletions are required for tumor development of familial or sporadic retinoblastoma.
- Onset of familial retinoblastoma is earlier than sporadic form. Inherited and sporadic retinoblastoma is shown in Fig. 30.37A and B.

Surgical Pathology: Retinoblastoma

Gross Morphology

- Endophytic retinoblastoma growth protrudes into the vitreous cavity. Exophytic tumor grows between retina and pigment epithelium.
- Tumor may exhibit endophytic as well as exophytic growth patterns.
- Gross morphology of retinoblastoma is shown in Figs 30.38 and 30.39.

Light Microscopy

- Tumor is composed of small round cells with hyperchromatic nuclei and scant cytoplasm, numerous mitoses and formation of Flexner-Wintersteiner rosettes.
- These rosettes show central lumen lined by pink material with nuclei away from lumen (Fig. 30.40).

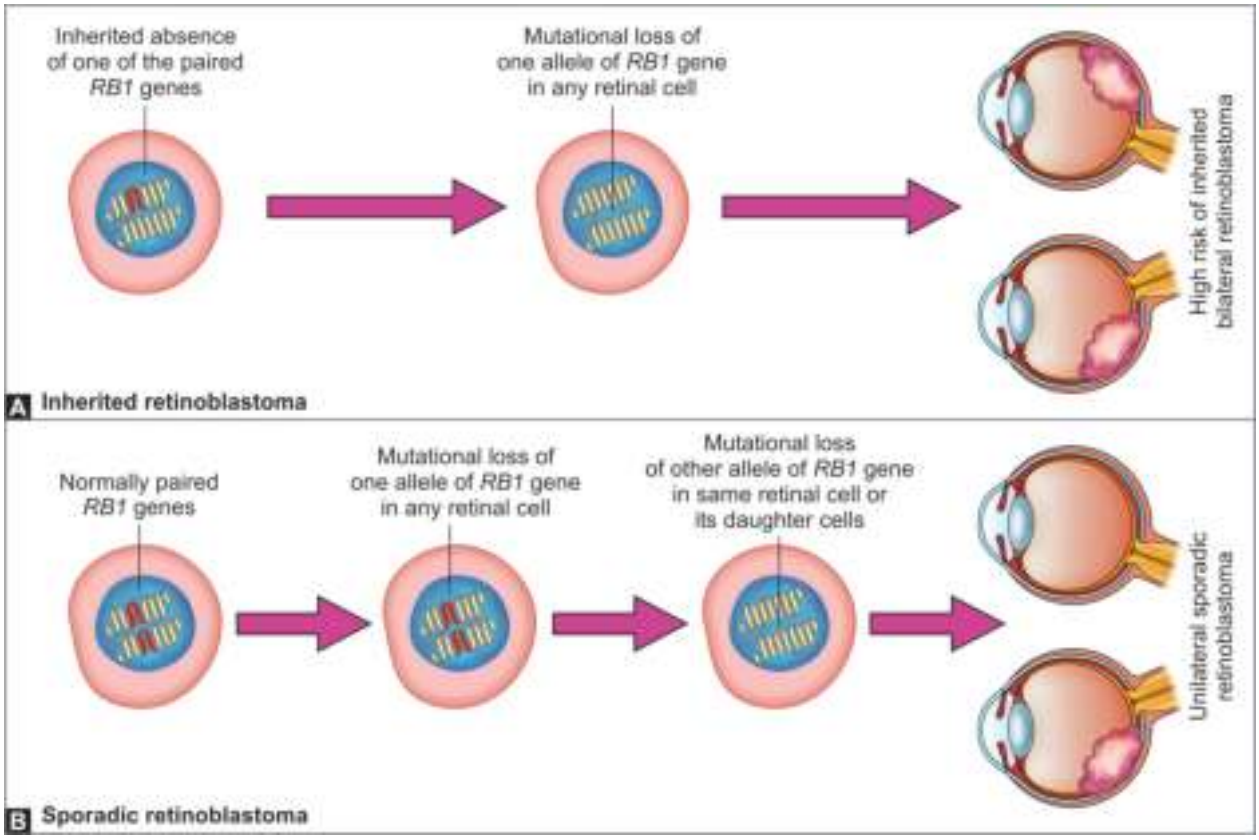


Fig. 30.37: Inherited and sporadic retinoblastoma. RB gene is a tumor suppressor gene located on long arm of chromosome 13. Mutation of RB1 gene predisposes to increased risk of development of retinoblastoma. (A) Germline mutation is one of the paired alleles of the RB1 gene is required to develop inherited bilateral retinoblastoma, (B) normal individuals without an inherited germline mutation of the RB1 gene have a low incidence of sporadic unilateral retinoblastoma.



Fig. 30.38: Retinoblastoma. The mother was alerted to the condition by the white color of the pupil. There was also some loss of vision.

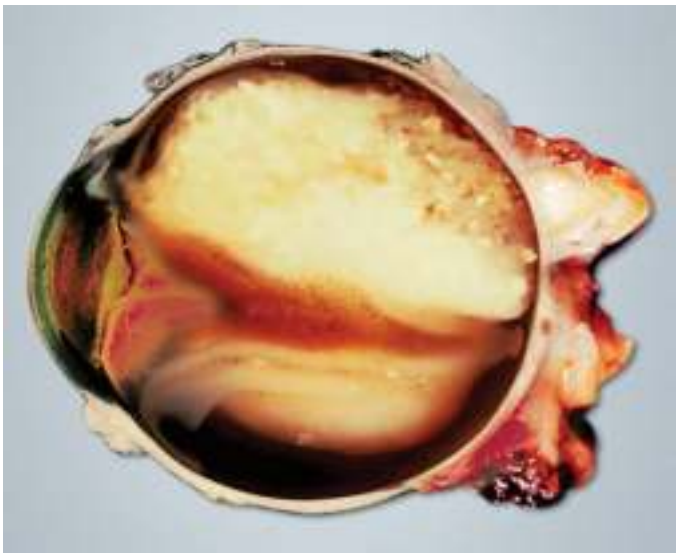


Fig. 30.39: Retinoblastoma. Tumor is filling the posterior chamber of the eye surgically excised. There was no spread along the subdural space around the optic nerve, no tumor in the other eye. There was no family history.

Immunohistochemistry Technique	
Panel of immunomarkers is used to analyze expression in retinoblastoma on histopathologic sections.	
Markers	**Expression**
▪ GFAP ▪ S-100 protein ▪ Neuron-specific enolase (NSE) ▪ Synaptophysin ▪ Myelin basic protein	▪ Positive ▪ Positive ▪ Positive ▪ Positive ▪ Positive

Mode of Spread

The tumor may fill the eye and spread locally into the brain via the optic nerve or systemically after invasion of the choroid. Distant metastases are demonstrated in cranial vault and skeletal system.

Management

Modern therapy of retinoblastoma is curative in and around 90% of cases. Some inherited cases also develop pinealoblastoma, so-called trilateral retinoblastoma.

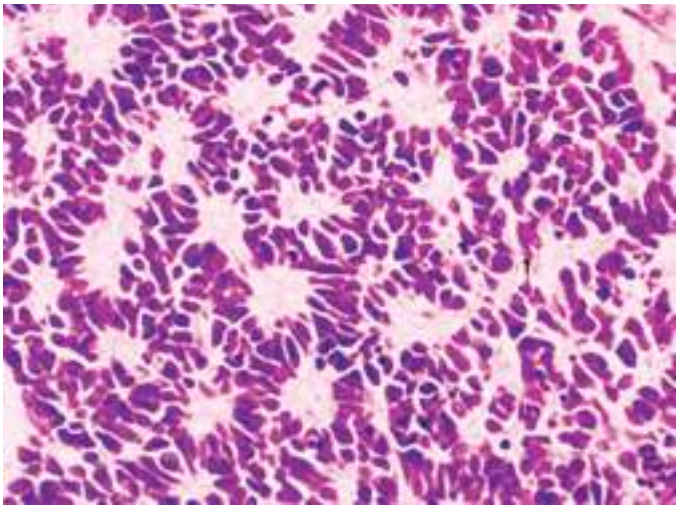


Fig. 30.40: Retinoblastoma shows small round cells with hyperchromatic nuclei and scant cytoplasm, numerous mitoses and formation of Flexner-Wintersteiner rosettes. These rosettes show central lumen lined by pink material with nuclei away from lumen (400X).

HYPOXIA-INDUCED BRAIN INJURY

CEREBRAL HYPOXIA

Neurons are most vulnerable cells of the central nervous system. Reactive gliosis is a normal response of the brain to injury and infection but is not visible on the cut surface of the brain at autopsy. The various cells of CNS are not equally sensitive to hypoxia and deficiency of nutrients. Cerebral hypoxia affects hippocampus neurons, cerebellar Purkinje cells, pyramidal neurons, and neurons within layers of III and V of neocortex (laminar necrosis).

CLINICAL FEATURES

Cerebral hypoxia causes loss of consciousness in less than a minute. Restoration of blood circulation and oxygenation in <1 minute leads to complete recovery.

- Interruption of blood circulation and oxygenation more than three minutes leads to brain damage and coma, with dilated pupils unresponsive to light, inert or rigid extremities, unresponsive to all stimuli, abolition of brain reflexes and ultimately no electrical activity on electroencephalogram (EEG).
- Causes of interruption to the blood flow and oxygen to the brain are hypotension, cardiac arrest, anesthesia complications during major surgical procedures, carbon monoxide poisoning, travelling high altitude, breathing in carbon monoxide and smoke, strangulation, head injury, and extreme bronchial asthma.

PATHOPHYSIOLOGY

Brain hypoxia symptoms range from mild to severe depending on the cause. Mild symptoms are temporary such as memory loss, difficulty in taking sound decisions and reduced ability to move body. Severe symptoms are seizures, coma and brain death. Cardiac arrest is a major cause of mortality and neurologic disability.

- During cardiac arrest, cessation of cerebral oxygen delivery O_2 occurs with resultant neuron ischemia and cell death within minutes. The cerebrum consumes 20–25% of cardiac output to maintain function. The brain is devoid of nutrient stores, and consequently neuroglycopenia and metabolic crisis occur within minutes after cardiac death leading to irreversible cell death. Reduced cerebral oxygen supply to brain manifests as reduced neuronal aerobic metabolism leading to decreased cellular adenosine triphosphate (ATP) production.
- Intracellular accumulation of calcium leads to mitochondrial toxicity, that further causes reduction of ATP production. Inability to sustain cellular respiration results in cell death and apoptosis. In addition, microvasculature endothelial dysfunction leads to a porous blood–brain barrier resulting in cerebral edema, formation of microthrombi and limitation of cerebral blood flow with exacerbation of cellular ischemia. Reduced cerebral oxygen delivery results in cessation of ATP production, causing cessation of energy-dependent ion channel function. Subsequent intracellular Na^+ accumulation leads to cytotoxic edema.
- Depletion of adenosine triphosphate leads to anaerobic metabolism, cerebral lactate accumulation, and intracellular acidosis. Additionally, cellular ischemia leads to intracellular Ca^{2+} influx through N-methyl-D-aspartate channels, which activates hydrolytic enzymes and mitochondrial dysfunction, thereby depleting adenosine triphosphate further. Finally, excitatory neurotransmitter release activates lipases and proteases, which leads to apoptosis. Brain structures especially susceptible to reduced cerebral oxygen supply include the hippocampi, thalami, cerebral cortex, corpus striatum, and cerebellar vermi, owing to highly metabolically active tissue.

Surgical Pathology: Cerebral Hypoxia

Light Microscopy

- Cerebral hypoxia of more than three minutes results in liquefactive necrosis in the brain. When the cells die, they are digested by lysosomes in the body. This digestion process results in the formation of pus-filled cysts.
- Affected neurons have pyknotic nuclei and an acidophilic cytoplasm, which stains intensely red with eosin (red is dead) in standard hematoxylin and eosin slides.

LABORATORY DIAGNOSIS

Brain hypoxia is diagnosed by blood oxygen concentration, skull, MRI scan, echocardiogram, electrocardiogram and electrocephalogram (electrical activity).

CEREBROVASCULAR DISORDERS

Cerebrovascular disorders are the most common group of CNS disorders; which include cerebral stroke, carotid stenosis, vertebral stenosis and intracranial stenosis, aneurysms, and vascular malformations. Restrictions in blood flow may occur from blood vessel narrowing, thrombus formation, embolus formation interrupting blood flow or blood vessel rupture. Cerebrovascular disorders rank after heart disease and cancer as the third major cause of death in the United States. Predisposing factors for cerebrovascular diseases are given in Table 30.33.

CEREBRAL INFARCTION

Cerebral infarction is more frequent than hemorrhage. Brain does not contain proteins; hence ischemia produces liquefactive necrosis and formation of cystic spaces containing creamy liquid necrotic cell debris by hydrolytic enzymes released by phagocytes.

- The cyst wall is formed by proliferating capillaries, neutrophils, macrophages and gliosis (proliferating neuroglial cells).

Table 30.33 Predisposing factors for cerebrovascular diseases

Type	Predisposing Factors	Common Sites
Cerebral infarction		
▪ Thrombus formation	▪ Atherosclerosis	▪ Arterial obstruction of internal and external carotid arteries at the origin in the neck, vertebral arteries, basilar arteries, middle cerebral arteries of circle of Willis
▪ Embolism	▪ Cardiac mural thrombi, valvular vegetation and fat emboli	▪ Middle cerebral is the most frequent site of embolic occlusion
Cerebral hemorrhage		
▪ Intracerebral hemorrhage	▪ Hypertension, coagulation disorders, hemorrhage within a malignant tumor	▪ Intracranial hemorrhage may result from rupture of Charcot-Bouchard aneurysms in a long-standing case of hypertension
▪ Subarachnoid hemorrhage	▪ Rupture of congenital berry aneurysm especially in hypertension	▪ Circle of Willis and bifurcation of the middle cerebral artery

- Arterial occlusion occurs either due to complicated atheromatous plaques in blood vessels supplying brain (bifurcation of carotid arteries), or emboli arising from cardiac mural thrombi, infected vegetations on cardiac valve, tumor emboli, bubbles of air, or droplets of fat into middle cerebral artery. Embolism is much less common than thrombosis.

PATHOPHYSIOLOGY

Liquefactive necrosis (colliquative necrosis) transforms the tissues into liquid viscous mass. It most often occurs as a result of ischemic necrosis of brain.

- Brain does not contain proteins; hence ischemia produces liquefactive necrosis.
- Hydrolytic enzymes liberated by neutrophils and macrophages digest the tissue resulting in transformation of the tissue into soft, circumscribed creamy liquid viscous.
- Later a cyst wall is formed. It is worth mentioning that ischemia causes coagulative necrosis in organs rich in proteins such as kidney, spleen and heart; except brain, that lacks proteins. Gross morphology and histologic features of cerebral infarct are given in Table 30.34.

CLINICAL FEATURES

Clinical features of cerebral infarction depend on the site of vascular obstruction and extent of collateral circulation. Arterial obstruction in this site causes contralateral paralysis, as well as motor and sensory defects and aphasias.

- **Carotid bifurcation and middle cerebral arteries occlusion:** These blood vessels are the most frequent sites of thrombotic occlusion as a result of complicated atherosclerotic plaques.

Table 30.34 Gross morphology and histologic features of cerebral infarct

Feature	Duration	Gross Morphology	Histologic Features
Acute cerebral infarct	Hours to few days	▪ Soft parenchyma with preservation of architecture and edematous infarct ▪ Poorly defined area of soft parenchyma; preservation of architecture and edematous infarct	▪ Red neurons and rarefaction of white mater within 12–24 hours ▪ Liquefactive necrosis of neurons. Mild neutrophilic infiltrate and extravasated blood cells as a result of reperfusion
Subacute (organizing) cerebral infarct	Hours to weeks	Partially liquefied infarcts with well-defined are of soft tissue, friable parenchyma; loss of architecture	Liquefactive necrosis of neurons and glial components drop out, marked macrophage infiltrate, reactive capillaries and gliosis at periphery
Chronic (remote) cerebral infarct	Months to years	Cystic cavity with collapse of surrounding tissue	▪ Cystic spaces lined by 'gliotic scar' without collagen, loss of parenchyma, sparing of subpial region as opposed to old contusion; with or without hemosiderin and macrophages ▪ Gliosis at the periphery

Hemorrhagic cerebral infarcts occur due to emboli or compression of the blood vessels. Nonhemorrhagic cerebral infarcts are caused by thrombi.

- **Middle cerebral arteries occlusion:** These blood vessels are the most frequent site of embolic occlusion. Emboli are arising from left side of heart. Patient presents with contralateral upper extremity weakness and ipsilateral facial weakness. Also, in right-handed individuals, the left side of the cerebral cortex is usually dominant, and verbal aphasias are caused by left-sided lesions in the great majority of cases.
- **Anterior cerebral artery occlusion:** Occlusion in anterior cerebral artery causes contralateral lower extremity weakness and altered mental status.
- **Posterior cerebral artery occlusion:** Occlusion in posterior cerebral artery causes contralateral visual loss.
- **Small cerebral arteries occlusion:** Obstruction of small cerebral arteries produce lacunar strokes involving motor or sensory areas. Pure motor lacunar stroke most often results from lesions affecting the internal capsule. Pure sensory lacunar stroke most often results from lesions affecting the thalamus.

Surgical Pathology: Cerebral Infarction

Gross Morphology

Brain tissue is creamy liquid viscous. It may show cyst formation (Fig. 30.41).

Light Microscopy

- Histologic examination of liquefactive necrotic tissue obtained from brain reveals cystic space contains necrotic cell debris.
- The cyst wall is formed by proliferation of capillaries, neutrophils, macrophages and gliosis (proliferating glial cells).

CEREBRAL HEMORRHAGE

Cerebral hemorrhage refers to hemorrhage into the tissue of cerebral hemispheres. It is most often complicated by long-standing hypertension, coagulation disorders, hemorrhage within a brain tumor and Charcot-Bouchard aneurysms. Hemorrhage most often occurs in the basal ganglia/thalamus; other favored sites include the pons, cerebellum, and frontal lobe white matter.

SUBARACHNOID HEMORRHAGE

Subarachnoid hemorrhage is commonly associated with rupture of a congenital berry aneurysm in the circle of Willis compounded by hypertension. Other causes are mycotic aneurysms, arteriovenous malformations and cavernous hemangiomas. A transient ischemic attack is a brief episode of impaired neurologic function caused by a brief disturbance in cerebral circulation. Mortality can occur in the first week in 30% of cases. Common sites of aneurysms formation in intracranial arteries linked to cardiovascular diseases are shown in Fig. 30.42.



Fig. 30.41: Cerebral infarct. This intermediate infarct of the frontal lobe shows liquefactive necrosis with formation of cystic spaces as resolution begins (arrow).

BERRY ANEURYSM

Berry aneurysm is rounded sac-like structure at the site of congenital defect in vessel wall involving only part of the circumference of a blood vessel. It throbs and pushes against surrounding structures. Mural thrombus is most often found within aneurysmal sac.

- The most common site of berry aneurysm formation is between the anterior communicating and the anterior cerebral arteries in the circle of Willis. It develops as saccular structure (1.0–1.5) at site of congenital defect in smooth muscle distribution at a branch point of the arterial wall.
- Risk factors for berry aneurysm are hemodynamic stress, hypertension and coarctation of aorta, autosomal dominant adult polycystic kidney disease, Marfan's syndrome and type 1 neurofibromatosis. Rupture of berry aneurysm is the most common cause of subarachnoid hemorrhage.

Clinical Features

Patient with berry aneurysm presents with history of severe headache, nuchal rigidity from irritation of the meninges. Aneurysm present on the anterior communicating artery may rupture into the third ventricle leading to massive intraventricular hemorrhage.

Complications

Cerebral infarction is the most common complication of berry aneurysm. Patient may develop hydrocephalus as a result of blockage by blood clot of the channels through

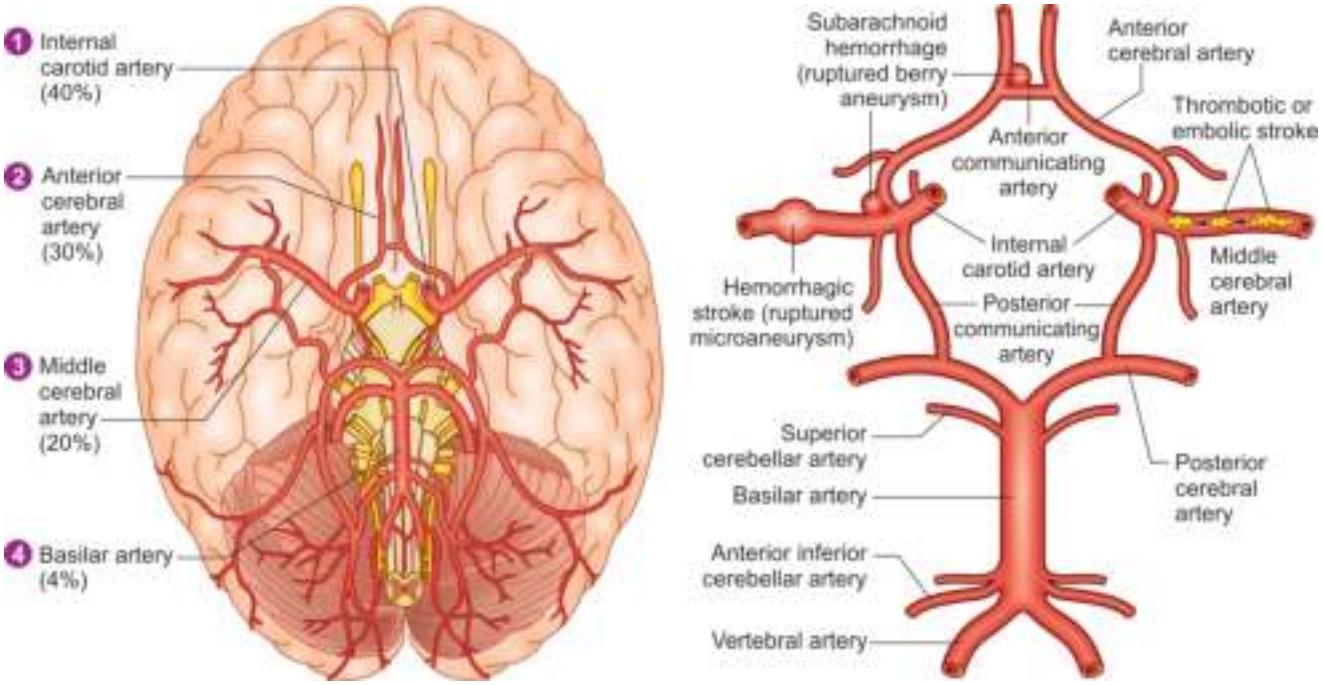


Fig. 30.42: Common sites of aneurysms formation in intracranial arteries linked to cardiovascular diseases.

Table 30.35 Sites for rupture of berry aneurysm in brain

Site of Aneurysm	Frequency
Junction of the internal carotid and the posterior communicating artery	40%
Junction of the anterior cerebral and anterior communicating artery	30%
Main bifurcation of the middle cerebral artery in the sylvian fissure	20%
Point where the basilar artery divides into the posterior cerebral artery	4%

which cerebrospinal fluid flows. Sites of rupture of berry aneurysm in brain are given in Table 30.35.

MYCOTIC ANEURYSM

Mycotic aneurysm is caused by infection of the artery wall. Most cases develop mycotic aneurysm due to impaction of infected emboli derived from cardiac vegetation occurring within first 5 weeks of development of infective endocarditis in 3–10% of patients.

- Causative organisms of infective endocarditis include pyogenic bacteria such as *Streptococcus pyogenes* and *Staphylococcus aureus*. True aneurysms result from fungal meningitis or fungal emboli from cardiac valves. Aspergillus is the species most commonly involved.
- Emboli from infective endocarditis may involve cerebral blood vessels, which result in hemorrhage in the basal ganglia, extending into the subarachnoid space.

ARTERIOVENOUS FISTULA

Arteriovenous (AV) fistulas are abnormal communications (direct shunt) between arteries and veins. Arteriography with thorostat dye establishes the diagnosis. It is often secondary to trauma.

- Penetrating knife injury is the most common cause of arteriovenous fistula.
- Most common sites are scalp, arm and leg vessels. Veins are arterialized.
- Patient develops increased circumference of the limbs. There is presence of bruits and thrills. Large arteriovenous fistulas reduce systemic resistance and bypass the microcirculation, hence increasing venous return to the heart and producing high output cardiac failure.

CAVERNOUS HEMANGIOMAS

Cavernous hemangiomas are located in the white matter of the brain. Patient presents in the 3rd to 5th decades with neurological symptoms such as epilepsy (38%), intracranial bleeding (23%), headache (28%) and focal neurological deficits.

TRANSIENT ISCHEMIC ATTACKS

Transient ischemic attacks are brief episodes of impaired neurologic function caused by a temporary disturbance of cerebral circulation due to breaking of small proportion of mural thrombus from underlying atheromatous plaque.

- Other causes of transient ischemic attacks are sudden decline in cardiac output and hypercoagulable states.
- Transient ischemic attacks are not associated with permanent damage, but are considered precursors to more serious occlusive events.

FAT EMBOLISM SYNDROME

Fat embolism is presence of emboli comprising of fat microglobules in the circulation. Fat embolism syndrome is most often a consequence of severe trauma with long bone fractures such as femur with abundant fatty marrow. It can also be seen with extensive trauma to fat laden tissues, burns, and very rarely with orthopedic procedures.

- On long bone fracture, bone marrow fatty fragments enter the circulation, lodge in small blood vessels of the lung, skin, kidneys, brain and other organs producing ischemia and hemorrhage. Fat embolic syndrome results in the clinical manifestations.
- Fat microglobules are converted into fatty acids, which damage vascular endothelium resulting in formation of platelet thrombi in areas of injury.
- Bronchoalveolar macrophages take up the fatty acids in fat embolism syndrome cases. Bronchoalveolar lavage should be done. Smears stained by fat stains demonstrate fat in the alveolar macrophages. Fat embolus in cerebral hemisphere is given in Fig. 30.43.

CLINICAL FEATURES

Patient develops potentially fatal fat embolism syndrome within 24–72 hours. Patient presents with pulmonary distress, cutaneous petechial hemorrhages, and various neurologic manifestations.

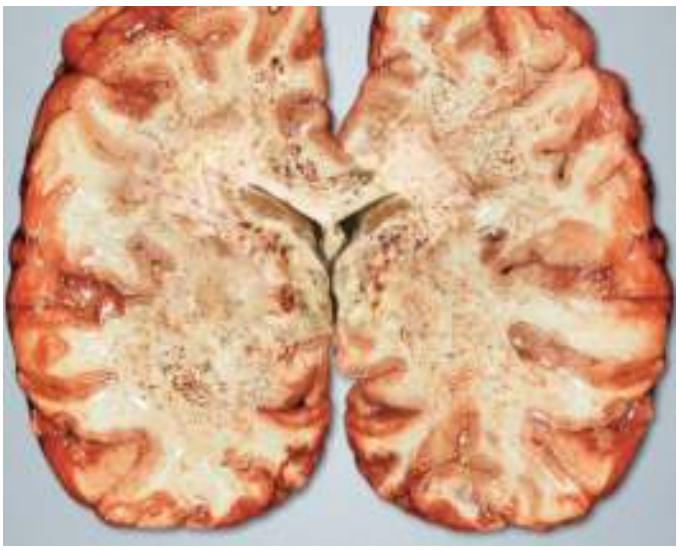


Fig. 30.43: Fat embolus in cerebral hemisphere. The patient was in a motorcycle accident and sustained multiple fractures of both legs. The fat emboli caused petechial hemorrhages in the white mater throughout the brain, resulting in coma and death. The macroscopic appearance of cerebral malaria is identical to this.

- **Pulmonary manifestations:** Patient develops dypnea and tachypnea is due to presence of fat microglobules in pulmonary capillaries causing hypoxemia.
- **Petechial hemorrhage:** Patient develops petechial hemorrhage over the chest and upper extremities due to thrombocytopenia from platelet adhesion to microglobules of fat.
- **Neurologic manifestations:** Numerous petechial hemorrhages are produced by fat emboli to the brain, particularly in white matter known as 'brain purpura'. Patient develops neurologic manifestations such as loss of consciousness, cerebral edema and herniation within a week in less than 10% of cases with fatal outcome.

HEAD INJURY

Head injuries are caused by penetrating wounds and nonpenetrating injuries. Penetrating wounds result in brain damage predisposing to infection of brain (meningitis, cerebral abscess). Brain injury caused by penetrating injuries producing impact at the site of injury is known as coup injury. Injury on the opposite side of the brain from the impact is known as contrecoup injury.

Contusions comprise both coup and cuntrecoup injuries. Location of epidural, subdural and intracerebral hematomas are shown in Fig. 30.44.

EPIDURAL HEMATOMA

An epidural hematoma is an arterial hemorrhage between the dura and the skull, which results from skull fracture of temporal bone and laceration of the middle meningeal artery. Patient presents with short period of consciousness (lucid interval), headache, vomiting, inability to move parts of the body followed by loss of consciousness and signs of cerebral compression. Complications of epidural hematoma may induce seizures. Epidural hematoma is shown in Fig. 30.45.

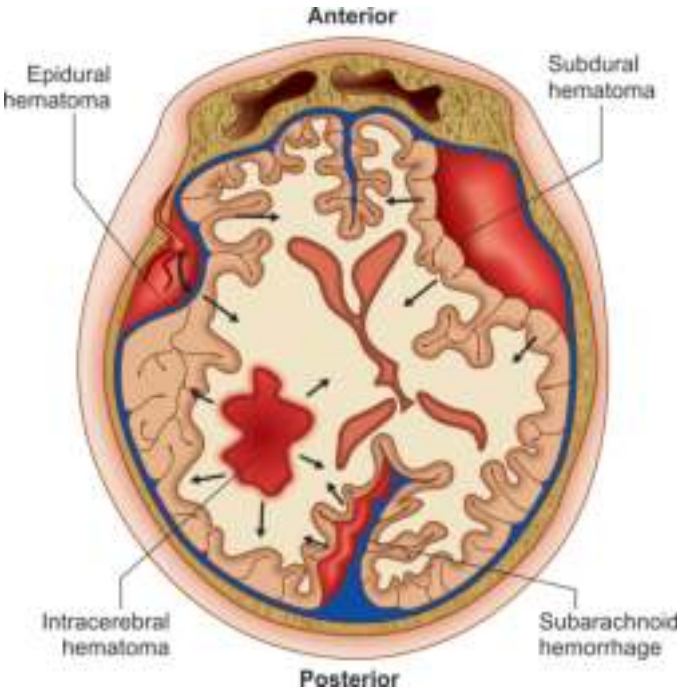


Fig. 30.44: Location of epidural, subdural and intracerebral hematomas.

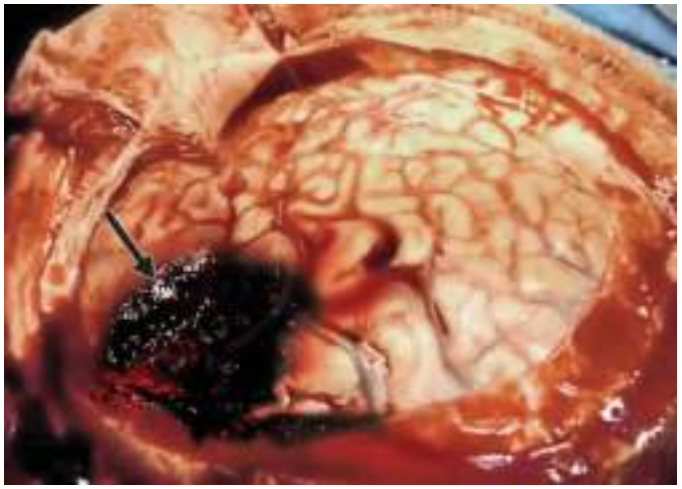


Fig. 30.45: Epidural hematoma. A blood clot is seen over the external surface of the dura. It occurs as a result of trauma to middle meningeal artery (arrow).

SUBDURAL HEMATOMA

A subdural hematoma is venous hemorrhage underneath the dura usually associated with traumatic brain injury. Bleeding in subdural space most often results from laceration of the bridging veins joining the cerebrum to venous sinuses within the dura. Patient presents with gradual signs of cerebral compression occurring hours, days, or weeks after injury. After cessation of bleeding, the resultant blood clot can slowly imbibe water, resulting in signs of increasing intracranial

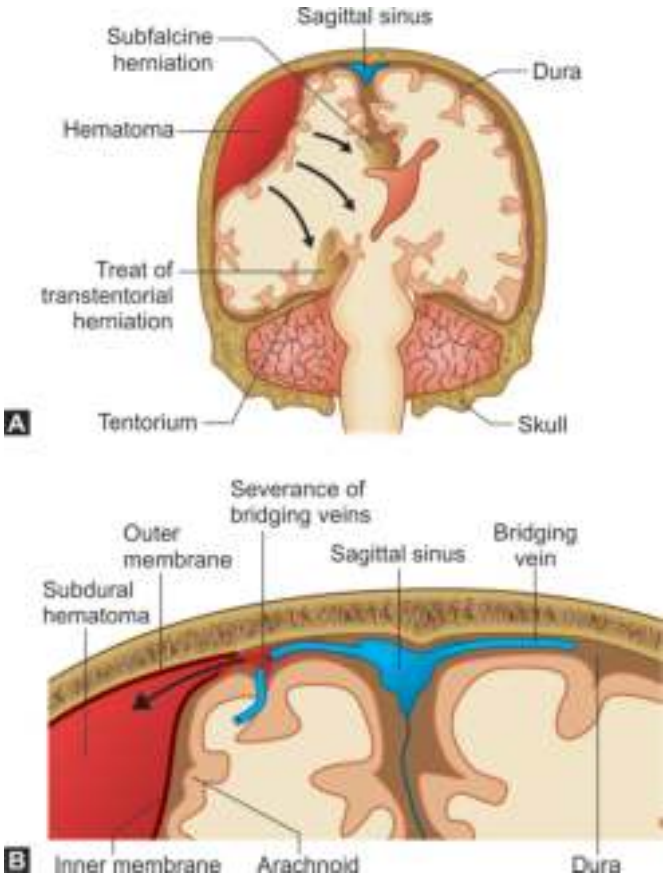


Fig. 30.46: Mechanism of bleeding in subdural hematoma.

pressure. Mechanism of bleeding in subdural hematoma is shown in Fig. 30.46A and B.

INTRACEREBRAL HEMORRHAGE

Intracerebral hemorrhage (ICH) is caused by bleeding within the brain tissue itself—a life-threatening type of cerebral stroke.

- Cerebral stroke occurs when the brain is deprived of oxygen and blood supply. Intracerebral hemorrhage is most commonly caused by hypertension, arteriovenous malformations, or head trauma (penetrating injury). Less common causes cerebral stroke are anticoagulant therapy, aneurysms, bleeding disorders (hemophilia, sickle cell anemia, disseminated intravascular coagulation, thrombocytopenia), highly vascular angiomas and metastatic tumors, amyloid angiopathy, alcohol and cocaine.
- Tiny arteries bring blood to areas deep inside the brain. Hypertension can cause thin-walled tiny arteries to rupture, and releasing blood into the brain tissue. Enclosed within the rigid skull, clotted blood and fluid buildup increases intracranial pressure that can crush the brain against the bone or cause it to shift

and herniate. Spillage of blood from the ruptured tiny blood vessel into the brain parenchyma is called cerebral stroke.

- **Laboratory diagnosis:** Patient is diagnosed by clinical history, family history, CT scan, MRI and angiography by administration of contrast medium into the blood-stream to view arteries of the brain. Diagnostic tests help to determine the source of bleeding.
- **Complications:** Cerebellar hemorrhage causing brain-stem compression and hydrocephalus are treated by surgical procedures (craniotomy, stereotactic clot aspiration) is to remove the intracerebral hematoma as soon as possible.
- **Management:** Patient with small hemorrhages and minimal neurological deficits are treated by medicines.

DEMYELINATING DISORDERS OF NERVOUS SYSTEM

MULTIPLE SCLEROSIS

Multiple sclerosis is the most common demyelinating disease of unknown etiology and characterized by destruction of myelin sheaths in the spinal cord and brain with relative preservation of axons. It is considered to be autoimmune disorder (probable viral etiology) due to increased incidence in HLA haplotypes (A3, B7, DR2, and DW2).

- Multiple sclerosis is associated with plaques scattered irregularly throughout the central nervous system.
- Common sites involved in multiple sclerosis are the optic nerve, brainstem, and paraventricular areas. CD4+ helper T cells, CD8+ cytotoxic T cells and macrophages infiltrate plaques; reactive gliosis occurs later. Peripheral nerves are not affected.
- Multiple sclerosis begins between 20 and 30 years of age, which most often affects men than women.
- Clinical course of multiple sclerosis is highly variable depending on the site of involvement. Patient may present with numbness, weakness of the lower extremities, visual disturbances and retrobulbar pain, sensory disturbances, and possible loss of bladder control. The classic Charcot triad (nystagmus, intention tremor, and scanning speech) is significant for diagnosis.
  - The classic CSF electrophoretic finding is that of multiple oligoclonal bands of immunoglobulin.
  - Clinical course of multiple sclerosis is characterized by exacerbations and remissions. Clinical course is progressive, leading to increasing disability.

ACUTE DISSEMINATED ENCEPHALOMYELITIS

Acute disseminated encephalomyelitis refers to wide-spread demyelination, which is preceded by viral illnesses such as measles, mumps, rubella, chickenpox, and is often known as post-infectious encephalitis. It may be due to delayed hypersensitivity reaction. The clinical course varies from a complete recovery to a fatal outcome.

GUILLAIN-BARRÉ SYNDROME

Guillain-Barré syndrome is an autoimmune disorder involving acute inflammation and destruction of myelin sheath of peripheral nerves by macrophages and lymphocytes. It is often preceded by viral infection, immunization, or allergic reactions. It most often occurs in young adults. Guillain-Barré syndrome has four variants. Acute inflammatory demyelinating polyradiculoneuropathy is the most common variant (90%).

- Acute motor axonal neuropathy occurs in children and has good prognosis. Acute motor sensory axonal neuropathy affects adults and has poor recovery rate. Miller Fisher syndrome is rare variant of Guillain-Barré syndrome involving only cranial nerves.
- Guillain-Barré syndrome usually starts in the extremities and moves toward the trunk, may result in respiratory failure. If cranial nerves are involved, patient has facial weakness, pain, and difficulty in speech's examination shows increased proteins concentration with only modest increase in cell count, which is an important diagnostic finding.
- Patients are administered immunomodulators to inhibit antibodies formation. Anticoagulants are given to reduce risk of deep vein thrombosis and pulmonary embolism. Nonsteroidal anti-inflammatory drugs are given to relieve pain.

HYDROCEPHALUS

Hydrocephalus denotes increased volume of cerebro-spinal fluid (CSF) within the cranial cavity. In infants, it is associated with (sometimes marked) enlargement of the skull. It is most often caused by obstruction to the CSF circulation by various mechanisms such as congenital malformations, inflammation, and tumors.

- Hydrocephalus can also result from overproduction of CSF by choroid plexus papilloma (rare). Intrauterine CMV infection is one of the agents

of TORCH syndrome, which crosses placenta to induce encephalitis in developing fetus during intrauterine life in proximity to the third ventricle and the aqueduct and the patient is prone to develop hydrocephalus. It can also occur as hydrocephalus *ex vacuo* without obstruction or increased CSF production in disorders characterized by decreased cerebral mass such as ischemic brain atrophy or advanced Alzheimer disease.

- Congenital hydrocephalus refers to an excessive amount of CSF and ventricular enlargement. It is caused by congenital atresia of the aqueduct of Sylvius.
- Histologic examination of the midbrain may disclose multiple atretic channels or an aqueduct narrowed by gliosis.

Clinical Pearls: Congenital Hydrocephalus

- **Internal hydrocephalus:** The increased volume of CSF is entirely within the ventricles.
- **External hydrocephalus:** The increased volume of CSF is confined to the subarachnoid space.
- **Communicating hydrocephalus:** Free flow of CSF occurs between the ventricles and the subarachnoid space.
- **Noncommunicating hydrocephalus:** Obstructed flow of CSF occurs from the ventricles to the subarachnoid space.

ARNOLD-CHIARI MALFORMATION

Arnold-Chiari malformation is characterized by downward displacement of the cerebellar tonsils and medulla through the foramen magnum. The cerebellar vermis is herniated below the level of the foramen magnum. Arnold-Chiari malformation results in pressure atrophy of displaced brain tissue. In addition, it causes hydrocephalus as a result of obstruction of the CSF outflow tract. Presence of a thoracolumbar meningomyelocele is almost always characteristic in this disorder.

CONGENITAL DISORDERS OF NERVOUS SYSTEM

NEURAL TUBE DEFECTS

Neural tube defects occur due to failure of closure of the neural tube linked with maternal folic acid deficiency. These neural defects can involve vertebrae or skull with or without involvement of the underlying meninges, spinal cord, or brain. In neural tube defects, an increase in fetoprotein in both the maternal serum and the amniotic fluid is noted. Maternal folic acid deficiency has been associated with an increased incidence of neural tube defects, and folic acid has, therefore, been approved for inclusion as a food supplement in commercial flour. Neural defects are shown in Fig. 30.47A to C.

SPINA BIFIDA

Spina bifida is neural tube defect, that occurs when the spine and spinal cord do not develop properly. Neural

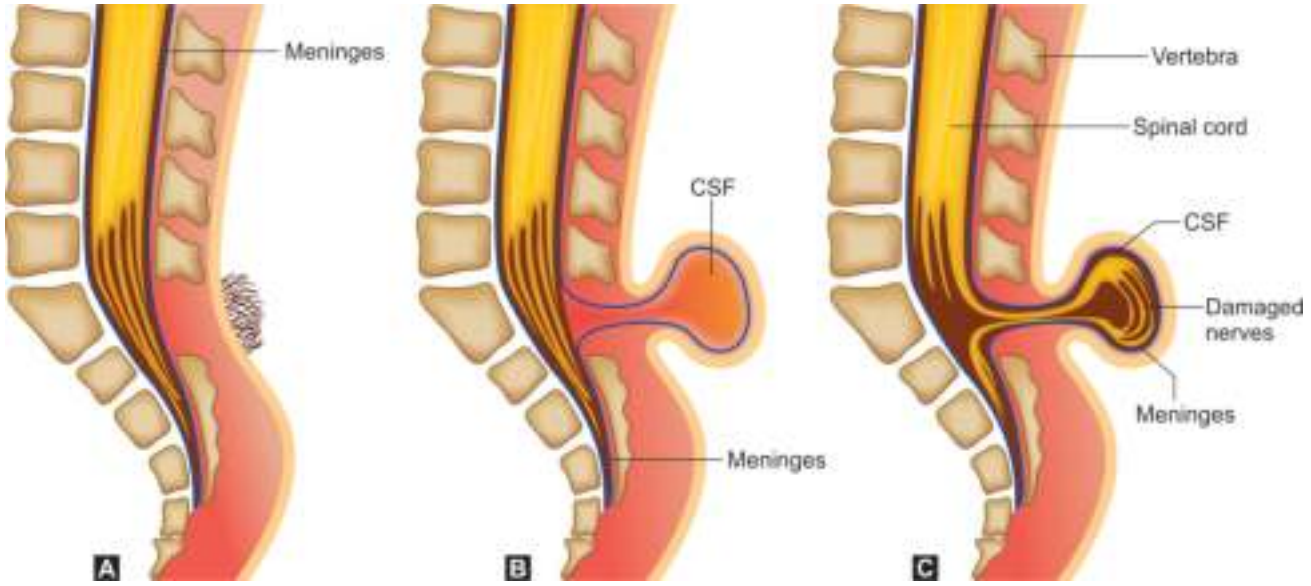


Fig. 30.47: Neural defects. (A) Spina bifida occulta, (B) meningocele, (C) myelomeningocele.

tube is the structure in a developing embryo that eventually becomes the baby's brain, spinal cord and the tissues that enclose them. Normally, the neural tube is formed during early pregnancy and closed by the 28th day after conception. In baby's spina bifida, a portion of the neural tube fails to close or develop properly causing defects in the spinal cord and in the bones of the spine. Spina bifida can range from mild to severe depending on the size of defects, location and complications. Surgery does not always resolve the defect.

- **Spina bifida occulta:** Spina bifida occulta is neural tube defect with no clinically apparent abnormalities. Vertebral arch defect is most often limited to one or two vertebrae.
- **Spina bifida cystica:** Spina bifida cystica is complicated by herniation of meninges through a defect.
- **Meningocele:** Meningocele is defect in which herniated membranes consist only of meninges, and spina bifida occulta does not manifest any apparent abnormalities.
- **Meningomyelocele:** Meningomyelocele is a neural tube defect in which portion of spinal cord along with meninges are protruded in the fluid filled herniated tissue. This neural tube defect exposes the spinal canal and causes the nerve roots to be entrapped.

ANENCEPHALY AND OTHER DEFECTS

Anencephaly is characterized by the absence of a major portion of the brain, skull and scalp, that occurs during embryonic development. It is a cephalic disorder, that results from neural tube defect. Anencephaly occurs when the rostral (head) end of the neural tube fails to close usually between the 23rd and 26th day following conception. It can also be caused by folic acid deficiency in pregnant women. Anencephaly can be diagnosed before birth through ultrasonography. The maternal serum α-fetoproteins screening and detailed fetal ultrasonography can be useful for screening to detect spina bifida.

- **Holoprosencephaly:** Holoprosencephaly is a microcephalic brain in which the interhemispheric fissure is absent. Infant rarely survives more than a day. Holoprosencephaly is characterized by microcephalic brain in which the interhemispheric fissure is absent. The horseshoe-shaped cerebral hemispheres have fused frontal poles, across, which the gyri show an irregular horizontal orientation.
- **Meroanencephaly:** Meroanencephaly is a rare form of anencephaly characterized by malformed cranial bones, a median cranial defect, and a cranial protrusion called area cerebrovasculosa, which denotes abnormal spongy, vascular tissue admixed with glial tissue ranging from thin membrane to large pseudocephalic tissue.
- **Hydromyelia:** Hydromyelia refers to an abnormal dilation of the central canal of the spinal cord, that creates a cavity in which cerebrospinal fluid can accumulate. As the cerebrospinal fluid builds up, and it can cause abnormal pressure on the spinal cord resulting in damage to nerve cells and their connections.
- **Syringomyelia:** Syringomyelia is a congenital malformation, in which a tubular cavitation (syrinx) extends for variable distances along the entire length of the spinal cord.
- **Rachischisis:** Rachischisis is a developmental birth defect involving the neural tube most often without a recognizable spinal cord. This anomaly occurs *in utero*, when the posterior neuropore of the neural tube fails to close by the 27th intrauterine day. As a consequence, the vertebrae overlying the open portion of the spinal cord do not develop properly and remain unfused and open, leaving the spinal cord exposed. Patient develops motor and sensory neurological deficits, recurrent infections and dysfunction of urinary bladder. Rachischisis usually occurs with anencephaly.
- **Polymicrogyria:** Polymicrogyria affects development of the human brain with formation of multiple small, irregular and randomly distributed gyri (microgyri) creating excessive folding of the brain leading to an abnormally thick cortex.
  - **Arrhinencephaly:** Arrhinencephaly is a congenital absence of rhinencephalon, the part of the brain that includes the olfactory bulbs and olfactory tracts.
  - **Lissencephaly:** Lissencephaly is a congenital disorder in which the cortical surface of the cerebral hemispheres is smooth or has imperfectly formed gyri. It occurs due to defective neuronal migration during the 12th to 24th weeks of gestation resulting in a lack of development of brain gyri and sulci.
  - **Pachygyria:** Pachygyria is a developmental disorder due to abnormal migration of neurons in the developing brain and nervous system. Brain has few broad and flat gyri.

AGENESIS OF CORPUS CALLOSUM

Agenesis of corpus callosum can be asymptomatic and is often found in association with other abnormalities.

TORCH COMPLEX

TORCH complex is a group of infections transmitted from the mother to the fetus.

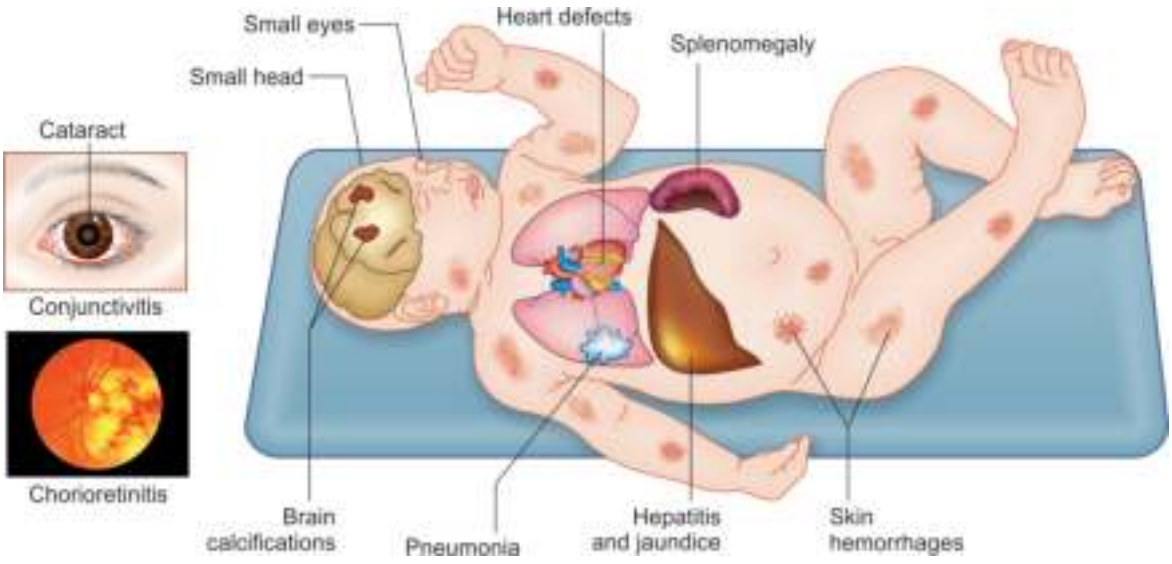


Fig. 30.48: TORCH complex infections. Fetuses infected in the first trimester by Toxoplasma, rubella, cytomegalovirus, herpesvirus or many other microbes have similar clinical findings as those illustrated in this figure.

- TORCH infections comprise *Toxoplasma gondii*, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV) infection including *Treponema pallidum*, human immunodeficiency virus, varicella, parvovirus B19 and enteroviruses. These infections can cause pregnancy wastage at any stage of gestation and TORCH complex syndrome is characterized by microcephaly, chorioretinitis, central nervous system (CNS) calcifications, petechial rash, hepatosplenomegaly, and thrombocytopenia.
- Intrauterine CMV infection is one of the agents of TORCH complex syndrome, which crosses placenta to induce encephalitis during intrauterine life in proximity to the third ventricle and the aqueduct. The patient is prone to develop hydrocephalus.
- Severe CMV infection can cause mental retardation, microcephaly, chorioretinitis, and hepatosplenomegaly; periventricular calcifications.
- For many of these pathogens of TORCH complex, treatment or prevention strategies are available. Early recognition, including prenatal screening, is key.
- Serological assays are important diagnostic tools. ELISA is highly specific and sensitive diagnostic technique of TORCH complex. Life-immunoassay is a new, cheaper and reliable diagnostic technique on the contrary on single panel strip. TORCH complex infections are shown in Fig. 30.48.

FETAL ALCOHOL SYNDROME

Fetal alcohol syndrome is the most common acquired cause of mental retardation in the United States associated with excessive maternal alcohol intake during pregnancy (Fig. 30.49). The common features

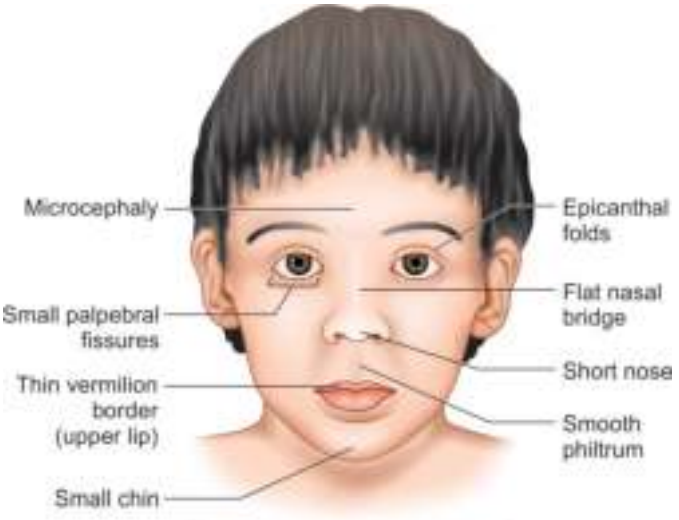


Fig. 30.49: Fetal alcohol syndrome.

of fetal alcohol syndrome include intrauterine growth retardation, mental retardation, neurologic impairment, microcephaly, and atrial septal defect. Children later suffer from mental retardation and minor facial dysmorphic features.

TUBEROUS SCLEROSIS SYNDROME

Tuberous sclerosis syndrome is an autosomal dominant disorder characterized by nodular proliferation of multinucleated atypical astrocytes forming tubers (small white nodules scattered in the cerebral cortex and periventricular areas), adenoma, sebaceoma of the skin, and angiomyolipoma of the kidney. Seizures and mental retardation beginning in infancy are characteristic.

Index

A

A subdural hematoma 2939
A whole chromosome paint (WCP) 1610
Abetalipoproteinemia 1951
Abnormal RBCs morphology and interpretations 1088
Abnormalities of placental attachment 2480
ABO and Hh blood group
  antigens 1425
  systems 1422
ABO antigens expression 1425
ABO blood group
  compatibility 458
  genetics 1423
ABO blood grouping 1466
ABO gene products 1424
ABO subgroups 1428
Abruptio placentae 2480
Acanthosis nigricans 2879
Accessory nipple 2545
Acetaminophen-induced hepatocellular injury 2058
Achalasia 1914
Achondroplasia 2716
Acid
  phosphatase stain 1189
  serum lysis test 1160
Acid-citrate-dextrose 1435
Acinic cell carcinoma 1896
Acne vulgaris 2877
Acquired
  atelectasis 1830
  atelectasis of lung 1830
  causes of hemolytic anemia 1110
  coagulation system disorders 1390
  disaccharidase deficiency 1950
  hypercholesterolemia 1646
  hypercoagulable
    state 274, 1410
    states associated with increased risk of thrombosis 278
  immunodeficiency syndrome 392
  myopathies 2797, 2805
  premalignant diseases 682
  sideroblastic anemia 1158
  thrombophilia (hypercoagulable state) 1410
Acral subungual and mucosal lentiginous melanoma 2851
Acrofacial vitiligo 2845
Acrosin 2323, 2374
Actinic keratosis 2857
Actinomadura madurae 1041
Actinomyces israelii 987
Actions of chemical mediators in type 1 hypersensitivity reaction 413
Activated
  B cells 344
  macrophages and their products 184
  partial thromboplastin time 1416
Activating tissue invasion and metastasis 742
Activation of leukocytes 144
Active immunity 313
Acute
  alcoholic steatohepatitis 2080
  appendicitis 1997
  bacterial
    endocarditis 1731
    prostatitis 2353
    thyroiditis 2592
  cervicitis 2390
  chest syndrome 1133
  cholecystitis 2123, 2132
  coronary syndrome 1697
  cystitis 2309
  diabetic complications 2567
  endocarditis 1732
  endometritis 2405
  erosive gastritis 1925
  erythroid leukemia (AML-M6, FAB) 1223
  fatty liver of pregnancy 2092
  fibrinous pericarditis 1708
  gouty arthritis 2727
  graft-versus-host disease (GVHD) 456
  icteric hepatitis 2048
  inflammation 131
  inflammatory response 130
  interstitial nephritis 2253
  iron poisoning 1096
  kidney disease 2190, 2242
  lymphangitis 1673
  lymphoblastic
    leukemia with 1193
      BCR-ABL1 rearrangement 1207
      E2A-PBX1 fusion gene 1208
      ETV6-RUNX1 rearrangements 1208
      iAMP21 alterations 1208
  mastitis 2488
  monoblastic/monocytic leukemia (AML-M5A, AML-M5B, FAB) 1222
  myelogenous leukemia 1211
    entities 1215
  myelogenous/lymphoblastic leukemias 647
  myelomonocytic leukemia (AMML/AML-M4 FAB) 1220
  necrotizing ulcerative gingivitis 1880
  occlusion of the vessel lumen 1655
  oophoritis 2429
  otitis media 1767
  pancreatitis 2132, 2138
  pericarditis 1744
  peritonitis 2002
  phase proteins and their functions 199
  phase reactants 1723
    synthesis 198
  promyelocytic leukemia (AML-M3, FAB) 1219
  pyelonephritis 2219, 2245, 2573
  respiratory distress syndrome 1815
  restrictive pulmonary diseases 1814
  rhinosinusitis 1757
  salpingitis 2426
  serous otitis media 1767
  sialadenitis 1886
  thyroiditis 2592
  transplant rejection by direct pathway 453
  tubular necrosis 2239
  vascular transplant rejection 452
  viral hepatitis 2036
Adamantinoma 2696
Adaptive cell-mediated immune response 313

Adaptive humoral immune response 312
Adaptive/specific immune response 306, 311, 331
Addison's disease 1880, 1881, 2635
Adenocarcinoma 1920, 1958
Adenocarcinoma of
extrahepatic bile ducts 2136
fallopian tubes 2428
lung 1860
Adenoid cystic carcinoma 1895
Adenoma gallbladder 2134
Adenoma of the nipple 2503
Adenomatoid odontogenic tumor 1902
Adenomatoid tumor 2349
Adenomatous polyposis coli tumor suppressor gene 759
Adenomyosis uteri 2408
Adenosquamous lung carcinoma 1864
ADH deficiency 2644
Adhesive pericarditis 1746, 1747
Adipocytic tumors 2743
Adjuvant 333
ADP receptors of platelets and their inhibition disorder 1377
Adrenal cortex 2631
hyperfunctioning 2633
Adrenal cortical insufficiency 2635
Adrenal gland
dysfunction associated myopathy 2806
metastasis 704
tuberculosis 997
Adrenal
hyperfunction 2633
medulla 2631
Adrenaline (epinephrine) 2631
Adrenocortical
adenoma 2640
carcinoma 2640
insufficiency 2635
Adrenogenital syndrome 2636
Adult
fibrosarcoma 632
Gaucher's disease (type 1) 2090
hemoglobin synthesis 1080
polycystic kidney disease 540, 2268
respiratory distress syndrome 1814
sclerosing rhabdomyosarcoma 2772
stem cells 204
T cell leukemia/lymphoma 1525
type of coarctation of the aorta 1755
Advanced gastric carcinoma 1937
Advantages of microarray analysis 1606
Adverse
blood transfusion reactions 1459
effects of immunosuppressive drugs 460
Afibrinogenemia 1401
Aflatoxin B1: natural carcinogenic agent 792
Aflatoxin-2,3-epoxide 793
African trypanosomiasis 1047
Agar gel electrophoresis 1632
Age of the patient with breast carcinoma 2530
Agenesis of corpus callosum 2942
Agonists promoting platelets aggregation 273
Agonists, promoters and inhibitors of platelets aggregation 273
Air embolism 290
Alagille syndrome 2031
Albinism 105, 2845
Albright syndrome 597
Albuminuria/proteinuria 2304
Alcian blue 1869
at pH 1557
Alcoholic (Laennec or nutritional) cirrhosis 2060, 2080
liver disease 2076
Alcohols 1550
Alder-Reilly anomaly 1070, 1175
Aliquoting of blood components 1450
Alkaline phosphatase 1558, 2020
Alkaptonuria 107, 557
Alkylation and DNA damage 505
Allergic (atopic) reactions 407
Allergic
polyps 1758
rhinitis (hay fever) 407
ALL-L1 (B cell or T cell) 1199
ALL-L2 (mostly T cell) 1199
ALL-L3 (mature B cell) 1199
Allogeneic hematopoietic stem cell transplantation 1278, 1351
Alloimmune hemolytic anemia 1144
Alport's syndrome 561, 2175, 2198
Alterations in autosome chromosome structure 515
Alveolar
rhabdomyosarcoma 1765, 2770
soft part sarcoma 634, 2789
Alzheimer's disease 10, 13
Ameloblastic
carcinoma 1904
fibroma 1902
fibrosarcoma 1904
odontoma 1903
Ameloblastoma (adamantinoma) 1903
Amenorrhea 2404
Amino acid metabolism disorders 557
AML-M0 with minimal differentiation 1215
AML-M1 without maturation 1218
Amniotic fluid aspiration syndrome in newborn 2478
Amniotic fluid embolism 291, 2478
Amoebic
colitis 1975
encephalitis 2894
liver abscess 2055
Amorphic 'O' gene 1424
Amorphous ground substance 219
Amphiarthroses joints 2723
Amyloid associated amyloidosis 464
Amyloid
light chain amyloidosis 461
stains 2091
Amyloidosis 460, 1881
liver 2091
of GIT 1909
Amylopectin 2089
Amyotrophic lateral sclerosis 10, 13
Anaerobic bacterial abscesses 1011
Anal
carcinoma 2001
fissure 2000
Anaphylactic shock 303
Anaphylaxis 408
Anaplasia 582
Anaplasmosis 1013
Anaplastic
astrocytoma, IDH-mutant 2912
large T cell lymphoma 1526
lymphoma kinase positive anaplastic large T cell lymphoma 1526
rhabdomyosarcoma 2769
seminoma 2333
thyroid carcinoma 2619
Anatomy and histology—prostate 2351
Anatomy and physiology of
female breasts 2482
female genital system 2372
Anatomy of
nervous system 2883
pancreas 2550
parathyroid glands 2625
penis 2363
thyroid gland 2586
Ancylostoma duodenale (nematode) 1050
Anderson disease (GSD type 4) 101, 549
Androblastoma of ovary 2466
Anemia of chronic disease 1160
Anemias 1084
Anencephaly and other defects 2942
Aneuploidy 513, 583, 713
Aneurysm
formation 1656
of sinus of Valsalva 1662
Aneurysmal bone cyst 1901, 2699
Aneurysms 1657, 1658
Angelman syndrome 572
Angina pectoris (>12 hours) 1699, 1710
Angiodysplasia of colorectal region 1978
Angiogenesis 181, 224
during tissue healing 738
in cancer susceptibility syndromes 741
Angioimmunoblastic T cell lymphoma 1523
Angiomatoid fibrous histiocytoma 2763
Angiomyofibroblastoma of vulva 2378
Angiosarcoma 632, 1682
breast 2527
liver 2117
of spleen 1536
Anionic sialoglycoproteins 2168
Anitschkow cells 198, 1717
Ankylosing spondylitis 2726
Ankyrin protein 1116
Anoikis resistance 871
Anorectal fistula 2000
Anovulatory cycle (failure of ovulation) 2405

Anterior pituitary gland 2641
  hypofunctioning 2642
Anthrax 986
Antibody-dependent cellular cytotoxicity 415
  disorders 415
Anticardiolipin antibodies 1410
Anticoagulant
  pathways 262
  preservatives used in blood bank 1435
Anticoagulant protein C system 262
Anticoagulant protein S system 262
Anticoagulants 1082
Anticoagulants used in laboratory 1083
Anti-D antibody (RhoGAM) administration 1432
Antidiuretic hormone (ADH) 2162
Anti-DNA antibody 2020
Anti-DNAase 1715
Anti-DNase-B/anti-hyaluronidase titer 1723
Antigen and immunogen 334
Antigen sequestration (ignorance) 438
Antigenicity 332
Antigen-responsive T cells and B cells 341
Antigens and antibodies in common ABO blood groups 1430
Antigens and immunogens 332
Antigens of Rh blood group system 1431
Antigens versus immunogens 335
Anti-HBc (both IgG and IgM) 2046
Anti-HBs antibody last marker in serum 2046
Antihuman globulin test (Coombs' test) 1151
Anti-inflammatory therapeutic agents 179
Anti-mitochondrial antibody 2020
Antineutrophilic cytoplasmic autoantibody-mediated glomerulonephritis 2184
Anti-nuclear antibody 2020
Antiphospholipid antibody syndrome 1410
Anti-receptor antibody-mediated type 2 hypersensitivity reaction 419
Antiretroviral therapy (ART) 397
Anti-smooth muscle antibody 2020
Antistreptococcal *O* titer (ASO titer) 1722
Antistreptolysin-*O* (ASO) 1715
Antistreptozyme test 1723
Antithrombin III 1365, 1408
  deficiency 277, 1408
  protein C and protein S deficiency 277
Antithrombotic and prothrombotic properties of vascular endothelium 253
α_1-Antitrypsin
  deficiency 103, 1810, 2088
  protein accumulation 103
Aortic
  coarctation 1755
  dissecting hematoma 1661
  dissection 271
  stenosis 1740
  valve insufficiency/regurgitation 1740
  valve stenosis 1740
APC/β-catenin signaling pathway 1992
Aphthous stomatitis 1881
Aplasia 34
Aplastic
  anemia 1155
  crisis 1133
Apocrine
  breast carcinoma 2525
  metaplasia 2491
Apolipoproteins (APO) and dyslipoproteinemias 1644
Apoptosis 19, 42, 70, 80
  during adult life 91
  during embryogenesis 90
  in diseases 92
  in health 90
  induced by viruses 92
  inhibits tumor growth 675
  regulatory
    genes 593, 709
    proteins 838
Apoptosis of
  cancer stem cells 93
  cells with accumulated misfolded proteins 93
  cells with damaged DNA 93
  neutrophils in acute inflammation 93
  parenchymal organ's cells due to obstruction in ducts 93
Apoptotic
  blebs 89
  bodies 90
Appendicular osteoporosis 2659
Apple green birefringence 109
Applications of
  chemiluminescence 1591
  immunohistochemistry 1561
  polarizing microscopy 1550
Applied anatomy of lungs 1779
Aqueduct of Sylvius 2885
Arachidonic acid metabolites synthesis by enzymatic pathways 164
Arias-Stella reaction 2407
Aromatase inhibitors 2545
Aromatic amines 791
Array
  CGH technique 1601
  comparative genomic hybridization technique 1601
Arrhythmias 1706
Arrhythmogenic right ventricular cardiomyopathy 1730
Arsenical keratosis 2857
Arterial and venous thrombi 283
Arterial supply and venous drainage of lymph node 1474
Arteriolosclerosis 1638, 1639
Arteriovenous fistula 1662
Arthritis of
  metabolic origin 2727
  probable autoimmune origin 2723
Arthroscopy 2723
Articular syndrome 75
Asabach-Merritt syndrome 1676
Asbestosis 1815, 1824
Asbestos-related pulmonary and extrapulmonary lesions 1825
Ascaris strongyloidiasis 1954
Ascending cholangitis and liver abscess 2057
Aschoff
  giant cells 197
  nodule 1717, 1721
Ascites 247, 2018, 2070
Ascomycota 1034
Aspartate aminotransferase (AST) 1713
Aspartylglucosaminuria 555
Aspergillus
  flavus 1036
  fumigatus 1036
  niger 1037
Asterixis (flapping tremors) 2016
Astrocytic tumors 2907
Asymptomatic
  inflammatory prostatitis 2353
  microscopic hematuria 2190
  proteinuria 2190
  silent gallbladder stones 2128
Ataxia-telangiectasia 390, 681, 695
Atelectasis neonatorum of lung (pulmonary collapse) 1830
Atelectasis or lung collapse 1830
Atelectasis: types 1830
Atheromatous plaque disruption 1655
Atherosclerosis 13, 98, 1676, 1691
Atherosclerotic
  aneurysm rupture 271
  aneurysms 1658, 1659
  plaques 75
Atopic dermatitis 408
ATP7B gene 2020, 2088
Atresia and stenosis of GIT 1908
Atrial
  myxoma 1751
  natriuretic factor 242, 2163
  septal defects 1754
  thrombi 1709
Atrophic gastritis 1925
Atrophy 17, 27
Attenuated familial adenomatous polyposis 691
Atypical and malignant glomus tumors 2777
Atypical
  alveolar hyperplasia 1864
  bronchopulmonary carcinoid tumor 645
  carcinoid tumor 1838
  chronic myelogenous leukemia, BCR-ABL1 negative 1254
  ductal hyperplasia 2493, 2507
Atypical
  fibroxanthoma 2762
  mitotic figure 619
Austin-Flint murmur 1660
Autocrine signaling 212
Autoimmune
  diseases 440
  enteropathy 1950
  hemolytic anemia 1141

hepatitis (lupoid hepatitis) 2053
myopathies 2806
oophoritis 2429
pancreatitis 2142
pericarditis 1709
sialadenitis 1887
Autoimmunity 306
Autologous and allogeneic hematopoietic stem cells 1456
Autologous HSC transplantation 1352
Autonomy 662
Autophagy 76
Autophagy and heterophagy 12
Autosomal dominant
disorders 539
inheritance 538
inherited cancer syndromes 688
Autosomal recessive
disorders 544
inheritance 538
Autosomal recessive inheritance linked to inherited cancer syndromes 695
Autosomes 480
Autosplenectomy 1133, 1532
AV node 1696
Avascular necrosis of femoral head 1133
Avidin–biotin complex (ABC) indirect method 1559
Axial osteoporosis 2659
Axillary
lymph nodes 2484, 2534
lymphatic drainage 2528
Azzopardi phenomenon 645

B

B cell activation and differentiation 367
B cell defects: primary immunodeficiency diseases 381
B cell immunologic tolerance in
central lymphoid organs 439
peripheral lymphoid organs 440
B cell lineage acute lymphoblastic leukemia with CRLF2 rearrangement and JAK2 kinase alterations 1209
B cell prolymphocytic leukemia 1310, 1521
B cells and humoral adaptive immune response 366
Babesiosis 1046
Bacterial and parasitic infections of liver 2053
Bacterial
carcinogenesis 806
contamination in stored blood 1449
infections 984
liver infections 2053
orchitis 2326
skin infections 2817
Balanced
chromosomal rearrangement 714
polymorphism 564
Ballooning degeneration of hepatocytes 2037, 2040
Band 3 (anion channel) protein 1116, 1118
Band 4.1 protein 1118
Band 4.2 protein (pallidin) 1116
Barium enema 1996
Barr body 522, 657
Barrett esophagus 683, 1913
linked to esophageal adenocarcinoma 686
Barriers for pathogens 317
Barriers in first and second line of defense in innate immunity 318
Bartholin cyst 2377
Basal cell
carcinoma of skin 635, 796, 2862
nevus syndrome 2865
Basal-like breast carcinoma 2518
Basaloid carcinoma 2002
Base analogue and intercalating agents: DNA damage 508
Basic laboratory assessment of metastases to brain 2907
Basidiomycota 1038
Basophilic stippling 1088
Basosquamous carcinoma 2865
Batten disease 29, 555
Bazex syndrome 796
BCL-1 cell cycle regulatory gene 2510
BCR-ABL1-like acute lymphoblastic leukemia 1208
Becker muscular dystrophy 560, 2801
Benign
epithelial breast tumors 2500
fibrous histiocytoma 2759
mesenchymal tumors 602, 613
metastasizing leiomyoma 2422
nephrosclerosis 2265
nodular hyperplasia of prostate gland 23, 2354
phyllodes tumor 2498
soft tissue tumors 2735
versus malignant tumors 606
Ber-EP4 monoclonal antibody 1869
Bernard-Soulier syndrome 1375, 1416
Berry aneurysm 1658, 1662, 2936
rupture 271
Berylliosis 1825
Bile
canalicular antibody 2020
canaliculi 2012
duct disorders 2092
juice: composition 2126
pigment 2305
salts 2305
Biliary
adenocarcinoma 2136
atresia 2031
cholestasis 2038
cirrhosis 2060, 2092
intraepithelial neoplasia 2135
Bilirubin
metabolism 2024
pigment 105
Biochemical
abnormalities in hepatic encephalopathy 2073
functions of vitamin A 972
genetic testing 577
parameter studies 1087
Biological therapy 2545
Biotin 981
Biphenotypic mixed lineage acute leukemias 1205
Birt-Hogg-Dubé syndrome 2282
Bite cells 1089, 1138, 1150
Blastomas-derived from immature embryonic cells resembling fetal analogue 604
Blastomyces dermatitidis 1037
Bleeding (purpura) associated with vascular disorders 1383, 1384
Bleeding and bruising 2075
Bleeding diathesis 269, 1367
associated with vascular disorders 1367
due to glycoprotein VI deficiency disorder 1377
Bleeding diathesis: diagnostic approach 1410
Bleeding time 1416
Blood clot
dissolution (lysis) 260, 1359
dissolution (thrombolysis) 267
retraction test 267, 1359, 1418
Blood collection, processing and storage 1434
Blood component
labeling 1446
transfusion 1454
Blood cross-matching (compatibility) test 1470
Blood
culture 1723
donation process 1434
examination 1415
group A 1428
group AB 1428
group B 1428
group O 1428
grouping by 1466
microplate method 1468
slide test 1467
test tube method 1467
plasma fractionation 1451
sampling 1151, 1632
supply of heart 1696
transfusion and blood component therapy 1452
transfusion
reactions 418
goals 1452
indications 1452
Blood transfusion-related
acute hemolytic reactions 1461
acute lung injury 1463
air embolism 1466
allergic reactions 1462
anaphylactic/anaphylactoid reactions 1462

circulatory overload 1463
clinical manifestations 1460
delayed hemolytic reactions 1462
dyspnea 1463
febrile nonhemolytic reactions 1462
graft-versus-host disease 1464
hemosiderosis 1465
hyperkalemia 1466
hypokalemia 1466
hypotension 1464
immune hemolytic reactions 1461
nonimmune hemolysis 1466
purpura 1465
sepsis 1464
thrombophlebitis 1466
Blood urea nitroge 2299
Blood vessels of brain 2884
Blue
bleb nevus syndrome 1676
bloaters tolerate hypoxia 1809
dome cysts 2491
nevus 2848
Boerhaave's syndrome 1916
Bombay phenotype and para-Bombay phenotype 1432
Bone and joint crises 1133
Bone lesions: location 2665
Bone marrow aspirate smear
examination 1090
preparation 1074
staining 1074
Bone marrow aspiration 1071
needles 1073
procedure 1074
sites 1074
Bone marrow
cellularity ranges for various groups 1075
examination 1107, 1117
failure syndromes 1154
HSCS harvesting 1348
hyperplasia at high altitude 22
stem cells 206
trephine biopsy 1076
fixation 1076
needles 1076
procedure 1076
processing technique 1077
section staining technique 1078
Bone metastasis 702
Bone scan (bone scintigraphy) 2536
Bone scintigraphy to evaluate bone metastases 2668
Bone specimen handling in surgical pathology 2729
Bone tumors: diagnostic approach 2661, 2663
Bony callus formation stage 236
Borderline
lepromatous leprosy 1004, 2821, 2823
mucinous tumor of ovary 2444
phyllodes tumor 2498
serous tumor of ovary 2441
tuberculoid leprosy 1004, 2821, 2823
tumors 616
tumors of low malignant potential 603
Bordetella pertussis (whooping cough) 987
Borrelia burgdorferi 1941
Borrelia burgdorferi-induced breast carcinogenesis 807
Botryoid rhabdomyosarcoma 2321, 2768
Botulism (*Clostridium botulinum*) 1012
Bouin's fluid 1551
Boutonnière deformity 2725
Bouveret's syndrome 2133
Bowel ischemia 40
necrosis (gangrene) 1657
Bowen disease (squamous cell carcinoma *in situ*) 2858
Bowen's disease of vulva 2378
Bowenoid papulosis of the genitalia 2858
Bowman's capsule 2168
Brain atrophy in
advancing age 28
disorders 29
Brain
infarct 297
metastasis 704
purpura 1833
tuberculoma—diagnosis 995
Branchial cyst 1774
BRCA1 and BRCA2 genes 759
BRCA1 tumor suppressor gene 2508
mutation 2506
BRCA2 gene 2147
BRCA2 tumor suppressor gene 2509
mutation 2506
Breast carcinoma 2503
index test 2543
histologic variants 2514
mode of spread, prognostic and predictive factors 2527, 2529
routes of metastasis 2527
Breast
ductal adenocarcinoma 622
hyperplasia during pregnancy 22
lump biopsy 2537
lumpectomy 2545
lumps and their anatomical correlation 2484
lumps: laboratory diagnosis 2534
sarcoidosis 2489
tuberculosis 2489
Brenner tumor of ovary 2450
Bridging necrosis 2018, 2038
of hepatocytes 2018
with periportal fibrosis 2040
Brilliant cresyl blue 1080, 1139
Broad categories of genes 590
Brodie abscess (chronic bone pyogenic abscess) 2654
Bromsulphthalein excretion 2030
Bronchi 1778
Bronchial
asthma 407, 409, 413, 1804, 1812
biopsy 1869
Bronchiectasis 1803, 1804, 1809, 1811
Bronchioles 1779
Bronchiolitis 1804
Bronchioloalveolar carcinoma 1864
Bronchoalveolar lavage 1869
Bronchopneumonia 1783, 1788, 1789
Bronchopulmonary
carcinoid tumor 1838
carcinoids 1837, 1838
Bronze diabetes 106
Brooke-Spiegler syndrome (familial cylindromatosis) 692
Brown atrophy of organs in
advancing age 28
disorders 29
Brown tumor of hyperparathyroidism 1905
Brushfield spots 519
Bruton's agammaglobulinemia 150
Bubble boy disease 388
Buccal choristoma 649
Budd-Chiari syndrome 247, 249, 2096
Buffy coat method to prepare platelet component 1439
Bullous pemphigoid 2843
Bundle of His and its branches 1696
Burkitt's lymphoma 659, 733, 1508, 1509
Burnt-out germ cell tumors of testis 2344
Burr cells 1089
Byssinosis 1827

C

'C peptide' analysis 2555
Cabot's ring 1088
Cachexia (irreversible wasting syndrome) 30
Cadherin-catenin linkage 220
Cadherins 5, 220
CagA 1923
effector molecules 1923
Calcific aortic stenosis 1741
Calcification in various tissues 2658
Calcifying
epithelial odontogenic tumor (Pindborg tumor) 1902
odontogenic cyst 1900
Calcitonin 2624, 2625
Calcium
absorption 2626
functions 2626
homeostasis 2163
metabolism: regulation 2625
oxalate and phosphate stones 2257
Calretinin 1764, 1869
CAM 5.2 1764
Campylobacter jejuni-induced colorectal carcinogenesis 807
Cancer
epidemiology and prevention strategies 676
immunosurveillance and immunoediting 910
stem cells evasion of immune destruction 751
stem cells to proliferate 596

Cancer-associated cachexia 898
Cancers in AIDS 399
Candida albicans (oral thrush) 1037, 1881, 2382
Candida albicans skin infection 2835
Candida glabrata 1037
Candida krusei 1037
Capillary
hemangioma 1675, 2873
microaneurysm 1663
Capsular drop 2219, 2573
Caput medusae around umbilicus 2070
Carbon tetrachloride-induced
liver injury 2059
hepatocellular injury 2059
Carbuncle 2377
Carcinoembryonic antigen 1587, 1869, 2624
Carcinogenesis 583, 765, 779
of skin cancers 2853
Carcinogenic agents linked to cancer 584
Carcinogenic agents: cellular interaction and carcinogenesis 779
Carcinogen-linked malignancies 779
Carcinoid
heart disease 1741, 1960
syndrome 1960
tumor 644, 1944, 1998, 2153
of ovary 2461
Carcinosarcoma 1898
of lung 1864
Cardiac
amyloidosis 468
arrhythmias 1706
cirrhosis 2096
hemolytic anemia 1148
hypertrophy and heart failure 1747
imaging techniques 1713
muscle stem cells 208
rhabdomyoma 1752
tamponade 1742
transplantation 1728
troponins (TnI and TnT) 1713
valves 1695, 1737
vegetations 1732
Cardiogenic
pulmonary edema 1836
shock 300, 1706
Cardiomyopathies 1725
Cardiovascular
manifestations 1656
syphilis 1010
Carney's syndrome 1961
Carnitine palmitoyltransferase deficiency associated myopathy 2805
Carnoy's fluid 1550
Caroli disease 2031, 2115
Carotid angiography 1775
Carotid body tumor 1775
Cartilaginous choristoma 649
Caseating granulomas 191
Caseous necrosis 62, 63
Caspase recruitment domain family member 19 1040
Castleman disease 1477
histologic variants 1478
Casts in urine 2305
Catalase 53
Catarrhal inflammation 177
Categories of
antigens 333
birefringence 1549
blood components 1452
cell adhesion molecules 219
gallbladder pigment stones 2130
gallstones 2126
IgE-mediated type 1 hypersensitivity reaction 407
shock 298
Caterpillar cells 1717
Caterpillar-like appearance 198
Cathepsin-D 1965
Cauliflower ear 1767
Causes of
acute tubular necrosis (ATN) 2240
anemia 1085
cholestasis 2027
disseminated intravascular coagulation 281
dysphagia 1909
edema 240
fat embolism 291
flea-bitten kidneys 2264
gastrointestinal tract bleeding 1909
hemoptysis 997
increased platelet count 1381
infarction depending on blockage of blood vessel 293
inherited and acquired abnormalities of hemostasis 268
malabsorption syndrome 1946
obstructive uropathy 2249
restrictive cardiomyopathy 1730
sea-blue histiocytosis 1344
secondary immunodeficiency diseases 392
Cavernous hemangioma 615, 2937
CD markers used for hematolymphoid neoplasms 1074
CD15 (LeuM1) 1869
CD4+ helper T cell and cell-mediated adaptive immune response 361
CD4+ helper T cells 342, 1167
CD4+ regulatory T cells 1167
CD4+, CD25+, FOXP3+ regulatory T (Treg) cells 343
CD8+ cytotoxic T cell and cell-mediated immune response 426
CD8+ cytotoxic T cells 342, 1168
CD8+ cytotoxic T cells and cell-mediated cytotoxicity 365
CD8+ cytotoxic T cells/NK cells (granzyme B/perforin) mediated pathway of apoptosis 87
cDNA microarray in breast cancer 2544
Celiac disease 1947
Cell
adhesion molecules 5, 219
cycle
checkpoints 665
in tissue homeostasis 208
grouping (forward grouping) 1467
junctions 6
polarity in tumors 654
regeneration: regulation 210
signaling 813
surface receptors 818
unit of life 4
Cell-associated pattern recognition receptors 326
Cell-derived chemical mediators 136, 161
Cell-mediated immune response 338
Cell-mediated immunity 306, 331
Cells
in the epidermis and dermis 2811
involved in chronic inflammation 181
of immune system 314
α Cells of islets of Langerhans 2551
Cellular adaptations 17
Cellular and molecular hallmarks of cancer 724
Cellular biology of aging 116
Cellular changes in acute inflammation 139
Cellular events: leukocytes recruitment to the sites of inflammation 138
Cellular
leiomyoma 2422
response to injurious stimuli 16
senescence 117
Cellulose acetate electrophoresis 1632
Cementifying fibroma 1905
Cementoma 1903
Central core (nucleocapsid) 2043
Central core myopathy 2804
Central
diabetes insipidus 2644
dogma of biology 497
giant cell granuloma 1905
Central nervous system
infections 2887
tumors 2896
venous thrombosis 285
Centriacinar emphysema 1807
Centrilobular
area 2011
necrosis 2015
Centromere 482
enumerating probes 1610
protein H 1869
Centronuclear myopathy 2804
Cerebral 2933
abscess 2895
edema 247
hemorrhage 2936
infarction 2934
malaria 2894
parenchymal compartment 2885
stroke 288
Cerebrospinal fluid
findings in tuberculous meningitis 999
flow/circulation 2885
synthesis 2885

Cerebrovascular
diseases 1657
disorders 2934
Ceruloplasmin 2020
Cerumic gland adenoma 1767
Cervical
carcinoma 2393
intraepithelial dysplasia 34
intraepithelial neoplasia 597, 2392
lymphadenopathy 1478, 1774
CFTr gene 1810
Chagas disease 1047
Chancroid (soft chancre) 987, 2365, 2383
Changes in autosome chromosome number 513
and structure 511
Changes in stored blood components 1447
Chaperone proteins 503
Chaperone-mediated autophagy 79
Characteristics of
an ideal tumor marker 913
leukocytes 1068
Charcot-Bouchard aneurysms 2936
Charcot-Leyden crystals 1070
in sputum 1812
Charcot-Marie-Tooth disease type 2 10
Chédiak-Higashi syndrome 7, 9, 151
CHEK2 tumor suppressor gene 2509
Chemical
agents induced cell injury 43
barriers 318
carcinogenesis 2853
carcinogenic agents 783
mediators with
chemotactic actions 156
combined vasoactive and chemotactic actions 156
nature of amyloid 461
Chemifluorescence 1590
technique 1592
Chemiluminescence technique 1591
Chemokines 183
involved in glomerulonephritis 2189
synthesis 170
Chemotaxis of leukocytes 143
Chemotherapy 933, 2545
Cherry hemangioma 2873
Cherubism 1905
Chest radiographs 1492
Chlamydia trachomatis 2383
Chlamydial infections 1013
Cholangiocarcinoma 2115
Cholangitis and liver abscess 2057
Cholecystitis 2123
Choledochal cyst 2125
Cholelithiasis (gallbladder stone disease) 2125
Cholelithiasis: clinical aspects 2128
Cholera 1976
Cholestasis 2024, 2025
Cholesteatoma 1768
Cholesterol and
bile acid homeostasis 122
its esters accumulation 98
Cholesterol
gallstones 2129
granuloma 1768
Cholesterolosis (strawberry gallbladder) 2124
Chondroblastoma 2674
Chondrocalcinosis (pseudogout) 2728
Chondrogenic tumors 2671
Chondroid syringoma 2869
Chondroma 615
Chondromas (enchondroma and periosteal chondroma) 2671
Chondromesenchymal hamartoma 2715
Chondromyxoid fibroma 2675
Chondrosarcoma 630
Chorioamnionitis 2479
Choriocarcinoma of
ovary 2456
testis 2342
Choriocarcinoma syndrome 2342
Choristoma 648, 649
Choroid plexus
papilloma 2920
tumors 2920
Chromatids 482
Chromatin 476
condensation 587
Chromoblastomycosis 1042
Chromogenic *in situ* hybridization 1607
Chromogranin A 1764
Chromophobe renal cell carcinoma 2283, 2290
Chromosomal abnormalities
and dysregulation of genes: mechanisms 711
in urothelial carcinoma 2317
Chromosomal disorders 511
caused by aneuploidy (numerical abnormalities) 513
Chromosomal translocations 516
Chromosome
banding techniques 483
deletion 719
duplication 720
inversion 720
structure 482
analysis (karyotyping test) 959
Chromosomes and alleles 1423
Chromothripsis (shattering of chromosomes) 720
Chronic
active
hepatitis 2039, 2040, 2048
myocarditis 1724
atrophic gastritis 1926
bronchitis 1803, 1804
carriers 2048
cervicitis 2390
cholecystitis 2124
cystitis 2309
endometritis and infertility 2406
gastritis 1925
type A/autoimmune gastritis 1926
type AB 1926
type B/non-autoimmune gastritis 1926
graft-versus-host disease 456
granulomatous disease of childhood 153, 1176, 1810
hepatitis 2048
hypoxic state and tumor invasion and metastasis 742
inflammation 180
inflammatory response 131
iron overload 1096
ischemic heart disease 1715
kidney disease 2191, 2228
laboratory findings and clinical manifestations 2230
kidney disease: medical/surgical management 2234
leg ulcers 1111, 1134
lobular hepatitis 2039
lymphocytic
leukemia 1142, 1301, 1514
leukemia/small lymphocytic lymphoma 1513, 1514
lymphoproliferative neoplasms 1300
mucocutaneous candidiasis 388
myelogenous leukemia (CML), BCR/ABL1 positive in
accelerated phase 1266
blast phase 1268
chronic phase 1263
myelogenous/lymphocytic leukemias 647
myelomonocytic leukemia 1249
myeloproliferative neoplasms 1255
narrowing of the vessel lumen 1655
neutrophilic leukemia 1279
obstructive pulmonary diseases 1803, 1841
obstructive sialadenitis 1887
otitis media 1768
pancreatitis 2141
pericarditis 1745
progressive external ophthalmoplegia 562
prostatitis 2353
pyelonephritis 2247
recurrent parotitis 1887
restrictive pulmonary diseases 1820
rhinosinusitis 1757
salpingitis 2427
sinusitis 1757
venous congestion
in liver 248, 2095
of lungs 250
viral hepatitis 2038
Churg-Strauss syndrome 1670
Chylothorax 1873
Chylous effusion 1674
Circulating immune complex mediated glomerulonephritis 2175
Cirrhosis 2009, 2059
Citrate
agar electrophoresis 1633
toxicity 1465

Citrate-phosphate-dextrose 1435
Citrate-phosphate-dextrose-adenine 1 1435, 1436
Cladosporium 1042
Classes of immunoglobulin 369
Classic
adenoma-colon carcinoma sequence with microsatellite instability signaling pathway 1994
carcinoid tumor 1838
dysgerminoma 2452
hemophilia 558
seminoma 2332
Classical Reed-Sternberg cells 1489, 1491
Classification of
acquired thrombocytopenias 1370
antibodies 1557
cholecystitis 2123
common benign and malignant tumors according to their origin 608
diabetes mellitus 2561
diabetic neuropathies 2582
endometrial stromal tumors 2420
immunodeficiency diseases 383
inherited hemolytic anemia 1108
inherited thrombocytopenias 1367
joints 2723
pathogenic fungi and diseases 1035
pneumonias 1783
RNA (ribonucleic acid) 502
Clear cell
adenocarcinoma of vagina 627, 2386
carcinoma in various anatomic sites 626
carcinoma of ovary 2448
chondrosarcoma 2680
endometrial carcinoma 2418
odontogenic carcinoma 1904
renal cell carcinoma 2283
sarcoma of kidney 2295
Cleft
lip 1908
palate 1880, 1908
Clinical
application of
hematopoietic growth factors 1064
liquid biopsy 946
complications of acute myocardial infarction 1711
consequences
in α_1-antitrypsin deficiency 2089
of cirrhosis 2073
course of
surface epithelial tumors of ovary 2438
mature cystic teratoma of ovary 2459
examination of breast lumps 2487
features and complications of Down syndrome (trisomy 21) 520
features of
fulminant acute liver failure 2016
liver disease 2016
rickets in children 976
findings in hemorrhagic disorders 270
manifestations
in chronic kidney disease 2232
in inflammation 201
of malabsorption syndrome 1947
Marfan syndrome 541
multiple myeloma 1326
oncology 896
pearls:
blood–brain barrier 2884
congenital hydrocephalus 2941
diagnostic approach of tuberculoma of the brain 2892
diagnostic criteria for systemic lupus erythematosus 2224
drug inhibitors of advanced glycation end products (AGES) 2571
Gilbert syndrome 2029
hereditary hyperbilirubinemia 2028
inflammatory bowel disease 1968
laboratory diagnosis of gallbladder stone disease 2134
procedural alert of glucose tolerance test 2584
protocols for reporting endometrial carcinoma on resection specimens 2419
risk factors for cervical carcinoma 2395
presentation of breast lumps 2486
significance of immunologic tolerance 438
significance of karyotyping 576
stages of transplant rejection 452
Clinically important
helminths 1043
protozoa 1043
type of edema 246
Cloacae 2655
Cloacae cortical defect in pyogenic osteomyelitis 2655
Clonal
anergy 438
hypereosinophilia 1294
hypereosinophilic syndrome 1169
selection in lymphoid organs 356
Closed
bone fracture 234
pneumothorax 1829
Clostridial infections 1011
Clostridium difficile 1952
Clot retraction 1364
test 1418
CML, BCR-ABL1 positive in chronic phase 1263
c-Myc oncogene (nuclear transcription factor) 2510
Coagulation
disorders in hepatic disease 1406
profile 1416
Coagulation system 1358, 1361, 1364
cascade 257, 261
disorders 1390
in hepatic disease 1406
inhibitors 1365
Coagulation/fibrinolysis system in fibrin-mediated glomerulonephritis 2188
Coagulative necrosis 59, 60
Coal pigment 108
Coal worker's pneumoconiosis 1815, 1822
Coarctation of aorta 1755
Cobblestone appearance of mucosa 1974
Cockayne's syndrome 120
Codman triangle 629
Codominant inheritance 539
Co-infection 2050, 2051
COL1A1 and COL1A2 genes 2717
Cold
agglutinin disease 1143
autoinflammatory syndrome 75
Collagen fibers 217
synthesis 181
Collagen IV 2168
Collagen vascular diseases 1827
Collagenous
colitis 1977
sprue 1948
Collecting duct carcinoma of Bellini 2283, 2293
Collection of
granulocytes by apheresis 1443
plasma by apheresis 1442
platelets by apheresis 1442
red blood cells by apheresis 1441
Colloid (mucinous) carcinoma of breast 2521
Colloid breast carcinoma 2521
Colloidal iron 1557
Colonoscopy 2004
Colony forming units 1058
Color changes in CSF suggestive of disease states 2888
Color Doppler ultrasonography 2536
Colorectal
adenocarcinoma 624
carcinoma 624, 1986
Combined gallstones 2127
Comedocarcinoma 2514
Common
causes of chronic inflammation 180
neoplasms and precursor lesions of gallbladder 2134
sites of arterial and venous thrombosis in antiphospholipid syndrome 278
variable immunodeficiency disease 387, 680, 1950
warts (verrucous vulgaris) 2828
Commonly used stains in histopathology 1554–1556
Comparative genome hybridization 579
technique 1600
Comparison between B cells and T cells 1071
Comparison of
autosomes and sex chromosomes 481
cardiac vegetations 1736, 1737
cardiogenic and noncardiogenic edema 246

constructive and facultative heterochromatin 484
different hypersensitivity reactions 405
DNA replication and gene transcription 495
euchromatin and heterochromatin 484
exudate and transudate 245
hepatotropic viruses 2052
mitosis and meiosis 483
purine and pyrimidine 478
secretors and nonsecretors 1430
structure of DNA and RNA 479
transcription and translation in human cell 496
X and Y chromosomes in XX and YY genotypes 486
Compartment syndrome 237
Compensated
liver cirrhosis 2066
shock stage 304
Compensatory
hyperplasia of hepatocytes following partial resection 24
hypertrophy in residual cardiac myocytes 27
mechanisms 1112
of hemolytic anemia 1112
Competitive ELISA 1615
Complement system 159, 322
actions 161, 323
activation 322
cascade and its actions 162
defects: primary immunodeficiency diseases 391
dysregulation-related glomerulopathy and other disorders 2187
pathways 322
mediated glomerulonephritis 2185
Complement-mediated with increased susceptibility to phagocytosis of target cells 417
Complete
bone fracture 234
hydatidiform mole 2470
mutagenic disorders 535
Complex
adenoma 2503
fibroadenoma of breast 2503
odontoma 1903
Complications of
atherosclerosis 1652
bone fracture healing 237
hypertension 1689
infective endocarditis 1732
lobar pneumonia 1788
Paget's disease of bone 2660
peptic ulcer disease 1930
pulmonary tuberculosis 1800
pyogenic
meningitis 2892
osteomyelitis 2655
tubercular osteomyelitis 2656
urolithiasis 2261
wound healing and tissue repair 231
Components of
flow cytometer 1593
immune system 313
innate immune system 316
Composite pheochromocytoma 2637
Composition of
bile juice 2126
edema fluid 243
Compound odontoma 1903
Compression atelectasis 1830
Compressive atelectasis of lung 1830
Compton scattering 798
Computed tomography scan 2536
Concentric cardiac hypertrophy 1691
Concept: central dogma of molecular biology 497
Conditions associated with
increased erythropoietin production 1063
splenomegaly 1532
Conduction system 1696
of heart 1696
Condyloma acuminatum 2365
Congenital
amegakaryocytic thrombocytopenia 1377
anomalies of penis and miscellaneous disorders 2363
disorder of
N-linked glycosylation (glycosidase 1 deficiency) and resistance to viral infections 567
nervous system 2941
dyserythroblastopoietic anemia 1159
type 1 1159
type 2 1160
type 3 1160
epulis 1880
fiber-type disproportion myopathy 2804
heart diseases and blood vessel malformations 1752
hypoplasia of breast 2545
hypothyroidism 2592
lactase deficiency 1950
lip pits 1880
lymphedema 243
melanocytic nevi 2846
myopathy 2804
nephrotic syndrome 2175
pyloric stenosis 1944
rubella syndrome 1028
syphilis 1010
Congestive
cardiomyopathy 1726
heart failure 1708
splenomegaly 251, 1531
Congo red stain 469
Conjugated bilirubin 105
Connective tissue mucins 1869
Consequences and clinical manifestations of atherosclerosis 1656
Constituents of approved blood bank anticoagulant preservative solutions 1435
Constitutional chromosome 3 translocation associated renal cell carcinoma 2283
Constrictive pericarditis 1746, 1747
Contact dermatitis 427
Contractile apparatus associated proteins 1587
Contraction atelectasis of lung 1830
Conventional
adult fibrosarcoma and other histologic variants 2757
alveolar rhabdomyosarcoma 2770
chondrosarcoma (grades 1, 2, 3) 2676, 2677
chordoma 2701
embryonal rhabdomyosarcoma 2768, 2769
hepatocellular carcinoma 628, 2105
intramedullary
chondroblastic osteosarcoma 2687
chondroblastoma-like osteosarcoma 2687
clear cell osteosarcoma 2687
fibroblastic osteosarcoma 2687
giant cell-rich osteosarcoma 2687
malignant fibrohistiocytoma-like osteosarcoma 2687
osteoblastic osteosarcoma 2687
osteosarcoma 2683
small cell-rich osteosarcoma 2687
osteosarcoma 629
Coombs' test 1117, 1142, 1151
Co-operation in immune cells against antigens 359
Copy number variations 569
Cor bovinum 1660
Core biopsy 2537
Cori disease (GSD type 3) 101, 549
Corona penetrating enzyme 2374
Corpus luteal cyst 2429
Corticotroph adenoma 2648
Cotton dye sirius red F3B 470
Councilman bodies 2015
in hepatocytes in viral hepatitis 92
Countercurrent mechanism and urine concentration 2164
Cowden
disease 623, 2506
syndrome 1982
Coxsackievirus infection 1027
CpG dinucleotide islands hypermethylation 765
Craniopharyngioma 2649
C-reactive protein analysis 1647
Creatine kinase-MB (CK-MB) 1712
Creatinine clearance 2299
Creeping fibrosis 2091
CREST syndrome 448, 1915
Cretinism (thyroid dwarf) 2593
Cribriform carcinoma of breast 2526
Cri-du-chat (5p minus, cat-cry) syndrome 522
Crigler-Najjar syndrome 2029
type I 2029
type II 2029
Crohn's disease 430, 1973

Cronkhite-Canada syndrome 1983
Cross-matching 459
Cryoglobulinemia vasculitis (ANCA negative) 1671
Cryoprecipitate 1457
  preparation 1449
Cryopreservation of spermatozoa 2324
Cryostat section technique 1546
Cryosupernatant plasma 1457
Cryptococcus neoformans 1038
Cryptorchidism 2351
Crystal
  violet stains 470
  in urine 2305
CSF findings in normal persons 2888
Curschmann spirals 1812
Cushing's syndrome 2633
Cutaneous
  angiosarcoma of breast 2527
  dermatophytes and lesions 1036
  dermatophytosis 1034
  leishmaniasis 1046
  metastases 2877
  wound healing and tissue repair by 227
    primary intention 228
    secondary intention 229
Cyanocobalamin (vitamin B_{12}) 981
Cyclin-dependent kinases in cell cycle 210
Cyclins and cyclin-dependent kinase 2510
Cyclooxygenase
  enzyme deficiency disorder 1377
  pathway 164
Cylindrical aneurysm 1658
Cystic
  diseases of kidney 2268
  disorders 1887
  fibrosis 546, 1757, 1810
    and resistance to cholera 565
    of pancreas 2144
  warts 2829
Cysticercosis 1049
  tapeworm (cestode) 1049
Cystine stones 2260
Cystinosis 556
Cystinuria 2254
Cystitis glandularis and/or cystitis cystica 2310
Cytochrome c 666
Cytogenetic analysis techniques 1190, 1191, 1596, 1598
Cytogenetic testing 575
Cytokeratins 1585, 1586
Cytokine
  milieu and effects in cancer-associated cachexia 899
  receptors 148
Cytokines 353, 1650
  synthesis 168
  principal source and cellular targets 324
Cytologic examination 924
Cytomegalovirus involving organs 1032
Cytoskeleton 6
Cytotoxic
  antibody-mediated glomerulonephritis 2184
  granule-dependent signaling pathway of natural killer cells 321

D

Dabska-type papillary intralymphatic angioendothelioma and retiform hemangioendothelioma 1679
DAF (decay accelerating factor) 1146
Damage-associated molecular patterns 144, 147, 330
Dane particle 2043
DDAVP (desmopressin acetate) 560, 1396
Deamination 504
Death receptor signaling pathway of natural killer cells 321
Decalcification 1552
Decalcifying solutions 1552
Decay accelerating factor (DAF) (CD55) 1146
Decline in immune responses and immunologic memory 375
Decompensated liver cirrhosis 2066
Decompression sickness (Caisson disease) 291
Decreased erythropoiesis 1723
Decreased pH in stored blood 1448
Dedifferentiated
  chondrosarcoma 2678
  liposarcoma 2747
Deep
  fibromatosis (desmoid type) 2754
  palmoplantar warts 2829
  soft tissue leiomyoma 2765
  vein thrombosis 247, 283, 285, 1672
    in legs 284
    thrombophlebitis 1672
Deeply located hemangioma including angiomatosis 1678
Defect in primary intention of wound healing 24
Defects in
  blood vessels 271
  chemotaxis of leukocytes 390
  hemostasis 269
  opsonization of injurious agents 390
  phagocytosis 391
  primary hemostasis 270
  adhesion of leukocytes 390
  primary hemostasis (platelet defects and von Willebrand disease) 270
Defects of secondary hemostasis (coagulation factor defects) 270
Deficiency of complement system cascade linked to diseases in human beings 391
Degenerative
  disease of joints 2726
Delayed wound healing 1395
Deletion in chromosome 515
Delphin lymph nodes 1478
Dematiaceous fungi 1040
Dementia with Lewy bodies 10
Demyelinating disorders of nervous system 2940
Denaturing of protein 503
Dendritic cells 320, 347
Dengue
  hemorrhagic fever 1380
  virus infection 1028
Dense granules 257
Dental
  caries 1898
  procedures 1395
Dentigerous cyst 1900
Dentigerous cyst (follicular cyst) 1900
Denys-Drash syndrome 694
Deoxyadenosylcobalamin 1101
Depurination 504
Deranged bilirubin metabolism in various disorders 2027
Dermatitis herpetiformis (herpes simplex virus II skin infection) 2829
Dermatofibroma 2871
Dermatofibrosarcoma protuberans 2756, 2871
Dermatomyositis 448
Dermatopathy 2598
Dermatophytosis (superficial fungal skin infections) 2831
Dermis and subcutaneous fat: tumors 2871
Dermis and subcutaneous tissue 2813
Dermoepidermal melanoma 2851
Desmin 1587
  myopathy 10
Desmoid tumor 233
Desmoplastic fibroma of bone 2702
Desmosomes 6
Desquamative interstitial pneumonia 1791
Detection of
  carrier 1397
  systems of immunohistochemistry technique 1558
Development of
  B cells and T cells 354
  kidneys 2156
  lymphoid organs 349
  urinary bladder and urethra 2156
Developmental
  anomalies of GIT 1908
  dysplasia of the hip 568
  stages of hematopoietic stem cells 1474
Dexamethasone suppression test 2649
Diabetes insipidus 2644
Diabetes mellitus 1646, 2560
  complications: cellular metabolic signaling pathways 2569
Diabetes mellitus type 2 567
Diabetes mellitus: classification 2560
Diabetes mellitus-related
  autoantibodies analysis 2585
  nonalcoholic fatty liver disease 2583

Diabetic
  autonomic nervous system neuropathy 2582
  capsular drop in Bowman's capsule 2577
  cardiovascular disease 2573
  cataract 2581
  cerebral stroke 2574
  fibrin cap in glomerular subendothelial region 2578
  glaucoma 2580
  glomerulopathy 2575
  glomerulosclerosis 2573
  infections 2582
  ketoacidosis 2567
  macrovascular complications 2573
  macular edema 2580
  mastopathy 2490
  mastopathy/lymphocytic mastitis 2583
  mononeuropathy (focal neuropathy) 2582
  nephropathy 2215, 2575
  neuropathy 2581
  ocular complications 2578, 2579
  peripheral
    neuropathy 2581
    vascular disease 2575
  proximal neuropathy (diabetic radiculopathy) 2582
  renal
    hyaline arteriolosclerosis 2577
    papillary necrosis 2576
  retinal ganglion cells neuropathy 2581
  retinopathy pathogenesis 2579
  tubulointerstitial disease 2578
  vascular disease (ANCA negative) 1671
Diagnostic approach and treatment of platelet disorders 1380
Diagnostic approach of
  anemia 1085, 1088
  cancer 915
  genetic disorders 573
  lung tumors 1868
Diagnostic criteria for blood transfusion-related acute lung injury 1463
Diagnostic criteria of
  celiac disease 1947
  Hodgkin's disease 1488, 1489
Diagnostic
  modalities of colon carcinoma 1996
  significance of bone marrow aspiration 1073
Diagonal ear lobe crease 1767
Diarrhea 1951
Diarthroses synovial joints 2723
Diastolic heart failure 1751
2,6-Dichlorophenolindophenol test 1154
Dietary requirements for red blood cell production 1064
Differences between
  Arthus reaction and serum sickness 423
  B cells and T cells 340
  T cell-dependent and T cell-independent antigens 337
  trisomy and monosomy 515
Differential diagnosis of jaundice 2032
Diffuse
  alveolar damage 1819
  astrocytoma, IDH-mutant 2910
  fibrosis of visceral layer 1875
  gastric carcinoma (linitis plastica) 627
  glomerulonephritis 2219
  glomerulosclerosis 2575
  infiltrative type gastric adenocarcinoma 1939
  large B cell lymphoma 1508, 1510, 1962
  leptomeningeal glioneuronal tumor 2924
  malignant mesothelioma 1875, 2003
  midline glioma, histone H3K27M-mutant 2911
  neurofibroma 2928
  proliferative glomerulonephritis 2178
  pulmonary fibrosis 1841
  renal cortical necrosis secondary to shock 2262
DiGeorge syndrome (congenital thymic hypoplasia) 387, 522, 1537, 2629
Digital
  examination 2004
  mammography 2534
Digits formation in fetus 91
Dimorphic fungi 1037
Dipalmitoylphosphatidylcholine 1779
Diphtheria 986
Direct
  bilirubin 2026
  conjugate-labeled antibody method for immunostaining 1558
  Coombs' test 1142, 1151
  DNA damage reversal mechanism 509
  ELISA 1612
    and indirect ELISA 1614
  immunofluorescence microscopy 1589
Direct-acting
  chemical carcinogenic agents associated human cancers 788
  genotoxic chemical carcinogenic agents 788
Disaccharidase deficiency 1950
Diseases caused by antibody-dependent cellular cytotoxicity (ADCC) mechanism in type 2 hypersensitivity reaction 416
Diseases caused by
  anti-receptor antibody-mediated type 2 hypersensitivity reaction 419
  type 3 hypersensitivity reaction 423
Disorders associated with
  elevated serum α-fetoprotein (AFP) 2339
  mitochondrial DNA mutations 563
Disorders causing myelophthisic anemia 1162
Disorders of
  adrenal glands 2631
  anus and perianal region 1999
  appendix 1997
  autosomal chromosome 518
  bones 2652
  cardiac valves 1737
  cytoskeleton 7
  ear 1766
  endocardium 1731
  endocrine pancreas 2550
  esophagus 1911
  exocrine pancreas 2137
  fallopian tubes 2426
  gallbladder 2122
  jaw 1898
  joints 2723
  large intestine 1963
  larynx 1769
  liver 2009
  myocardium 1724
  neck 1772
  oral cavity 1879
  ovaries 2428
  parathyroid glands 2625
  penis 2363
  pericardium 1741
  peritoneum 2002
  pituitary gland 2641
  prostate gland 2351
  salivary glands 1886
  sex chromosome 523
  small intestine 1945
  testes 2322
  thyroid gland 2586
  urinary bladder 2308
  uterine
    cervix 2389
    endometrium 2403
    myometrium 2421
  vagina 2381
  vulva 2375
  X and Y chromosomes 487
Disorders related to single-gene mutation 535
Disruption of hormones inactivation 2074
Dissecting hematoma 1658, 1661, 1691
Disseminated intravascular coagulation 201, 279, 1379, 1403
  in amniotic fluid embolism 292
Dissolution of thrombus 281
Distal (paraseptal) emphysema 1807
Distal adenocarcinomas 2137
Distant metastases of breast carcinoma 2529
Distinguishing
  features of benign and malignant tumors 651
  self- and nonself-antigen 451
Distribution of colorectal carcinoma in decreasing frequency 1989
Diversion colitis 1977
Diverticular disorders 1966
Diverticulitis 1967
Diverticulosis 1966
Diverticulum 1966
DNA
  content analysis of breast carcinoma of the tumor 2532

damage
repair systems 503
mechanisms 504
ladder
pattern 94
remains the hallmark of apoptosis 94
laddering 733
ligase enzyme 491
methylation 765
mismatch repair genes 709
ploidy and cytogenetics in
embryonal carcinoma 2339
seminomas 2333
Sertoli cell tumors 2345
teratomas 2342
yolk sac tumors 2340
polymerase 491, 2043
probe design 1607
probes 1610
repair 673, 1587
enzymes 673
genes 591, 593
mechanisms 508
pathways 673
protects cellular genomes from genotoxic stresses 673
proteins maintaining genome integrity 839
replication 487, 588
process 491
sequencing methods 1616
vaccine 377
viruses 1030
Donor selection and exclusion criteria 1434
Double contrast barium enema 2006
Down syndrome (trisomy 21) 518
Dressler's syndrome 1709
Drug allergy 408
Drug-induced
autoimmune hemolytic anemia or thrombocytopenia 417
hemolytic anemia 1143
hepatitis 2031
hypersensitivity vasculitis 424
interstitial pulmonary fibrosis 1826
thrombocytopenia 1378
vasculitides involving skin (ANCA positive) 1670
Drugs and toxins induced
hepatocellular injury 2057
liver injury 2057
Drugs
associated with interstitial fibrosis 1826
used in leukapheresis 1443
Dry gangrene 65
Dubin-Johnson syndrome 2029
Duchenne muscular dystrophy 560, 2801
Duct ectasia 2491
Ductal carcinoma *in situ* of breast 2514
Ductal carcinoma: basic concept 2514
Ductal epithelial hyperplasia 2493
Ductus arteriosus 1755
Duodenal
atresia 1956
peptic ulcer 1927
Duplication of
chromosome 515
small intestine 1946
Durck's granulomas 195
D-xylose tests 2006
Dysbiosis of gut microbiota
and colorectal carcinogenesis 778
mechanism 778
Dyserythropoiesis 1066
Dysfibrinogenemia 1401
Dysfibrinolysis 277
Dysfunctional
leukocytes 7, 8
uterine bleeding 2405
Dysgerminoma
of ovary 2451
with syncytiotrophoblastic element 2452
with yolk sac tumor 2452
Dysmenorrhea 2405
Dysphagia 1911
Dysplasia in 17, 32
bronchial epithelium 34
mesenchymal tissues 597
Dysplastic
melanocytic nevus 2847
nodules in liver 2104
Dystrophic calcification 67, 113, 1656

E

Early
clinical manifestations in myxedema 2595
congenital syphilis 1010
gastric carcinoma 1937
phase reactions in bronchial asthma 1813
pre-B cell lineage acute lymphoblastic leukemia 1204
T-precursor acute lymphoblastic leukemia 1209
Ebola virus and Marburg virus infection 1029
E-cadherin cell adhesion molecule analysis 2541
E-cadherins 2510
Eccrine spiradenoma 2869
Echinococcus granulosus 2055
Ectopic
kidney 2273
pregnancy 2477
thyroid tissue 2620
Eczematous dermatitis 2818
Edible shellfish 2041
EDNRB gene 1964
Edward syndrome (trisomy 18) 520
EGFR gene amplification 723
Ehlers-Danlos
disease 231
syndrome 542, 1385, 1661
Elastin fibers 218
Electrocardiogram 1723
Electron microscope (EM) 928, 1547
Electronic based blood cross-matching 1472
Electrophoresis 1632
Embolism 289
Embryology of
female breasts 2482
female genital system 2372
pancreas 2550
Embryonal carcinoma of
ovary 2454
testis 2337
rhabdomyosarcoma 2768
tumor with multilayered rosettes 2921
Embryonic
hemoglobin 1080
stem cell therapy 206
stem cells 204
Emery-Dreifuss muscular dystrophy 2803
Emigration 140
Emphysema 1803, 1804, 1806
Empty sella syndrome 2644
Empyema 1872
gallbladder 2123
Enabling replicative potential (immortality) 735
Encephalitis 2894
Endocarditis in 1719
intravenous drug abuse 1734
prosthetic valves 1734
rheumatic heart disease 1735
Endocarditis of carcinoid syndrome 1736
Endocervical polyp 2391
Endocrine glands atrophy in disorders 29
Endocrine glands–proteins associated amyloidosis 466
Endocrine
myopathies 2806
signaling 213
Endocytosis of thyroglobulin colloid into thyroid follicular cells 2588
Endogenous
antigens 334
melanin pigmentation 1880
Endometrial
carcinoma 2411
hyperplasia 23, 2406
during menstrual cycle 21
polyp 2409
stromal tumors 2420
Endometrioid
adenocarcinoma 2446
carcinoma of ovary 2447
Endometriosis (chocolate cysts) 2431
Endophytic papilloma 1760
Endophytic/inverted urothelial neoplasia 2312
Endoplasmic reticulum 12
Endoplasmic reticulum hypertrophy in liver by
excessive alcohol consumption 27
phenobarbital 27
Endopredict breast cancer prognostic test 2544

Endoscopic retrograde cholangiography (ERCP) 2134
Enhancer and silencer regulatory region of DNA 528
Enneking staging system 2671
Enterochromaffin cells 1959
Enzyme-linked immunosorbent assay 1612
  test 414
  technique 1612
Enzymes involved in DNA
  nucleotide–excision repair 761
  DNA replication 490
Enzymopathies and resistance to malaria 565
Eosinophil 320, 1166
Eosinophilia 1168
Eosinophilic
  cystitis 2310
  gastroenteritis 1950
  granuloma 1346, 1827
  myocarditis 1724
  peritonitis 2003
  pneumonia 1827
Eosinophils 187, 1069
Ependymal tumors 2919
Ependymoma with RELA fusion positive 2919
Epidemic typhus 1013
Epidemiology of cancer 675
Epidermal
  growth factor and transforming growth factor-α 210
  growth factor receptor (EGFR) 2533
  inclusion cyst 234
  keratinous cysts 2879
Epidermis 2810
  tumors 2857
Epidermodysplasia verruciformis 2858
Epidermoid carcinoma 2001
Epidermolysis bullosa simplex 10
Epidural
  hematoma 2938
  space 2885
Epigenetic alterations 595
Epigenetics in cancer 867
Epiphrenic diverticulum 1914
Epispadias 2363
Epithelial
  membrane antigen 1586
  precancerous lesions 2857
Epithelial–mesenchymal transition 744, 746, 870
Epithelioid
  glioblastoma 2917
  hemangioendothelioma 632, 1680
  leiomyoma 2422
  malignant peripheral nerve sheath tumor 2930
  sarcoma 2788
  trophoblastic tumor 2475
Epsilon aminocaproic acid (EACA) 1396
Epstein-Barr virus
  induced human cancers 803
  specific antibodies assay 1178
Equine gait 1394
ER and HER2/neu negative molecular pathway 2513
ER, PR and HER2/neu triple negative basal-like molecular pathway 2513
Erdheim-Chester disease 2714
Erysipelothricosis-erysipeloid 986
Erythema
  marginatum 1722
  multiforme 1881, 2841
  nodosum leprosum 1006, 2825
Erythropoiesis 1064
Erythropoietin 1061
  secreting tumors 1064
E-selectin (CD62E) 141
Esophageal
  achalasia 1914
  diverticulum 1914
  squamous cell carcinoma 1918
  stricture 1915
  varices 1672, 1915, 2070
Esophagus 1911
Essential (primary) hypertension 568, 1685
Essential
  morphologic features of chronic inflammation 180
  thrombocythemia 1281, 1382
Estrogen and progesterone receptors analysis 2539
Estrogen positive molecular pathway (luminal A and luminal B) 2512
Ethylenediamine tetra-acetic acid and trisodium citrate 1083
Ethylenediaminetetra-acetic acid 1083
Etiological classification of glomerular diseases 2173
Etiology of large hepatic vein thrombosis 2097
Etiopathogenesis 13, 1403, 1788
Euglobulin lysis time 1419
Evaluation of basic hematologic studies 1087
Evasion of apoptosis (programmed cell death) 731
Ewing sarcoma 2693
Examples of
  frameshift mutations associated disorders 533
  virulence factors produced by pathogenic bacteria 984
Excessive fibrosis 232
Exchange of gases 1779
Executioner/effectors caspases 89
Exocrine and endocrine pancreatic tumors 2145
Exocrine glands atrophy in cystic fibrosis 30
Exocrine tumors 2145
Exocytosis of T4 and T3 and binding to TBG in blood stream 2589
Exogenous
  antigens 334
  pigments 108
Exons 527
Exons (coding sequence) and introns (noncoding sequence) in gene 526
Exophthalmos 2598
Exophytic
  carcinomas 1937
  papillomas 1760
Expansion of myocardial infarct area 1710
Expiratory reserve volume 1782
Exposure
  of host to antigens 358
  to chemicals 1841
  to ionizing radiation 1841
Exstrophy of urinary bladder 2311
Extending inflammation in biliary tree 2133
External
  genital organs 2373
  hemorrhoids 2000, 2070
Extracellular
  fluid compartment 315
  matrix 215, 1651
    functions 216
Extracorpuscular hemolytic anemia 1108
Extrahepatic
  bile duct adenocarcinoma 2136
  biliary atresia/stenosis 2031
  jaundice 2033
  obstructive jaundice 2033
Extramedullary hematopoiesis 1122
Extranodal marginal zone
  B cell lymphoma (MALT lymphoma) 1507
  lymphoma 1519
  NK cell/T cell lymphoma 1527
Extrapulmonary tuberculosis 994
  in order of frequency 998
Extraskeletal
  mesenchymal chondrosarcoma 2795
  myxoid chondrosarcoma 2794
  osteosarcoma 2793
Extravasation of cancer stem cells 747
Extravascular hemolysis 1111
  involving RBCs destruction 1112
Extrinsic
  death receptor pathway of apoptosis 82
  pathway of
    blood coagulation system 258
Exudate 139, 244
  formation 137
Exudative (invasive) diarrhea 1952
  effusions 1873
  pharyngitis 1881

F

Fabry disease 561
Facial clefts 1879
Facioscapulohumeral muscular dystrophy 2802
Factor I (fibrinogen) deficiency 1400
Factor II (prothrombin) deficiency 1401
Factor IX concentrate 1458
Factor V (labile factor) deficiency 1401
Factor V Leiden (RS6025) 1409

Factor V Leiden mutation 275
Factor VII (stable factor) deficiency 1402
Factor VIII (antihemophilic globulin) 1392
Factor VIII assay 1396
Factor VIII concentrate 1457
Factor X (Stuart-Prower factor) deficiency 1402
Factor XI (plasma thromboplastin antecedent) deficiency 1402
Factor XII 261
Factor XIII (fibrin stabilizing factor) deficiency 1403
Factors
adversely affecting bone fracture healing 237
affecting wound healing and tissue repair 229
favoring and reducing iron absorption 1091
influencing infarct formation 292
removed by therapeutic plasmapheresis 1443
Familial
adenomatous polyposis coli 1936, 1985
atypical multiple mole melanoma syndrome 694
cancer syndrome 583
focal segmental glomerulosclerosis 2176
glomerular diseases 2175
hemophagocytic lymphohistiocytosis 681
Hodgkin's disease 697
hypercholesterolemia 540, 1644, 1645
medulloblastoma 696
melanoma 694
membranoproliferative glomerulonephritis 2176
nondiabetic end stage renal disease 2176
pancreatic carcinoma 697
testicular germ cell tumors 697
Family of cell surface adhesion molecules 220
Fanconi
anemia 696, 1156, 1162
syndrome 2254
Fasting blood glucose 2584
Fat
embolic syndrome 237
embolism syndrome 289, 1832, 2938
necrosis in 63, 2489
breast 64
female breast 63
pancreas 64
necrosis of breast 2489
stains 2080
Fate of thrombus 281
Fat-soluble vitamins 970
Fatty
change (steatosis) in liver 97, 2077
cysts 2079
Features of regulated cell death (RCD) 69
Feces examination 2006
Felty syndrome 2726
Female breasts 2482
uterus and vaginal atrophy 28
Female pseudohermaphrodite 572
Fenton reaction 54, 55
Fernandez reaction 1008, 2826
Ferritin 1092
Ferroportin 1092
Ferroptosis 76
Fertilization and development of embryo 2373
Fetal
alcohol syndrome 2943
ejection reflex 2374
hemoglobin 1080
estimation 1125
synthesis 1122
FGF3 (fibroblast growth factor 3) 2510
Fiberoptic bronchoscopy 1869
Fibrin
cap 2573
clot formation 260
degradation products 273
glue and platelet gel 1458
Fibrinogen degradation products (FDPs) and D-dimer tests: interpretation 1419
Fibrinoid necrosis 66, 1689
of arterioles 112, 1639
Fibrinolytic system 158, 262, 1364
Fibrinous inflammation 175
Fibrinous or serofibrinous pericarditis 1744
Fibrinous pericarditis 1692
with or without effusion 1708
Fibroadenoma 2494
of breast 2494
Fibroblast growth factor 3 (FGF-3) 2510
Fibroblastic growth factor-β 211
Fibroblastic/myofibroblastic tumors and other lesions 2750
Fibroblasts 189, 2168
Fibrocongestive splenomegaly 1531, 1750
Fibrocystic disease of breast 2490
histopathologic changes 2491
Fibroepithelial polyp 2879
Fibroepithelioma of Pinkus 2865
Fibrogenic bone tumors 2702
Fibrohistiocytic tumors 2759
Fibrolamellar hepatocellular carcinoma 2112
Fibroma
group lesions 1905
of ovary 2465
Fibromatosis colli 2751
Fibronectin 212, 217
Fibro-osseous pseudotumor of digits 2792
Fibrosarcoma 632
Fibrosis of peritoneum 2003
Fibrous dysplasia 1905
of bone 1880, 2710
Fibrovascular polyps 1916
FIGLU test 1104
Filiform warts 2829
Filopodia 140, 871
Finding eligible donor–recipient match 458
Fine needle aspiration cytology 2537, 2623
Firm adhesion of leukocytes 141
First line of defense 316
Fistulous tract 1974
Fixatives 1550
Flapping tremors 2073
Flat
mucosal lesions 1937
urothelial lesions with atypia 2313
warts 2829
Flexible sigmoidoscopy 2004
Flow cytometer
Flow cytometry 95, 1117, 1154, 1592, 1593
technique 1154, 1593
test 1117
Fluorescein 1558
Fluorescein labeled proaerolysin (FLAER) test 1146, 1147
Fluorescence *in situ* hybridization 578, 1606
Fluorescent spot test 1139
Focal
hepatic necrosis 2015
liver necrosis 2015
segmental glomerulosclerosis 2178, 2206
Folate (folic acid) 981
Folic acid 1100
in DNA synthesis 1102
Follicular
cyst 2429
lymphoma 1508, 1515
thyroid
adenoma 2605
carcinoma 2610
Food
allergens 408
poisoning 1952
Fordyce's granules 1880
Foregut and hindgut carcinoid tumors 644
Foreign body
giant cell 196
granulomas 195
Formaldehyde 1550
Forms of thyroid dysgenesis 2621
Four classes of normal cellular regulatory genes 590
Fragile X syndrome 562
Frameshift mutations associated disorders 533
Freckle (ephelis) 2846
Free immunoglobulin functions 373
Freezing, thawing and deglycerolizing red blood cells 1451
French Federation of Comprehensive Cancer Center Grading System of soft tissue tumors 2737
Frequency of breast lumps 2484
Fresh frozen plasma 560, 1457
preparation 1449
Frozen section technique 1545, 2538
Frozen/cryostat section technique 2538
Fulminant
acute hepatitis with massive hepatic necrosis 2048
hepatitis 2042
myocarditis 1724

Functional (acalculous) gallbladder disease 2128
Functional categories of
chemical mediators 156
mutations in tumor suppressor genes and associated cancers 847
Functional
disorders of spleen 1531
residual capacity 1782
zones of liver acinus (lobule) 2011
Functions of
B cells and T cells 354
erythropoietin 1063
the liver and clinical manifestations due to altered functions 2013
skin 2810
Fundic gland polyps 1931
Fungal
diseases 1034
infections 1759
meningitis 2893
sinusitis 1759
skin infections 2831
Fusiform aneurysm 1658

G

G protein-coupled transmembrane receptors 328
G6PD deficiency 1137
Galactosemia 551
Galectin 3 2511, 2533
Galectin 3 encoded by
4GALS3 gene 2511
lGALS3 proto-oncogene 2533
Gallbladder 2122, 2137
adenocarcinoma 2135
adenoma 2134
black pigment stones 2130
brown pigment stones 2130
cholesterol stones 2129
mixed stones 2129
pigment stones 1111, 1134, 2130
Gallbladder stones
categories 2126
complications 2131
Gallstone ileus 1957, 2132
Gamna-Gandy bodies 251
Ganglion cyst 2729
Gangrene 64
Gangrenous inflammation 177
Gap junction channels 6
Gardner's syndrome 1985
Gardnerella vaginalis 2382
Gas gangrene (*Clostridium perfringens*) 1012
Gasdermin-D 74
Gastric
adenocarcinoma 1932
adenomatous polyps 1931
carcinoma 1932, 1940
hamartomatous polyps 1931
hyperplastic polyps 1931
MALT lymphoma 1941
neuroendocrine tumors 1944
peptic ulcer 1927
polyps 1931
Gastrinoma 2152
Gastroesophageal reflux disease 1912
Gastrointestinal stromal tumor 1921, 1942, 1960, 2773
(benign/low-grade and malignant) 2773
Gastrointestinal tract
amyloidosis 468
bleeding 1395
disorders: diagnostic approach 2004
Gatekeeper tumor suppressor genes 592
Gaucher's
cells 1534, 2090
disease 552, 1090, 1339, 2090
involving spleen 1534
type 1 to type 3 1340
Gel electrophoresis technique 1634
Gene
amplification 721
expression, transcription, translation and protein folding 492
expression: DNA replication, transcription and translation 588
mutation 529, 593, 1138
structure 526, 587
General
characteristics of transformed cancer stem cells 596
features of RNA (ribonucleic acid) 502
Generalized lymphadenopathy 1479
Generation and differentiation of stem cells 205
Genes
dysregulation and carcinogenesis 721
involved in human DNA repair 506
on Y chromosomes involved in spermatogenesis 486
Genetic and epigenetic alterations and cancers 781
Genetic
code 497
disorders and
breast carcinoma 2506
faulty DNA repair systems 511
disorder conferring resistance to infectious diseases 564
factors in celiac disease 1948
polymorphism 568
predisposition 1715
Genetics and molecular biology 479
Genital herpes simplex virus 2 (HSV-2) infection 2376
Genital leiomyoma 2765
Genitourinary system bleeding 1394
Genome imprinting disorders 572
Genomic instability 593, 695, 2853
Genomic instability (mutated phenotype) 756
Geographic and racial/ethnic differences in cancer epidemiology 678
Germ cell tumors of
nervous system 2931
ovary 2451
testis 2328
Germline gene mutation 593, 595
Gestational
choriocarcinoma 2473
diabetes mellitus 2478, 2566
complications in neonates 2567
maternal complications 2566
trophoblastic neoplastic disorders 2473
trophoblastic disease 2468
GFAP 2484
Ghon's focus in lung 1799
Giant
Aschoff's bodies 1718
axonal neuropathy 10
Giant cell tumor of bone (osteoclastoma) 2698
Giant cells in various pathologic disorders 197
Giant juvenile
fibroadenoma of breast 2496
hypertrophy of breast 2545
Giardiasis 1953
Gilbert's syndrome 2029
Gingival cyst 1900
Giuffrè-Tsukahara syndrome 562
Glandular
epispadias 2363
odontogenic cyst 1900
Glanzmann disease (thrombasthenia) 1375, 1416
Gleason grading of prostatic adenocarcinoma 2362
Gleason
grading system 2360
staging system 2360
Glial
choristoma 649
fibrillary acidic protein 2484
Glisson's capsule 2017
Global
glomerulosclerosis 2178
impact of cancer 676
Globin chain electrophoresis 1154
Globulomaxillary cyst 1900
Glomerular
basement membrane 2168
diseases of kidney 2166
Glomerular diseases: clinical syndromes 2190
Glomerular
filtration 2163
filtration rate assessment 2298
permeability barriers 2169
rate 2169
Glomeruloid hemangioma 615
Glomerulonephritis:
molecular pathogenesis 2178
terminology 2170
Glomerulus structure 2166
Glomus tumor 2776
Glucagonoma 2152
Glucocerebrosidase 1534

Glucocorticoid-remediable aldosteronism 1686, 2634
Glucose homeostasis 122
Glucose-6-phosphate dehydrogenase deficiency disorder 1137
Glucose-6-phosphate dehydrogenase deficiency and pyruvate kinase deficiency disorders 1137
Glucose-6-phosphate dehydrogenase enzyme deficiency 565
Glucosuria 2304
Glucuronyl transferase 2025
Glutamine metabolism 751
Glutaminolysis 751
Glutamyl transpeptidase 2020
Glutaraldehyde 1550
Glutathione 53
Glycocalyx 1362
Glycogen storage disease 99, 547, 2089
GSD type 10 551
GSD type 9 550
GSD type 8 561
due to hepatic phosphorylase kinase deficiency 102
type 0 100, 547
type 10 102
type 4 2089
type 5 101
type 9 102
Glycolytic/glucose auto-oxidation pathway and formation of advanced glycation end products (AGEs) 2571
Glycoprotein IIb/IIIa 1362
Glycosaminoglycans (GAGs) 219
Glycosylated hemoglobin (HbA1C)
analysis 1646
for routine glycemic control 2584
GM2 gangliosidosis type 2 (Sandhoff disease) 555
Goblet cell carcinoid 1959
Goiter 2600
Goitrogenic drugs 2600
Goltz syndrome 562
Gomori methenamine stain 2057
Gonadoblastoma of ovary 2467
Gonococcal arthritis 2728
Good immunogens versus poor immunogens 334
Goodpasture syndrome 417, 450, 1827, 2201
Gorlin syndrome 796
Gout 8
Gouty
arthritis 2727
tophus 2727
Grading and staging 2530
system of breast carcinoma 2530
systems 927
Grading of iron stores on bone marrow aspirate 1076
Graft-versus-host disease 455
Granular cell tumor 1921, 2779
Granulation tissue formation 223
α-Granules 257
Granulocyte component 1440
Granuloma
inguinale 987, 2364, 2376
pyogenicum 1885, 2881
Granulomatous
cystitis 2309
gastritis 1926
inflammation 180, 189
of spleen 1533
type 4 hypersensitivity reaction 429
liver abscess 2054
lymphadenitis 1476
mastitis 2489
orchitis 2326
serositis 2002
Granulosa cell tumor of ovary 2463
Granzyme 321, 751
Granzyme B 88
Graves' disease 246, 419, 450, 2596
Gray platelet syndrome (platelet α-granule defect) 1376
Grocott's methenamine silver stain 1759
Grouping and characteristics of chromosomes 482
Growth factors 814, 1650, 1651
synthesis 171
Growth hormone insensitivity (Laron syndrome) 2644
Growth signal autonomy (unrestricted cell proliferation) 725
GTP-binding proteins (G proteins) 827
Guardian of genome 592
Guillain-Barré syndrome 430, 2940
Gummatous necrosis 66
Gut microbiome-induced colorectal carcinoma 778
Gut-associated lymphoid tissue 350
Gynecomastia 2018, 2547

H

Haber-Weiss reaction 54, 55
Haemophilus ducreyi 2383
Hairy cell leukemia 1308, 1507, 1521
Ham test *in vitro* 1147
Hamartoma 648
Hamartomatous polyps 1931
Hamazaki-Wesenberg bodies 108
HAMP gene 1092
Hand and foot syndrome 1132, 1133
Handling of surgical specimens 1552
Hand-Schüller-Christian disease (multifocal LCH) 1346, 2713
Haploinsufficiency 719
Hapten-carrier molecule 332
Harelip (cleft lip) 1880
Hartnup disease 2255
Hashimoto's thyroiditis 415, 2589
Hassall corpuscles 1537
HBeAg 2046
HbH disease 1094
HBV-DNA 2046
Healing in myocardial infarct 67
Heart 1695
Heart failure cells 250
Heinz bodies 1088, 1138
Helicobacter pylori tests 2006
Helicobacter pylori-induced
gastric carcinogenesis 806
gastrointestinal tract disorders 1922
GIT cancer 778
GIT malignancies 1925
Helly's fluid 1551
Helmet cells 1089
Hemangioblastoma 615, 1765, 2930
Hemangioma liver 2101
Hemangiopericytoma, solitary fibrous tumor of soft tissue and related lesions 2774
Hemarthrosis 1394, 1397
Hematocele 2350
Hematocrit
packed cell volume 1080
values 1079
Hematogenous route of metastasis 701, 2528
Hematology pearls: red blood cell antigens on RBC membrane 1427
Hematolymphoid malignancies 647
Hematopoiesis during
intrauterine life 1057
postnatal period 1057
Hematopoietic
cells 1058
growth factors in clinical practice 1062
microenvironment 1057
progenitor cells collection 1445
stem cell
harvesting 1347
lineages 1058
transplantation 1347
transplantation in the recipients 1352
used for transplantation 1455
transcription factors 1061
Hematoxylin and eosin staining 1553
Hematuria 2305
Hemidesmosomes 6
Hemifacial hypertrophy 1880
Hemochromatosis 106, 1880, 2083
induced cirrhosis 2060
Hemoglobin 1080
Hemoglobin C
disorder 1136
inclusions 1088
Hemoglobin D disorder 1136
Hemoglobin disorders 1082
Hemoglobin E disorder 1136
Hemoglobin electrophoresis 1126, 1134, 1151, 1632
Hemoglobin electrophoresis
patterns 1152
technique 1632
Hemoglobin H
disease (β_4) 1129
inclusions 1088
Hemoglobin synthesis 1080
Hemoglobinemia 1113

Hemoglobinopathies and resistance to malaria 564
Hemoglobinuria 1114
Hemolysis of red blood cells in stored blood 1448
Hemolytic
  anemia 1108
  blood transfusion reactions 1145
  disease of fetus (erythroblastosis fetalis) or newborn 418
  disease of newborn 1144
  jaundice 105, 1111, 2032
  transfusion reactions 1145
  uremic syndrome 1148, 1379, 2201
Hemopericardium 1742
Hemophilia A 1392
Hemophilia B 1397
Hemorrhage into atheromatous plaque 1655
Hemorrhagic (red) infarct in loose-textured organs 60
Hemorrhagic
  cystitis 2310
  disease of newborn 1405
  disorders 268
  hypovolemic shock 298
  inflammation 177
  pericarditis 1744
Hemorrhagic/red infarct of testis or ovaries 297
Hemorrhoids in rectal submucosa 2070
Hemosiderin pigment 106
Hemosiderinuria 1120, 1151
Hemosiderosis 106
Hemosiderosis and susceptibility to typhoid fever 567
Hemosiderosis of spleen 1533
Hemostasis 251, 1355, 1356
Hemothorax 1874
Henoch-Schönlein purpura (ANCA negative) 1384, 1670, 2201
Heparin 1084
Heparin-induced thrombocytopenia syndrome 279
Hepatic
  angiosarcoma 2117
  artery 2011
  encephalopathy 2018, 2071
  epithelioid hemangioendothelioma 2118
  hemangioma 2101
  lymphangioma 2102
  necrosis: histologic patterns 2017
  sinusoids 2011, 2012
  vasculature 2011
  vein 2011
Hepatitis 2033
Hepatitis A virus (HAV) infection 1027, 2040
Hepatitis B
  core antigen 2043
  virus infection 2043
Hepatitis Be antigen (HBeAg) 2043
Hepatitis C virus infection 1030, 2048
Hepatitis D virus infection 2050
Hepatitis E virus infection 2051
Hepatitis G virus (HGV) infection 2052
Hepatoblastoma 2116
Hepatocellular
  adenoma 2100
  carcinoma 1064, 1162
  disease 2015
  jaundice 2032
  nodules 2103
Hepatocyte membrane antibody 2020
Hepatocytes 2011
Hepatomegaly due to malarial parasite 2057
Hepatorenal syndrome 2074
Hepatotropic viruses 2040
Hepcidin 1092
Hephaestin 1092
HER2/neu
  enriched molecular pathway 2513
  enrich breast carcinoma 2518
  growth factor receptor analysis 2540
  proto-oncogene 2533
Hereditary
  angioneurotic edema 391
  diffuse gastric carcinoma 692
  hemorrhagic telangiectasia 540, 1384
  hyperbilirubinemia 2028
  hypercoagulable state 1408
  leiomyomatosis and renal cell carcinoma 692
    syndrome 2282
  nonpolyposis
    colon cancer 1986
    colorectal cancer 691, 1936
  ovalocytosis 1118
  papillary renal cell carcinoma 693
  persistence of fetal hemoglobin 1129
  retinoblastoma 690
  sideroblastic anemia 1157
  spherocytosis 541, 1115
  stomatocytosis 1119
  thrombophilia 274, 1407
    hypercoagulable state 1408
Hermansky-Pudlak syndrome 1376
Herpes simplex virus 1 infection 1881
Herpes simplex virus 2 2365
Hers disease (GSD type 6) 101, 550
Heterochromatin and euchromatin regions in chromosomes 587
Heterologous antigens 334
Heterophagy 42
Heterotropic
  gastric mucosa 1945
  pancreas 1946
Heterozygous
  HbS/HbC disorder 1136
  HbE disorder 1137
  inheritance 595
Hexokinase II activation signaling pathway driven glycolytic metabolic products overload 2572
Hexosamine biosynthetic signaling pathway 2570
HFE gene 2087
Hh gene and ABO phenotypes 1424
Hiatus hernia 1914
Hibernoma 2745
High performance liquid chromatography 1126, 1134, 1152, 1627
High resolution MRI 1713
Highly reactive hydroxyl free radical 153
High-risk squamous cell carcinoma of skin 2860
Hirschsprung's disease 1963
Histochemical stains 469, 1554
Histogenesis of neoplasms 596
Histoid leprosy 2824
Histoid leprosy—rare variant of lepromatous leprosy 1006
Histologic changes in bronchial asthma 413
Histologic variant of
  breast carcinoma 2529
  classic Hodgkin's disease (HD) 1496
Histological patterns of liver necrosis 2017
Histology of
  nervous system 2884
  skin biopsy in leprosy 1003
Histone methylation 585
Histone octamer
  core proteins 585
  protein modifications 766
Histone tails 585
Histoplasma capsulatum 1037
HIV vaccine 404
HIV/AIDS-related malignant tumors 400
Hodgkin disease 1142, 1488
  morphologic subtypes 1495
Holoblastic cleavage 2374
Homocysteinemia 1647
Homogentisic acid pigment 107
Homologous
  recombination repair mechanism 510
  hemoglobin E disorder 1137
Hormonal
  carcinogenesis 807
  therapy 931
Hormone
  receptor status of breast carcinoma 2532
  therapy 2545
Hormones and breast development 2484
Horner's syndrome 1859
Horseradish peroxidase 1558
Horseshoe kidney 2273
Host
  defense against cancer 910
  three lines of defense 316
How to approach clinical problems 125
Howell-Jolly bodies 1066, 1088
Human
  adenovirus infection 1034
  chromosomes 480
  deoxyribonucleic acid (DNA) and cancer 584
Human
  gene structure and mutations 526
  genetic disorders 535
  genome 472
    project 475
  immunodeficiency virus infection 1030
  papillomavirus infection 1032, 2375

rotavirus infection 1030
T cell leukemia virus type 1 infection 1030
Humoral immunity 306, 331
Hunter syndrome
MPS type 1 disease 551
MPS type 2 disease 560
Hutchinson's type of neuroblastoma 2639
Hutchinson-Gilford progeria syndrome 119
Hyaline
arteriolosclerosis 1639, 1640, 2219
arteriosclerosis 2573
body myopathy 2804
Hyalinizing trabecular tumor 2606
Hyaluronidase 1715, 2323, 2374
Hydatid cyst in liver 2055
Hydatid cyst—tapeworm (cestode) 1049
Hydatidiform mole 2468
Hydrocele 2350
Hydrocephalus 2940
Hydrogen peroxide 56
Hydrolysis (deamination and depurination) 504
Hydronephrosis 2261
Hydropericardium 1742
Hydrops fetalis 552, 1129
Hydrothorax 247, 1874
Hydroxyl radical 55
11β-Hydroxylase gene 1686
Hyperacute transplant rejection 452
Hyperaldosteronism 2633
Hyperdiploid acute lymphoblastic leukemia 1207
Hyperdiploid versus hypodiploid multiple myeloma 1318
Hyperemia 247
Hyperemia and congestion 247
Hyperestrinism in women 2075
Hyperglycemia and cyclooxygenase (COX) enzyme activation 2571
Hyperhomocystinemia 1410, 1713
Hyper-IgE syndrome (Job syndrome) 681
Hyperlipidemia 1641
Hyperosmolar hyperglycemic state 2568
Hyperparathyroidism 2626
Hyperpigmentation
disorders 105
overhealed wound 234
Hyperplasia 17, 21, 582
Hyperplastic
arteriolosclerosis 1639, 2266
ileocecal tuberculosis 1954
polyps 1931, 1981
Hypersensitivity 306
pneumonitis 425, 1827
reactions 404
Hypersplenism 1532
Hypertension 1646, 1683
Hyperthyroidism 2596
Hypertrophic
cardiomyopathy 1728
osteoarthropathy 2729
scar 232
Hypertrophy 17, 24
Hypochlorous ($HOCl^-$) free radical 153
Hypochlorous acid 56
Hypodiploid acute lymphoblastic leukemia 1207
Hypofibrinogenemia 1401
Hypoparathyroidism 2629
Hypopigmentation disorders 105
Hypoplasia 34
of megakaryocytes 1379
Hypoplastic left heart syndrome 1756
Hypospadias 2363
Hyposplenism 1532
Hypothyroidism 2592
Hypovolemic shock 298
Hypoxia-induced brain injury 2933
Hypoxia-inducible factor synthesis 175

I

Iatrogenic
hypopituitarism 2643
meningitis 2894
Idiopathic
giant cell myocarditis 1724
pulmonary fibrosis 1826
Idiosyncrasy 2058
IgA
immunoglobulin 373
nephropathy (Berger's disease) 424, 2199
IgD immunoglobulin 373
IgE immunoglobulin 373
IgG immunoglobulin 372
IgM immunoglobulin 373
Imaging diagnostic techniques: pulmonary thromboembolism 1832
Imbalanced chromosomal rearrangements 719
Immature teratoma 2341
of ovary 2459
Immature/transitional B cells in bone marrow 343
Immediate and late response of allergic reactions 412
Immediate spin blood cross-matching 1472
Immune
cells 339
of innate immune system 319
checkpoint molecules in cancers 881
complex formation and sites of deposition 422
status in leprosy 1002, 2822
system 306
thrombocytopenic purpura 1371
Immune-mediated
acute-phase reactants 199
hemolytic anemia 1140, 1141
Immunoadsorption/selective adsorption 1446
Immunoassay 1154
Immunodeficiency
diseases 380
disorders of thymus gland 1537
states in cancer epidemiology 680
Immunodominant sugar 1425
Immunofluorescence microscopy 1589
Immunogenicity 332
Immunohistochemistry
protocol 1560, 1561
technique 1557, 2539
Immunologic tolerance 338, 432
Immunology of transplant rejection 451
Immunophenotyping features of classic variant of Hodgkin's disease 1495
Immunosuppressive drugs 459
used in transplant recipient 460
Immunotherapy 934
Impairment of hepatic venous drainage (chronic venous congestion of liver) 2095
Impetigo 2818
Important
human DNA and RNA virus-induced diseases 1014
platelet plasma membrane glycoproteins 257
In situ (noninvasive) breast carcinoma 2504
In situ
hybridization 1606
immune complex mediated glomerulonephritis 2174, 2178
Inactivation of pathogens in stored blood 1450
Inborn errors of metabolism induced cirrhosis 2061
Incisional hernia 232
Inclusions in red blood cells 1088
Incomplete or partial bone fracture 234
Incontinentia pigmenti 562
Increased concentration of factor
I 276, 277
IX 276
VIII 276
XI 277
Increased excretion of
stercobilinogen 1113
urobilinogen 1113
Increased
iron stores 1113
serum bilirubin 1113
Incubation osmotic fragility 1117
Indeterminate leprosy 1004, 2822
Indian childhood cirrhosis 2091
Indian leprosy classification 1001, 2820
Indications of
blood component transfusion 1452, 1453
bone marrow aspiration 1073
therapeutic apheresis 1444, 1445
Indicators of defective mismatch repair 673
Indirect
bilirubin 2026
Coombs' test (indirect antiglobulin test) 1142
ELISA 1614
immunofluorescence microscopy 1590
Indirect or sandwich method for immunostaining 1558

Indirect-acting chemical carcinogenic agents associated human cancers 789
Induced pluripotent stem cell therapy 206
Induction and sustained tumor angiogenesis 737
Ineffective erythropoiesis 1066
Infantile
fibrosarcoma (congenital fibrosarcoma) 2755
Gaucher's disease (type 2) 2090
polycystic kidney disease 2269
type of coarctation of the aorta 1755
Infarction 292
Infections and atherosclerosis 1647
Infectious
dermatitis 2817
mononucleosis 1177, 1476
Infective
arthritis 2728
endocarditis 1731
Inferior vena cava syndrome 1673
Inflammasome 74
Inflammation and infection 1757
Inflammation of pinna and external auditory meatus 1767
Inflammatory
bowel disease 1968
breast diseases 2488
carcinoma of breast 2520
cells involved in glomerulonephritis 2188
dermatitis 2838
clinical entities 2840
overview 2838
disorders of breast 2488
disorders–prostate 2353
fibroid polyp 1931
myopathies 2805
polyps 1916, 1982
Inflammatory disorders of
fallopian tubes 2426
ovaries 2428
penis 2364
testis 2326
uterine endometrium 2405
vulva 2375
Influenza virus infection 1026
Inguinal hernia 1957
Inheritance pattern analysis by pedigree construction 573
Inherited
ataxia-telangiectasia 2506
cancer syndromes 1935
coagulation system disorders 1390, 1392
hypercoagulable state 274
myopathies 2797, 2798
thrombotic disorders 274
Inhibitors of
coagulation 1365
platelets aggregation 273
tumorigenesis 685
Initiator/inducer caspases 88
Injured tissue repair by fibrosis 67
Injury to vascular endothelium and vasoconstriction 1358
Innate (inborn, natural, nonspecific) immune response 311
Innate (nonspecific) immune response 306
Innate lymphoid cells 348
Insensitivity to growth inhibitory (suppressor) signals (inactivation/biallelic loss of tumor suppressor genes) 727
Insertional mutagenesis 724
Insertions and deletions within coding sequences 532
Inspiratory reserve volume 1782
Insulin
actions 2555
degradation 2554
hormone 2552
release 2554
signaling pathways 2557
synthesis 2552
Insulinoma 2152
Insulin-resistant type 2 diabetes mellitus 419
Integrins 5, 140
Interferons synthesis 170
Interleukin-1 and tumor necrosis factor-α 212
Interleukins synthesis 168
Interleukins, source and their actions 169
Intermediate filaments 9
soft tissue tumors 2735
Internal hemorrhoids 2000, 2070
Internalized factors analysis of breast carcinoma 2532
Interpretation 1152
Interstitial
cystitis 2309
pneumonia 1784, 1789, 1790
Intestinal
epithelial stem cells 207
hemorrhagic/red infarct 296
intussusception 1956
lymphangiectasia 1950
obstruction 1967
tuberculosis 995
type of gastric adenocarcinoma 625, 1938
ulcers in typhoid fever 1955
Intracanalicular pattern 2496
Intracellular accumulation 20
Intracerebral hemorrhage 2939
Intracranial compartments 2884
Intraductal and invasive prostatic adenocarcinoma 2356
Intraductal papilloma of breast 2500
Intraepithelial
lymphocytes 322
neoplasia (carcinoma *in situ*) 1770
route of metastasis 707
Intrarenal acute kidney disease 2243
Intrathoracic manifestations 1852
of bronchial carcinoma 1853
Intravasation, transport and arrest of CSCs 747
Intravascular
hemolysis 1111
hemolytic anemia 1108
RBCs destruction 1113
Intraventricular space 2885
Intrinsic
factor 1101
mitochondrial pathway of apoptosis 86
pathway of blood coagulation system 259
Introns 527, 534
Intussusception 1968
Invadopodia 871
Invasive
breast carcinoma 2504
ductal carcinoma of breast 2515, 2516, 2547
hydatidiform mole 2472
lobular carcinoma of breast 2516
micropapillary breast carcinoma 2523
urothelial carcinoma 2314
Invasiveness of breast carcinoma 2529
Inversion of chromosome 515
Involucrum 2654
Involucrum (reactive viable bone formation) 2654
Involution of embryonic Wolffian or Müllerian structures 90
Iodide trapping 2587
Iodination of tyrosine 2588
Ionizing radiation-induced
carcinogenesis—mechanisms 797
DNA damage 507
Iron
deficiency anemia 1093
metabolism 1090
Irradiation of blood components 1451
Irregular emphysema 1806, 1807
Irreversible cell injury 18, 35
cell death 48
Irreversible decompensated shock stage 304
Ischemia-reperfusion-induced cell injury 41
Ischemic
acute tubular necrosis 2240
heart disease 1696
optic neuropathy 2581
Islet cell tumors 2152
Isochromosome 517
Isoelectric focusing (IEF) 1153
Isolated gonadotropin deficiency 2644

J

Jacob syndrome 514
trisomy XYY 526
Jacobsen syndrome (platelet α-granule defect) 1377
Janeway's lesions 1733
Jaundice 2018, 2024
Jaw 1898
Jenner's stain 1084
John Cunningham virus (JC virus) infection 1032
Johnsen score system for quantifying spermatogenesis 2324

Jones diagnostic criteria 1722
Juvenile (aggressive) ossifying fibroma 1905
Juvenile
coli 1986
Gaucher's disease (type 3) 2090
hypertrophy of the left breast 2545
myelomonocytic leukemia 1253
papillomatosis of breast 2498
polyposis
coli 1986
syndrome 692
rectal polyp 1982
xanthogranuloma 2760
Juxtacrine signaling 213
Juxtaglomerular
apparatus (JGA) 2162
cells 2162

K

Kallmann syndrome 2644
Kaposi sarcoma 399, 632, 1680, 1884, 2874
Kaposiform hemangioendothelioma 1679
Kartagener's syndrome (immotile cilia syndrome) 7, 8, 1757
46, XX karyotype
female with normal ovaries and uterus but male or ambiguous genitalia 570
testicular disorder of sexual development 570
Karyotyping 575
Kawasaki disease 1668
Kayser-Fleischer ring 2088
Kearns-Sayre syndrome 562
Keloid 232, 2751
Keratinocyte apoptosis in epidermis 92
Keratoacanthoma of skin 2861
Keratocystic odontogenic tumor (odontogenic keratocyst) 1900
Kernicterus 105
Ketone bodies 2304
Key features of lymphocytes 355
Ki-67 (MB-1) analysis 1592
Kikuchi disease 1477
Kimura's disease 1477
Klatskin tumor 2136
Kleihauer-Betke test (acid elution test) 1154
Klinefelter syndrome 514
trisomy 47, XXY 524
Knudson two-hit hypothesis 671
Köhler bone disease 2718
Kreberg's stain 1869
Krukenberg tumor 706
metastatic tumor 2432
Kupffer cell 2012, 2075, 2090
hyperplasia 2040
Kwashiorkor (edematous malnutrition) 965

L

Labeled streptavidin–biotin (LSAB) indirect method 1559
Labile cells 21, 662
Labile cells/continuous dividing cells 203
Laboratory
findings in von Willebrand disease 1400
tests for primary and secondary hemostasis 1412
Laboratory diagnosis:
B cell prolymphocytic leukemia 1521
hairy cell leukemia 1521
Laboratory diagnosis of
acute monoblastic/monocytic leukemia (AML-M5A and AML-M5B, FAB) 1223
acute myelogenous leukemia M3 (AML-M3, FAB) 1220
amyloidosis 469
anemia of chronic disease 1161, 1162
aplastic anemia 1156
Bernard-Soulier syndrome 1376
cardiac hemolytic anemia 1148
chronic eosinophilic leukemia, NOS (CEL, NOS) 1298
chronic lymphocytic leukemia 1303
chronic neutrophilic leukemia 1280
dengue hemorrhagic fever 1380
disseminated intravascular coagulation 1404
essential thrombocythemia 1283
factor V (labile factor) deficiency 1402
factor XIII deficiency 1403
Fanconi anemia (FA) 1162
G6PD deficiency disorder 1138
gastric carcinoma 1940
Gaucher's disease 1341
hemoglobin H disease 1129
hemolytic disease of newborn 1145
hemolytic uremic syndrome 1149
hemophilia A 1395
hemophilia B 1397
hereditary sideroblastic anemia 1157
hereditary spherocytosis 1117
Hodgkin's disease 1493
hydrops fetalis 1129
immune thrombocytopenic purpura 1373
infective endocarditis 1733
Letterer-Siwe disease 1346
mantle cell lymphoma—spillover in blood 1312
mastocytosis (mast cell disease) 1300
multiple myeloma 1332, 1522
myelophthisic anemia 1161, 1162
Niemann-Pick disease (NPD) 1343
ovarian tumors 2467
paroxysmal nocturnal hemoglobinuria (PNH) 1146
pernicious anemia 1107
primary myelofibrosis 1291
secondary thrombocytosis 1382
sickle cell trait 1135
thrombophilia 275
thrombotic thrombocytopenic purpura (TTP) 1379
urolithiasis 2262
Waldenström macroglobulinemia 1337
warm antibody-mediated hemolytic anemia 1142
β-thalassemia major 1125
β-thalassemia minor 1127
Lactate dehydrogenase (LDH) 1713
Lactating adenoma of breast 2501
Lactiferous ducts and sinuses 2486
Lactotrope adenoma (prolactinoma) 2647
Lacunar Reed-Sternberg cells 1489
Lacunar type Reed-Sternberg cells 1491
Lambert-Eaton myasthenic syndrome 419, 2807
Lamellipodia 871
Laminar blood flow 1360
Laminin 217, 2168
Langerhans cell histiocytosis 1344, 2712
Langerhans cell histiocytosis (LCH): clinical forms 2713
Langerhans' cells of skin 348
Langhans' giant cells 196
Lardaceous spleen 111
Large
bowel infarction/ischemic bowel disease 1977
cell lung carcinoma 1864
ducts of female breasts 2486
Laron syndrome 2644
Laryngeal tuberculosis 999
Larynx 1778
Laser microtomy 1546
Late
clinical manifestations in myxedema 2595
congenital syphilis 1010
phase reactions in bronchial asthma 1813
Latent benign syphilis 1010
Lateral periodontal cyst 1900
Layers of immunologic tolerance 436
12-lead electrocardiogram (ECG) 1699
Leber's hereditary optic neuropathy 563
Left-sided
colorectal carcinoma 1995
heart failure 1748
Leg vein thrombosis 1709
Legg-Calvé-Perthes disease 2718
Leigh disease 563
Leiomyoma 1921
Leiomyoma (fibroid/fibromyoma/myoma) 2421
Leiomyosarcoma 633, 2424
of vagina 2388
Leishman stain 1084
Leishmania 1046
Lentigo
maligna melanoma 2851
simplex 2846
Lepromatous leprosy 1003, 1006, 2822, 2824
Lepromin test 1007, 2826
Lepros (Hansen's disease) 2820
Leprosy classification 2820
Leptin 966
Leptocytes 1089
Leptomeningeal route of metastasis 707
Lesch-Nyhan syndrome 561, 2727
Lethargy and other manifestations 201
Letterer-Siwe disease (acute disseminated LCH) 1345, 2713

Leukemia 647, 1179
disease burden impact 1192
Leukocyte adhesion deficiency
disorder 1176
type 1 1176
type 2 1176
Leukocyte adhesion
type 1 (LAD-1) deficiency 142
type 2 (LAD-2) deficiency 142
Leukocyte
alkaline phosphatase stain 1189
esterase stain 1189
myeloperoxidase stain 1189
Leukocyte-induced injury 154
Leukocytosis 1168
Leukopenia 1172
Leukoplakia 1885
of vulva 2378
Leukopoiesis 1067, 1164
Leukoreduction before blood storage 1450
Leydig cell tumor of testis 2346
Libman-Sacks endocarditis 1734
Lichen
planus 1881, 2842
sclerosus 2378
Licorice 1686
Liddle syndrome 1685
Life cycle of SARS-COV-2 1020
Li-Fraumeni syndrome 593, 623, 690, 759, 1936, 2506
Light chain
cast nephropathy in multiple myeloma 2254
multiple myeloma 1315
Limbal stem cells in cornea 208
Limb–girdle muscular dystrophy 2803
Limitations of microarray analysis 1606
Limiting plates 2011
Lingual thyroid 2620
Lipedema 246
Lipid
breakdown products and membrane damage 58
homeostasis 122
peroxides 56
pneumonia 1792
Lipofuscin pigment 104
Lipogranulomas 2490
Lipoleiomyoma 2422
Lipoma 2744
Liposarcomas 630
Lipoxygenase 12 enzyme activation signaling pathway 2572
Lipoxygenase pathway 166
Liquefactive necrosis in
brain 60
organ's abscess 61
Liquid biopsy
protocol and steps 945
cell-free DNA technology 944
Lisch nodules 543
Listeriosis 986
Liver
acinus (lobule) 2010
amyloidosis 111, 467
biopsy 2022
cirrhosis and portal hypertension 2059
in α_1-antitrypsin deficiency 2089
manifestations 1133
metastasis 701, 2119
stem cells 206
transplantation 2016
Lobar pneumonia 1783, 1785, 1788, 1789
Lobular carcinoma 2515
in situ 2515
of breast 2515
Local
effects of tumors on the host 897
invasion: first step in metastasis 698
tumor invasion route of metastasis 2528
Localized (Arthus reaction) type 3 hypersensitivity reaction 423
Localized lymphadenopathy 1479
Locally malignant tumors 635
Location and frequency of gastric carcinoma 1934
Locations of metastases in nervous system 2907
Locus specific identifier (LSI) probe (most versatile) 1610
Long noncoding RNA (LNCRNA) 529
Long or short umbilical cord 2479
Long-term complications of
diabetes mellitus 2573
pancarditis 1722
Looser's zones 2658
Loss of
cell adhesion molecules 2533
heterozygosity 719, 731
Low-grade
central osteosarcoma 2689
fibromyxoid sarcoma variant of adult fibrosarcoma 2757
malignant peripheral nerve sheath tumor (low-grade MPNST) 2928
Low-platelet count and clinical manifestations 1372
L-selectin (CD62L) 141
Ludwig angina 1881
Lumen formation in hollow organs 91
Luminal 'A' breast carcinoma 2511, 2512, 2518
Luminal 'B' breast carcinoma 2511, 2512, 2518
Lumpectomy 2545
Lung
abscess 1793
adenocarcinoma 625, 1849, 1860
capacities 1781
tumors 1837
volumes and capacities 1782
Lungs 1779
Lupus
anticoagulant 1410
nephritis 424, 2224
Lyme
arthritis 2729
disease 1011, 2729
Lymph node
metastasis 700
micrometastases 2528
non-neoplastic disorders 1475
organization 1474
skip metastases 2528
Lymphangiectasia 1950
Lymphangioleiomyomatosis 1828
Lymphangioma 1677
liver 2102
Lymphatic
filariasis 1048
route of metastasis 698, 2528
system 315, 1673
Lymphedema 243, 244, 246, 1673
praecox 243
tarda 243
Lymphocyte homing receptor 6
Lymphocyte-depletion variant 1497
Lymphocyte-rich classic variant of Hodgkin's disease 1496
Lymphocyte-rich variant 1496
Lymphocytes 184
Lymphocytic–histiocytic (L&H) Reed-Sternberg cells 1489, 1492
Lymphocytopenia 1173
Lymphocytosis 1171
Lymphogranuloma venereum 2364, 2376
Lymphoid
hyperplasia 1476
neoplasms 1479
organs and tissues 315
tissues/organs 349
Lymphoma 647, 1921
Lymphomas
associated with HIV 399
of the gastrointestinal tract 1910
Lymphomatoid
granulomatosis 1671
granulomatous 401
Lymphopoiesis 1167
Lymphovascular invasion of breast carcinoma 2530
Lyon hypothesis 522, 576
Lyonization 657
Lysosomal
associated proteins 11
disorders 12
enzymes in platelets 258
integral proteins 11
luminal proteins 11
storage disease 2090
storage disorders 133
Lysosomal-dependent cell death 79
Lysosomes 11
Lyst gene 7
Lysyl oxidase 218

M

Macroautophagy 79
Macrocytic anemia with
megaloblastic erythropoiesis 1097
normoblastic erythropoiesis 1098

Macrometastases 700
Macronodular cirrhosis 2061
Macrophage
activation 182
inhibitory factor 1816
origin and distribution 181
products and their actions 185
proliferation and immobilization in tissues 183
Macrophages 320, 345
Macrophages and lymphocytes interaction 187
Macrovesicular steatosis 2079
Macula densa 2162
Madurella grisea 1041
Maffucci syndrome 615
Magnesium, ammonium, calcium phosphate stones (struvite stones) 2260
Magnetic resonance
cholangiopancreatography 2134
imaging 2536
of bone tumors 2668
Magnification mammography 2536
Maintenance of
stem cells 206
stored blood at lower temperature 1448
Major blood group system, their genes and locus on chromosome 1429
Major classes of
HLA genes 351
human plasma lipoproteins 1642
Major
components of the host defenses 317
damage-associated molecular patterns (DAMPs) in microbes 147
diagnostic criteria 1718
family of cell adhesion molecules 5
functions of vitamins and deficiency syndrome 971
histocompatibility complex 350
Jones diagnostic criteria 1718
leukocyte–endothelial cell adhesion molecules 143
molecular pathways in evolution of breast carcinoma 2511
pathogen-associated molecular patterns (PAMPs) in microbes 145
proteins present in lysosomes 11
types of DNA damage 504
Malabsorption syndrome 1946
Malabsorptive disorders 1946
Malakoplakia of urinary bladder 2310
Malassezia furfur 1038
Male
hypogonadism 2351
impotence 2351
pseudohermaphrodite 571
pseudohermaphroditism 2351
sterility 7
Malformations of CVS 1756
Malignant
epithelial tumors 604, 617
fibrous histiocytoma of bone 2705
mesenchymal tissue 604
mesenchymal tumors 629
mesothelioma 1875
nephrosclerosis 2265
peripheral nerve sheath tumor 2784, 2929, 2930
phyllodes tumor 2498
pleural tumors 1875
soft tissue tumors 2735
tumors 603
of colon 1986
vascular tumors 631
Mallory hyaline (Mallory-Denk body) 9, 103
Mallory-Denk bodies 628
Mallory-Weiss syndrome 1916, 2075
Malrotation of small intestine 1946
Mammaglobin analysis 2542
Mammalian sirtuin system 120
Mammaprint gene expression profile test 2542
Mammary
duct ectasia 2488
tuberculosis 999
ultrasonography 2536
Management of
atheromatous plaque 1657
urolithiasis 2262
Mannose receptors 328
Mannose-binding lectin (MBL-2) 1715
Mantle cell lymphoma 1508, 1518
spillover in blood 1312
Manufacture of DNA vaccine 377
Maple syrup urine disease 557
Marasmus 964
March hemolytic anemia 1150
Marfan's syndrome 218, 219, 231, 541, 1661
Marginal zone lymphoma 1518, 1519
Margination of leukocytes 141
Markers of cell proliferation in breast carcinoma 2531
Maroteaux-Lamy disease 552
Marrow aspiration needles 1073
Mary Joseph nodule in periumbilical region 1940
Massive (fulminant)
hepatic necrosis 2015
liver necrosis 2015
Massive
blood transfusion 1459
splenomegaly 2070
Mast cells 188, 321
preformed and newly synthesized chemical mediators and their actions 412
Mastocytosis (mast cell disease) 1299
Matrix metalloproteinases 227
Mature B cell
lineage acute lymphoblastic leukemia 1204
non-Hodgkin's lymphoma 1506
Mature
cystic teratoma of ovary 2457
insulin 2554
solid teratoma of ovary 2459
T cell and NK cell lymphomas 1523
teratoma 2341
Maturity-onset diabetes mellitus of the young 2565
Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome 571
May-Grunwald stain 1084
May-Hegglin anomaly 1070, 1175
Mazabraud syndrome 597, 2710
McArdle disease (GSD type 5) 549
McCallum plaque 1721
Mean corpuscular hemoglobin concentration (MCHC) 1079
Mean corpuscular volume (MCV) 1079
Measles virus infection 1029
Mechanisms of severe combined immunodeficiency (SCID) 389
Meckel's diverticulum 1945
Meconium ileus 1956
Median palatal cyst 1900
Medullary
breast carcinoma 2520
carcinoma thyroid 2618
sponge kidney 2271
thyroid carcinoma 645, 2615
Medulloblastoma 2921
Megaloblastic
anemia 983, 1097
classification 1097
Megaloblastic
macrocytic anemia 1097
Melanin pigment 104
Melanoblasts 2845
Melanocytes 2845
Melanocytic
neoplasms 2846
nevus (common mole) 2846
Melanoma 1764, 1921, 2848, 2849
Melanoma of
skin 796
vagina 2388
vulva 2380
Melanoma: clinical variants 2849
Melanosomes 2845
Membrane inhibitor of reactive lysis (MIRL) 1146
Membranoproliferative glomerulonephritis 2178, 2212
Membranous glomerulonephritis 425, 2209
Memory T cell 343, 1168
MEN IIa syndrome (sipple syndrome) 2617
MEN IIb syndrome (Gorlin syndrome) 2617, 2637
Mendelian (monogenic) disorders (single-gene mutation) 536
Mendelian traits 539
Ménétrier disease 1935, 1944
Meningeal tumors 2923
Meninges 2884
Meningioma 2923
Meningitis 2887
Meningovascular syphilis (neurosyphilis) 1010
Menstrual cycle 2403
disorders 2404

Mercaptans 2073
Mercurials 1550
Merkel cell carcinoma 2867
MERRF syndrome 564
Mesangial cells 2168
Mesenchymal
  chondrosarcoma 2679
  nonmeningeal tumors 2930
Mesenchymal–epithelial transition (MET) 744, 871
Mesenteric granulomatous lymphadenitis 1998
Mesothelioma 1876
Metabolic
  alterations in inflammation 201
  bone disease 2657
  liver disorders 2076
  myopathies 2805
  pathways in the red blood cells 1067, 1068
Metachromatic
  leukodystrophy 556
  stains 469, 1557
Metaphase
  CGH technique 1601
  comparative genomic hybridization technique 1601
  FISH technique 1599
  fluorescence *in situ* hybridization for chromosomal karyotyping technique 1599
Metaplasia 17, 30, 582, 2002
  in neoplasms 32
  of peritoneum 2002
Metaplastic carcinoma of breast 2524
Metastases
  in spleen 1536
  to nervous system 2907
Metastasis multi-step process 886
Metastasis-promoting genes 599
Metastatic bone disease 2718
  in adults and children 2720
Metastatic
  calcification 113, 115, 2627
  cancer-induced meningitis 2894
  pancreatic tumors 2153
  suppressor genes 599
  tumor suppressor gene mutations and associated cancers 601
  tumors (liver metastases) 2119
Metazoa (helminths) 1048
Methods for DNA sequences 1616
Methods of
  ABO blood grouping 1467
  detection of tumor markers 914
Methyl
  green pyronine special stain for plasma cells 1333
  violet stain 470
Methylene blue 1139
MHC (HLA) class I and II molecules associated diseases 352
MHC (HLA)
  class I molecules 352
  class II molecules 352
Micro- and macronodular cirrhosis 2062
Microangiopathic hemolytic anemia 1148
Microarray
  analysis technique: application and technology 1603
  application and technology 1603
  studies in breast cancer 2544
Microautophagy 77
Microbial carcinogenic agents associated human cancers 782
Microbial pathogens causing lung abscess 1793
Microcolumn chromatography 1153
Microfilaments 7
Micrometastases 700
Micronodular and macronodular cirrhosis 2062
Microorganisms demonstrated in bone marrow 1076
MicroRNA (miRNA) 502, 528, 767
MicroRNAs and epigenetics in cancer diagnosis 776
MicroRNAs targeting the hallmarks of cancer 777
Microsatellite instability 2416
Microscopic
  polyangiitis (ANCA positive) 1670
  polyarteritis 1670
Microspherocytes 1088
Microtomy 1552
Microtubules 8
Microvesicular steatosis 2079
Microvillus inclusion disease 1950
Mid-borderline leprosy (BB) 2821
Mid-diastolic murmur 1739
Midzonal necrosis 2015
Migratory polyarthritis 1719
Mikulicz syndrome 1302
Mild hemophilia A 559
Miliary tuberculosis 989, 1802
Milroy disease 1673
Minimal
  change disease 2205
  residual disease 658, 1211
Minor Jones diagnostic criteria 1718
Mirizzi's syndrome 2133
MIRL (membrane inhibitor reactive lysis) 1146
Miscellaneous
  disorders of joints 2729
  non-neoplastic disorders of skeletal muscle 2806
  skin disorders 2877
  soft tissue tumors of uncertain lineage 2785
  terminology related to neoplasms 648
Mismatch repair mechanism 509
Missense mutation 532
Mitochondria 10
Mitochondrial
  damage 50
  deafness 562
  DNA 480
  DNA mutations associated disorders 562
  encephalopathy, lactic acidosis and stroke-like episodes 563
  gene mutations associated disorders 11
  hypertrophy 11
  inheritance 539
  myopathy 2804
  permeability transition 50
    pore driven necrosis 69
Mitotic cell count analysis 1592
Mitral valve
  insufficiency/regurgitation 1739
  prolapse 1740
  stenosis 1738
Mitsuda reaction 1008, 2827
Mixed
  cellularity variant 1498
  connective tissue disease 448
  gallbladder stone 2126
  genitalia and sex organs (46, XX ovotesticular disorder of sexual differentiation) 571
  germ cell tumors of
    ovary 2463
    testis 2344
  Müllerian tumor 2421
  patterns penile carcinoma 2368
  somatotroph–lactotroph adenoma 2649
  vitiligo 2845
Mode of spread 1492
  of surface epithelial carcinomas of ovary 2438
Moderate hemophilia A 559
Modes of inheritance of mendelian disorders 538
Modification of
  blood components 1450
  cellular components 1456, 1457
Modified radical mastectomy 2545
Molds 1036
Molecular basis of
  cancer 708
  tumor markers 914
Molecular diagnostics
  in oncology 939
  uses in oncology 941
Molecular genetic testing 577, 2623
  in neuroblastoma 2638
  methodology 578
Molecular mimicry and autoimmunity 335
Molecular mimicry and rheumatic fever 336
Molecular pathogenesis of
  colorectal carcinoma 1988
  Crohn's disease 1974
  urolithiasis 2256
Molecular pathways of breast carcinoma 2517

Molecular regulators of hematopoietic stem cell (HSC) fate 1063
Molecular subtypes of breast carcinoma 2511
Molecular techniques: methodologies 943
Molecules expression in tumor microenvironment 881
Molluscum contagiosum 2827
Mönckeberg's
arteriosclerosis 1638
medial calcific sclerosis 1638
Monitoring
autoimmune disorders in diabetic patients 2585
diabetic cardiovascular diseases 2585
diabetic nephropathy 2585
long-term diabetic complications 2585
Monoclonal
gammopathy of undetermined significance 1320, 1338
immunoglobulin deposition disease of kidney 2222
properties of malignant neoplasms 656
Monocyte/macrophage
half-life span 182
recruitment 183
Monocytes 1166, 1167
Monocytopenia 1173
Monocytosis 1171
Monomorphic adenoma 1893
Mononuclear
phagocytic system disorders 1339
Reed-Sternberg cells 1491, 1489
Monospot test 1178
Monostotic
fibrous dysplasia 2710, 2711
lesion 2660
Morpheaform basal cell carcinoma 2864, 2865
Morphologic changes in apoptosis and diagnostic techniques 733
Morphologic changes: myocardial infarction 1705
Morphologic
lung lesions in tuberculosis 1800
patterns of
acute inflammation 175
necrosis 59
subtypes of Hodgkin's disease 1495
Morquio syndrome (MPS type 4 disease) 552
Mosaic
down syndrome 518
Klinefelter syndrome 518
turner syndrome 518
Mosaicism 513
Most common
metastatic tumors to bone 2662
sites of aortic aneurysms and their etiology 1658
Mucicarmine 1557, 1869
Mucin types and their origin 1557
Mucinous
borderline tumor of ovary 616
cystadenocarcinoma of ovary 2444, 2445
cystadenoma of ovary 2443
surface epithelial tumors 2443
Muckle-Wells syndrome 75
Mucocele of 1887
appendix 1998
gallbladder 2125, 2133
Mucocutaneous leishmaniasis 1046
Mucoepidermoid carcinoma 1893
Mucolipidosis 556
Mucopolysaccharide storage diseases (mucopolysaccharidosis type 2) 551
Mucormycosis 1039, 2837
Mucormycosis—clinical manifestations 1040
Mucosa-associated lymphoid tissue (MALT) 350
Multicystic
mesothelioma 2003
renal dysplasia 2272
Multidrug-resistant tuberculosis 989
Multifocal bone metastatic disease 2719
Multilocular cystic renal cell carcinoma 2288
Multinodular goiter 2601
Multiple
biliary hamartomas (von Meyenburg complex) 2102
endocrine
neoplasia 1 1929, 2153
neoplasia 2 693
myeloma 647, 1313, 1522, 2706
and other monoclonal gammopathies 1313
organs failure in septic shock 302
polyposis syndromes 1985
sclerosis 430, 2940
subtypes of lymphocytes 1070
Multiplex
ligation-dependent probe amplification 579
metaphase fluorescence *in situ* hybridization for chromosomal karyotyping technique 1599
Multistep
carcinogenesis: mechanisms 784
process of chemical carcinogenesis 786
Mumps
orchitis 2326
virus infection 1029
Mural thrombi 1710
with possible embolization 1709
Mural thrombus 1655
Muscle-specific actin 1587
Muscular dystrophies 2798
Mutations within noncoding sequences (introns) 534
Myasthenia gravis and resistance to rabies virus 566
Mycetoma 2836
madura foot 1040
Mycobacterium leprosy (Hansen's disease) 1001
Mycobacterium
tubercle bacillus 1794
tuberculosis 988
Mycosis fungoides 1528
Mycotic aneurysm 1662, 2937
rupture 271
Myelin figures 48
Myelodysplastic syndrome unclassifiable 1241
Myelodysplastic syndrome with
excess blasts 1240
isolated deletion of chromosome 5q 1240
multilineage dysplasia 1239
ring sideroblasts 1239
single lineage dysplasia 1238
Myelodysplastic syndromes 1238
Myelodysplastic/myeloproliferative neoplasms: entities 1249
Myeloid metaplasia 32
Myeloperoxidase deficiency 1176
Myelophthisic anemia 1161
Myocardial
infarct 295
infarction 287, 1701
rupture 1708
Myocarditis 1719, 1724
Myocardium 1719
Myoclonic epilepsy with ragged-red fibers (MERRF) syndrome 563
Myoepithelial cell immunomarkers 2542
Myoepithelioma 1893
Myogenin 1588
Myoglobin 1588
Myopathies 2797
Myopericytoma family of tumors 2775
Myositis ossificans 2792, 2807
Myotonic muscular dystrophy 2802
Myotubular myopathy 2804
Myrid leprosy classification 1001, 2820
Myxedema 246, 2595
Myxofibrosarcoma 2758
variant of adult fibrosarcoma 2757
Myxoid liposarcoma 2748
Myxoma (intramuscular myxoma and juxtaarticular myxoma) 2785

N

Nails structure 2813
Naïve B cells 344
Naïve CD4+ helper T cell activation and differentiation 363
Naked eye single tube red cell osmotic fragility test (NESTROFT) 1125
Nasal
adenocarcinoma 1765
plasmacytoma 1765
Nasopalatine duct cyst 1900
Nasopharyngeal
angiofibroma 1764
carcinoma 1762
Natural killer cells controlling human viral infections 187

Natural killer cells kill viruses and cancer stem cells 366
Natural killer T cells 1168
Necroptosis 70
Necrosis 18
Necrotic cell versus apoptotic cell 19
Neisseria meningitidis 987
Nelson syndrome 2644
Nemaline myopathy 2804
Neonatal alloimmune thrombocytopenia 1374
Neonatal
  cholestasis 2030
  diabetes mellitus 2565
  hepatitis of unknown etiology 2032
  respiratory distress syndrome 1817
    in amniotic fluid embolism 292
Neoplasia 583
Neoplastic disorders of adrenal
  cortex 2640
  medulla 2636
Neoplastic disorders of
  anal canal 2001
  blood vessels 1674
  heart 1751
  kidney 2274
  large intestine 1979
  lung 1837
  male breasts 2547
  mesothelium 2349
  ovary 2431
  parathyroid glands 2630
  penis 2366
  pituitary gland 2645
  spleen 1534
  testis 2327
  thyroid gland 2602
  uterine cervix 2391
  uterine endometrium 2409
  uterine myometrium 2421
  prostate 2355
  liver 2098
  nodules in liver 2105
  polyps 1983
Nephritic factors (Ne-AP and Ne-CP) involved in glomerulonephritis 2189
Nephritic syndrome 2190, 2191
Nephroblastic and cystic tumors 2274
Nephrocalcinosis 2255
Nephrogenic diabetes insipidus 2644
Nephrosclerosis 2263
Nephrosialidosis 2176
Nephrotic syndrome 2190, 2202
Nephrotoxic acute tubular necrosis 2242
Nervous system
  amyloidosis 468
  tumors: overview 2896
Neural and perineural tumors and other lesions 2778
Neural
  stem cells in brain 206
  tube defects 568, 2941
  tumors of skin 2876
Neuregulin gene 1964
Neuroblastoma 2638
Neurocysticercosis 2894
Neurodermatitis 2819
Neuroendocrine
  cells 1959
  tumors 643, 1958, 2866
    of cervix 2402
Neurofibroma, neurofibromatosis type 1 and malignant change in neurofibroma 2780, 2928
Neurofibromatosis 1880
  type 1 543, 693, 2928
  type 2 543, 693
Neurofibromin 543
Neurogenic shock 303
Neuroglial cells in
  CNS 2884
  peripheral nervous system 2884
Neuroglial
  tissue 2884
  apoptosis and formation of red neurons in brain 92
  intermediate filaments 9
Neurons 2884
Neuron-specific enolase (NSE) 1764
Neuropeptides synthesis 174
Neurotensin 2113
Neutropenia (agranulocytosis) 1173
Neutrophil and macrophage lysosomal enzymes 164
Neutrophilia 1168
Neutrophils 189, 320, 1069, 1165
Nevoid basal cell carcinoma syndrome (Gorlin-Goltz syndrome) 693
New methylene blue 1080
Newly synthesized chemical mediators of mast cells 411
Next-generation sequencing
  in breast cancer 2544
  technique 953
Niacin (vitamin B_3) 980
Niemann-Pick disease 554, 1090, 1341, 2090
  type A 1342
  type B 1342
  type C 1342
  variants 554
Niemann-Pick type C disease and resistance to flaviviruses such as Ebola and Marburg viruses 566
Nijmegen breakage syndrome 681, 696
Nipple and areola eczema 2534
Nipple
  discharge 2487
  retraction 2487, 2516
Nitric oxide 56
  synthesis 173
Nitroblue tetrazolium slide test 153
Nitrogen base pair 480
Nitrosamines 791
N-Myc gene amplification 723
Nocardia 986
Nodal marginal zone lymphoma 1519
Nod-like receptors 327
Nodular
  basal cell carcinoma 2864
  fasciitis 2752
  glomerulosclerosis 2219
  hidradenoma 2868
  lymphocytic–histiocytic predominant variant of Hodgkin's disease 1499
  melanoma 2849, 2851
  regenerative hyperplasia 2097
  sclerosis classic variant of Hodgkin's disease 1497
Nomenclature of hemoglobin 1081
Nonalcoholic fatty liver disease 2082
Nonbacterial thrombotic endocarditis 1735
Noncardiogenic pulmonary edema 1836
Noncaseating granulomas 193, 1974
Nonchromaffin enteroendocrine cells 1959
Noncirrhotic portal hypertension 2097
Noncoding regulatory RNA 528
Noncomedocarcinoma 2515
Noncompetitive ELISA 1615
Nongranular lacis cells 2162
Nonhematopoietic cells 1059
Nonhemorrhagic hypovolemic shock 298
Non-Hodgkin's lymphoma 1142, 1500
Nonhomologous end joining repair mechanism 511
Noninfectious granulomatous oophoritis 2429
Noninfective endocarditis 1731
Noninvasive papillary urothelial neoplasia 2312
Nonmelanocytic cutaneous neoplasms 2853
Non-metastatic systemic effects of cancer on the host 898
Non-mutational epigenetic reprogramming in cancer stem cells 764
Non-neoplastic disorders of
  fallopian tubes 2427
  mesothelium 2350
  skeletal muscle 2796
  uterine endometrium 2406
Non-neoplastic
  infiltrative disorders of spleen 1534
  melanocytic disorders and melanocytic neoplasms 2845
  pleural disorders 1872
Nonodontogenic tumors 1905
Non-pitting edema 246
Non-professional APCs 346
Nonprogressive shock stage 304
Nonproliferative
  diabetic retinopathy 2580
  fibrocystic changes 2491
  glomerulonephritis 2177
Nonsecretory multiple myeloma 1318
Nonsense mutation 531
Nonspecific
  granulomatous prostatitis 2353
  interstitial pneumonias 1791
Nonsteroidal anti-inflammatory drugs (NSAIDs) 1929

Nonstructural proteins of severe acute respiratory distress syndrome (SARS)-COV-2 virus for COVID-19 disease 1019
Non-syndromic hereditary Wilms' tumor 694
Nontoxic goiter 2600
Nonwedge-shaped splenic infarcts 1533
Noradrenaline (norepinephrine) 2633
Normal breast-like
breast carcinoma 2512, 2518
molecular pathway 2513
Normal cell proliferation and tissue growth regulation 203
Normal endothelial hemostasis 1355
Normoblastic macrocytic anemia 1098
Norovirus (Norwalk virus) infection 1027
Northern blot technique 1622
Norwalk virus 1952
Nose 1778
NOTCH1
gene 2510
oncogene 2510
Notochordal tumors 2701
Nottingham modification of the bloom 2530
Novel serum, urine and tissue biomarkers 2300
NTHL1-associated polyposis disorder 695
Nuclear
Barr bodies 522
DNA and mitochondrial DNA 476
pores 487
Nucleosome 476, 585, 587
Nucleotide base pairs in DNA 476
Nucleotide-excision repair mechanism 509
Numerical alterations in chromosomes 713
Nutritional disorders 964

O

Obesity 966
Obesity-associated health problems 968
Obliterative endarteritis 1659
Obstructive
atelectasis of lung 1830
disorders of gastrointestinal tract 1908
jaundice 2131
Occult blood 2006
Occupational exposure 1841
Ochronosis of joints 2728
Oculopharyngeal muscular dystrophy 2803
Odontogenic
carcinoma 1904
keratocyst 1900
myxoma 1903
tumors 1901
Odontoma 1903
Odynophagia 1911
Olfactory neuroblastoma 1764
Oligodendroglial tumors 2918
Oligodendrogliomas 2918
Oligodontia-colorectal cancer syndrome 692
Oligohydramnios 2479
Oligonucleotide microarray in breast cancer 2544
Ollier disease 615
Omenn syndrome 388
Onchocerciasis 1051
Oncocytic
papillomas 1761
thyroid neoplasms 2614
Oncocytoma 1892
Oncogenes 591, 840
Oncogenes, mode of activation and associated tumors 840
Oncogenesis 582, 662
Oncogenic RNA viruses associated cancers 801
Oncotic necrosis 35, 42
Open
biopsy 1869, 2537
bone fracture 234
pneumothorax 1828
Opportunistic
infections in AIDS 397
pulmonary infections 1791
Opsonin receptors on leukocytes 148
Opsonins 322
Oral
choristomas 649
glucose tolerance test 2584
lesions in systemic disorders 1881
Organ transplants 352
Organification of
thyroglobulin 2588
nucleus and its DNA 487
thrombus 281
Organohalogen compounds 792
Organs involved in
acute GVHD 457
amyloidosis 466
chronic GVHD 457
Organs with
parallel blood supply 293
single arterial supply with rich anastomoses 293
Origin of thrombi causing embolization in various organs 289
Osgood-Schlatter disease 2718
Osmotic
diarrhea 1952
fragility 1117
Osseous
choristoma 649
metaplasia in myositis ossificans 32
Ossifying fibroma 1905
Osteitis fibrosa cystica 2658
Osteoarthritis 2726
Osteoblastic lesions in metastatic bone disease 2720
Osteoblastoma 2682
Osteoblasts 2652
Osteochondroma 2673
Osteoclastic giant cell-rich bone tumors and cystic lesion 2697
Osteoclasts 2652
Osteocytes 2652
Osteofibrous dysplasia (ossifying fibroma) 597, 2712
Osteogenesis imperfecta 2716
Osteogenic tumors 2680
Osteoid osteoma 2681
Osteolytic lesions in metastatic bone disease 2720
Osteolytic/osteoblastic lesions in metastatic bone disease 2721
Osteoma 616
Osteomalacia 976, 2657
Osteomyelitis 1134
Osteonecrosis (avascular necrosis) 2718
Osteopetrosis 2717
Osteophytes (bony spurs) 2726
Osteoporosis 2659
Osteosclerotic multiple myeloma 1318
Other blood group systems 1433
Other viral infections 1881
Otitis media with effusion (glue ear) 1768
Otosclerosis 1768
Outcome of
acute inflammation 177
necrosis 66
Ovalocytes 1088
Ovarian cysts 2429
Ovaries 2372
Oxidant stress 1138
Oxidation of iodide 2587
Oxidative DNA damage 506
Oxygen-dependent microbial killing 153
Oxygen-derived free radicals 54
synthesis 172
Oxygen-independent microbial killing 154

P

P53 tumor suppressor gene mutation 2506
Pacemaker cells of Cajal 1942
Paget's disease of
bone (osteitis deformans) 2659
breast 2518
vulva 2379
Pagetoid spread melanoma 2851
Painful breasts 2486
Pale (ischemic) infarct in solid organs 60
Pale and hemorrhagic infarcts 60
Pale/white
infarct 295
myocardial infarct undergoing hemorrhagic/red infarct 297
Palmar erythema 2075
Panacinar emphysema 1807
Pancarditis 1718, 1719
Pancoast lung tumor 1859
Pancreas 2137
Pancreatic
adenocarcinoma 2146, 2147
duct adenocarcinoma 2146
heterotropic rests 1944
islets of Langerhans 2550

Pancreatitis 1930, 2137
Pancreatoblastoma 2152
Pancytopenia 1172
Panel of reactive antibody test 459
Pantothenic acid (vitamin B_5) 980
Papillae in renal pyramids 2158
Papillary (chromophil) renal cell carcinoma 2283
Papillary and polypoid (bullous) cystitis 2310
Papillary
  breast carcinoma 2522
  hidradenoma of vulva 2378
  necrosis 2219, 2573
  thyroid carcinoma 2606
Papilloma in various organs 610
Para-Bombay phenotype 1433
Paracrine signaling 212
Paraesophageal hernia 1914
Paraffin embedding of processed tissue 1552
Parafollicular cells or C cells of thyroid gland 2586
Paraganglioma (carotid body tumor) 1775
Paraganglioma (neuroendocrine tumor) 646
Paralytic ileus 1957
Paraneoplastic syndrome 901, 1540
  in lung cancer 1853
Paraovarian or paratubal cysts 2431
Paraproteinemia-induced platelet dysfunction 1380
Parasite associated with pancreatic carcinoma 2147
Parasite species and geographical distribution of schistosomiasis 1051
Parasite-induced carcinogenesis 807
Parasitic
  encephalitis 2894
  liver infections 2055
Parathormone (PTH) 2625
Parathyroid
  adenoma 612, 2630
  carcinoma 2631
  glands dysfunction associated myopathy 2806
Parietal epithelial cells of Bowman's capsule 2168
Paris-Trousseau syndrome (platelet α-granule defect) 1376, 1377
Parkinson's disease 13
Parosteal osteosarcoma 2691
Paroxysmal
  cold hemoglobinuria 1143
  nocturnal hemoglobinuria 392, 1146
Partial
  hydatidiform mole 2472
  pressure of gas 1781
Parvovirus B19 infection 1034
PAS stain 1557
Passive immunity 313
Patau syndrome (trisomy 13) 520
Patch stage in Kaposi sarcoma 2875
Patchy atelectasis of lung 1830
Patent ductus arteriosus (PDA) 1755
Pathogen recognition receptors 326
Pathogen-associated molecular patterns 144, 329
Pathogenesis of 13
  alcoholic liver disease 2077
  atherosclerosis 1647
  autoimmune diseases 440
  cholelithiasis 2127
  Graves' disease 2597
  Hashimoto's thyroiditis 2589, 2590
  immune complex-mediated tissue injury and organs damage 420
  multinodular goiter 2601
  PNH 1146
  simple nontoxic goiter 2600
  tuberculosis 989
Pathogens
  associated with diarrhea 1952
  induced liver cell cancers 2106
Pathologic
  adrenal gland hypertrophy 26
  atrophy 29
  classification of penile carcinoma 2367
  findings in organs in shock 298
  hyperplasia 22
  hypertrophy 26
  metaplasia 31
Pathology pearls:
  adipocytic tumors 2750
  analgesic interstitial nephritis 2254
  cervical intraepithelial neoplasia 2392
  chronic kidney disease 2230
  components of the glomerulus 2168
  desmosomal cadherins—desmocollin and desmoglein 2812
  diabetes mellitus-related nonalcoholic fatty liver disease 2583
  diabetic nephropathy 2219
  diagnostic criteria of neurofibromatosis type 1 2928
  diffuse renal cortical necrosis 2262
  endometrial stromal tumors 2421
  evolution of cervical carcinoma 2396
  family of insulin receptor substrates 2555
  fibrocystic disease of breast 2491
  focal segmental glomerulosclerosis 2208
  functional zones of liver acinus 2011
  gastric glands in body of stomach 1922
  genetic disorders associated with renal cell carcinoma 2282
  glut family of glucose transporters in human tissues 2557
  Goodpasture syndrome 2201
  hematopoietic stem cells processing 1350
  HER2/neu enriched molecular pathway 2513
  human papillomavirus (HPV) oncoproteins role in cervical carcinoma 2396
  immune complex-mediated glomerulonephritis 2184
  immunoperoxidase staining 1559
  increased serum α-fetoprotein (AFP) tumor marker in various disorders 2111
  inflammatory disorders of breast 2488
  insulin-mediated GLUT4 (glucose transporter 4) 2559
  intraductal papilloma of breast 2500
  ischemic acute tubular necrosis 2242
  key features of vitamin D 2657
  light chain cast nephropathy in multiple myeloma 2254
  lupus nephritis 2225
  lymph nodes isolation in mastectomy specimens 2529
  membranous glomerulonephritis 2212
  metabolic alterations in diabetes mellitus 2584
  metastases (secondary tumors) involving kidneys 2274
  metastatic mucinous carcinoma to ovaries 2446
  minimal change disease 2205
  mode of spread with gastric carcinoma 1940
  molecular genetic alterations in HCC 2110
  morphologic phases of Paget's disease of bone 2660
  myoepithelial cells 2505
  neurofibromatosis type 2 2927
  nonproliferative and proliferative diabetic retinopathy 2580
  papillary lesions of breast 2524
  pathogenesis of acute tubular necrosis 2241
  pathophysiology of pemphigus vulgaris 2843
  poststreptococcal glomerulonephritis (PSGN) 2197
  protocol for reporting hepatocellular carcinoma 2110
  protocol for reporting lymph node in hepatocellular carcinoma 2111
  rapidly progressive glomerulonephritis (RPGN) categories 2228
  recent advances of colorectal carcinoma stem cell biology 1989
  renal amyloidosis 2222
  renal oncocytoma 2281
  risk factors for urolithiasis 2256
  sequence of events: luminal 'B' of breast carcinoma 2512
  sporadic and familial/hereditary medullary thyroid carcinoma 2617
  steatosis in HCV infection 2050
  thyroid hormones synthesis and regulation 2587
  Toker cells versus Paget cells in differential diagnosis 2520
  type 1 membranoproliferative glomerulonephritis (type 1 MPGN) 2214
  type 2 membranoproliferative glomerulonephritis (type 2 MPGN) 2215
  type 3 membranoproliferative glomerulonephritis (type 3 MPGN) 2215
  von Hippel-Lindau disease associated clear cell renal cell carcinoma 2283

Pathophysiologic findings in hypovolemic, cardiogenic and endotoxic shock 298
Pathophysiology of 13
edema 241
embolism 289
Helicobacter pylori-induced gastrointestinal disorders 778
liver cirrhosis 2062
thrombocytopenia 1371
transplant rejection 451
viral hepatitis 2037
Pathways involved in hematopoiesis 1064
Patterns of
acute viral hepatitis 2037
liver necrosis 2015
renal transplant rejection 452
Paul and Bunnell test 1178
Peau d'orange skin 2534
Pediatric renal cell carcinoma with Xp11.2 translocation and TFE3 fusions 2283, 2292
Pedigree construction 573
Pedunculated polyps 650
Pelger-Hüet anomaly 1070, 1176, 2098
Pelvic inflammatory disease 2383
Pemphigus vulgaris 420, 450, 1881, 2842
Penile
intraepithelial neoplasia 2366
invasive squamous cell carcinoma 2366
syphilis 2364
Pepper type of neuroblastoma 2639
Peptic ulcer disease 1927
Percutaneous transhepatic cholangiography (PTC) 2134
Perforated gallbladder 2132
with peritonitis 2132
Perforin 751
Perforin and granzymes synthesis 426
Perforin polymerizes 321
Perfusion scintigraphy 1713
Pericanalicular pattern 2496
Pericardial effusion 247, 1742
Pericarditis 1743
Pericardium 1741
Pericytic/perivascular tumors 2774
Periductal mastitis 2488
Perihilar adenocarcinomas 2137
Perineural and leptomeningeal routes of metastasis 707
Perineural spread of cancers 707
Perineuroma (sclerosing, intraneural, soft tissue and reticular perineuromas) 2783
Periodic acid–Schiff stain 470, 1189
Periodontal disease 1899
Periosteal
chondrosarcoma 2676
osteosarcoma 2691
Peripheral blood
HSCs harvesting 1348
smear examination 1087
Peripheral neuropathy in vitamin B_{12} deficiency 1103
Peripheral tissue resistance to insulin by NEFAs 2564
Peripheral vascular disease 1657
limb infarction 288
Periportal
area 2011
necrosis 2015
piecemeal necrosis of hepatocytes 2017
Perisinusoidal
endothelial cells 2062
stellate cells (Ito cells) 2012
Peritoneal cavity seeding 706
Perls stain 1090
Perls Prussian blue staining: principle 1075
Permanent cells 21, 662
nondividing cells 204
Pernicious anemia 420, 1105, 1935
Peroxidase enzyme deficiency 2600
Peroxidase-antiperoxidase
method 1559
indirect method 1559
Peroxynitrite 56
Peutz-Jeghers syndrome 1880, 1936, 1981
Phaeohyphomycosis 1041
Phagocytic receptors 148
Phagocytosis and clearance of the injurious agents 149
Pharmaceutical drugs used to reduce/prevent bleeding in hematologic disorders 1458
Pharynx 1778
Phenotypic plasticity and disrupted differentiation 763
Phenylketonuria (PKT) 557
Phenylketonuria and lower risk for miscarriages 566
Pheochromocytoma 646, 2636
Philadelphia chromosome 516, 659
Phimosis 2364
Phospholipase 1925
Photomultiplier tubes 1595
PHOX2B gene 1964
Phyllodes tumor of breast 2496
Physical examination 1087
Physiologic
atrophy 28
hyperplasia 21
hypertrophy 25
metaplasia 31
myocardial hypertrophy 26
skeletal muscle hypertrophy 25
uterine hypertrophy during pregnancy 25
Physiological jaundice of newborn 2025
Physiology of
pancreas 2550
parathyroid glands 2625
PI3K/AKT signaling pathway 2557
Piecemeal necrosis 2040
Pigment accumulation 104
Pigmented basal cell carcinoma 2864
Pilar leiomyoma 2765
Pilocytic astrocytoma 2917
Pilomatrixoma 2870
Pilonidal sinus 2000
Pituitary
adenoma 612, 2645
gland 2641
hyperfunction 2599
PKHD1 gene 2031
Placenta
accreta 2480
increta 2480
percreta 2480
previa 2480
Placental
disorders 2479
malperfusion 2479
site trophoblastic tumor 2474
Plague 987
Plaque stage in Kaposi sarcoma 2875
Plasma
cells 344
component 1438
fractionation products 1451, 1458
haptoglobin 1150
haptoglobin level 1114
hemoglobin 1151
hemopexin 1114, 1151
methemalbumin 1114
products/recombinant factor VIII concentrates used in hemophilia 1397
protein component changes in stored blood 1449
protein-derived chemical mediators 158
Plasmablastic
lymphoma 1520
multiple myeloma 1319
Plasmacytoma 1765
Plasma-derived chemical mediators 136
Plasminogen 1363
activators 267, 1364
Platelet
activation 264
aggregation inhibitors 264
agonists 264
changes in stored blood 1448
component transfusion 1458
count 1415
derived growth factor 1360
disorders of receptors and signal transduction 1377
function tests 1416
neutralizing test 1410
phospholipid plasma membrane defects 1375
plug formation (primary hemostasis) 265
primary hemostatic plug formation 1358
secretory products involved in glomerulonephritis 2188
Platelet storage
changes 1449
pool defects deficiency (platelet dense granules defect) 1376
Platelet structure 256
components and their functions 258
Platelet-activating factor synthesis 174

Platelet-derived agonists and their receptors 264
Platelet-rich plasma method to prepare platelet component 1439
Platelets 189, 1360
  adhesion 1360
  aggregation 273, 1360
Platelet-type von Willebrand disease 1377
Pleomorphic
  adenoma 1889
  liposarcoma 2749
  lung carcinoma 1864
  Reed-Sternberg cells 1491, 1489
Pleomorphic rhabdomyosarcoma 2771
Pleural
  cavity seeding 706
  diseases 1872
  effusion 1872
  fibrosis 1874
  plaques 1874
Pleuritis 1872, 1874
Plexiform
  fibrohistiocytic tumor 2762
  neurofibroma 2876, 2928
Ploidy and cytogenetics in chorio-carcinoma 2343
Plummer-Vinson syndrome 1093
Pneumoconiosis 1820
Pneumonias 1782, 1785
  in immunocompromised persons 1785
Pneumothorax and atelectasis lung 1828
Point mutation (substitution in single nucleotide base pair) within coding sequences 530
Point mutation in proto-oncogene 723
Polarizer and analyzer 1550
Polarizing
  light microscopy 1548, 1636
  microscope 1549
Poliovirus infection 1027
Poly-ADP-ribosyl-polymerase (PARP) 509
Polyarteritis nodosa 424, 448, 1666
Polychromasia 1088
Polycyclic
  aromatic hydrocarbons 508, 790
  ovary syndrome (Stein-Leventhal syndrome) 2429
Polycythemia vera 1285
Polyembryoma of ovary 2462
Polygenic and multifactorial disorders 567
Polymerase chain reaction technique 1616
Polymerase proofreading-associated polyposis 691
Polymerized human serum albumin (PHSA) 2044
Polymorphic microbiomes and cancer 777
Polymorphous low-grade adenocarci-noma 1897
Polymyositis 448
Polyol pathway activation (sorbitol/aldose reductase activation pathway) 2569
Polyostotic
  fibrous dysplasia 597, 2710, 2711
  lesions 2660
Polyploidy 714
Polyps in various organs 650
Pompe disease (GSD type 2) 100, 548
Pooling of
  blood components 1450
  platelets in massive splenomegaly 1378
Poor prognostic factors for primary brain tumors 2907
Poorly differentiated thyroid carcinoma 2619
Porcelain gallbladder 2132
Portal
  hepatitis 2039
  hypertension 2068
    with portal vein obstruction 2069
  triad 2011
  vein 2011
  vein thrombosis 285
Portal of entry of pyogenic bacteria in nervous system 2890
Portosystemic shunts 2069
Port-wine stain 2873
Positive O-dimer 1832
Positron emission tomography-computed tomography (PET-CT) scan of bone tumors 2668
Positron emission tomography scanning 2537
Possible synthesis sites of factor VIII 1392
Postcricoid webs (rings) 1915
Posterior pituitary gland 2641
  hypofunctioning 2644
Postmortem
  autolysis 66
  blood clots 283
Post-necrotic cirrhosis 2060
Post-primary pulmonary (secondary) tuberculosis 990
Postrenal acute kidney disease 2243
Poststreptococcal glomerulonephritis 425, 2192
Post-transfusion purpura 1378
Postulates of the clonal selection hypothesis 356
Prader-Willi syndrome 572
Preauricular sinus 1766, 1767
Pre-B cell lineage acute lymphoblastic leukemia 1204
Precursor
  B cell and T cell NHLs 1504
  lesions 1842
  lung lesions 1842
Prediabetes stage 2560
Predisposing factors 2660
Predisposing factors for gallbladder
  cholesterol stones 2129
  pigment stones 2130
Preferred lymph node biopsy sites in Hodgkin's disease 1494
Preformed chemical mediators of mast cells 410
Pregnancy associated non-neoplastic disorders 2475
Pregnancy luteoma of ovary 2467
Preparation of blood components from whole blood 1436
Prerenal acute kidney disease 2243
Presbycusis 1769
Pretransfusion common blood bank tests 1467
Pretransfusion modifications of blood cellular components 1456
Prevention of transplant rejection 459
Priapism 1133, 2364
Primary
  adenocarcinoma 2321
  aldosteronism 2634
  amenorrhea 2404
  amyloidosis 109
  biliary cirrhosis 2092
  brain tumors 2902
  central chondrosarcoma 2676
  CNS lymphoma 2931
  glioblastoma multiforme, IDH-wild-type 2913
  glomerular diseases 2174
  gout 2727
  hemochromatosis 106
  hemostasis (platelet hemostatic plug formation) 263
  hemostatic plug formation 1360
  hyperparathyroidism: bone lesions 2626, 2627
  hypopituitarism 2642
  hypothyroidism 2595
  immune response 375
  immunodeficiency diseases 381, 384
  intestinal tuberculosis 1954
  lymphoid organs 349
  malignant tumors of lung: overview 1839
  myelofibrosis 1288
  neoplasms 1751
  nervous system tumors 2896
  osteoporosis 2659
  pleural effusion lymphoma 1876, 1878
  pneumothorax 1829
  pulmonary malignant tumors 1839
  pulmonary tuberculosis of Ghon's complex 990
  sclerosing cholangitis 2094
  squamous cell carcinoma 2320
  structure of protein 502
  syphilis 1010
  tuberculosis (Ghon's complex in lung) 1799
  tuberculosis (primary complex) 1799
  tuberculous (Ghon's complex) 989
  urothelial neoplasms 2311
  viral myocarditis 1724
Primary and secondary
  immune responses 374
  lymphoid organs 349
Primary/clonal thrombocytosis 1381
Primary/hereditary hemo-chromatosis 2085
Primase enzyme 491
Primitive neuroectodermal tumors 2931

Principal characteristics of
benign and malignant tumors 606
carcinoma and sarcoma 609
Prinzmetal angina 1701
Pro-B cell lineage acute lymphoblastic leukemia 1204
Procoagulant substance secreting malignant tumors 280
Proctoscopy 2004
Professional APCs 346
Prognosis in histologic variants of osteosarcoma 2689
Progression
of tumorigenesis 685
to chronic inflammation 178
Progressive
decompensated shock stage 304
morphologic changes in myocardial infarction 1707
staining 1554
Proinflammatory and anti-inflammatory cytokines synthesis role in septic shock 301
Proinsulin 2553
Proliferative
changes in breasts 2491
diabetic retinopathy 2580
fibrocystic changes 2491
glomerulonephritis 2177
Prolymphocytic leukemia 1507
Promoter regulatory region of DNA 528
Promotion of tumorigenesis 685
Proofreading 491
Propagation of thrombus 281
Properties of antigens 333
Proposed mechanism of thrombosis in antiphospholipid antibody syndrome 278
Prosigna kit test (PAM50) risk of recurrence score 2543
Prostate-specific antigen 2358
Prostatic adenocarcinoma with
acinar pattern 2359
ductal pattern 2359
Prostatic
carcinoma of other histologic types 2359
intraepithelial neoplasia 2355
valve endocarditis 1734
Protease 1925
Protease (cathepsin D) overexpression 2533
Protein
kinase C activation signaling pathway 2569
misfolding and aggregation 503
Protein C 1365, 1408
deficiency 277, 1408
folding 502
Protein S deficiency 277, 1409
Protein-calorie malnutrition 964
Proteins as biomarkers 958
Proteoglycan 219
Proteolysis of thyroglobulin colloid in lysosome 2588
Prothrombin complex concentrate 1458
Prothrombin G20210A gene mutation 1410
Prothrombin time and international normalized ratio 1416
Proto-oncogenes 591, 709, 839
Protozoa 1044
Pruritus ani 2001
Psammomatous melanocytic schwannoma 2782
P-selectin (CD62P) 141
Pseudoangiomatous stromal hyperplasia 2499
Pseudoarthrosis 2729
Pseudochylous effusion 1873
Pseudohermaphrodite 571
Pseudohypoparathyroidism 2630
Pseudomembranous
colitis 1974
inflammation 177
Pseudomyxoma peritonei 2004
Pseudo-obstruction syndrome 1966
Psoriasis 24, 2840
Psoriatic arthritis 2726
PTEN tumor suppressor gene 2510
Pulmonary
alveolar proteinosis 1828
angiogram 1832
edema 247, 1834
embolism 284, 286
hamartoma 1837
hemorrhagic/red infarct 296
hypertension 1833
manifestations 1693
metastasis 701
stenosis 1741
thromboembolism 283, 1831
tuberculosis 990
consequences 993
morphological patterns 992
valve stenosis 1741
vascular disorders 1831
vasculitis 1836
volumes 1781
Pulmonic valve stenosis 1741
Pure
gall calculus 2126
gallbladder stone 2126
Leydig cell tumor of ovary 2466
neuritic leprosy 1003
red cell anemia 1156
red cell aplasia (erythroblastopenia) 1156
Purkinje fibers 1696
Purpose to analyze cancer biomarkers 939
Purulent (suppurative) inflammation 176
Purulent exudate 1744
Purulent or suppurative pericarditis 1744
Pus cells in urine 2305
Pyelonephritis 2244
Pyogenic
bacterial liver abscess 2053
meningitis and age group predilection 2888
osteomyelitis in adults 2653
osteomyelitis in children and adolescents 2653
osteomyelitis in neonates 2653
Pyogenicum granuloma 233
Pyonephrosis 2261
Pyothorax 1872
effusion lymphoma 1878
Pyridoxine (vitamin B_6) 980
Pyroptosis 42, 74
Pyruvate kinase
deficiency disorder 1139
enzyme deficiency 565

Q

Q fever (*Coxiella burnetii*) infection 1014
Qualitative
acquired platelet disorders 1378
fluorescence polymerase chain reaction 579
hemoglobinopathies 1082
inherited platelet disorders 1375
Quantitative
hemoglobinopathies 1082
platelet disorders (thrombocytopenias) 1367
variations in leukocytes 1168
Quaternary structure of protein 503
Quebec platelet syndrome (platelet α-granule defect) 1377

R

Rabies virus infection 1029
Racemose/cirsoid aneurysm 1658
Radiation
colitis 1976
reactions and DNA damage 506
therapy 930, 2545
Radiation-induced
carcinogenesis 793
interstitial pulmonary fibrosis 1826
interstitial lung disease 1826
Radicular cyst (periapical cyst) 1900
Radioactive iodine uptake and thyroid imaging 2623
Radioallergosorbent test (RAST) 413
Radiographic features of bone tumors 2666
Radionuclide
scan 1832, 2607
scanning (99mTc) stannous pyrophosphate 1713
Ramsay Hunt syndrome 1767
Random
blood glucose 2584
mutagenesis 536
Rapid massive blood transfusion-related reactions 1465
Rapidly progressive (crescentic) glomerulonephritis 2178, 2226
RAS (from rat sarcoma virus) gene 2510
RAS (RAT sarcoma) oncogene 2510

RAS/RAF/MEK/ERK (MAPK) signaling pathway 2559
Raynaud's phenomenon (ANCA negative) 1671
RB tumor suppressor gene 2510
RBC autohemolysis test 1119
Reactive
lymphoid hyperplasia 1476
oxygen species 52, 55
pleural fibrosis 1874
splenic disorders 1533
vascular proliferation 1674
Recent 2019 WHO classification of breast tumors 2494
Recent 2020 WHO classification of bone tumors 2661
Recent 2020 WHO classification of soft tissue tumors 2732
Recent advances:
liver biopsy specimen handling in surgical pathology 2153
molecular genetic techniques in breast cancer 2542
Receptor tyrosine kinases 598
Recombinant
plasma protein production 1451
tissue plasminogen activator (tPA) 1364
used in clinical practice 268
Rectal mucosal prolapse 2000
Recurrent
jaundice during pregnancy 2028
myocardial infarction 1710
pyogenic granulomas with satellitosis 195
Red blood cell
changes in stored blood 1448
component transfusion 1455
distribution width 1079
fragmentation syndromes 1148
inclusions in hematologic disorders 1090
indices 1079
storage changes 1448
Red blood cell-associated antigenic polymorphism and resistance to malaria 565
Red cell indices 1079
Red pulp of spleen 1531
Reduction of ATP and 2,3 diphosphoglycerate (2,3-DPG) in stored blood 1448
Reed-Sternberg cell variants 1489
Reed-Sternberg cells 198, 1482, 1489
Refractive index 1550
Refractory sprue 1948
Regenerating capacity of cells 662
Regeneration of
liver 238
nervous system 238
specific organs 238
Regenerative
nodules in liver 2103
stain 1554
Regulated cell death (RCD) 68
Regulation of
apoptosis by gene products 82
coagulation system 1388
hematopoiesis 1060
iron metabolism 1090
selectin family 141
thyroid hormones 2586
Regulatory
noncoding RNAS 534
regions in DNA: promoters, enhancers and silencers 527
Rejuvenation of red blood cells 1451
Relapsing polychondritis 1767
Removal of blood components by apheresis 1441
Renal
agenesis 2272
amyloidosis 466, 2219
angiomyolipoma (hamartoma) 2296
atrophy in disorders 30
biopsy evaluation and interpretation 2234
cell carcinoma 2281
clear cell variant 626
function tests 2297
hypertension 1657
infarct 295
infarction 2263
lesions in diabetic nephropathy 2573
osteodystrophy 2658
papillary necrosis 1133, 2253
tubular functional disorders 2254
vein thrombosis 285
Rendu-Osler-Weber disease 2098
Renin-angiotensin-aldosterone system 241, 2162
Renovascular hypertension 1686
Reperfusion-induced myocardial injury 1705
Repetitive DNA sequence causing polymorphism 569
Reprogramming energy metabolism (Warburg effect) 748
Residual volume 1782
Respiratory
disorders 1781
distress syndrome in the infants 1817
system 1778
system amyloidosis 468
Responses of innate immune system 328
Restenosis of coronary arteries 1656
Restrictive
cardiomyopathy 1729
pulmonary diseases 1814
Reticulocyte count 1080
Reticuloendothelial cells distribution in various tissues 183
Retinoblastoma tumor suppressor gene 759
RET-PTC oncogene 2607
Retroperitoneal
hemorrhage 1394
leiomyoma 2765
Rett syndrome 561
Reverse transcription polymerase chain reaction (RT-PCR) 495
Reversible and irreversible cell injury 35
Reversible cell injury 18, 45
Reversible cell injury versus irreversible cell injury 45
Reye syndrome 2092
Rh blood group system 1431
Rh blood grouping 1468
Rh factor incompatibility during pregnancy 1432
Rhabdomyolysis 2807
Rhabdomyoma 2767
Rhabdomyosarcoma 633
Rheumatic fever and rheumatic heart disease 1715
Rheumatoid arthritis 420, 430, 449, 1142, 1827, 2723
Rheumatoid nodules 2725
Rhinorrhea 1758
Rhinoscleroma 1766
Rhinosporidiosis 1765, 1766
Rhinosporidium seeberi 1765
Rhodamine 1558
Riboflavin (vitamin B_2) 980
Riboflavin functions 980
Ribonucleic acid 477
biomarkers 956
Ribosomal rNAS (rRNAS) 502
Richardson grading system 2530
Richter syndrome 1512
Rickets 975, 2657
Rickettsial infections 1013
Ridley-Jopling leprosy classification 1003, 2821
Riedel's thyroiditis 2592
Right ventricular infarct 1710
Right-sided colon
cancer 1995
carcinoma 1995
Right-sided endocarditis in carcinoid syndrome 1736
Right-sided heart failure 1750
Ring chromosome 517
Risk factors for
atherosclerosis 1697
colorectal carcinoma 1989
development of gastric carcinoma 1933
development of osteosarcoma 2684
esophageal adenocarcinoma 1920
RNA vaccine 380
Robertsonian translocation 517, 718
Rocky Mountain spotted fever 1013
Role of hormones in
breasts development 2484
hematopoiesis 1063
Role of
hypoxia-inducible factor (HIF) in angiogenesis 742
immunohistochemistry technique in primary surface epithelial tumors of ovary and Krukenberg tumor of ovary 2438
Rolling of leukocytes 141
Romanowsky stains 1084

Rosai-Dorfman disease 2716
Rota virus 1952
Roth's retinal hemorrhages 1733
Rothmund-Thomson syndrome 696
Rotor's syndrome 2030
Rouleaux formation 1088
Rubella virus infection 1028
Rugger Jersey appearance 2658
Russell
  bodies 103
  viper venom time test 1410

S

SA node 1696
Saccharopolyspora rectivirgula 1827
Saccular aneurysm 1658
Saddle embolus 1832
Salient features of ovarian masses 2435
Salivary gland 1886
Salmonella typhi infection (typhoid fever) 988
Salmonella typhimurium 1952
Salpingitis isthmica nodosa 2427
Salt and pepper chromatin 645
Sandwich ELISA 1614
Sanfilippo syndrome (MPS type 3 disease) 551
Sarcoidosis 1826
SARS-CoV-2 vaccines 1026
Scabies 2820
Scanning electron microscopy (SEM) 1548
Schatzki ring 1915
Schaumann bodies 194
Scheie syndrome (MPS type V disease) 552
Schilling test 1107
Schindler disease 556
Schistosomiasis in liver 2057
Schneiderian sinonasal papillomas 1759
Schwannoma (neurilemmoma) 2926
Schwannoma and neurofibromatosis type 2 2781
Scleroderma 447, 1827, 1914
Sclerosing
  adenosis 2493
  epithelioid fibrosarcoma variant of adult fibrosarcoma 2757
  lymphocytic lobulitis 2490
  odontogenic carcinoma 1904
Sclerotherapy 2070
Scot syndrome 1376
Scurvy purpura 1383
Sea-blue histiocytosis 1343
Seborrheic keratosis 2866
Second line of defense 317
Secondary
  aldosteronism 2634
  amenorrhea 2404
  amyloidosis 109, 110
  biliary cirrhosis 2093
  bone tumors (metastatic bone disease) 2718
  central chondrosarcoma 2676
  glioblastoma multiforme, IDH-mutant 2915
  glomerular diseases 2175
  gout 2727
  hemochromatosis 106, 2087
  hemostasis (stable hemostatic plug formation) 266
  hyperparathyroidism 2628
  hypertension 1686
  hypothyroidism 2595
  immune response 375
  immunodeficiency diseases 392
  intestinal tuberculosis 1954
  lymphedema 243
  lymphoid organs 349
  messenger systems 2533
  neoplasms 1751, 2004
  osteoporosis 2659
  peripheral chondrosarcoma 2676
  pneumothorax 1829
  pulmonary malignant tumors 1864
  structure of protein 502
  syphilis 1010
  thrombocytosis 1382
  thrombocytosis and essential thrombocythemia 1381
  thyroid tumors 2619
  tuberculosis 989, 1799
Secretors and nonsecretors 1428
Secretory
  breast carcinoma 2525
  diarrhea 1952
  multiple myeloma 1315
Seeding of cancer stem cells and transcoelomic route of metastasis 706
Segmental glomerulosclerosis 2178
Selected
  autoimmune diseases with autoantibodies reacting only with antigens in the affected organs 442
  autosomal
    dominant disorders 539
    recessive disorders 544
  examples of molecular diagnostics in malignant tumors 940
  monoclonal antibody-based drugs 376
    used in various disorders 376
Selectins 5
Selection of donor for obtaining HSCs 1350
Selective
  IGA deficiency 386, 681
  IGG subclass deficiency 387
  IGM deficiency 386
Semen (seminal fluid) 2324
Semen analysis 2324
Seminoma
  of testis 2332
  with syncytiotrophoblastic component 2333
  with yolk sac component 2333
Senile purpura 1384
Sentinel lymph node biopsy 2538
Septic (infective) infarcts 294
Septic shock 201, 300
Sequence of events of metastatic cascade 886
Sequestration crisis 1133
Sequestrum (necrotic bone) formation 2654
Serine protease inhibitors (SERPINs) 262
Serofibrinous pericarditis 1719
Serous
  borderline tumor of ovary 616
  cystadenocarcinoma of ovary 2442
  cystadenoma of ovary 2440
  endometrial carcinoma 2418
  inflammation 175
  pericarditis 1744
  surface epithelial tumors 2440
Serpentine aneurysm 1658
Serrated polyps 1980
Sertoli cell tumor (androblastoma) of testis 2345
Sertoli-Leydig cell tumor of ovary 2466
Serum
  alkaline phosphatase 2359
  cardiac enzymes 1712
  ceruloplasmin 2020, 2021
  creatinine 2300
  ferritin 2087
  grouping (reverse grouping) 1467
  haptoglobin 1139
  lactic dehydrogenase 1139
  prostate-specific acid phosphatase 2359
  sickness 424
  TSH, T4 and T3 levels 2622
  tumor markers in prostatic adenocarcinoma 2358
Sessile
  polyps 650
  serrated adenomas 1985
  serrated adenomas/polyps with dysplasia 1981
Seven α-helical transmembrane G protein-coupled receptors 148
Several types of tissues/organs transplantation 451
Severe
  acute respiratory syndrome coronavirus 2 (SARS-COV-2) for COVID-19 pandemic 1017
  biliary colic 2131
  combined immunodeficiency syndrome 388
  congenital anomalies of kidneys and urinary tract 2272
  congenital neutropenia 681
  hemophilia A 558
  respiratory distress syndrome 1819
Severity of SARS-COV-2 (COVID-19) disease 1022
Sex chromosome 484
  deviation 522
  disorder of sexual differentiation [children may have either one X chromosome (X0) or an extra chromosome (XXY)] 571

Sex cord-stromal tumors of
ovary 2463
testis 2345
Sexually transmitted infections 2364
Sézary syndrome 1529
Sheehan's syndrome 2478, 2643
Shepherd's crook deformity 2711
Shigella sonnei 1952
Shock 297
Shunting of blood in left renal vein 2070
Sickle cell anemia and
resistance to malaria 564
susceptibility to pneumococcal infections 567
Sickle cell 1088
crisis 1132
disease 544, 1129, 1130
disorders and other hemoglobinopathies 1129
nephropathy 2263
thalassemia syndrome 1136
trait 1135
Sickling test 1134, 1151, 1162
Sideroblastic anemia 1157
Siderotic nodules 1088
Siderotic nodules/Pappenheimer bodies 1088
Signal
relaying junctions 6
transduction proteins 827
Signaling mechanisms of cell growth 212
Silent
carrier of α-thalassemia 1128
mutation 531
Silicone breast implants 2489
Silicosis 1815, 1823
Silo filler's disease 1827
Simon's focus in lungs in secondary tuberculosis 992
Simond's disease 2643
Simple
bone cyst 1901, 2709
bruising 1384
nontoxic goiter 2600
Single cell or group of cells necrosis of hepatocytes 2017
Single nucleotide polymorphisms 569
Single-gene mutation disorders and inheritance pattern 535
Single-gene mutation disorders induced due to various defects 535
Single-nucleotide polymorphism (SNP) technique 1603
Sinonasal
adenocarcinoma 1765
endophytic papilloma 1760
hemangioblastoma 1765
melanoma 1764
oncocytic papilloma 1761
plasmacytoma 1765
undifferentiated carcinoma 1762
papillomas 1759
Sinus histiocytosis 1476
Sinusoidal obstruction syndrome (veno-occlusive disorder) 2097
SIP1 gene 1964
SIRT1 and metabolic diseases 121
Sirtuins in health and disease 120
Site of hematopoiesis 1057
Site-directed mutagenesis 536
Sjögren syndrome 448, 1887, 2725
Skeletal disease in multiple myeloma—pathophysiology 1329
Skeletal muscle
atrophy 2806
atrophy in disorders 29
hypertrophy 2807
satellite cells 208
structure 2796
tumors 2767
Skin
amyloidosis 468
appendageal tumors and hamartomatous of the skin 2868
biopsy 2538
disorders 2814
epidermis stem cells 207
histology and skin disorders 2809
rashes (erythema marginatum) 1718, 1722
scratch testing 413
Skin-associated lymphoid tissue (SALT) 350
Skip metastases 2528
Skipping lesions 1974
Sliding hiatal hernia 1914
Sly syndrome (MPS type 7 disease) 552
SMADA4 tumor suppressor gene 2147
Small bowel infarction 1957
Small cell
lung carcinoma 645, 1848, 1854
osteosarcoma 2690
Small
lymphocytic lymphoma/CLL 1513, 1514
nuclear RNAs (SNRNAs) 502
vessel vasculitides 1668
Smoldering multiple myeloma (asymptomatic multiple myeloma) 1318
α-Smooth muscle actin 2484
Smooth muscle tumor of uncertain malignant potential (STUMP) 2423
Smooth muscle tumors 2765
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) 1117
Sodium dodecyl sulfate polyacrylamide gel electrophoresis 1160
Soft tissue
bleeding 1394
chondroma 2794
giant cell tumor with low malignant potential 2763
leiomyoma 2765
leiomyosarcoma 2766
tumor specimen handling in surgical pathology 2807
tumors 2732
Solenoid 476, 587
Solid variant of alveolar rhabdomyosarcoma 2770
Solid variant of vascular leiomyoma 2765
Solitary and multifocal bone disease 2719
Solitary
and multifocal bone metastatic disease 2719
neurofibroma 2928
osseous/extraosseous plasmacytoma 1320
renal cyst 2270
Soluble
factors involved in glomerulonephritis 2189
mediators of innate immune system 322
Somatic
mosaicism 517
mutations 534
Somatotroph adenoma 2647
Some examples of chronic granulomatous inflammation 190
Some important cells of the immune system 340
Southern blotting technique 658, 659
Special
granuloma formation 195
stains 1554
tests on bone marrow cells to diagnose hematologic malignant disorders 1074
Specific
autoimmune diseases 441
immunoglobulins 1458
Specimen requirements for molecular diagnostics in cancer 941
Spermatocele 2350
Spermatocytic seminoma of testis 2335
Spermatogenesis 2323
Spider nevi 2075
Spina bifida 2941
Spindle cell
hemangioma 1677
rhabdomyosarcoma 2768, 2769
Spindled malignant peripheral nerve sheath tumor 2930
Spitz nevus 2848
Spleen 350, 1531
amyloidosis 111, 467
involvement in amyloidosis 1534
structure 1531
Splenectomy 1531
Splenic
infarct 296, 1533
lymphoma with villous lymphocytes 1313
marginal zone B cell lymphoma 1507
marginal zone lymphoma 1520, 1535
rupture 1533
sequestration crisis and autosplenectomy 1133
Splenomegaly 1531
Splenosis 1533

Sporadic and familial medullary thyroid carcinoma 2615
Sporadic medullary carcinoma 2617
Sporothrix schenckii 1042
Spurious thrombocytosis 1381
Squamous cell carcinoma in various tissues 620
Squamous cell carcinoma of
cervix 621
larynx 1771
lung 1856
skin 620, 795, 2858
vagina 2385
vulva 2379
Squamous cell endometrial carcinoma 2418
Squamous cell
lung carcinoma 621, 1849, 1856, 1858
papilloma 1881
Squamous epithelium metaplasia to become columnar epithelium in Barrett's esophagus 32
Squamous metaplasia in
bronchi lined by columnar epithelium 31
cervix 31
conjunctiva 32
pancreatic ducts 32
tissues/organs 31
urothelium lining urinary bladder 31
Squamous
odontogenic tumor 1902
papilloma 1770, 1916
Stabilized fibrin clot formation 262
Stable
angina 1699
bone fracture 234
cells 21, 662
cells/quiescent cells (facultative mitotic cells) 204
Stages of
B cell development 367
development of red blood cells 1067
maturation of myeloid cells 1069
primary hemostasis 1358
shock 304
lymphoid neoplasms 1487
Staging system 2468
Stargardt disease 29
Start and stop codons 529
Status of axillary lymph nodes in breast carcinoma 2529
Steatohepatitis 2080
Stellate (Ito) cells 2062
Stem cell therapy in clinical practice 206
Stem cells in tissue homeostasis 204, 206
Steps of DNA repair mechanisms 509
Stercoral ulcer 1977
Sticky platelet syndrome 278
Still disease (juvenile rheumatoid arthritis) 2726
Stomatocytes 1089
Stomatostatinoma 2153
Stone granuloma 2261
Storage conditions for blood components 1447
Straggler (stray) chromosomes 666
Streptokinase enzyme 281
Stress response protein 27 (heat shock protein 27) 2533
Stricture 1974
String sign 1974
Stromal
breast tumors 2494
desmoplasia in solid malignant tumors 652
Strongyloidiasis 1048
Structural
alterations in chromosomes 714
fibrous proteins 217
Structure and functions of platelet 1072, 1362, 1363
Structure of
female breasts 2482
immunoglobulin 372
spermatozoon 8
Struma ovarii 2599
monodermal teratoma 2460
Sturge-Weber syndrome 2873
Subacute
bacterial endocarditis 1731
combined degeneration of spinal cord 1103
Subacute
endocarditis 1732
granulomatous thyroiditis 2591
painless lymphocytic thyroiditis 2591
Subarachnoid hemorrhage 2936
Subcellular endoplasmic reticulum hypertrophy in liver 27
Subcutaneous
edema 247
necrosis 66
nodules 1718, 1722
Subdural space 2885
Subendocardial
infarct 1705, 1712
myocardial infarct 1705
Submassive
hepatic necrosis 2015
liver necrosis 2015
Sucrose hemolysis test *in vitro* 1147
Sudan black stain 1189
Sudden cardiac death 1708
Sugar-phosphate backbone 488, 584
Superantigens 335
Superantigens activate naïve CD4+ helper T cell 365
Superficial
basal cell carcinoma 2864
fibromatosis (Dupuytren's disease or contracture) 2753
spreading melanoma 2849, 2851
Superinfection 2050, 2051
Superior vena cava syndrome 1673
Superoxide
anion (O_2^-) 55
dismutase 53
Suppurative
lymphadenitis 1475
thrombosis of portal vein 2057
Supravital staining 1154
Surface epithelial inclusion cysts 2431
Surface epithelial tumors of ovary 2435
Surfactant phosphatidylcholine (lecithin) 1830
Surgical pathology:
acanthosis nigricans 2880
acinic cell carcinoma 1897
acquired renal cystic disease 2271
acute cholecystitis 2123
acute erosive gastritis 1925
acute pyelonephritis 2246
acute viral hepatitis 2037
adamantinoma of bone 2697
adenocarcinoma of small intestine 1958
adenoid cystic carcinoma 1896
adenoma of the nipple 2503
adenomas of anterior pituitary gland 2646
adenomatoid tumor 2349
adenomyosis uteri 2408
adrenocortical adenoma 2640
adrenocortical carcinoma 2640
adult fibrosarcoma 2757
adult polycystic kidney disease 2269
adult sclerosing rhabdomyosarcoma 2772
adult T cell leukemia/lymphoma 1525
alcoholic cirrhosis 2081
alveolar rhabdomyosarcoma 2770
alveolar soft part sarcoma 2790
amyloidosis of liver 2091
anal carcinoma 2002
anaplastic astrocytoma, IDH-mutant 2913
anaplastic large T cell lymphoma (ALK positive) 1526
anaplastic thyroid carcinoma 2619
aneurysmal bone cyst 2700
angioimmunoblastic T cell lymphoma 1524
angiomatoid fibrous histiocytoma 2763
angiomyofibroblastoma of vulva 2378
angiosarcoma of breast 2527
apocrine carcinoma of breast 2525
Arias-Stella reaction 2407
atypical and malignant glomus tumors 2778
atypical fibroxanthoma 2762
autoimmune enteropathy 1950
basal cell carcinoma of skin 2864
benign fibrous histiocytoma 2759
benign nephrosclerosis 2265
benign nodular hyperplasia of prostate gland 2354
borderline mucinous tumor of ovary 2444
borderline serous tumor of ovary 2441
botryoid rhabdomyosarcoma of urinary bladder 2322
botryoid rhabdomyosarcoma of vagina 2387
Brenner tumor of ovary 2450
Budd-Chiari syndrome 2096
bullous pemphigoid 2844
Burkitt's lymphoma 1510

burnt-out germ cell tumors of testis 2344
capillary hemangioma 2874
carcinoid tumor of ovary 2462
cerebral infarction 2936
cervical carcinoma 2397
cholangiocarcinoma 2115
cholesterolosis 2125
chondroblastoma 2675
chondroid syringoma 2870
chondromesenchymal hamartoma 2715
chondromyxoid fibroma 2676
choriocarcinoma of ovary 2456
choriocarcinoma of testis 2343
choroid plexus papilloma 2920
chromophobe renal cell carcinoma 2291
chronic atrophic gastritis type A 1926
chronic endometritis 2406
chronic lobular hepatitis 2039
chronic pyelonephritis 2250
chronic salpingitis 2427
chronic venous congestion of liver 2095
clear cell adenocarcinoma of vagina 2387
clear cell carcinoma of ovary 2449
clear cell renal cell carcinoma 2285
collecting duct carcinoma of Bellini 2294
colloid carcinoma of breast 2521
colorectal adenocarcinoma 1995
common warts (verrucous vulgaris) 2829
complete hydatidiform mole 2471
condyloma acuminatum 2365
conventional chordoma 2701
conventional chondrosarcoma 2677
conventional hepatocellular carcinoma 2107
conventional intramedullary osteosarcoma 2687
craniopharyngioma 2650
cribriform carcinoma of breast 2526
Crohn's disease 1974
cystitis glandularis 2310
dedifferentiated chondrosarcoma 2679
dedifferentiated liposarcoma 2747
deep fibromatosis 2754
dermatitis herpetiformis (herpes simplex virus II infection) 2830
dermatofibroma 2871
dermatofibrosarcoma protuberans 2756, 2872
dermatophytosis 2834
diffuse astrocytoma, IDH-mutant 2911
diffuse infiltrative type of gastric carcinoma (linitis plastica) 1939
diffuse large B cell lymphoma 1512
diffuse leptomeningeal glioneuronal tumor 2926
diffuse midline glioma, histone H3K27M-mutant 2912
drug-induced hepatocellular injury 2058
dysgerminoma of ovary 2451
dysplastic melanocytic nevus 2847
eccrine spiradenoma 2869
eczematous dermatitis 2818
embryonal carcinoma of ovary 2455
embryonal carcinoma of testis 2338
embryonal rhabdomyosarcoma 2769
emphysema 1808
endocervical polyp 2392
endometrial hyperplasia 2406
endometrial polyp 2411
endometrioid adenocarcinoma of ovary 2447
endometriosis 2409
ependymoma 2919
ependymoma with RELA fusion positive 2920
epidermal keratinous cyst 2879
epithelioid glioblastoma 2917
epithelioid hemangioendothelioma 2118
epithelioid sarcoma 2789
epithelioid trophoblastic tumor 2475
Erdheim-Chester disease 2715
esophageal adenocarcinoma 1920
esophageal squamous cell carcinoma 1919
Ewing sarcoma 2694
extrahepatic biliary atresia/stenosis 2031
extranodal marginal zone lymphoma of MALT 1520
extranodal NK cell/T cell lymphoma 1527
extraskeletal mesenchymal chondrosarcoma 2795
extraskeletal myxoid chondrosarcoma 2794
fat necrosis of breast 2489
fatty change in liver 2079
fibroadenoma of breast 2496
fibrocongestive splenomegaly 1532
fibrocystic disease of breast 2491
fibroma of ovary 2465
fibromatosis colli 2751
fibrous dysplasia of bone 2711
flat urothelial lesions with atypia 2314
follicular lymphoma 1516
follicular cyst in ovary 2429
follicular thyroid adenoma 2605
follicular thyroid carcinoma 2613
gallbladder adenocarcinoma 2135
gallbladder pigment stones 2131
gastric MALT lymphoma 1941
gastrointestinal stromal tumors 1943, 2773
genital herpes simplex virus 2 infection 2377
gestational choriocarcinoma 2473
giant cell tumor of bone 2698
Gilbert syndrome 2029
glioblastoma multiforme with a primitive neuroectodermal tumor component 2918
glomus tumor 2776
gouty arthritis 2727
granular cell tumor 2779
granuloma inguinale 2376
granulomatous mastitis 2489
granulosa cell tumors of ovary 2463
Graves' disease 2598
gynecomastia 2547
Hashimoto's thyroiditis 2590
hemangioblastoma 2930
hemangiopericytoma and solitary fibrous tumor 2775
hepatic angiosarcoma 2117
hereditary nonpolyposis colon cancer 1986
hibernoma 2745
high-grade surface osteosarcoma 2692
Hirschsprung's disease 1965
HPV-related epithelial changes in vulva 2376
hydatid cyst in liver 2056
hyperplastic polyps in colon and rectum 1981
hypertrophic cardiomyopathy 1728
idiopathic giant cell myocarditis 1725
immature teratoma of ovary 2460
impetigo 2819
Indian childhood cirrhosis 2091
infantile fibrosarcoma 2755
infantile polycystic kidney disease 2269
intestinal tuberculosis 1954
intestinal type of gastric adenocarcinoma 1938
intraductal papilloma of breast 2500
invasive ductal carcinoma (NOS) of breast 2517
invasive ductal carcinoma of male breast 2547
invasive hydatidiform mole 2473
invasive micropapillary carcinoma of breast 2523
invasive urothelial carcinoma 2317
ischemic bowel disease 1978
juvenile xanthogranuloma 2761
Kaposi sarcoma 2875
keloid 2751, 2882
keratoacanthoma 2862
Krukenberg tumor 2432
Langerhans cell histiocytosis 2714
leiomyoma 2422
leiomyosarcoma of vagina 2388
Leydig cell tumor of testis 2346
lichen planus 2842
lichen sclerosus 2378
lipoma 2744
liver biopsy in HBV infection 2048
liver cirrhosis 2064
liver in Gaucher's disease 2090
liver in Niemann-Pick disease 2090
liver metastases 2122
lobular carcinoma of breast 2516
low-grade central osteosarcoma 2689
lymph node biopsy in mantle cell lymphoma (MCL) 1312
lymphocyte-rich classic variant of Hodgkin's disease 1496
malignant fibrous histiocytoma of bone 2705
malignant nephrosclerosis 2265
malignant peripheral nerve sheath tumor 2785, 2929

mammary duct ectasia 2488
mantle cell lymphoma 1518
massive hepatic necrosis 2017
mature cystic teratoma of ovary 2458
mature solid teratoma of ovary 2459
medullary carcinoma of breast 2520
medullary sponge kidney 2271
medullary thyroid carcinoma 2617
medulloblastoma 2921
melanocytic nevus 2847
melanoma 2851
melanoma of vagina 2389
meningioma 2923
Merkel cell carcinoma 2867
mesenchymal chondrosarcoma 2679
metaplastic carcinoma of breast 2524
metastases to brain 2907
metastatic bone disease 2721
mixed cellularity classic variant of Hodgkin's disease 1498
mixed Müllerian tumor 2421
molluscum contagiosum 2828
mucinous cystadenocarcinoma of ovary 2445
mucinous cystadenoma of ovary 2443
mucocele of gallbladder 2133
mucoepidermoid carcinoma 1894
multicystic renal dysplasia 2272
multinodular goiter 2602
multiple myeloma 2707
myopericytoma family of tumors 2776
myositis ossificans 2792
myxofibrosarcoma 2758
myxoid liposarcoma 2748
nerve sheath myxoma 2784
neuroblastoma 2638
neuroendocrine tumor 1921
neurofibroma and neurofibromatosis type 2 2782
neurofibroma, neurofibromatosis type 1 and malignant change in neurofibroma 2928
nodal marginal zone lymphoma 1519
nodular fasciitis 2752
nodular hidradenoma 2869
nodular lymphocytic–histiocytic predominant variant of Hodgkin's disease 1499
nodular sclerosis classic variant of Hodgkin's disease 1497
nonalcoholic fatty liver disease 2083
ochronosis of joints 2728
oligodendroglioma 2918
oncocytoma of salivary glands 1893
osteoarthritis 2727
osteoblastoma 2683
osteochondroma 2674
osteofibrous dysplasia 2712
osteoid osteoma 2682
osteoma 2681
osteopetrosis 2717
Paget's disease of breast 2519
Paget's disease of vulva 2379
pancreatic adenocarcinoma 2149
papillary (chromophil) renal cell carcinoma 2288
papillary carcinoma of breast 2523
papillary hidradenoma of vulva 2378
papillary thyroid carcinoma 2607
paraovarian or paratubal cysts 2431
paraovarian or paratubal cysts in ovaries 2431
parathyroid adenoma 2631
parathyroid carcinoma 2631
parosteal osteosarcoma 2691
partial hydatidiform mole 2472
pemphigus vulgaris 2843
penile squamous cell carcinoma 2368
peptic ulcer disease 1929
periductal mastitis 2488
perineuroma 2783
periosteal osteosarcoma 2692
pheochromocytoma 2637
phyllodes tumor of breast 2497
pilocytic astrocytoma 2917
pilomatrixoma 2871
placental site trophoblastic tumor 2475
plasmablastic lymphoma 1520
pleomorphic adenoma 1890
pleomorphic liposarcoma 2749
pleomorphic rhabdomyosarcoma 2771
plexiform fibrohistiocytic tumor 2762
plexiform neurofibroma 2877
polycystic ovary syndrome (Stein-Leventhal syndrome) 2430
polyembryoma of ovary 2462
precursor B cell lymphoblastic leukemia/lymphoma 1504
primary adenocarcinoma of urinary bladder 2321
primary biliary cirrhosis 2093
primary bone marrow lymphoma 2708
primary glioblastoma multiforme, IDH-wildtype 2914
primary sclerosing cholangitis 2095
prostatic adenocarcinoma 2359
psammomatous melanotic schwannoma 2783
psoriasis 2841
pyogenic meningitis 2891
rapidly progressive glomerulonephritis (RPGN) 2228
rectal mucosal prolapse 2001
renal angiomyolipoma 2296
renal oncocytoma 2281
renal papillary necrosis 2253
retinoblastoma 2932
rheumatoid arthritis 2726
salpingitis isthmica nodosa 2427
Schwannoma (neurilemmoma) 2927
seborrheic keratosis 2866
secondary biliary cirrhosis 2094
secondary glioblastoma multiforme, IDH-mutant 2916
secretory carcinoma of breast 2526
seminoma of testis 2332
serous cystadenocarcinoma of ovary 2442
serous cystadenoma of ovary 2441
Sertoli cell tumor of testis 2345
simple bone cyst 2710
simple nontoxic goiter 2600
small cell osteosarcoma 2690
soft tissue chondroma 2794
soft tissue giant cell tumor with low malignant potential 2763
soft tissue leiomyoma 2765
soft tissue leiomyosarcoma 2766
solitary renal cyst 2271
spermatocytic seminoma of testis 2335
splenic marginal zone lymphoma 1535
squamous cell carcinoma in oral cavity 1883
squamous cell carcinoma of skin 2860
squamous cell carcinoma of urinary bladder 2320
squamous cell carcinoma of vagina 2385
struma ovarii 2461
superficial fibromatosis 2753
surface epithelial inclusion cysts in ovaries 2431
synovial sarcoma 2787
tabes dorsalis 2896
telangiectatic osteosarcoma 2690
teratoma of testis 2341
testicular diffuse large B cell lymphoma 2347
thymoma 1539
traumatic neuroma 2779, 2877
tubal endometriosis 2427
tubercular osteomyelitis 2656
tubercular pyelonephritis 2252
tubular adenomas 1983
tubular carcinoma of breast 2522
type 1 endometrioid carcinoma 2416
type 2 nonendometrioid carcinoma 2418
ulcerative colitis 1972
undifferentiated pleomorphic sarcoma 2764
uterine endometrium during menstrual cycle 2404
uterine leiomyosarcoma 2424
vaginal intraepithelial neoplasia 2384
viral encephalitis 2895
Warthin's tumor 1891
well-differentiated liposarcoma/atypical lipomatous tumor 2746
well-differentiated papillary mesothelioma 2003
Whipple disease (tropical sprue) 1953
Wilms' tumor (nephroblastoma) 2278
Wiskott-Aldrich syndrome 1537
xanthogranulomatous pyelonephritis 2252
xanthoma 2761
yolk sac tumor of testis 2339
yolk sac tumor of ovary 2453
Surgical
specimens 1552
treatment 930

Swan-neck 2725
Sweat chloride 546
Sweet syndrome 1212
Swiss
  cheese disease 2498
  type SCID 388
Sydenham's chorea (St. Vitus dance) 1722
Symplastic (bizarre) leiomyoma 2422
Symptomatic
  gallbladder stone disease 2128
  gallstones disease 2128
Synarthroses joints 2723
Syndrome of
  apparent mineralocorticoids excess (AME) 1686
  inappropriate ADH secretion 2644
Synovial sarcoma 634, 2786
Synthesis of
  chemical mediators in varying quantity in septic shock stages 301
  coagulation factors 1387
  factor VIII 1392
  immunoglobulin 368
  thyroid hormones 2586
Syphilis 1009
Syphilitic (luteic) aneurysm 1659
Syphilitic
  aortitis 1660
  orchitis 2326
Systemic
  complications of sickle cell disease 1131
  effects of inflammation 198
  immune-complex-mediated type 3 hypersensitivity reaction-induced diseases 424
  inflammatory response 201
  lupus erythematosus 415, 444, 1142, 1827
  venous thrombosis 1710

T

γ and δ T cells 343
T cell- and B cell-mediated immune responses 353
T cell and cell-mediated adaptive immune response 361
T cell immunologic tolerance in
  central lymphoid organs 435
  peripheral lymphoid organs 436
T cell lineage acute lymphoblastic leukemia with genetic abnormalities 1209
T cell-/NK cell-mediated glomerular injury 2185
Tabes
  dorsalis in tertiary syphilis 2896
  mesenterica 1954
Takayasu arteritis pulseless disease 1666, 1668, 1670
Target cells in various disorders 1126
Taruis' disease (GSD type 7) 550
Tattoo ink pigment 108
Tay-Sachs disease and resistance to tuberculosis 566
Telangiectatic osteosarcoma 2690
Telomere attrition 117
Telomeres 483, 587
Telomeres and senescence 675
Temporal (cranial/giant cell) arteritis 1665
Tenosynovial giant cell tumor localized type 2760
Tension pneumothorax 1829
Teratoma of testis 2340
Teratoma with somatic malignant transformation 2341
Teratomas of ovary 2457
Terminal
  deoxynucleotidyl transferase 1189
  duct lobular unit 2482, 2486
Termination of acute inflammatory response 155
Terminology
  of edema and effusions 240
  used by geneticist 472
  used for chromosomal appearances and abnormalities 512
  used for number of chromosomes in the cell 481
Tertiary
  hyperparathyroidism 2629
  structure of protein 502
  syphilis 1010
Testicular
  atrophy 2351
  plasmacytoma 2348
Tetany (tetanic seizure) 2629
Tetrahydrofolate 1100
Tetralogy of Fallot 1756
Thalassemia 1120
  β-Thalassemia 1127, 1128
    intermedia 1094
    major 1094
    trait 1094
Thalassemia and resistance to malaria 565
The main angiogenic proteins involved in angiogenesis 226
The nucleus and DNA replication 487
Theca luteal cysts 2431
Thecoma of ovary 2465
Therapeutic
  apheresis 1443
  erythrocytapheresis 1445
  fluid replacement 1445
  leukapheresis 1445
  plasma exchange 1443
  splenectomy 1531
Thermophilic actinomyces 1827
Thiamine (vitamin B_1) 979
Thioflavin T and thioflavin blue stains 470
Third line of defense 317
Thoracic vena cava syndrome 1673
Three categories of intracellular accumulations 20
Three histologic variants of diffuse large B cell lymphoma (DLBCL) 1511
Thrombi in
  cardiac chambers 288
  arteries 288
Thrombin (precursor of prothrombin) 260, 273
Thrombin time 1417
Thromboangiitis obliterans 1668
Thrombocytopenia with absent radii bone (tar syndrome) 1377
Thromboembolism 282, 1656, 2478
Thrombogenesis 272
Thrombophilia (hypercoagulable state) 1407
Thrombophilias in approximate descending order of probability 276
Thrombophlebitis 1734
Thromboplastin 701
Thrombopoiesis 1070, 1360, 1366
Thrombosis 272
Thrombospondin 1363
Thrombotic
  disorders 274
  microangiopathy 2266
  thrombocytopenic purpura 1149, 1379
Thromboxane A_2 273
Thromboxane A_2 receptor defect disorder 1377
Thrombus
  embolization 281
  formation 1651
Thymic
  carcinoma 1540
  neoplasms 1538
Thymoma 1538
Thymus hyperplasia 1537
Thyroglobulin 2624
Thyroglobulin synthesis by thyroid follicular cells 2587
Thyroglossal
  cyst 1774
  duct cyst 1775, 2620
Thyroid
  anaplastic carcinoma 2624
  disorders: diagnostic approach 2621
  follicular cells 2586
  function tests 2621
Thyroid gland 2586
  anomalies 2620
  dysfunction associated myopathy 2806
  hormone binding proteins 2622
  hormones in circulation and tissues 2589
  hormones synthesis 2587
  hormones: actions 2589
  hyperplasia 22
  hyperplasia in Graves' disease 23
Thyroiditis 2589
Tidal volume 1782
Tinea
  capitis 2834
  corporis and tinea cruris 2834
  pedis 2835
  unguium 2835

Tissue
decalcification 1552
destruction 181
factor 260
factor pathway inhibitor 1365
fixatives 1550
healing by fibrosis 181
microarray 1625
microarray—FISH technique 1610
microarray technique 1625
processing 1625
processor 1552
proliferative activity 203
repair 202
typing 458
organ transplantation immunology 450
Tissues/organs transplant rejection 430
Tobacco smoking 1647, 1839
Toll-like receptors on leukocytes 146
Tonsils 350
TORCH complex 2942
Total anomalous pulmonary venous return 1756
Total lung capacity 1782
Touton giant cells 197
Toxemia of pregnancy involving kidneys 2267
Toxic
effects of oxidized LDL molecules 1649
myopathies 2806
nodular goiter (Plummer's disease) 2596
shock syndrome 2378
Toxoplasma gondii encephalitis 2895
Tp53 gene mutation associated with familial and sporadic cancers 672
Tp53 tumor suppressor gene 759, 2509
mutation 2506
Trachea 1778
Tracheoesophageal fistula (TEF) 1908, 1911
Traction diverticulum 1914
Traditional serrated polyps 1981
Transaminases 2020
Transbronchial biopsy 1869
Transcription 498
Transcription, translation and protein folding 496
Transfer RNA (tRNA) 502
Transferrin 1092
Transforming growth factor-β 211
Transfusable plasma components 1457
Transient
hypogammaglobulinemia of infancy 387
ischemic attacks 2937
jaundice 2025
thrombocytosis 1381
Transitional cell carcinoma of urinary bladder 625
Transitional epithelium metaplasia to glandular epithelium 32
Translation 501, 590
Transmigration of leukocytes 142
Transmission electron microscopy 1547, 1548
Transmural
infarct 1712
infarction 1704
myocardial infarct 1704
Transport of iodide into thyroid follicular cavity 2588
Transportation of blood components 1450
Transposition 594
Transposition of the great vessels 1756
Transthyretin protein associated amyloidosis 465
Transudate 139, 244
Transudative effusions 1872
Transversion 594
Traumatic neuroma 238, 2779, 2877
Tree bark appearance 1660
Trephine bone biopsy 1076
Treponema pallidum 2383
Trichinosis 1051
Trichobezoar 1944
Trichomonas vaginalis 1045, 2382
Tricuspid
insufficiency 1741
valve insufficiency 1741
Trinucleotide repeat mutations 533
Triple
assessment of breast lumps 2537
negative basal-like breast carcinoma 2512
Triple X (47, XXX syndrome) and other multi-X chromosome anomalies 526
Trisodium citrate 1084
Tropical sprue and bacterial overgrowth 1952
Truncating the genome by homozygous deletions 719
Truncus arteriosus communis 1756
Tubal
ectopic pregnancy 2427
endometriosis 2427
Tubercular
arthritis 2729
granulomas 1802
infection 989, 1795
infection and disease 1795
intestinal ulcer 1954
osteomyelitis 2656
pyelonephritis 2252
stricture of intestine 1954
Tuberculin skin test—type 4 hypersensitivity reaction 429
Tuberculin test 1795
Tuberculoid leprosy 1003, 1005, 2822
Tuberculosis in HIV 1794
cases 1803
patients 993
Tuberculosis
disease 989
epididymo-orchitis 2326
Tuberculous
lymphadenitis 999
meningitis 994, 2892
osteomyelitis 996
pericarditis 999
peritonitis 999
pyelonephritis 996, 997
salpingitis 999
Tuberous sclerosis 1986
complex 543
syndrome 2943
Tubular
adenoma of breast 2501
adenomas 1983
breast carcinoma 2521
diseases of kidney 2239
Tubulointerstitial diseases of kidney 2244
Tubulovillus adenomas 1984
Tumor
angiogenesis 598, 738
classification hierarchy 606
development, invasion and metastasis 601
dormancy and reactivation at secondary sites 747
growth patterns 2367
initiation 785
invasion and metastasis: molecular mechanisms 868
lysis syndrome 934
macroscopic morphology 926
markers: diagnostics in clinical practice 913
metastatic cascade 599
microenvironment 879
monoclonal properties: analysis techniques 655
necrosis factors synthesis 170
nomenclature 602
progression 787
promotion 786
size of breast carcinoma 2530
suppressor
caretaker genes 847, 854
gatekeeper genes 847, 850
gene 592, 709, 846
gene mutations 2533
genes: genome stability enablers 759
promoting inflammation 761
Tumors metastasizing to lungs 1864
Tumors of
blood vessels 1683
testis 2347
sellar region 2930
TUNEL assay 95
Turcot's syndrome 1985
Turner syndrome (monosomy 45, X/XO) 525
Two-dimensional echocardiography 1713
Tympanosclerosis 1768
Type 1
diabetes mellitus 429, 2560
endometrioid adenocarcinoma 2411
endometrioid carcinoma 2412, 2415
heparin-induced thrombocytopenia syndrome 279
hypersensitivity reaction 404, 406
Type 2
diabetes mellitus 2564
heparin-induced thrombocytopenia syndrome 279

hypersensitivity reaction 404, 415, 1715
nonendometrioid carcinoma 2411, 2417
Type 3 hypersensitivity reaction 404, 420
Type 4 hypersensitivity reaction 404, 425, 1716
Type 5
hypersensitivity reaction 431
stimulatory hypersensitivity reaction 404
Type and causes of shock 298
Type of
bone fractures 234
electrophoresis 1632
immunofluorescence 1589
platelet disorders 1367
diarrhea 1952
HSC transplantation 1351
infarct 294
Typhoid
fever 1955
intestinal ulcers 1955

U

Ulcerated carcinoma 1937
Ulceration in wound 232
Ulcerative colitis 1971
Ultramicrotomy 1546
Ultrasound or CT-guided percutaneous needle biopsy 1869
Ultraviolet radiation-induced
carcinogenesis 794
DNA damage 507
Umbilical cord
blood HSCs harvesting 1350
HSC transplantation 1352
Umbilical hernia 1957
Undifferentiated
endometrial carcinoma 2418
pleomorphic sarcoma 2764
Unstable angina (crescendo or preinfarction angina) 1700
Urachal
diverticulum 2311
sinus 2311
Urate nephropathy 2253, 2727
Urea cycle 2160
Urease 1924
Uremia-induced platelet dysfunction 1380
Uric acid stones 2260
Urinary bladder diverticula 2311
Urinary tract
infection 2261
obstruction 2247
Urine analysis 2300
Urinothorax 1874
Urobilinogen 2305
Urolithiasis 2255
Urothelial
carcinoma 2311, 2316
neoplasms reporting protocol: transurethral resection and cystectomy specimens 2320
Uterine
cervix 2389
leiomyoma 613

V

VacA exotoxin 1923
Vaccination of patients at high-risk group require specific vaccinations 376
Vaccines 377
immunization 376
preparation 377
Vagina 2373
Vaginal intraepithelial neoplasia 2384
Varicella zoster virus skin infection (chickenpox) 2830
Varicocele 1672, 2350
Varicose veins in legs 1671
Variola (smallpox) 1030
Various types of electrophoresis 1635
Vascular changes in
benign hypertension 1688
malignant hypertension 1688
the inner retina in diabetic patients 2579
Vascular
diseases of kidney 2262
disorders of liver 2095
endothelial component 1360
growth factor 212
leiomyoma (angioleiomyoma) 2765
response in acute inflammation 134
Vascular tumors of 615, 1921
skin 2873
soft tissue 2774
Vascular-occlusive episodes 1132
Vasculitis 1663, 1666, 1668, 1670
involving kidneys 2268
Vasculogenesis 224
Vasoactive
amines 161
intestinal peptide tumor 2153
Vaso-occlusive crisis 1132
Vater's syndrome 1915
Veins 1671
Velamentous insertion and vasa previa 2479
Veno-occlusive disorder 2097
Venous
thrombi 278, 283
thromboembolism 282
variant of vascular leiomyoma 2765
Ventral hernia 1957
Ventricular
aneurysm 1709
mural thrombi 1709
septal defects 1754
Verruciform penile carcinoma 2368
Verrucous carcinoma of vagina 2386
Vertical growth
melanoma 2851
penile carcinoma 2368
Vesicoureteral reflux 2247
Vibrio cholerae 1952
Villus adenomas 1984
Vim Silverman needle biopsy 2022
Vincent angina 1880
Viral
carcinogenesis 799
cervicitis 2391
encephalitis 2895
hepatitis 2036
infections 1014
meningitis 2893
oncogenes 592
sialadenitis 1887
skin infections 2827
Viral-associated epithelial precancerous lesions 2858
Virchow node 599
Visceral
epithelial cells of Bowman's capsule 2168
leishmaniasis 1046
Vital
capacity 1782
signs observed in patients 125
Vitamin A 970
and normal vision 971
Vitamin B_{12}
deficiency 981, 1101, 1103
functions 981
DNA synthesis 1102
Vitamin B-complex 979
Vitamin C (ascorbic acid) 978
Vitamin D 974, 2657
deficiency: causes 2626
related disorders 975
functions 974
metabolism: regulation 2626
sources 2626
storage sites 2626
transport 2626
Vitamin D3 (calcitriol): functions 2626
Vitamin E 976
Vitamin K 977
Vitamins deficiency 970
Vitiligo 105, 2845
universalis 2845
vulgaris 2845
Vocal cord nodule 1770
Volume reduction from blood components 1450
Volvulus 1968
von Gierke disease 100
GSD type 1 548
von Gieson stain 470
von Hippel-Lindau
disease 543, 693
syndrome 2874
von Meyenburg complex 649
von Willebrand
disease 1397
factor 261, 270, 1367
Vulnerability of tissues to hypoxia 293
Vulvar cysts 2377

W

WAGR syndrome, Denys-Drash syndrome, and nonsyndromic hereditary Wilms' tumor 694
Wald's visual cycle and color vision 973
Waldenström macroglobulinemia 1337
Warm antibody-mediated
  autoimmune hemolytic anemia 417
  hemolytic anemia 1141, 1142
Warthin's tumor 1891
Warthin-Finkeldey giant cells 198
Warts with focal epithelial hyperplasia 2829
Washing of blood components 1451
Waterhouse-Friderichsen syndrome 302, 2635
Watershed areas 35, 40
  in brain 297
Watershed regions 293, 1977
Water-soluble vitamins 978
Watson-Crick pairing 488
WBCs qualitative anomalies 1070
Wedge-shaped splenic infarct 1533
Wegener's granulomatosis (ANCA positive) 1669, 1758, 2201
Well differentiated
  papillary mesothelioma 2003
  liposarcoma/atypical lipomatous tumor 2745
Wells
  diagnostic criteria of pulmonary embolism 286
  score system for deep vein thrombosis (DVT) 285
Werner's syndrome 119, 696
Wet gangrene 61, 65
Whipple disease 1953
  tropical sprue 1952
White blood cell
  changes in stored blood 1449
  component transfusion 1455
White
  pulp of spleen 1531
  sponge nevus 1880
WHO
  grade I meningiomas 2923
  grade II meningiomas (atypical meningiomas) 2923
  grade III meningiomas (anaplastic meningiomas) 2923
  leprosy classification 1002, 2820
WHO classification of
  G6PD deficiency 1138
  lung tumors 1842
  mutant G6PD isoenzymes 1138
  tumors of the oral cavity 1882
Whole blood
  composition 1437
  transfusion 1455
Whole genome sequencing analysis 952
Whole-exome sequencing 579
Williams-Campbell syndrome 1810
Wilms' tumor (nephroblastoma) 2274
Wilson's disease 2020, 2088
  induced cirrhosis 2060
Wiskott-Aldrich syndrome (X-linked disorder) 8, 389, 681, 1376, 1537
Wolf-Hirschhorn syndrome 719
Wound
  contraction 227
  contracture 233
  healing and malignant transformation 234
  healing in surgical wound 228
  infection 233
Wright's stain 1084

X

X chromosome inactivation 523
Xanthine stones 2261
Xanthogranulomatous
  prostatitis 2354
  pyelonephritis 2251
Xanthoma 2761, 2881
Xenomammography 2536
Xeroderma pigmentosum 696, 796
X-inactivation 657
X-linked (sex-linked) recessive disorders 538
X-linked agammaglobulinemia (Bruton agammaglobulinemia) 381
X-linked dominant
  disorders 561
  hypophosphatemic rickets 561
  inheritance 539
  protoporphyria 562
X-linked
  hyper-IgM syndrome 386
  lymphoproliferative (XLP) syndrome 387
  macrothrombocytopenia with dyserythropoiesis 1377
X-linked recessive
  disorders 557
  inheritance 538
X-linked SCID 388
XYY syndrome 514

Y

Y chromosome 485
Yeast 1037
Yellow fever virus infection 1027
Yersinia enterocolitica 1998

Z

Zenker's
  diverticulum 1914
  fixative 1550
  necrosis in skeletal muscle 66
Ziehl-Neelsen (Wade-Fite) 2824
Zollinger-Ellison syndrome 1929
Zona
  fasciculata 2631
  glomerulosa 2631
  lysin or acrosin 2374
  occludens (tight junctions) 6
  reticularis 2631
Zonal liver necrosis 2015
Zonula adherens (cell-to-cell junctions) 6
Zygomycota 1038
  members 1039

Textbook of Pathology

Systemic Pathology & Molecular Diagnostics

2nd Edition

Volume II

Second Edition of ***Textbook of Pathology*** has been designed for undergraduate and postgraduate medical students, and aspirants for postgraduate courses and related health sciences.

Textbook of Pathology has three sections: (a) general pathology, (b) hematology, (c) systemic pathology and molecular diagnostics. It provides comprehensive coverage of etiology, molecular pathogenesis of the disorders, clinical manifestations, clinicopathological correlation and diagnostic approach.

The chapters of this textbook have been rewritten and shared with the eminent teachers, and young promising postgraduate residents across nation. After taking their valuable suggestions, chapters have been finalized to meet the need of young generation of pathologists.

An unrivaled collection of high-resolution lavishly colored illustrations/photographs of surgical pathology and hematolymphoid disorders have complemented the clear concise text.

Salient Features

- Textbook has unrivaled collection of high-resolution lavishly 1901 colored figures and 1719 tables complementing the clear concise text.
- Textbook has clinical photographs, schematic illustrations, flow diagrams, gross morphologic and microscopic photographs of surgical specimens, and photographs of peripheral blood smear, bone marrow aspirate smear and bone marrow trephine biopsy section to understand clinicopathologic correlation of human disorders and diagnostic approach.
- Textbook has reader-friendly large font-size of the text for quick revision of chapters.
- Revised WHO classification of solid neoplasms and hematolymphoid malignancies is given in this textbook.
- Chapters on 'Cellular–Molecular Diagnostic Techniques in Clinical Practice' and 'Blood Banking and Transfusion Practices'.
- Recent concepts in cellular biology with key points of the disorders are given in boxes in the chapters.
- Immunohistochemistry of surgical specimens and immunophenotyping of hematolymphoid neoplasms have been given in tabulated forms.

Vinay Kamal has more than three-decade of teaching experience at India's premier institution, while serving as Director Professor, Department of Pathology, Maulana Azad Medical College, New Delhi. An alumnus of Post Graduate Institute of Medical Sciences (PGIMS), Rohtak, Haryana, he has also served in the premier institute. He has published articles in national and international journals. Budding medical professionals Dr Anubhav and Dr Vigyat have put sincere efforts in the completion of *Textbook of Pathology*.

Dedicated to Education
CBSPD
CBS Publishers & Distributors Pvt Ltd
4819/XI, Prahlad Street, 24 Ansari Road, Daryaganj, New Delhi 110 002, India
E-mail: delhi@cbspd.com, customercare@cbspd.com; Website: www.cbspd.com
New Delhi | Bengaluru | Chennai | Kochi | Kolkata | Lucknow | Mumbai
Hyderabad | Jharkhand | Nagpur | Patna | Pune | Uttarakhand