Paniker textbook of medical parasitology pdf free download

CK Jayaram Paniker MD Emeritus Medical Scientist, Indian Council of Medical Research, New Delhi, India Sougata Ghosh MD DCH Professor, Department of Microbiology, Medical College, Kolkata, West Bengal, India In this following post we share a PDF link of PANIKER'S TEXTBOOM OF MEDICAL PARASITOLOGY PDF free by clicking the link given below. The PDF link can be easily accessible at the end section of this post. We hope you will find it helpful for your studies. The aim of the contents of the book remains the same in this edition is compact yet informative and useful for both graduate and postgraduate students. This present edition is also designed in a colorful format, which can be easily read and comprehended. Paniker's Textbook of Medical Parasitology – 8th edition. In the current edition, many new tables, flow charts, and photographs of specimens and microscopic view pictures have been added for better comprehension of the subject. Recent advances such as vaccinology of malaria and leishmaniasis, malarial drug resistance, new treatment protocols of different parasitic infections are the salient features of the book. Features of Paniker's Textbook of Medical Parasitology PDF: Written as per the latest curriculum of MBBS and MD (Microbiology). Rearranges all the chapters in a sequential format and thoroughly updated and expanded to include new data. Adds the recent advances in each topic such as vaccine trials in Leishmania and Malaria, drug resistance in malaria. The new and fresh look reinvigorates the reading experience. Includes many new photographs, tables and flow charts. Rewrites the laboratory diagnosis, treatment protocols, and prophylaxis completely. Describes important concepts for the preparations of postgraduate examination in separate boxes. Presents Key Points in a box format at the end of each chapter for last-minute revision. Arranges the whole content of the book in a bulleted format and uses subheads for increased readability. At the end of each chapter, the must-know facts are given as "Key Points" inbox formats for the quick recapitulation. Important multiple-choice questions (MCQs) and review questions from various university examinations' papers have been added to test and reinforce understanding of the topics by the students. Netter's Sport Medicine PDF DOWNLOAD THE ECG MADE EASY PDF 9th EDITION DOWNLOAD FREE Download Paniker's Textbook of Medical Parasitology PDF 8th Edition free: you can easily downloadPANIKER'S TEXTBOOK OF MEDICAL PARASITOLOGY 8TH EDITION PDF free by clicking the link given below. If the link is nit responding kindly inform us through comment section. We will fixed it soon. Click to download pdf " NOTE: We do not own copyrights to these books.

 Author(s): CK Jayaram Paniker Publisher: Jaypee Brothers Medical Publishers, Year: 2018 Description: In the current edition, many new tables, flow charts and photographs of specimens and microscopic view pictures have been added for better comprehension of the subject. Recent advances such as vaccinology of malaria and leishmaniasis, malarial drug resistance, new treatment protocols of different parasitic infections are the salient features of the book. The aim of the contents of the book remains the same in this edition, that is compact yet informative and useful for both graduate and postgraduate students. Like the last edition, the present edition is also designed in a colourful format, which can be easily read and comprehended. Important points and terms have been highlighted by making them bold and italic. At the end of each chapter, the must-know facts are given as â Key Pointsâ in box formats for quick recapitulation. Important multiple-choice questions (MCQs) and review questions from various university examinationsâ papers have been added to test and reinforce understanding of the topics by the students. . Customer Reviews, including Product Star Ratings help customers to learn more about the product and decide whether it is the right product for them. To calculate the overall star rating and percentage breakdown by star, we donâ€™t use a simple average. Instead, our system considers things like how recent a review is and if the reviewer bought the item on Amazon. It also analyzed reviews to verify trustworthiness. Learn more how customers reviews work on Amazon AntoniE Van Leeuwenhoek Born: 24.10.1632 - Died: 26.8.1723 Delft, Holland This man, born poor, with little education, a draper in his hometown of Delft had surprising visitors! They included great men of science as well as the Royalty like the Tsar Peter the Great, Frederick the Great of Prussia and King James II of England. This was due to his hobby of grinding fine lenses through which he looked at various objects and brought forth the wonder world of small things that none had seen before. He kept clear descriptions and accurate drawings of what he saw and communicated them to the Royal Society in London. A strict check convinced the Society of their authenticity. The unlettered Antonie was elected a Fellow of the Royal Society! The papers sent by him over decades can still be seen in the Philosophical Transactions of the Royal Society. The discoveries he made are legion. He described the first protozoan pathogen Giardia. He also discovered many types of bacteria, human and animal spermatozoa, and eggs of various animals realizing their importance in reproduction. He could not recognize the significance of the different types of bacteria, and to him, they were just 'little animalcules'. His fault was in being much before the time, for it took two centuries more for people to accept the microbial origin of infectious diseases. But that should not deter us from acknowledging the great contributions made by Leeuwenhoek to Biology and many other branches of Science. He was truly the Founder of Microbiology. Paniker's Textbook of Medical Parasitology Paniker's Textbook of Medical Parasitology SEVEnth EDition Ck Jayaram Paniker MD Formerly Director and Professor of Microbiology and Principal, Medical College Calicut Dean, Faculty of Medicine, Calicut University, Kerala, India Emeritus Medical Scientist Indian Council of Medical Research, new Delhi, India Revised and Edited by Sougata Ghosh MD, DCH Professor, Department of Microbiology Medical College, Kolkata, West Bengal, India Former Faculty IPGME&R and School of Tropical Medicine, Kolkata, West Bengal, India ® JAYPEE BROTHERS MEDICAL PUBLISHERS (P) LTD New Delhi • London • Philadelphia • Panama ® Jaypee Brothers Medical Publishers (P) Ltd Headquarters Jaypee Brothers Medical Publishers (P) Ltd 4838/24, Ansari Road, Daryaganj New Delhi 110 002, India Phone: +91-11-43574357 Fax: +91-11-43574314 Email: Overseas Offices J.P. Medical Ltd 83, Victoria Street, London SW1H 0HW (UK) Phone: +44-2031708910 Fax: +02-03-0086180 Email: Jaypee-Highlights Medical Publishers Inc City of Knowledge, Bld. 237, Clayton Panama City, Panama Phone: +507-301-0496 Fax: +507-301-0499 Email: Jaypee Medical Inc The Bourse 111 South Independence Mall East Suite 835, Philadelphia, PA 19106, USA Phone: + 267-519-9789 Email: Jaypee Brothers Medical Publishers (P) Ltd 17/1-B Babar Road, Block-B, Shaymali Mohammadpur, Dhaka-1207 Bangladesh Mobile: +08801912003485 Email: Jaypee Brothers Medical Publishers (P) Ltd Shorakhute, Kathmandu Nepal Phone: +00977-9841528578 Email: jaypee.ne Website: www.jaypeebrothers.com Website: www.jaypeedigital.com Paniker's Textbook of Medical Parasitology First Edition Second Edition Third Edition Fourth Edition Fifth Edition Sixth Edition Seventh Edition : : : : : : : 1988 1989, Reprint : 1991 1993, 1997, Reprint : 1999 2002, Reprint : 2003, 2004 2007, Reprint : 2011 2013 ISBN 978-93-5090-534-0 Printed at Preface to the Seventh Edition The current edition of this book is written in a new user-friendly format in contrast to the classic narrative style of Dr. Paniker's Textbook of Medical Parasitology that has served medical students and teachers for more than 25 years since 1988. Considering the advancement in the field of Parasitology, I have updated the text thoroughly , incorporating the recent epidemiological data and new diagnostic methods especially the molecular techniques and current treatment modalities. Almost all chapters have been revised and few new chapters like Pnemocystis jirovecil, Microsporidia, and Balantidium coli are added. The main emphasis of the current edition is to make the text more comprehensive, colorful, and student-friendly. Diagrams of life cycle have been redrawn in a manner to facilitate the students reproduce them during examinations. Several new tables, flowcharts, and easy-to-remember boxes are given to equip the students for better answering of theory and oral questions during examinations. More microscopic view pictures, photographs of specimens, and diagnostic images have been added in a manner to favor better visible impressions of parasitic diseases. I have included "Key points" of important parasites in box formats to highlight "must know facts" that are pertinent to the topic. Important MCQs and review questions carefully selected from various university examination papers have been added to test and reinforce understanding of the topic by the student. The aim of the book remains to be compact, yet informative, and useful for both undergraduate and postgraduate students. My endeavor will be successful, if the book is found to be useful for faculty and students. Expressions and emotions fail to find words to express thanks to my parents. I thank them for a being a constant source of inspiration and motivation. I am grateful to all the colleagues in my department for their valuable suggestions during preparing the texts. I am especially indebted to the Director and Staff of School of Tropical Medicine, Kolkata for providing mounted specimens. I gratefully acknowledge the help of Mr Jitendar P. Vij (Group Chairman), Mr Ankit Vij (Managing Director), Mr Tarun Duneja (Director Publishing) and Mr Sabyasachi Hazra for their professional help and guidance during the project. The insight and skills of Dr Sakshi Arora (Chief Development Editor) along with her team helped in polishing this book to best meet the needs of students and faculty alike. Lastly I acknowledge the support extended by my family members during revising the book. All suggestions are welcome and may be emailed to: Sougata Ghosh Preface to the First Edition Parasitic infections continue to account for a large part of human illness. Antimicrobial drugs and vaccines that have made possible the effective control of most bacterial and viral diseases have not been as successful against parasitic infections. The numbers of persons afflicted by parasites run into many millions. Malaria still affects over 500 millions, pinworm and whipworm 500 millions each, hookworm 800 millions and roundworm a billion persons. Filariasis, leishmaniasis and schistosomiasis remain serious public health problems. Infections due to opportunist parasites are becoming increasingly evident in the affluent countries. In recent years there has been a resurgence in the study of parasitic infections. Much new knowledge has been gained making possible precise diagnosis and more effective control of parasites and the diseases they cause. This textbook attempts to present the essential information on parasites and parasitic diseases, with emphasis on pathogenesis, epidemiology, diagnosis and control. Every effort has been made to incorporate recent advances in the subject. It is hoped that medical students, teachers and physicians will find this book useful. Their comments and suggestions for improvement of the book will be most welcome. Shanthi, East Hill Road Calicut, Kerala 673 006 CK Jayaram Paniker Contents

General Introduction: Parasitology 1 Introduction Parasites Medical parasitology deals with the parasites, which cause human infections and the diseases they produce. ¾¾ It is broadly divided into 2 parts— € Protozoology € Helminthology. ¾¾ The pioneer Dutch microscopist, Antonie von Leeuwenhoek of Holland in 1681, first introduced single lens microscope and obeserved Giardia in his own stools. ¾¾ Louis Pastuer in 1870, first published scientific study on a protozoal disease leading to its control and prevention during investigation of a epidemic silk worm disease in South Europe. ¾¾ A seminal discovery was made in 1878 by Patrick Manson about the role of mosquitoes in filariasis. This was the first evidence of vector transmission. ¾¾ Afterwards, Laveran in Algeria discovered the malarial parasite (1880), and Ronald Ross in Secunderabad and Calcutta in India, showed its transmission by mosquitoes (1897). A large number of vectorborne disease have since then been identified. ¾¾ By midtwentieth century, with dramatic advances in antibiotics and chemotherapy, insecticides and antiparastic drugs, and improved lifestyles, all infectious diseases seemed amenable to control. Parasites are living organisms, which depend on a living host for their nourishment and survival. They multiply or undergo development in the host. ¾¾ The term 'parasite' is usually applied to Protozoa (unicellular organisms) and Helminths (multicellular organisms) (Flowchart 1.1). ¾¾ Parasites can also be classified as: € Ectoparasite: Ectoparasites inhabit only the body surface of the host without penetrating the tissue. Lice, ticks, and mites are examples of ectoparasites. The term infestation is often employed for parasitization with ectoparasites. € Endoparasite: A parasite, which lives within the body of the host and is said to cause an infection is called an endoparasite. Most of the protozoan and helminthic parasites causing human disease are endoparasites. € Free-living parasite: It refers to nonparasitic stages of active existence, which live independent of the host, e.g. cystic stage of Naegleria floweri. ¾¾ Endoparasites can further be classified as: € Obligate parasite: The parasite, which cannot exist without a host, e.g. Toxoplasma gondii and Plasmodium. 2 Textbook of Medical Parasitology Flowchart 1.1: Type of parasites €€ €€ €€ Facultative parasite: Organism which may either live as parasitic form or as free living form. Accidental parasites: Parasites, which infect an unusual host are known as accidental parasites. Echinococcus granulosus infects man accidentally, giving rise to hydatid cysts. Aberrant parasites: Parasites, which infect a host where they cannot develop further are known as aberrant or wandering parasites, e.g. Toxocara canis (dog roundworm) infecting humans. Host Host is defined as an organism, which harbors the parasite and provides nourishment and shelter to latter and is relatively larger than the parasite. ¾¾ The host may be of the following types: €€ Definitive host: The host, in which the adult parasite lives and undergoes sexual reproduction is called the definitive host, e.g. mosquito acts as definitive host in malaria. The definitive host may be a human or any other living being. However, in majority of human parasitic infections, man is the definitive host (e.g. filaria, roundworm, hookworm). €€ Intermediate host: The host, in which the larval stage of the parasite lives or asexual multiplication takes place is called the intermediate host. In some parasites, 2 different intermediate hosts may be required to complete different larval stages. These €€ €€ €€ are known as first and second intermediate hosts, respectively. Paratenic host: A host, in which larval stage of the parasite remains viable without further development is referred as a paratenic host. Such host transmits the infection to another host. Reservoir host: In an endemic area, a parasitic infection is continuously kept up by the presence of a host, which harbors the parasite and acts as an important source of infection to other susceptible hosts, e.g. dog is the reservoir host of hydatid disease. Accidental host: The host, in which the parasite is not usually found, e.g. man is an accidental host for cystic echinococcosis. Parasites with man as intermediate or secondary host ••Plasmodium spp. ••Babesia spp. ••Toxoplasma gondii ••Echinococcus granulosus ••Echinococcus multilocularis ••Taenia solium ••Spirometra spp. Zoonosis The word zoonosis was introduced by Rudolf Virchow in 1880 to include the diseases shared in nature by man and animals. General Introduction: Parasitology ¾¾ Later, in 1959, the World Health Organization (WHO) defined zoonosis as "those diseases and infections, which are naturally transmitted between vertebrate animals and man". ¾¾ It is of following types: €€ Protozoal zoonoses, e.g. toxoplasmosis, leish-maniasis, balantidiasis, and cryptosporodiasis €€ Helminthic zoonoses, e.g. hydatid disease, taeniasis €€ Anthropozoonoses: Infections transmitted to man from lower vertebrate animals, e.g. cystic echinococcosis €€ Zooanthroponoses: Infections transmitted from man to lower vertebrate animals, e.g. human tuberculosis to cattle. Host-parasite relationships are of following types (Flowchart 1.2): ¾¾ Symbiosis ¾¾ Commensalism ¾¾ Parasitism. Life Cycle of Parasites ¾¾ Direct life cycle: When a parasite requires only single host to complete its development, it is called as direct life cycle, e.g. Entamoeba histolytica requires only a human host to complete its life cycle. ¾¾ Indirect life cycle: When a parasite requires 2 or more species of host to complete its development, the life cycle is called as indirect life cycle, e.g. malarial parasite requires both human host and mosquito to complete its life cycle. Sources of Infection €€ Protozoa Helminths ••Entamoeba histolytica Ascaris lumbricoides ••Giardia lamblia Enterobius vermicularis ••Trichomonas vaginalis Trichuris trichiura ••Balantidium coli Ancyclostoma duodenale ••Cryptosporidium parvum Necator americanus ••Cyclospora cayetanensis Hymenolepis nana ••Isospora belli ••Microsporidia Parasites having indirect life cycle Parasite Host-parasite Relationships ¾¾ Contaminated soil and water: Parasites having direct life cycle Soil polluted with embryonated eggs (roundworm, whipworm) may be ingested or infected larvae in soil, may penetrate exposed skin (hookworm) Protozoa Plasmodium spp. Babesia Leishmania Trypanosoma brucei Trypanosoma cruzi Toxoplasma gondii Cestodes Taenia solium Taenia saginata Echinococcus granulosus Trematodes Fasciola hepatica Fasciolopsis buski Schistosoma spp. Nematodes Trichinella spiralis Wuchereria bancrofti Brugia malayi Dracunculus medinensis Flowchart 1.2: Host-parasite relationships Definitive host Intermediate host Female Anopheles mosquito Tick Man, dog Man Man Man Cat Triatomine bug Man Man Man Dog Pig Cattle Man Man Man, pig Man Snail Snail Snail Man Man Pig Mosquito Man Man Mosquito Cyclops Man Sandfly Tsetse fly 3 4 Textbook of Medical Parasitology Infective forms of parasites present in water may be ingested (cyst of amoeba and Giardia) €€ Water containing the intermediate host may be swallowed (cyclops containing guineaworm larva) €€ Infected larvae in water may enter by penetrating exposed skin, (cercariae of schistosomes) €€ Free-living parasites in water may directly enter through vulnerable sites (Naegleria may enter through nasopharynx). ¾¾ Food: €€ Ingestion of contaminated food or vegetables containing infective stage of parasite (amoebic cysts, Toxoplasma oocysts, Echinococcus eggs) €€ Ingestion of raw or under-cooked meat harboring infective larvae (measly pork containing cysticercus cellulosae, the larval stage of Taenia solium). ¾¾ Insect vectors: A vector is an agent, usually an arthropod that transmits an infection from man to man or from other animals to man, e.g. female Anopheles is the vector of malarial parasite. Vectors can be: €€ Biological vectors: The term biological vector refers to a vector, which not only assists in the transfer of parasites but the parasites undergo development or multiplication in their body as well. They are also called as true vectors. Example of true vectors are: ^^ Mosquito—Malaria, filariasis ^^ Sandflies—Kala-azar ^^ Tsetse flies—Sleeping sickness ^^ Reduviid bugs—Chagas' disease ^^ Ticks—Babesiosis. €€ Mechanical vectors: The term mechanical vector refers to a vector, which assists in the transfer of parasitic form between hosts but is not essential in the life cycle of the parasite. Example of Mechanical vectors is: ^^ Housefly—amoebiasis €€ In biological vectors, a certain period has to elapse after the parasite enters the vector, before it becomes infective. This is necessary because the vector can transmit the infection only after the parasite multiplies to a certain level or undergoes a developmental process in its body. This interval between the entry of the parasite into the vector and the time it takes to become capable of transmitting the infection is called the extrinsic incubation period. ¾¾ Animals: €€ Domestic: ^^ Cow, e.g. T. saginata, Sarcocystis ^^ Pig, e.g. T. solium, Trichinella spiralis ^^ Dog, e.g. Echinococcus granulosus ^^ Cat, e.g. Toxoplasma, Opisthrorchis. Wild: ^^ Wild game animals, e.g. trypanosomiasis ^^ Wild felines, e.g. Paragonimus westermani ^^ Fish, e.g. fish tapeworm ^^ Molluscs, e.g. liver flukes ^^ Copepods, e.g. guineaworm. ¾¾ Other persons, which may be carriers of the parasite or patients, e.g. all anthroponotic infections, vertical transmission of congenital infections. ¾¾ Self (autoinfection) €€ Finger-to-mouth transmission, e.g. pinworm €€ Internal reinfection, e.g. Strongyloides. €€ Parasites causing autoinfection ••Hymenolepis nana ••Enterobius vermicularis ••Taenia solium ••Strongyloides stercoralis ••Capillaria philippinensis ••Cryptosporidium parvum Modes of Infection ¾¾ Oral transmission: The most common method of transmission is through oral route by contaminated food, water, soiled fingers, or fomites. Many intestinal parasites enter the body in this manner, the infective stages being cysts, embryonated eggs, or larval forms. Infection with E. histolytica and other intestinal protozoa occurs when the infective cysts are swallowed. ¾¾ Skin transmission: Entry through skin is another important mode of transmission. Hookworm infection is acquired, when the larvae enter the skin of persons walking barefooted on contaminated soil. Schisto somiasis is acquired when the cercarial larvae in water penetrate the skin ¾¾ Vector transmission: Many parasitic diseases are transmitted by insect bite, e.g., malaria is transmitted by bite of female Anopheles mosquito, filariasis is transmitted by bite of Culex mosquito. A vector could be a biological vector or a mechanical vector. ¾¾ Direct transmission: Parasitic infection may be transmitted by person-to-person contact in some cases, e.g. by kissing in the case of gingival amoebae and by sexual intercourse in trichomoniasis. ¾¾ Vertical transmission: Mother to fetus transmission may take place in malaria and toxoplasmosis. ¾¾ Iatrogenic transmission: It is seen in case of transfusion malaria and toxoplasmosis after organ transplantation. General Introduction: Parasitology Pathogenesis Parasitic infections may remain inapparent or give rise to clinical disease. A few organisms, such as E. histolytica may live as surface commensals, without invading the tissue. ¾¾ Clinical infection produced by parasite may take many forms—acute, subacute, chronic, latent, or recurrent. ¾¾ Pathogenic mechanisms, which can occur in parasitic infections are: € Lytic necrosis: Enzymes produced by some parasite can cause lytic necrosis. E. histolytica lyses intestinal cells and produces amoebic ulcers. € Trauma: Attachment of hookworms on jejunal mucosa leads to traumatic damage of villi and bleeding at the site of attachment. € Allergic manifestations: Clinical illness may be caused by host immune response to parasitic infection, e.g. eosinophilic pneumonia in Ascaris infection and anaphylactic shock in rupture of hydatid cyst. € Physical obstruction: Masses of roundworm cause intestinal obstruction. Plasmodium falciparum malaria may produce blockage of brain capillaries in cerebral malaria. € Inflammatory reaction: Clinical illness may be caused by inflammatory changes and consequent fibrosis e.g. lymphadenitis in filariasis and urinary bladder granuloma in Schistosoma haematobium infection. € Neoplasia: A few parasitic infection have been shown to lead to malignancy. The liver fluke, Clonorchis may induce bile duct carcinoma, and S. haematobium may cause urinary bladder cancer. Immunity in Parasitic Infection Like other infectious agents, parasites also elicit immuno responses in the host, both humoral as well as cellular (Fig. 1.1). But immunological protection against parasitic infections is much less efficient, than it is against bacterial or viral infections. Several factors may contribute to this. ¾¾ Compared to bacteria and viruses, parasites are enormously larger or more complex structurally and antigenically, so that immune system may not be able to focus attack on the protective antigens. ¾¾ Many protozoan parasites are intracellular in location, and this protects them from immunological attack. Several protozoa and helminths live inside body cavities This location limits the efficiency of immunological attack. Fig. 1.1: Eosinophils surrounding schistosomulum (An example of immune attack in bloodstream) ¾¾ Once the parasitic infection is completely eliminated, the host becomes again susceptible to reinfection. This type of immunity to reinfection is dependent on the continued presence of residual parasite population and is known as 'Premunition'. ¾¾ Antibodies belonging to different immunoglobulin classes are produced in response to parasitic infections. Selective tests for IgM are helpful in differentiating current infections from old infections. ¾¾ Excessive IgE response occurs in helminthiasis. A characteristic cellular response in helminth parasite is eosinophilia both local and systemic (Fig. 1.1). ¾¾ Parasites have evolved to be closely adapted to the host and most parasitic infections are chronic and show a degree of host specificity. For example, malarial parasites of human, bird, and rodents are confined to their own particular species. ¾¾ Parasites like trypanosomes exhibit antigenic variation within the host. This genetic switch protects them from antibodies. Similar mechanism may be operative in the recrudescences in human malaria. Parasites exhibiting antigenic variations •¾Trypanosoma brucei gambiense •¾Trypanosoma brucei rhodesiense •¾Plasmodium spp. •¾Giardia lamblia ¾ ¾ Some parasites adopt antigenic disguise. Their surface antigens are so closely similar to host components that they are not recognized as foreign by the immune system. Some infections may produce immunodeficiency due to extensive damage to the reticuloendothelial system, as in case of visceral leishmaniasis. 5 6 Textbook of Medical Parasitology Table 1.1: Parasite Escape Mechanisms made in identifying protective antigens in malaria and some other infections, with a view to eventual development of prophylactic vaccines. Parasite escape mechanisms Example Intracellular habitat Malarial parasite, Leishmania Encystment Toxoplasma Trypanosoma cruzi Resistance to microbial phagocytosis Leishmania Masking of antigens Schistosomes Variation of antigen Trypanosomes Plasmodium spp. Suppression of immune response Malarial parasite Trichinella spiralis Schistosoma mansoni Interference by polyclonal activation Trypanosomes Sharing of antigens between parasite and hostmolecular mimicry Schistosomes Continuous turnover and release of surface antigens of parasite Schistosomes The fact that immunity normally plays an important role in the containment of parasitic infections is illustrated by the florid manifestations caused by opportunistic parasites such as Pnemocystis jirovecii and T. gondii, when the immune response is inadequate as in acquired immunodeficiency syndrome (AIDS) and other immunodeficiencies. Laboratory Diagnosis Most of the parasitic infection cannot be conclusively diagnosed. On the basis of clinical features and physical examination laboratory diagnosis depends upon: ¾¾ Microscopy ¾¾ Culture ¾¾ Serological test ¾¾ Skin test ¾¾ Molecular method ¾¾ Animal inoculation ¾¾ Xenodiagnosis ¾¾ Imaging ¾¾ Hematology. Microscopy All animal pathogens, including parasitic protozoa and worms have evolved effective mechanism to avoid elimination by the host defence system as described in Table 1.1. An appropriate clinical specimen should be collected for definitive diagnosis of parasitic infections. ¾¾ Following specimens are usually examined to establish a diagnosis: € Stool € Blood € Urine € Sputum € Cerebrospinal fluid (CSF) € Tissue and aspirates € Genital specimens. Vaccination Stool Examination No effective vaccine for humans has so far been developed against parasites due to their complex life cycles, adaptive responses, and antigenic variation, great progress has been Examination of stool is very important for the detection of intestinal infections like Giardia, Entamoeba, Ascaris, Ancylostoma, etc. Immune Evasion Table 1.2: Parasites and Their Developmental Stages Found in Stool Cysts/Trophozoites Eggs Entamoeba histolytica Giardia lamblia Balantidium coli Sarcocystis spp. Isospora belli Cyclospora cayetanensis Cryptosporidium parvum CESTODES Taenia spp. Hymenolepis nana Hymenolepis diminuta Dipylidium caninum Diphyllobothrium latum TREMATODES Schistosoma spp. Fasciolopsis buski Fasciola hepatica Fasciola gigantica Clonorchis sinensis Gastrodiscoides horminis Heterophyes heterophyes Metagonimus yokogawai Opisthorchis spp. NEMATODES Trichuris trichiura Enterobius vermicularis Ascaris lumbricoides Ancyclostoma duodenale Necator americanus Trichostrongylus orientalis Larvae Adult worms Strongyloides stercoralis Taenia solium Taenia saginata Diphyllobothrium latum Ascaris lumbricoides Enterobius vermicularis Trichinella spiralis General Introduction: Parasitology Cysts and trophozoites of E. histolytica, G. lamblia can be demonstrosted in feces. Eggs of roundworm and tapeworm are also found in stool. The larvae are found in the feces in S. stercoralis infection (Table 1.2). For further details refer to Chapter 24. Blood Examination Examination Examination of blood is of vital importance for demonstrating parasites which circulate in blood vessels (Table 1.3). Malarial parasite is confirmed by demonstration of its morphological stages in the blood. Table 1.3: Parasites Found in Peripheral Blood Film in intestinal aspirates. Trophozoites of E. histolytica can be detected in liver pus in cases of amoebic liver abscess. Genital Specimen Examination Trophozoites of T. vaginalis are found in the vaginal and uretheral discharge. Eggs of E. vermicularis are found in anal swabs. Culture Some parasites like Leishmania, Entamoeba, and Trypanosoma can be cultured in the laboratory in various axenic and polyxenic media. Protozoa Nematodes Serological Tests • • • • • • • • Serological tests are helpful for the detection and surveillance of many protozoal and helminthic infections. These tests are basically of 2 types: ¾¾ Tests for antigen detection, ¾¾ Tests for antibody detection. Plasmodium spp. Babesia spp. Tryponosoma spp. Leishmania spp. Wuchereria bancrofti Brugia malayi Loa loa Mansonella spp. Urine Examination The characteristic lateralspined eggs of S. haemtobium and trophozoites of T. vaginalis can be detected in urine. Micro filaria of W. bancrofli are often demonstrated in the chylous urine. Parasites found in urine •¾Schistosoma haemtobium •¾Wuchereria bancrofti •¾Trichomonas vaginalis Antigen Detection Malaria antigen like P. falciparum lactate dehydrogenase (pLDH) and histidinerich protein 2 (HRP2) are detected by rapid immunochromatographic test. Filarial antigens are detected in current infection by enzymelinked immuno sorbent assay (ELISA) (Table 1.4). Table 1.4: Antigen Detection in Parasitic Diseases • Galactose lectin antigen Entamoeba histolytica • Giardia specific antigen 65 Giardia lamblia • WKK and rk39 antigen Leishmania donovani Sputum Examination • HRP2 antigen Plasmodium falciparum The eggs of P. westermani are commonly demonstrated in the sputum specimen. Occasionally, larval stages of S. stercoralis and A. lumbricoides may also be found in sputum. • Vivax specific pLDH Plasmodium vivax • 200 KD Ag and OG4C3 antigen Wuchereria bancrofti Cerebrospinal Fluid Examination Some protozoa like T. brucei, Naegleria, Acanthamoeba, Balamuthia, and Angiostrongylus can be demonstrated in the CSF. Tissue and Aspirates Examination The larvae of Trichinella and eggs of Schistosoma can be demonstrated in the muscle biopsy specimens. By histopathological examination of brain, Naegleria and Acanthamoeba can be detected. In Kalaazar, Leishman Donovan (LD) bodies can be demonstrated in spleen and bone marrow aspirate. Trophozoites of Giardia can be demonstrated Antibody Detection The following antibody detection procedures are useful in detecting various parasitic infection like amoebiasis, echinococcosis, and leishmaniasis in man: ¾¾ Complement fixation test (CFT) ¾¾ Indirect hemagglutination (IHA) ¾¾ Indirect immunofluroscent antibody test (IFA) ¾¾ Rapid immunochromatography test ¾¾ ELISA test. Skin Test Skin tests are performed by injecting parasitic antigen intradermally and observing the reaction. In immediate 7 8 Textbook of Medical Parasitology hypersensitivity reaction, wheal and flare response is seen within 30 minutes of infection, whereas erythema and induration seen after 48 hours of injection is called as delayed hypersensitivity reaction. Important Skin tests done in parasitology •¾Casoni's test done in Hydatid disease •¾Montenegro test or Leishmanin test done in Kalaazar •¾Frenkel's test done in Toxoplasmosis •¾Fairley's test done in Schistosomiasis •¾Bachman intradermal test done in Trichinellosis Molecular Diagnosis Molecular method most frequently used to diagnose human parasitic infection are DNA probes, polymerase chain reaction (PCR), and microarray technique. These tests are very sensitive and specific. Animal Inoculation It is useful for the detection of Toxoplasma, Trypanosoma, and Babesia from the blood and other specimens. Xenodiagnosis Some parasitic infection like Chagas' disease caused by T. cruzi can be diagnosed by feeding the larvae of reduviid bugs with patients blood and then detection of amastigotes of T. cruzi in their feces. Imaging Imaging procedures like Xray, ultrasonography (USG) computed tomography (CT) scan and magnetic resonance Imaging (MRI) are now being extensively used for diagnosing various parasitic infection like neurocysticercosis and hydatid cyst disease. Hematology Anemia is frequently seen in hookworm infection and malaria. Eosinophilia is frequently present in helminthic infections. Hypergammaglobulinemia occurs in visceral leishmaniasis. Leukocytosis is seen in amoebic liver abscess. Key points •¾Leeuwenhoek in 1681, first observed the parasite Giardia in stools. Laveran in 1880, discovered malarial parasite and Ronald Ross in 1897 showed the transmission of malaria by mosquitoes. •¾Protozoa belong to Kingdom Protista and helminths belong to Kingdom Animalia. •¾Definitive host: The host in which the adult stage lives or the sexual mode of reproduction takes place. •¾Intermediate host: The host in which the larval stage of the parasite lives or the asexual multiplication takes place. •¾Zoonoses: Diseases which can be transmitted to humans from animals, e.g., malaria, leishmaniasis, trypanosomiasis, and echinococcosis. •¾Parasites like trypanosomes exhibit antigenic variation within the host. •¾Parasites like Ascaris and Echinococcus cause allergic manifestations in the host. •¾Innate immunity against parasite may be genetic or by nonspecific direct cellmediated or by complement activation. •¾Aquired immunity in parasitic infections is by generating specific antibodies and effector T cells against parasitic antigens. •¾Diagnosis of parasitic infections are made by direct identification of parasite in specimens like stool, blood, urine, bonemarrow, CSF, sputum, etc. •¾Serological tests are also useful in diagnosis by detection of parasitespecific antibody and antigen. •¾Other diagnostic modalities include imaging, molecular methods like PCR, skin test, and xenodiagnosis. Review Questions 1. Write short notes on: (a) Parasites (c) Hostparasite relationship (e) Immune evasion mechanism of the parasites. (b) Host (d) Zoonoses 2. Discuss briefly the laboratory diagnosis of parasites. 3. Describe immunity in parasitic infections. 4. Differentiate between: (a) Direct and indirect life cycle (b) Definitive host and intermediate hosts Protozoa Protozoa General Features Single-celled eukaryotic microorganisms belonging to kingdom protista are classified as Protozoa (Greek Protos: first; zoon: animal). The single protozoal cell performs all functions. Most of the protozoa are completely nonpathogenic but few may cause major diseases such as malaria, leishmaniasis, and sleeping sickness. Protozoa like Cryptosporidium parvum and Toxoplasma gondii are being recognized as opportunistic pathogens in patients affected with human immunodeficiency virus (HIV) and in those undergoing immunosuppressive therapy. Protozoa exhibit wide range of size (1–150 µm), shape, and structure; yet all possess essential common features. The differences between protozoa and metazoa are given in Table 2.1. Structure The typical protozoan cell is bounded by a trilaminar unit membrane, supported by a sheet of contractile fibrils enabling the cell to move and change in shape. by producing pseudopodia is called as the ectoplasm. It also helps in respiration, discharging waste material, and in providing a protective covering of cell. Endoplasm: The inner granular portion of cytoplasm that contains nucleus is called endoplasm. The endoplasm shows number of structures—the golgi bodies, endoplasmic reticulum, food vacuoles, and contractile vacuoles. Contractile vacuoles serve to regulate the osmotic pressure. Nucleus The nucleus is usually single but may be double or multiple; some species having as many as hundred nuclei in a single cell. The nucleus contains one or more nucleoli or a central karyosome. Table 2.1: Differences Between Protozoa and Metazoa Protozoa Metazoa Morphology Unicellular; a single 'cell-like unit' Multicellular; a number of cells, making up a complex individual Physiology A single cell performs all the functions: reproduction, digestion, respiration, excretion, etc. Each special cell performs a particular function Example Amoeba Tapeworm Cytoplasm It has 2 portions: Ectoplasm: Outer homogeneous part that serves as the organ for locomotion and for engulfment of food 2 9 10 Textbook of Medical Parasitology Reproduction Terminologies used In Protozoology • Chromatoid Body Extranuclear chromatin material is called chromatoid body (e.g., as found in Entamoeba histolytica cyst). • Karyosome It is a DNA containing body, situated peripherally or centrally within the nucleus and found in intestinal amoeba, e.g. E. histolytica, E. coli. • Kinetoplast Non-nuclear DNA present in addition to nucleus is called kinetoplast. It is seen in trypanosomes. Flagellum orginates near the kinetoplast. Point of origin of flagellum is called as basal body. • Cilia These are fine, needle-like filaments, covering the entire surface of the body and are found in ciliates, e.g. Balantidium coli. • Trophozoite (Trophos: nourishment) Active feeding and growing stage of the protozoa is called the trophozoites. It derives nutrition from the environment by diffusion, pinocytosis, and phagocytosis. Reproduction can be: Asexual reproduction Sexual reproduction. Reproduction usually occurs asexually in protozoans; however, sexual reproduction occurs in cillates and sporozoas. Asexual Reproduction Binary fission: It is a method of asexual reproduction, by which a single parasite divides either longitudinally or transversally into two or more equal number of parasites. Mitotic division of nucleus is followed by division of the cytoplasm. In amoebae, division occurs along any plane, but in flagellates, division is along longitudinal axis and in ciliates, in the transverse plane (Fig. 2.1). Multiple fission or schizogony: Plasmodium exhibits schizogony, in which nucleus undergoes several successive divisions within the schizont to produce large number of merozoites (Fig. 2.1). Endodyogeny: Some protozoa like Toxoplasma, multiply by internal budding, resulting in the formation of two daughter cells. Sexual Reproduction The chromatin may be distributed along periphery (peripheral chromatin) or as condensed mass around the karyosome. Conjugation: In ciliates, the sexual process is conjugation, in which two organisms join together and reciprocally exchange nuclear material (e.g. Balantidium coli). Fig. 2.1: Asexual reproduction in protozoans Protozoa Gametogony or syngamy: In sporozoa, male and female gametocytes are produced, which after fertilization form the zygote, which gives rise to numerous sporozoites by sporogony (e.g. Plasmodium). Life Cycle Apicomplexa Microspora Ciliophora The important protozoan pathogens of human are summarized in Table 2.3. Phylum Sarcomastigophora Single Host: Protozoa like intestinal flagellates and cillates require only 1 host, within which they multiply asexually in trophic stage and transfer from one host to another by the cystic form. Second host: In some protozoa like Plasmodium, asexual method of reproduction occurs in one host (man) and sexual method of reproduction in another host (mosquito). Phylum Sarcomastigophora has been subdivided into 2 subphyla based on their modes of locomotion. Sarcodina (Sarcos meaning flesh or body): It includes those parasites, which have no permanent locomotory organs, but move about with the aid of temporary prolongations of the body called pseudopodia (e.g. Amoebae). Mastigophora (Mastix, meaning whip or flagellum): It includes those protozoa which possess whip-like flagella (e.g. Trypanosoma and Trichomonas). Amoebae Classification of Protozoa Protozoan parasites of medical importance have been classified into kingdom Protista, subkingdom Protozoa which is further divided into the following four phyla (Table 2.2): Sarcomastigophora These protean animalcules can assume any shape and crawl along surfaces by means of foot-like projections called pseudopodia (literally meaning false feet). They are structurally very simple and are believed to have evolved from the flagellates by the loss of the flagella. Two groups of amoebae are of medical importance. Table 2.2: Classification of Protozoa Phylum Sarcomastigophora Subphylum Superclass Class Mastigophora Zoomastigophora(having one or rea more flagella) Subclass Order Kinetoplastida Suborder Trypanosomatina Retortamonadida Diplomonadida Enteromonadina Diplomonadina Trichomonadida Trichomonadida Sarcodina (pseudopodia present) Rhizopoda Lobosea Gymnamoebia Amoebida Tubulina Acanthopodina Apicomplexa Sporozoea Coccidia Schizopyrenida Eucoccidia Piroplasmia Vestibuliferia Piroplasmida Trichostomastida Eimeriina Haemosporina Ciliophora Microspora 11 Kinetofragminophorea Microsporea Microsporida Trichostomatina Apansporoblastina Genus Trypanosoma Leishmania Retortamonas Chilomastix Enteromonas Giardia Trichomonas Dientamoeba Entamoeba Endolimax Iodamoeba Acanthamoeba Naegleria Cryptosporidium Isospora Sarcocystis Toxoplasma Plasmodium Babesia Balantidium Enterocytozoon Encephalitozoon Microsporum 12 Textbook of Medical Parasitology Table 2.3: Principal Protozoan Pathogen of Man Species Habitat Disease Entameba histolytica Large intestine Amoebic dysentery, amoebic liver abcess Naegleria fowleri CNS Amoebic meningoencephalitis Acanthamoeba CNS, eye Encephalitis, keratitis Giardia lamblia Small intestine Malabsorption, diarrhea Trichomonas vaginalis Vagina, urethra Vaginitis, urethritis Trypanosoma brucei Blood, lymphnode, CNS Sleeping sickness Trypanosoma cruzi Macrophage of bone marrow, nerves, heart, colon etc. Chagas' disease Leishmania donovani Reticuloendothelial system Kala-azar, Post kala azar dermal leishmaniasis Leishmania tropica Skin Cutaneous leishmaniasis (oriental sore) Leishmania braziliensis Naso-oral mucosa Mucocutaneous leishmaniasis (Espundia, Chiclero's ulcer) Plasmodium spp. RBC Malaria Babesia microti RBC Babesiosis Isospora belli Intestine Diarrhea in AIDS Cryptosporidium parvum Intestine Diarrhea in AIDS Balantidium coli Large intestine Dysentery Amoebae of the alimentary canal: The most important of these is E. histolytica, which causes intestinal and extraintestinal amoebiasis. Amoebae are also present in the mouth. Potentially pathogenic free-living amoebae: Several species of saprophytic amoebae are found in soil and water. Two of these, Naegleria and Acanthamoeba are of clinical interest because they can cause eye infections and fatal meningoencephalitis. Flagellates These protozoa have whip-like appendages called flagella as the organs of locomotion. The fibrillar structure of flagella is identical with that of spirochetes and its has been suggested that they may have been derived from symbiotic spirochetes, which have become endoparasites. In some species, the flagellum runs parallel to the body surface, to which it is connected by a membrane called the undulating membrane. Flagellates parasitic for man are divided into 2 groups: Kinetoplastida: These possess a kinetoplast from which a single flagellum arises. They are the hemoflagellates comprising the trypanosomes and Leishmania, which are transmitted by blood-sucking insects and cause systemic or local infections. Flagellates without kinetoplast: These bear multiple flagella. Giardia, Trichomonas, and other luminal flagellates belong to this group. Because most of them live in the intestine, they are generally called intestinal flagellates. Phylum Apicomplexa Phylum Apicomplexa was formerly known as Sporozoa. Members of this group possess, at some stage in their life cycle, a structure called the apical complex serving as the organ of attachment to host cells. They are tissue parasites. They have a complex life cycle with alternating sexual and asexual generations. To this group, belongs the malarial parasites (suborder: Haemosporina, Family: Plasmodiidae), Toxoplasma, Sarcocystis, Isospora, and Cryptosporidium (under the Suborder: Eimeriina), Babesia (under the subclass: Piroplasma), and the unclassified Pneumocystis jirovecii. Phylum Ciliophora These protozoa are motile by means of cilia, which cover their entire body surface. The only human parasite in this group is Balantidium coli, which rarely causes dysentery. Phylum Microspora Phylum Microspora contains many minute intracellular protozoan parasites, which frequently cause disease in immunodeficient subjects. They may also cause illness in the immunocompetent, rarely. Protozoa Important points to remember • • • Only protozoan parasite found in lumen of human small intestine: Giardia lamblia. Largest protozoa: Balantidium coli. Most common protozoan parasite: Toxoplasma gondii. The zoological classification of protozoa is complex and is subject to frequent revisions. The classification described in the chapter is an abridged version of the classification proposed in 1980 by the Committee on Systematics and Evolution of the Society of Protozoologists, as applied to protozoa of medical importance. Key points of Protozoa • • • • • • • • • • • Protozoa are single-celled, eukaryotic microorganisms consisting of cell membrane, cytoplasm, and nucleus. Some protozoa have kinetoplast and flagella or cilia. Amoebae move about with temporary prolongations of the body called pseudopodia. Hemoflagellates comprising of Trypanosoma and Leishmania possess a single flagellum and kinetoplast. Luminal flagellates like Giardia and Trichomonas bear multiple flagella without kinetoplast. Balantidium coli belongs to the Phylum Ciliophora, which is motile by cilia that cover its entire body surface. Trophozoites are active feeding and growing stage of protozoa. Cysts are resting or resistant stage of protozoa bounded by tough cell wall. Protozoa multiply by both asexual and sexual modes of reproduction. Malaria parasite, Toxoplasma, and Cyptosporidium belong to Phylum Apicomplexa or Sporozoa, which possess apical complex at some stage of their life cycle and have a complex life cycle with alternating sexual and asexual generations. Microspora are intracellular protozoan parasites, which cause disease in immunodeficient patients. Review Questions 1. Define Protozoa and describe their general characteristics. 2. Write short notes on: (a) Classification of Protozoa 3. Differentiate between Protozoa and Metazoa. 13 (b) Reproduction in Protozoa 14 Textbook of Medical Parasitology Amoebae Amoebae are structurally simple protozoans which have no fixed shape. They are classified under Phylum: Sarcomastigophora, Subphylum: Sarcodina, Superclass: Rhizopoda and Order: Amoebida. ¾¾ The cytoplasm of amoeba is bounded by a membrane and can be differentiated into an outer ectoplasm and inner endoplasm. ¾¾ Pseudopodia are formed by the amoeba by thrusting out ectoplasm, followed by endoplasm. These are employed for locomotion and engulfment of food by phagocytosis. ¾¾ Reproduction occurs by fission and budding. Cyst is formed in unfavorable conditions and is usually the infective form for vertebrate host (e.g. Entamoeba histolytica). Table 3.1: Classification of Amoebae Intestinal amoebae Freeliving amoebae Entamoeba histolytica Entamoeba dispar Entamoeba coli Entamoeba polecki Entamoeba hartmanni Entamoeba gingivalis Endolimax nana Iodamoeba butschlii Naegleria fowleri Acanthamoeba spp. Balamuthia mandrillaris Note: All intestinal amoebae are nonpathogenic, except Entamoeba histolytica Note: All freeliving amoebae are opportunistic pathogens 3 ¾¾ Amoebae are classified as either free-living or intestinal amoebae (Table 3.1). ¾¾ A few of the free-living amoebae occasionally act as human pathogens producing meningoencephalitis and other infections, e.g. Naegleria and Acanthamoeba. ¾¾ The parasitic amoebae inhabit the alimentary canal. Entamoeba Histolytica History and Distribution E. histolytica was discovered by Lösch in 1875, who demonstrated the parasite in the dysenteric feces of a patient in St. Petersburg in Russia. ¾¾ In 1890, William Osler reported the case of a young man with dysentery, who later died of liver abscess. ¾¾ Councilman and Lafleur in 1891 established the pathogenesis of intestinal and hepatic amoebiasis and introduced the terms 'amoebic dysentery' and 'amoebic liver abscess'. ¾¾ E. histolytica is worldwide in prevalence, being much more common in the tropics than elsewhere. It has been found wherever sanitation is poor, in all climatic zones from Alaska (61°N) to straits of Magellan (52°S). ¾¾ It has been reported that about 10% of world population and 50% of the inhabitants of developing countries may be infected with the parasite. ¾¾ The infection is not uncommon even in affluent countries, about 1% of Americans being reported to be infected. Amoebae ¾¾ While the majority of infected humans (80–99%) are asymptomatic, invasive amoebiasis causes disabling illness in an estimated 50 million of people and causes 50,000 deaths annually, mostly in the tropical belt of Asia, Africa, and Latin America. ¾¾ It is the third leading parasitic cause of mortality, after malaria and schistosomiasis. •¾Epidemiologically, India can be divided into 3 regions, depending on the prevalence of intestinal amoebiasis. High prevalence states (>30%): Chandigarh, Tamil Nadu, and Maharashtra. Moderate prevalence states (10–30%): Punjab, Rajasthan, Uttar Pradesh, Delhi, Bihar, Assam, West Bengal, Andhra Pradesh, Karnataka, and Kerala. Low prevalence states (94%) and highly specific (>95%) for the diagnosis of amebic liver abscess (Flowchart 3.3B). ¾¾ Radioisotope Immunity Infection with invasive strains includes both humoral and cellular Immune responses. Local and systemic antibodies can be demonstrated within a week of invasive infection. Infection confers some degree of protection as evidenced by the very low frequency of recurrence of invasive colitis and liver abscess in endemic areas. The course and

severity of amoebiasis does not seem to be affected by human immunodeficiency virus (HIV) infection. Serological response is hardly ever seen in infection with non-invasive zymodemes. Treatment Three classes of drug are used in the treatment of amoebiasis. ¾¾ Luminal amoebicides: Diloxanide furoate, iodoquinol, paromomycin, and tetracycline act in the intestinal lumen but not in tissues. ¾¾ Tissue amoebicides: Emetine, chloroquine, etc. are effective in systemic infection, but less effective in the intestine. Dosage of chloroquine in amoebic liver abscess is 1 g for 2 days followed by 5 g daily for 3 weeks. ¾¾ Both luminal and tissue amoebicides: Metronidazole and related compounds like tinidazole and ornidazole act on both sites and are the drug of choice for treating amoebic colitis and amoebic liver abscess. Amoebae A B 23 C Fig. 3.7: Schematic diagram of the morphological forms of Entamoeba coli (Heidenhain's hematoxylin Magn. X 2000). A. Vegetative form; B. Binucleate cyst; C. Eight-nucleate cyst Note: Although metronidazole and tinidazole act on both the sites but neither of them reach high levels in the gut lumen; therefore, patients with amoebic colitis or amoebic liver abscess should also receive treatment with a luminal agent (paromomycin or iodoquinol) to ensure eradication of infection (Table 3.3). Paromomycin is the preferred agent. Table 3.3: Recommended Dosages of Antiamoebic Drugs Drug Dosage Duration (in days) Amoebic colitis or Amoebic liver abscess Tinidazole 2 g/day orally 3 Metronidazole 750 mg three times a day, orally or IV 5–10 Paromomycin 30 mg/kg 4 times a day ,orally in 3 divided doses 5–10 Iodoquinal 650 mg orally ,three times day 20 Intestinal amoebiasis individuals with documented E. histolytica infection should also be treated because of the risks of developing amoebic colitis or amoebic liver abscess in the future and risk of transmitting the infection to others. Paromomycin or iodoquinol in the doses listed in the Table 3.3 should be used in these cases. ¾¾ Oral rehydration and electrolyte replacement should be done wherever necessary. ¾¾ Asymptomatic Prophylaxis General prophylaxis is as for all fecal-oral infections. Food and water have to be protected from contamination with human exc reta. ¾¾ Detection and treatment of carriers and their exclusion from food handling occupations will help in limiting the spread of infection. ¾¾ Health education and inclusion of healthy personal habits helps in control. NONPATHOGENIC INTESTINAL AMOEBA Entamoeba Coli E. coli was first described by Lewis (1870) and Cunnigham (1871) in Kolkata and its presence in healthy persons was reported by Grassi (1878). ¾¾ It is worldwide in distribution and a nonpathogenic commensal intestinal amoeba. ¾¾ It is larger than E. histolytica about 20–50 µm with sluggish motility and contains ingested bacteria but no red cells. ¾¾ The nucleus is clearly visible in unstained films and has a large eccentric karyosome and thick nuclear membrane lined with coarse granules of chromatin (Fig. 3.7A and B). ¾¾ Cysts are large, 10–30 µm in size, with a prominent glycogen mass in the early stage. The chromatoid bodies are splinterlike and irregular. The mature cyst has 8 nuclei (Fig. 3.7C). ¾¾ The life cycle is the same as in E.histolytica except that it remains a luminal commensal without tissue invasion and is nonpathogenic. 24 Textbook of Medical Parasitology ¾¾ It is global in distribution. ¾¾ Only the trophozoite is found; Fig. 3.8: Trophozoite of Entamoeba hartmanni Entamoeba Hartmanni E. hartmanni occurs wherever E. histolytica is found. It is now considered to be a separate species of nonpathogenic commensal intestinal amoeba. ¾¾ It is much smaller than E. histolytica, the trophozoite measuring 4–12 µm and cyst 5–10 µm in size (Fig. 3.8). ¾¾ Trophozoites do not ingest red cells and their motility is less vigorous. ¾¾ The cyst resembles that of Endolimax nana. Diffential features of cyst and trophozoites of E. coli, E. hartmanni, and E. hislolytica are shown in Table 3.4. Entamoeba Gingivalis E. Gingivalis was the first amoeba of humans, discovered by Gros in 1849. the cystic stage being apparently absent. ¾¾ The trophozoite is about 10–20 µm, actively motile with multiple pseudopodia. ¾¾ The cytoplasm contains food vacuoles with ingested bacteria, leuocytes, and epithelial cells. ¾¾ Nucleus is round with central karyosome lined by coarse chromatin granules. ¾¾ The amoeba lives in gingival tissues and is abundant in unhygienic mouths. It is a commensal and is not considered to cause any disease. ¾¾ It is transmitted by direct oral contact. ¾¾ E. gingivalis have been found in bronchial washings and vaginal and cervical smears, where it can be mistaken for E. histolytica. Endolimax Nana This common commensal amoeba is widely distributed. ¾¾ It lives in the human intestine. ¾¾ The trophozoite is small (nana: small), less than 10 µm in size with a sluggish motility (Fig. 3.9A). ¾¾ The nucleus has conspicuous karyosome connected to nuclear membrane by one or none coarse strands. ¾¾ The cyst is small, oval, and quadrinucleate with glyocgen mass and chromidial bars, which are inconspicuous or absent (Fig. 3.9B). ¾¾ It is non-pathogenic. Table 3.4: Differential Features of Intestinal Entamoebae E. histolytica E. coli E. hartmanni Size (µm) 12–60 20–50 4–12 Motility Active Sluggish Active Pseudopodia Finger-shaped, rapidly extruded Short, blunt slowly extruded Finger-shaped, rapidly extruded Cytoplasm Clearly defined into ectoplasm and endoplasm Differentiation not distinct Clearly defined into ectoplasm and endoplasm Inclusions RBCs present, no bacteria Bacteria and other particles, no RBCs Bacteria and other particles, no RBCs Nucleus Not clearly visible in unstained films Visible in unstained films Not visible in unstained films Karyosome Small, central Large, eccentric Small, eccentric Nuclear Membrane Delicate, with fine chromatin dots Thick, with coarse chromatin granules Coarse chromatin granules Size (µm) 10–15 10–30 5–10 Nuclei in mature cyst 4 8 4 Glycogen mass Seen in uninucleate, but not in quadrinucleate stage Seen up to quadrinucleate stage Seen in uninucleate, but not in quadrinucleate stage chromidial 1–4 with crounded ends Splinter like with angular ends Many with irregular shape Trophozoite Cyst Amoebae A 25 B Fig. 3.9: Endolimax nana; A. Vegetative form; B. Quadrinucleate cyst Iodamoeba Buetschlii This is widely distributed, though less common than E. coli and E. nana. ¾¾ The trophozoite is small, 6–12 µm, with conspicuous nucleus (Fig. 3.10A). ¾¾ The prominent karyosome is half the size of the nucleus, having bull's eye appearance. ¾¾ The cyst is oval, uniucleate, and has a prominent iodine staining glycogen mass (iodophilic body). Hence, the name 'Iodamoeba'. It is non-pathogenic (Fig. 3.10B). The comparative morphology of amoebae infecting humans is illustrated in Fig. 3.11. PATHOGENIC FREE-LIVING AMOEBAE Among the numerous types of free-living amoebae found in water and soil, a few are potentially pathogenic and can cause human Infections. ¾¾ Primary amoebic meningoencephalitis (PAM) – caused by amoeboflagellate Naegleria (the brain eating amoeba). Fig. 3.11: Comparative morphology of amoebae infecting humans, showing trophozoite and cyst stages, as well as enlarged representation of their nuclear structure ¾¾ Granulomatous amoebic encephalitis (GAE) and chronic amoebic keratitis (CAK) – caused by Acanthamoeba. A few instances of GAE caused by lyptomyxid amoeba like Balamuthia have also been reported. While PAM and CAK occur in previously healthy individual, GAE has been associated with immunodeficient patients. The term amphizoic has been used for organisms such as these, which can multiply both in the body of a host (endozoic) and in free-living (exozoic) conditions. A Naegleria Fowleri B Fig. 3.10: Iodamoeba buetschlii; A. Vegetative form; B. Cyst It is the only species of genus Naegleria, which infects man. N. fowleri causes the disease primary amoebic meningi encephalitis (PAM), a brain infection that leads to destruction of brain tissue. 26 Textbook of Medical Parasitology History and Distribution N. fowleri is named after Fowler, who along with Carter described it first from Australia in 1965. ¾ N. fowleri is a heat-loving (thermophilic) amoeba that thrives in warm water at low oxygen tension and is commonly found in warm freshwater (e.g. lakes, rivers, and springs) and soil. ¾ It is world wide in distribution. ¾ In the last 10 years from 2002 to 2011, 32 infections were reported in the US, and in India, a total of 17 cases have been reported so far. Morphology N .fowleri occurs in 3 forms: ¾ Cyst ¾ Amoeboid trophozoite form ¾ Flagellate trophozoite form Trophozoite Stage The trophozoites occur in 2 forms, amoeboid and flagellate. Amoeboid form The amoeboid form is about 10–20 µm, showing rounded pseudopodia (lobopodia), a spherical nucleus with big endosome, and pulsating vacuoles. € With electron microscopy, vacuole appear to be densely granular in contrast to highly vacuolated body of amoeba and are called as amoebostomes. They are used for engulfing RBCs and WBCs and vary in number, depending on the species. € Amoeboid form is the feeding, growing, and replicating form of the parasite, seen on the surface of vegetation, mud, and water. € It is the invasive stage of the parasite and the infective form of the parasite. € € € € The cyst is 7–10 µm in diameter and has a smooth double wall. They are the resting or the dormant form and can resist unfavorable conditions, such as drying and chlorine up to 50 ppm. The trophozoites can withstand moderate heat (45°C), but die at chlorine levels of 2 ppm and salinity of 0.7%. Cysts and flagellate forms of N. fowleri have never been found in tissues of cerebrospinal fluid (CSF). Life Cycle Typically, infection occurs when people go swimming or diving in warm freshwater river or ponds and poorlymaintained swimming pools or nasal irrigation using contaminated tap water (Fig. 3.12). ¾¾ The life cycle of N. fowleri is completed in the external environment. ¾¾ The amoeboid form of trophozoite multiplies by binary fission. ¾¾ Under unfavorable conditions, it forms a cyst and which undergoes excystation in favorable conditions. ¾¾ Flagellate form of trophozoite helps in the spread of N. fowleri to new water bodies. Since the amoeboid form is the invasive stage, hence, the flagellate forms revert to amoeboid forms to become infective to man. Flagellate form The biffagellate form occurs when trophozoites are transferred to distilled water. € This transformation of trophozoites to biflagellate pear-shaped form occurs within a minute. € The flagellate can revert to the amoeboid form, hence N. fowleri is classified as amoeboflagellate. Cyst Stage Trophozoites encyst due to unfavorable conditions like food deprivation, dessication, cold temperature, etc. Fig. 3.12: Life cycle of Naegleria fowleri Amoebae 27 Table 3.5: Differential features of Naegleria and Acanthamoeba Naegleria Acanthamoeba Disease Primary amoebic meningoencephalitis (PAM) Granulomatous amoebic encephalitis (GAE) and keratitis Portal of entry Nose Upper Respiratory tract (?), cornea Clinical course Acute Subacute or chronic Pathogenecity Acute suppurative inflammation Granulomatous inflammation Morphological forms 3 stages: trophozoite, cyst and flagellate form 2 stages: trophozoite and cyst flagellate form absent Trophozoite 10–20 µm, with a single pseudopodia 20–50 µm, with spine-like pseudopodia Cyst 7–10 µm, round with smooth wall 15–25 µm, polygonal double-walled with wrinkled surface Nuclear division By promitosis, nucleolus divides, nuclear membrane persists Nuclear membrance dissolves WBC in CSF Predominantly neutrophils Predominantly lymphocytes Pathogenecity and Clinical Features Culture Patients are mostly previously healthy young adults or children. ¾¾ Human infection comes from water containing the amoebae and usually follows swimming or diving in ponds. ¾¾ The amoebae invade the nasal mucosa and pass through the olfactory nerve branches in the cribriform plate into the meninges, and brain to initiate an acute purulent meningitis and encephalitis, called as primary amoebic meningo encephalitis (PAM) . ¾¾ The incubation period varies from 2 days to 2 weeks. ¾¾ In the incubation period, the patient experiences anosmia. ¾¾ The disease advances rapidly, causing fever, headache, vomiting, stiff neck, ataxia, seizure, and coma. ¾¾ Cranial nerve palsies, especially of the third, fourth, and sixth nerves have also been documented. ¾¾ The disease almost always ends fatally within a week (average 5 days). N. fowleri can be grown in several kinds of liquid axenic media or non-nutrient agar plates coated with Escherichia coli. Both trophozoites and cysts occur in culture. Laboratory Diagnosis The diagnosis of PAM is based on the finding of motile Naegleria trophozoites in wet mounts of freshly-obtained CSF. Cerebrospinal Fluid Examination The CSF is cloudy to purulent, with prominent neutrophilic leucocytosis, elevated protein, and low glucose, resembling pyogenic meningitis. ¾¾ Wet film examination of CSF may show trophozoites. ¾¾ Cysts are not found in CSF or brain. ¾¾ At autopsy, trophozoites can be demonstrated in brain histologically by immunofluroscent staining. Molecular Diagnosis Newer tests based on polymerase chain reaction (PCR) technology are being developed. Treatment The drug of choice is amphotericin-B intravenously. It can also be instilled directly into the brain. ¾¾ Treatment combining miconazole and sulfadiazine has shown limited success, only when administered early. ¾¾ More than 95% cases of PAM are fatal despite of treatment. Acanthamoeba Species A. culbertsoni (formerly, Hartmanella culbertsoni) is the species most often responsible for human infection but other species like A. polyphagia, A. castalleni, and A. astromyx have also been reported. Distribution This is an opportiunistic protozoan pathogen found worldwide in the environment in water and soil. ¾¾ Approximately, 400 cases have been reported worldwide. Morphology Acanthamoeba exists as active trophozoite form and a resistant cystic form. 28 Textbook of Medical Parasitology ¾¾ The trophozoite is large, 20–50 µm in size and characterized by spine-like pseudopodia (acantho podia). ¾¾ It differs from Naegleria in not having a flagellate stage and in forming cysts in tissues (Table 3.5). ¾¾ The polygonal double-walled cysts are highly resistant. ¾¾ The cysts are present in all types of environment, all over the world. Life Cycle ¾¾ Both trophozoites and cysts are infective. ¾¾ Human beings acquire by inhalation of cyst or trophozoite , ingestion of cysts, or through traumatized skin or eyes (Fig. 3.13). ¾¾ After inhalation of aerosol or dust containing trophozoites and cysts, the trophozoites reach the lungs and from there, they invade the central nervous system through the blood stream, producing granulomatous amoebic encephalitis (GAE). Fig. 3.13: Life cycle of Acanthamoeba culbertsoni usually occurs in patients with immunodeficiency, diabetes, malignancies, malnutrition, systemic lupus erythematosus (SLE), or alcoholism. ¾¾ The parasite spreads hemotogenously into central nervous system. Subsequent invasion of the connective tissue and induction of proinflammatory responses lead to neuronal damage that can be fatal within days. ¾¾ A postmortem biopsy reveals severe edema and hemorrhagic necrosis. typically occurs in persons with a compromised immune system. € GAE is believed to follow inhalation of the dried cysts. € The incubation period is long and the evolution of the illness is slow. € Clinical picture is that of intracranial space-occupying lesions with seizures, pareses, and mental deterioration. ¾¾ Disseminated infection: In immunocomprised states like acquired immunodeficiency syndrome (AIDS), a widespread infection can affect skin, lungs, sinuses, and other organs independently or in combination. Clinical Disease Laboratory Diagnosis Pathogenesis and Clinical Features ¾¾ Infection It presents chiefly as 2 chronic conditions—keratitis and encephalitis. ¾¾ Acanthamoeba keratitis: An infection of the eye that typically occurs in healthy persons and develops from the entry of the amoebic cyst through abrasions on the cornea. € Majority of such cases have been associated with the use of contact lenses. € The picture resembles that of severe herpetic keratitis with a slow relapsing course, but the eye is severely painful in the amoebic infection. € Unilateral photophobia, excessive tearing, redness and foreign body sensation are the earliest signs and symptoms; disease is bilateral in some contact lens users. € Keratitis and uveitis can result in permanent visual impairment or blindness. ¾¾ Granulomatous amoebic encephalitis(GAE): It is a serious infection of the brain and spinal cord that ¾¾ Diagnosis of amoebic keratitis is made by demonstration of the cyst in corneal scrapings by wet mount, histology and culture. Growth can be obtained from corneal scarpings inoculated on nutrient agar, overlaid with live or dead Escherichia coli and incubated at 30°C. ¾¾ Diagnosis of GAE is made by demonstration of trophozoites and cysts in brain biopsy, culture, and immofluroscence microscopy using monoclonal antibodies. € CSF shows lymphocytic pleocytosis, slightly elevated protein levels, and normal or slightly decreased glucose levels. € CT scan of brain provides inconclusive findings. Treatment In acanthamoeba keratitis, current therapy involves topical administration of biguanide or chlorhexadine with Amoebae or without diamidine agent. In severe cases, where vision is threatened, penetrating keratoplasty can be done. No effective treatment is available for GAE. Multidrug combinations including pentamidine, sulfadiazine, rifampicin, and fluconazole are being used with limited success. Balamuthia Mandrillaris B. mandrillaris, a leptomixid free-living amoeba, is a newly identified species reported to cause GAE. Morphology It exists in amoeboid trophozoite stage. The flagellate stage is absent. ¾¾ It is relatively large (12–60 µm), irregular in shape, and actively motile by broad pseudopodia. ¾¾ Cyst of B. mandrillaris are usually spherical (6–20 µm), surrounded by a three-layered cyst wall—outer irregular ectocyst, a middle mesocyst and an inner endocyst round wall. Under light microscopy, it appears to have two walls—an outer irregular wall and an inner smooth wall. ¾¾ Infection is trasmitted through respiratory tract skin lesions; or eyes. ¾¾ Life cycle is similar to that of Acanthamoeba spp. Clinical Disease It causes granulomatous amoebic encephalitis in both healthy and immunocompromised hosts particularly in children and elderly. Laboratory Diagnosis Laboratory diagnosis is done by identifying trophozoites of B. mandrillaris in the CSF and trophozoites and cysts in brain tissue. PCR also gives reliable diagnosis. Key points of Amoebae •¾E. histolytica is found in human colon and is mainly asymptomatic. •¾Cyst contains glycogen mass and 1–4 chromatid bars. •¾Pathogenic strains are identified by genetic markers and zymodeme analysis. •¾Stools: In amoebic dysentery, stool are copious foul smelling, brownish black often with blood-streaked mucus. •¾Amoebic ulcers: Typical ulcers are discrete, flask-shaped, with ragged undermined margin, found in caecum and sigmoido-rectal region. •¾Amoebic granuloma or amoeboma may develop from chronic ulcers. •¾Extraintestinal complications: Amoebic hepatitis and liver abscess are the most common. •¾Abscesses in other organs such as lung, brain, spleen, and genitourinary tract may result from hematogenous spread or by direct spread from hepatic lesion. •¾Diagnosis: By demonstration of trophozoites and cyst in stool and also by serological tests and imaging techniques in hepatic amoebiasis. •¾Treatment: By Metronidazole or tinidazole along with parmomycin, diloxanide furoate, or chloroquine. •¾E. hartmanni, E. coli, E. gingivalis, E. nana, and Iodamoeba are commensals and non-pathogenic amoebae. •¾Naegleria and Acanthamoeba are pathogenic free-living amoeba. •¾N. fowleri occurs in 3 forms – cyst, trophozoite, and flagellate. It causes PAM. •¾Acanthamoeba species cause amoebic keratitis and also GAE in immuono-compromised subjects. Review Questions 1. Describe briefly the life cycle and laboratory diagnosis of Entamoeba histolytica. 2. Write short notes on: (a) Extraintestinal amoebiasis 3. Differentiate between: (a) Amoebic dysentery and bacillary dysentery (b) Naegleria and Achanthamoeba 29 (b) Free-living amoebae (b) Entamoeba histolytica and Entamoeba coli 30 Textbook of Medical Parasitology Intestinal, Oral, and Genital Flagellates Parasitic protozoa, which possess whip-like flagella as their organs of locomotion are called as flagellates and classified as— Phylum: Sarcomastigophora Subphylum: Mastigophora Class: Zoomastigophora (mastix: whip) ¾¾ Depending on their habitat, they can be considered under: € Lumen-dwelling flagellates: Flagellates found in the alimentary tract and urogenital tract (Table 4.1). € Hemoflagellates: Flagellates found in blood and tissues (Table 4.1). ¾¾ Most luminal flagellates are nonpathogenic commensals. Two of them cause clinical diseases—Giardia lamblia, which can cause diarrhea and Trichomonas vaginalis, which can produce vaginitis and urethritis. 4 Giardia Lamblia History and Distribution It is one of the earliest protozoan parasite to have been recorded. ¾¾ The flagellate was first observed by Dutch scientist Antonie von Leeuwenhoek (1681) in his own stools. ¾¾ It is named 'Giardia' after Professor Giard of Paris and 'lamblia' after Professor Lamble of Prague, who gave a detailed description of the parasite. ¾¾ It is the most common protozoan pathogen and is worldwide in distribution. ¾¾ Endemicity is very high in areas with low sanitation, especially tropics and subtropics. Visitors to such places frequently develop traveller's diarrhea caused by giardiasis through contaminated water. Table 4.1: Flagellates Group Parasites Habitat Lumendwelling flagellates Giardia lamblia Trichomonas vaginalis Trichomonas tenax Trichomonas hominis Chilomastix mesnili Enteromonas hominis Retortamonas intestinalis Dientamoeba fragilis Duodenum and jejunum Vagina and urethra Mouth Large intestine (caecum) Large intestine (caecum) Large intestine (colon) Large intestine (colon) Large intestine (caecum and colon) Hemoflagellates Leishmania spp. Trypanosoma brucei Trypanosoma cruzi Reticuloendothelial cells Connective tissue and blood Reticuloendothelial cells and blood Intestinal, Oral, and Genital Flagellates 31 Habitat G. lamblia lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine. Protozoans found in small intestine •¾Giardia lamblia •¾Isospora belli •¾Cyclospora caytenensis •¾Cryptosporidium parvum •¾Sarcocystis hominis and suihominis Morphology Fig. 4.1: Giardia lamblia in duodenal fluid wet preparation. Magnification X 1500 It exists in 2 forms: ¾¾ Trophozoite (or vegetative form) ¾¾ Cyst (or cystic form). Trophozoite The trophozoite is in the shape of a tennis racket (heartshaped or pyriform shaped) and is rounded anteriorly and pointed posteriorly (Figs. 4.1 and 4.2A and B). ¾¾ It measures 15 µm x 9 µm wide and 4 µm thick. ¾¾ Dorsally, it is convex and ventrally, it has a concave sucking disc, which helps in its attachment to the intestinal mucosa. ¾¾ It is bilaterally symmetrical and possesses. € 1 pair of nuclei € 4 pairs of flagella € Blepharoplast, from which the flagella arise (4 pairs) € 1 pair of axostyles, running along the midline A Two sausageshaped parabasal or median bodies, lying transversely posterior to the sucking disc. ¾¾ The trophozoite is motile, with a slow oscillation about its long axis, often resembling falling leaf. € B Cyst It is the infective form of the parasite (Fig. 4.2C). ¾¾ The cyst is small and oval, measuring 12 µm x 8 µm and is surrounded by a hyaline cyst wall. ¾¾ Its internal structure includes 2 pairs of nuclei grouped at one end. A young cyst contains 1 pair of nuclei. ¾¾ The axostyle lies diagnonally, forming a dividing line within cyst wall. ¾¾ Remnants of the flagella and the sucking disc may be seen in the young cyst. C Fig. 4.2: Trophozoite. A. Ventral view; B. Lateral view; C. Quadrinucleate Cyst 32 Textbook of Medical Parasitology Life Cycle Pathogenicity and Clinical Features Giardia passes its life cycle in 1 host. Infective form: Mature cyst. Mode of transmission: € Man acquires infection by ingestion of cysts in contaminated water and food. € Direct persontoperson transmission may also occur in children, male homosexuals, and mentally ill persons. € Enhanced susceptibility to giardiasis is associated with blood group A, achlorhydria, use of cannabis, chronic pancreatitis, malnutrition, and immune defects such as 19A deficiency and hypogamma globulinemia. ¾ Within half an hour of ingestion, the cyst hatches out into two trophozoites, which multiply successively by binary fission and colonize in the duodenum (Fig. 4.3). ¾¾ The trophozoites live in the duodenum and upper part of jejunum, feeding by pinocytosis. ¾¾ During unfavorable conditions, encystment occurs usually in colon (Fig. 4.3). ¾¾ Cysts are passed in stool and remain viable in soil and water for several weeks. ¾¾ There may be 200,000 cysts passed per gram of feces. ¾ Infective dose is 10–100 cysts. G. lamblia is typically seen within the crypts of duodenal and jejunal mucosa. It does not invade the tissue, but remains tightly adhered to intestinal epithelium by means of the sucking disc. ¾ They may cause abnormalities of villous architecture by cell apoptosis and increased lymphatic infiltration of lamina propria. ¾ Variant specific surface proteins (VSSP) of giardia play an important role in virulence and infectivity of the parasite. ¾ Often they are asymptomatic, but in some cases, Giardia may lead to mucus diarrhea, fat malabsorption (steatorrhea), dull epigastric pain, and flatulence. The stool contains excess mucus and fat but no blood. ¾ Children may develop chronic diarrhea, malabsorption of fat, vitamin A, protein, sugars like xylose disaccharides, weight loss, and spruelike syndrome. ¾ Occassionally, Giardia may colonize the gall bladder, causing biliary colic and jaundice. ¾ Incubation period is variable, but is usually about 2 weeks. Protozoan parasites causing diarrhea •¾Giardia lamblia •¾Cyclospora cayetanensis •¾Isospora belli Fig. 4.3: Life cycle of Giardia lamblia ¾ Entamoeba histolytica Cryptosporidium parvum Intestinal, Oral, and Genital Flagellates 33 Flowchart 4.1: Laboratory diagnosis of Giardia lamblia Laboratory Diagnosis ¾¾ The sensitivity of the test is 95% and specificity is 100%, Stool Examination ¾¾ The Giardiasis can be diagnosed by identification of cysts of Giardia lamblia in the formed stools and the trophozoites and cysts of the parasite in diarrheal stools (Flowchart 4.1). ¾¾ On macroscopic examination fecal specimens containing G. lamblia may have an offensive odor, are pale colored and fatty, and float in water. ¾¾ On microscopic examination, cysts and trophozoites can be found in diarrheal stools by saline and iodine wet preparations. ¾¾ Often multiple specimens need to be examined and concentration techniques like formal ether or zinc acetate are used. In asymptomatic carriers, only the cysts are seen. Enterotest (String test) A useful method for obtaining duodenal specimen is enterotest. A coiled thread inside a small weighted gelatin capsule is swallowed by the patient, after attaching the free end of the thread in the check. The capsule passes through the stomach to the duodenum. After 2 hours, the thread is withdrawn, placed in saline, and is mechanically shaken. The centrifuged deposit of the saline is examined for Giardia. The use of enterotest is not recommended because of the very high cost of the test. when compared to conventional microscopy. test may be used for quantification of cysts and in epidemiological and control studies, but not for routine use. Antibody detection IIF test and ELISA are used to detect antibodies against Giardia. ¾¾ Demonstration of antibodies is useful in the epidemio logical and pathophysiological studies. ¾¾ These tests cannot differentiates between recent and past infection and lack sensitivity and specificity. Molecular Method DNA probes and polymerase chain reaction (PCR) have been used to demonstrate parasitic genome in the stool specimen (Flowchart 4.1). Treatment Metronidazole (250 mg, thrice daily for 5–7 days) and tinidazole (2 g single dose) are the drugs of choice. ¾¾ Cure rates with metronidazole are more than 90%. ¾¾ Tinidazole is more effective than metronidazole. ¾¾ Furuzolidone and nitazoxamide are preferred in children, as they have fewer adverse effects. ¾¾ Parmomycin, an oral aminoglycoside can be given to symptomatic pregnant females. Note: Only symptomatic cases need treatment. Serodiagnosis Antigen detection Enzyme-linked immunosorbent assay (ELISA), immuno chromatographic strip tests and indirect immunofluroscent (IIF) tests using monoclonal antibodies have been developed for detection of Giardia antigens in feces (Flowchart 4.1). ¾¾ The presence of antigen indicates active infection. ¾¾ Commercially available ELISA kits (ProSpec T/Giardia kit) detects Giardia-specific antigen 65 (GAS 65). Prophylaxis Giardiasis can be prevented by following measures: ¾¾ Proper disposal of waste water and feces. ¾¾ Practice of personal hygiene like hand-washing before eating and proper disposal of diapers. ¾¾ Prevention of food and water contamination. Community chlorination of water is ineffective for inactivating cysts. Boiling of water and filtration by membrane filters are required. 34 Textbook of Medical Parasitology Key points of Giardia lamblia Trichomonas Vaginalis •¾Giardia History and Distribution is the only protozoan parasite found in the lumen of the human small intestine (duodenum and jejunum). •¾Trophozoites are pearshaped, bilaterally symmetrical with 2 nuclei, 4 pairs of flagella, and a ventral concave sucking disc. They exhibit motility resembling a 'falling leaf'. •¾Ellipsoid cysts contain 4 nuclei with remnants of flagella. •¾Infective form: Ellipsoid cysts. •¾Clinical features: Mostly asymptomatic but in some cases may cause diarrhea, dull epigastric pain, and malabsorption. Stool contains excess mucus but no blood. •¾Diagnosis: By microscopic demonstration of tropho zoites or cysts in stool, enterotest, and serodiagnosis by ELISA (ProSpec T/Giardia antigen assay). •¾Treatment: Metronidazole and tinidazole are the drugs of choice. Trichomonas Trichomonas differs from other flagellates, as they exist only in trophozoite stage. Cystic stage is not seen. ¾¾ Genus Trichomonas has 3 species, which occur in humans (Fig. 4.4). € T. vaginalis (Fig. 4.4 A) € T. hominis (Fig. 4.4 B) € T. tenax (Fig. 4.4 C) A T. vaginalis was first observed by Donne (1836) in vaginal secretion. ¾ Prevalence of trichomoniasis varies from 5% patients at hospitals to 75% in sexual workers. Morphology It is pear-shaped or ovoid and measures 10–30 µm in length and 5–10 µm in breadth with a short undulating membrane reaching upto the middle of the body (Fig. 4.4A). ¾ It has four anterior flagella and fifth running along the outer margin of the undulating membrane, which is supported at its base by a flexible rod, costa. ¾ A prominent axostyle runs throughout the length of the body and projects posteriorly like a tail. ¾ The cytoplasm shows prominent siderophillic granules, which are most numerous alongside the axostyle and costa. ¾ It is motile with a rapid jerky or twitching type movement. Habitat In females, it lives in vagina and cevix and may also be found in Bartholin's glands, urethra, and urinary bladder. In males, it occurs mainly in the anterior urethra, but may also be found in the prostate and preputial sac. B Fig. 4.4: Trichomonas species. A. T. vaginalis; B. T. hominis; C. T. tenax C Intestinal, Oral, and Genital Flagellates Life Cycle Life cycle of T. vaginalis is completed in a single host either male or female. Mode of transmission: €€ The trophozoite cannot survive outside and so infection has to be transmitted directly from personto-person. Sexual transmission is the usual mode of infection. €€ Trichomoniasis often coexists with other sexually trans mitted diseases; like candidiasis, gonorrhea, syphillis, or human immunodeficiency virus (HIV). €€ Babies may get infected during birth. €€ Fomites such as towels have been implicated in transmission. ¾¾ Trophozoites divide by binary fission. ¾¾ As cysts are not formed, the trophozite itself is the infective form. ¾¾ Incubation period is roughly 10 days. Protozoa transmitted by sexual contact ••Trichomonas vaginalis ••Giardia lamblia ••Entamoeba histolytica Pathogenesis T. vaginalis particularly infects squamous epithelium and not columnar epithelium. It secretes cystine proteases, lactic acid, and acetic acid, which disrupt the glycogen levels and lower the pH of the vaginal fluid. ¾¾ It is an obligate parasite and cannot live without close association with the vaginal, urethral, or prostatic tissues. ¾¾ Parasite causes petechial hemorrhage (strawberry mucosa), metaplastic changes, and desquamation of the vaginal epithelium. ¾¾ Intracellular edema and so called chicken-like epithelium, is the most characteristic feature of trichomoniasis. Clinical Features Infection is often asymptomatic, particularly in males, although some may develop urethritis, epididymitis, and prostatitis. ¾¾ In females, it may produce severe pruritic vaginitis with an offensive, yellowish green, often frothy discharge, dysuria, and dyspareunia. Cervical erosion is common. Endometritis and pyosalpingitis are infrequent complications. ¾¾ Rarely, neonatal pneumonia and conjunctivitis have been reported in infants born to infected mothers. 35 ¾¾ The incubation period of trichomoniasis is 4 days to 4 weeks. Laboratory Diagnosis Microscopic examination ¾¾ Vaginal or urethral discharge is examined microscopically in saline wet mount preparation for characteristic jerky and twitching motility and shape. In males, trophozoites may be found in urine or prostatic secretions. ¾¾ Fixed smears may be stained with acridine orange, papanicolaou, and Giemsa stains. ¾¾ Direct fluroscent antibody (DFA) is another method of detection of parasite and is more sensitive than the wet mount. Culture Culture is recommended when direct microscopy is negative and is considered as a 'gold standard' as well as the most sensitive (95%) method for the diagnosis of T. vaginalis infection. ¾¾ It grows best at 35°–37°C under anaerobic conditions. The optimal pH for growth is 5.5–6.0. ¾¾ It can be grown in a variety of solid or liquid media, tissue culture, and eggs. Cysteine-peptone-liver-maltose (CPLM) medium and plastic envelope medium (PEM) are often used. Serology ELISA is used for demonstration of T. vaginalis antigen in vaginal smear using a monoclonal antibody for 65-KDA surface polypeptide of T. vaginalis. Molecular method DNA hybridization and PCR are also highly sensitive (97%) and specific (98%) tests for the diagnosis of trichomoniasis. Treatment Simultaneous treatment of both partners is recommended. ¾¾ Metronidazole 2 g orally as a single dose or 500 mg orally twice a day for 7 days is the drug of choice. ¾¾ In patients not responding to treatment with standard regime, the dose of metronidazole may be increased or it may be administered parenterally. ¾¾ In pregnancy, metronidazole is safe in second and third trimesters. Prophylaxis Prevention is same as for other sexually transmitted diseases. 36 Textbook of Medical Parasitology ¾¾ Avoidance of sexual contact with infected partners and use of barrier method during intercourse prevent the disease. ¾¾ Patient's sexual partner should be tested for T. vaginalis when necessary. Trichomonas Tenax T. tenax, also known as T. buccalis, is a harmless commensal which lives in mouthin the periodontal pockets, carious tooth cavities, and less often in tonsillar crypts. ¾¾ It is smaller (5–10 µm) than T. vaginalis. ¾ It is transmitted by kissing, through salivary droplets, and fomites. There are sporadic reports of its involvement in respiratory infections and thoracic abcesses. ¾¾ Better oral hygine rapidly eliminates the infection and no therapy is indicated. Trichomonas Hominis T. hominis measures 8–12 µm, pyriform-shaped, and carries 5 anterior flagella and an undulating membrane that extends the full length of the body. ¾¾ It is a very harmless commensal of the caecum. ¾¾ Microscopic examination of stool will reveal motile trophozoite of T. hominis. ¾¾ Transmission occurs in trophic form by fecaloral route. Key points of Trichomonas •¾Trichomonas occurs only in trophozoite form, which is pearshaped, with 5 flagella and an undulating membrane. •¾The motility is rapid jerky or twitching type. •¾Habitat: Vagina and cervix in female and urethra in males. •¾Clinical features: Often asymptomatic in males. In females, it leads to pruritic vaginitis with greenish yellow discharge, strawberry mucosa and dysuria. •¾Diagnosis: By wet mount microscopy of vaginal or uretheral discharge, Culture (gold standard), PCR, and by demonstration of antigen in vaginal smear by ELISA. •¾Treatment: Metronidazole is the drug of choice and simultaneous treatment of both partners is recommended. Fig. 4.5: Trophozoite and egg of Chilomastix mesnili ¾¾ A distinct spiral groove is seen on one side of the nucleus. ¾¾ The cysts are lemon-shaped having a spiral projection at the anterior end. It measures 5–10 µm in length and 4–6 µm in breadth and is surrounded by a thick cyst wall. ¾¾ Both trophozoites and cysts are demonstrated in the semiformed stool. ¾¾ It is a harmless commensal of caecum where the organism feeds on bacteria and food debris. Since infection is acquired through ingestion of cysts, prevention depends on improved personal hygine. Enteromonas Hominis E. hominis is a non-pathogenic commensal that lives in large intestine, mainly in the caecum. ¾¾ It exists in 2 forms: trophozoite and cyst (Fig. 4.6). ¾¾ The trophozoite is pear-shaped, with 3 anterior and 1 posterior flagella. Chilomastix Mesnili This occurs as trophozoites and cysts (Fig. 4.5). ¾¾ The trophozoite is pear-shaped measuring 5–20 µm in length and 5–10 µm in breadth. ¾¾ At the anterior end, it has a spherical nucleus. Fig. 4.6: Trophozoite and cyst of Enteromonas hominis Intestinal, Oral, and Genital Flagellates It measures 5–10 µm in length and 3–6 µm in breadth. € The cytoplasm contains numerous bacteria and an anteriorly placed nucleus but no cytostoma. € It shows jerky forward movements. ¾¾ The cyst is oval in shape, measuring 5–8 µm in length and 4–6 µm in breadth. € It contains 2–4 nuclei. € The cyst of E. hominis may mimic a twonucleated cyst of E. nana. ¾¾ Infection occurs through fecaloral route by ingestion of cysts in contaminated food and water. ¾¾ Diagnosis is made by identification of trophozoites or cysts in the stool by ironhematoxylin stain. 37 € Retortamonas Intestinalis Wenyon and O' Connor first observed the parasite in stool in Egypt. ¾¾ R. intestinalis is a small nonpathogenic flagellate found in the large intestine. ¾¾ It also exists in 2 forms—trophozoite and cyst. ¾¾ The trophozoite is elongagted, pyriform in shape, measuring 5–10 µm in length and 3–4 µm in breadth. € The cytoplasm is granular and vacuolated. € It has a cleftlike cytosome, spherical nucleus, and central karyosome. € Two minute blepharoplasts are present near nucleus, from which two flagella originate. € The trophozoite multiplies by binary fission. ¾¾ The cyst is ovoid or pyriform in shape, measuring 6 µm in length and 3 µm in breadth. ¾¾ Water and food contaminated by cysts are the main source of infection. ¾¾ Diagnosis is made by identifying the cysts and tropho zoites in the direct wet mount and iron hemtoxylin stained specimen of stool. Dientamoeba Fragilis D. fragilis was previously considered as an amoeba but has now been reclassified as an amoeboflagellate, based on electron microscopic study and antigenic similarity to Trichomonas. Fig. 4.7: Trophozoite of Dientamoeba fragilis ¾¾ It is unique as it has only trophozoite stage but no cyst stage. name Dientamoeba fragilis is derived from the binucleate nature of trophozoite (Dientamoeba) and the fragmented appearance (fragilis) of its nuclear chromatin. ¾¾ It is seen worldwide and is reported to be the most common intestinal protozoan parasite in Canada. ¾¾ It lives in colonic mucosal crypts, feeding on bacteria. It does not invade tissues, but may rarely ingest RBCs. ¾¾ The trophozoite is 7–12 µm in diameter. It is motile with broad hyaline leaflike pseudopodia. They have 1–4 nuclei; the binucleate form being the most common (Fig. 4.7). The nuclear chromatin is present as 3–5 granules in the center, with no peripheral chromatin on the nuclear membrane. ¾¾ In the absence of cyst stage, its mode of transmission is not clear. Possibly, it is transmitted from person toperson by the fecaloral route or by the eggs of Enterobius vermicularis and other nematodes, which may serve as a vector. ¾¾ Formerly believed to be nonpathogenic, it has now been associated with a variety of symptoms like intermittent diarrhea, abdominal pain, flatulence, anorexia, nausea, malaise, and fatigue. ¾¾ High incidence is seen among children between 2 and 10 years of age. ¾¾ Laboratory diagnosis is made by demonstration of trophozoites in stool. At least 3 stool specimens should be collected over a period of 7 days. ¾¾ Metronidazole, iodoquinol, paromomycin, and tetracycline have been used for treatment. ¾¾ The Review Questions 1. Describe briefly the life cycle and laboratory diagnosis of Giardia lamblia. 2. Write Short notes on: (a) Trichomonas vaginalis (b) Dientamoeba fragilis 38 Textbook of Medical Parasitology Hemoflagellates The blood and tissue flagellates belong to the family Trypanosomatidae. The family consists of 6 genera, of which 2 genera Trypanosoma and Leishmania are pathogenic to humans. Zoological Classification of Flagellates Phylum Subphylum Class Order Family : : : : : Sarcomastigophora Mastigophora Kinetoplastidea Trypanosomatida Trypanosomatidae 5 the blepharoplast to surface of the body is known as axoneme. A free flagellum at the anterior end traverses on the surface of the parasite as a narrow undulating membrane (Fig. 5.1). ¾¾ Hemoflagellates exist in two or more of four morphological stages. These forms were formerly called the leishmanial, leptomonad, crithidial, and trypanosomal stages. But as these names are also given to different genera within the family, they were changed to amastigote, promastigote, epimastigote and trypomastigote. The names of the stages are formed by the suffix mastigote, combined with various prefixes, referring to the arrangement of the flagella in relation to General Characteristics ¾¾ They live in the blood and tissues of man and other vertebrate hosts and in the gut of the insect vectors. ¾¾ Members of this family have a single nucleus, a kinetoplast, and a single flagellum (Fig 5.1). ¾¾ Nucleus is round or oval and is situated in the central part of the body. ¾¾ Kinetoplast consists of a deeply staining parabasal body and adjacent dotlike blepharoplast. The parabasal body and blepharoplast are connected by one or more thin fibrils (Fig 5.1). ¾¾ Flagellum is a thin, hairlike structure, which originates from the blepharoplast. The portion of the flagellum, which is inside the body of the parasite and extends from Fig. 5.1: Basic morphology of hemoflagellates Note: Parabasal body and blepharoplast together constitute the kinetoplast Hemoflagellates 39 Table 5.1: Differences between Various Morphological Stages of Hemoflagellates Amastigote Promastigote Epimastigote Trypomastigote Morphological characteristics Rounded or ovoid, without any external flagellum. The nucleus, kinetoplast, and axial filaments can be seen. The axoneme extends upto the anterior end of the cell Lanceolate in shape. Kinetoplast is anterior to the nucleus (antenuclear kineloplast) near the anterior end of the cell, from which flagellum emerges. There is no undulating membrane Elongated, with the kinetoplast placed more posteriorly, though close to and in front of the nucleus (juxtanuclear kinetoplast). The flagellum runs alongside the body as a short undulating membrane, before emerging from the anterior end This stage is elongated, spindle shaped with a central nucleus. The kinetoplast is posterior to the nucleus (postnuclear kinetoplast) and situated at the posterior end of the body. The flagellum runs alongside the entire length of the cell to form a long undulating membrane before emerging as a free flagellum from the anterior end Seen in Trypanosoma cruzi and Leishmania as intracellular form in vertebrate host It is the infective stage of Leishmania, found in the insect vector as well as in cultures invitro It is the form in which Trypanosoma brucei occur in salivary gland of the vector tsetse fly and Trypanosoma cruzi in the midgut of the vector reduviid bug. Note: This stage is lacking in Leishmania. This is the infective stage of trypanosomes found in arthropod vector and in the blood of infected vertebrate. Note: This stage is lacking in Leishmania Schematic illustration N = Nucleus; P = Parabasal body; B = Blepharoplast; A = Axoneme; U = Undulating membrane; F = Flagellum. Note: Besides the stages described in the table, some transitional stages have been recognized. These include the sphaeromastigote, a motile round form with free flagellum, which is a transitional stage from amastigote to promastigote, seen in the genus Trypanosoma and the paramastigote, a transitional form leading to the infective promastigote in Leishmania the position of the nucleus and its point of emergence from the cells (Table 5.1). ¾¾ Staining characteristics of trypanosomes: For smears of body fluids, Romanowsky's Wrights stain, Giemsa stain, and Leishman's stain are suitable for identifying internal structures. The cytoplasm appears blue, the nucleus and flagellum appear pink, and the kinetoplast appears deep red. For tissue section, hematoxylineosin staining is done for demonstrating structures of the parasite. ¾¾ All members of the family have similar life cycles. They all require an insect vector as an intermediate host. ¾¾ Multiplication in both the vertebrate and invertebrate host is by binary fission. No sexual cycle is known. Trypanosomes General Characters All members of the genus Trypanosoma (trypanes: to bore, soma: body), exist at sometime in their life cycle, as trypomastigote stage with an elongated spindleshaped body, central nucleus, a posterior kinetoplast, and long undulating membrane. Volutin granules are found in cytoplasm. Some trypanosomes such as T. cruzi assume amastigote forms in vertebrate hosts. In addition to the typical forms, cells with atypical features are frequently found, a condition known as polymorphism. ¾¾ Trypanosoma pass their life cycle in 2 hosts— vertebrate hosts (definitive hosts) and insect vectors (intermediate hosts). The vector becomes infective to the vertebrate host only after an extrinsic incubation period, during which the parasite undergoes development and multiplication. ¾¾ In the vector, the trypanosomes follow one or two modes of development and are accordingly classified into 2 groups—Salivaria and Stercoraria. € Salivaria (anterior station): In salivaria, the trypano somes migrate to mouth parts of the vectors, so that infection is transmitted by their bite (inoculative transmission). Examples are T. gambiense and T. rhodesiense causing African trypanosomiasis, which are transmitted by the bite of tsetse flies. € Stercoraria (posterior station): In stercoraria, the trypanosomes migrate to the hindgut and are 40 Textbook of Medical Parasitology Classification of Trypanosomes Trypanosomes Infecting Man •¾Trypanosoma brucei complex, causing African trypanosomiasis or sleeping sickness, subspecies are: Trypanosoma brucei gambiense: causing West African sleeping sickness. Trypanosoma brucei rhodesiense: causing East African sleeping sickness. •¾Trypanosoma cruzi, causing South American trypanosomiasis or Chagas' disease. •¾Trypanosoma rangeli, a nonpathogenic trypanosome causing human infection in South America. Trypanosomes of Animals •¾Trypanosoma brucei brucei, causing the economically important disease 'nagana' in African cattle. •¾Trypanosoma evansi, causing the disease 'surra' in horses, camels, and elephants. It is transmitted mechanically by biting flies and also by vampire bats. This infection is found in India. •¾Trypanosoma equiperdum, causing 'stallion's disease' in horses and mules. It is transmitted by sexual contact, without the need for an insect vector. •¾Trypanosoma lewisi, causing harmless infection of rats all over the world. The vector is rat flea. A trypanosome resembling Trypanosoma lewisi was reported from Madhya Pradesh in India in peripheral blood of 2 persons with shortterm fever. passed in feces (stercorian transmission), e.g. T. cruzi causing Chagas' disease, which is acquired by rubbing the feces of the vector bug into the wound caused by its bite and T. lewisi, the rat trypanosome, which is transmitted by ingestion of feces of infected rat fleas. ¾¾ Distribution: Human trypanosomiasis is strictly restricted to certain geographical regions; the African and South American trypanosomiasis being seen only in the respective continents. This is due to the vector being confined to these places alone. € African trypanosomiasis (sleeping sickness) € South American trypanosomiasis (Chagas' disease) Trypanosoma Brucei Gambiense (West African Trypanosomiasis) History and Distribution Trypanosomiasis is believed to have been existing in tropical Africa from antiquity (Fig. 5.2). Fig. 5.2: Geographical distribution of trypanosomiasis in Africa. Lines indicate areas endemic for Trypanosoma gambiense and dots represent Trypanosoma rhodesiense ¾¾ Trypanosome was first isolated from the blood of a steamboat captain on the Gambia river in 1901 (hence, the name gambiense) by Forde. ¾¾ Dulton, in 1902, proposed the name Trypanosoma gambiense. ¾¾ It is endemic in scattered foci in West and Central Africa between 15°N and 18°S latitudes. Habitat Trypanosomes live in man and other vertebrate host. They are essentially a parasite of connective tissue, where they multiply rapidly and then invade regional lymph nodes, blood, and finally may involve central nervous system. Morphology Vertebrate Forms In the blood of vertebrate host, T. brucei gambiense exists as trypomastigote form, which is highly pleomorphic. ¾¾ It occurs as a long slender form, a stumpy short broad form with attenuated or absent flagellum, and an intermediate form. ¾¾ The trypomastigotes are about 15–40 µm long and 1.5– 3.5 µm broad. ¾¾ In fresh blood films, trypomastigotes are seen as color less, spindleshaped bodies that move rapidly, spining around the red cells. ¾¾ In smears stained with Giemsa or other Romanowsky's stain, the cytoplasm appears pale blue and the nucleus appears red. The kinetoplast appears as a deep red dot Hemoflagellates and volutin granules stain deep blue. The undulating membrane appears pale blue and the flagellum red. Insect Forms In insects, it occurs in 2 forms: ¾¾ Epimastigotes ¾¾ Metacyclic trypomastigote forms. Antigenic Variation Trypanosomes exhibit unique antigenic variation of their glycoproteins. ¾¾ There is a cyclical fluctuation in the trypanosomes in the blood of infected vertebrates after every 7–10 days. ¾¾ Each successive wave represents a variant antigenic type (VAT) of trypomastigote posssesing variant surface specific antigens (VSSA) or variant surface glycoprotein (VSG) coat antigen. ¾¾ It is estimated that a single trypanosome may have as many as 1,000 or more VSG genes, that help to evade immune response. Besides this, trypanosomes have other mechanisms also that help them to evade host immune responses. Life Cycle T. brucei gambiense passes its life cycle in 2 hosts. Vertebrate host: Man, game animals, and other domestic animals. Invertebrate host: Tsetse fly. Both male and female tsetse fly of Glossina species (G. palpalis) are capable of transmitting the disease to humans. These flies dwell on the banks of shaded streams, wooded savanna, and agricultural areas. Infective form: Metacyclic trypomastigote forms are infec tive to humans. Mode of transmission: ¾¾ By bite of tsetse fly ¾¾ Congenital transmission has also been recorded. Reservoirs: Man is the only reservoir host, although pigs and others domestic animals can act as chronic asymptomatic carriers of the parasite. Development in Man and Other Vertebrate Hosts ¾¾ Metacyclic stage (infective form) of trypomastigotes are inoculated into a man (definitive host) through skin when an infected tsetse fly takes a blood meal (Fig. 5.3). ¾¾ The parasite transforms into slender forms that multiply asexually for 1–2 days before entering the peripheral blood and lymphatic circulation. ¾¾ These become 'stumpy' via intermediate forms and enter the blood stream. 41 ¾¾ In chronic infection, the parasite invades the central nervous system. ¾¾ Trypomastigotes (short plumpy form) are ingested by tsetse fly (male or female) during blood meal. Development in Tsetse Fly ¾¾ In the midgut of the fly, short stumpy trypomastigotes develop into long, slender forms and multiply. ¾¾ After 2–3 weeks, they migrate to the salivary glands, where they develop into epimastigotes, which multiply and fill the cavity of the gland and eventually transform into the infective metacyclic trypomastigotes (Fig. 5.3). ¾¾ Development of the infective stage within the tsetse fly requires 25–50 days (extrinsic incubation period). ¾¾ Thereafter, the fly remains infective throughout its life of about 6 months. Pathogenecity and Clinical Features T. brucei gambiense causes African trypanosomiasis (West African sleeping sickness). The illness is chronic and can persist for many years. ¾¾ There is an initial period of parasitemia, following which parasite is localized predominantly in the lymph nodes. ¾¾ A painless chancre (trypanosomal chancre) appears on skin at the site of bite by tsetse fly, followed by intermittent fever, chills, rash, anemia, weight loss, and headache. ¾¾ Systemic trypanosomiasis without central nervous system involvement is referred to as stage I disease. In this stage, there is hepatosplenomegaly and lymphadenopathy, particularly in the posterior cervical region (Winterbottom's sign). ¾¾ Myocarditis develops frequently in patients with stage I disease and is especially common in T. brucei rhodesiense infections. ¾¾ Hematological manifestations seen in stage I include anemia, moderate leucocytosis, and thrombocytopenia. High levels of immunoglobulins mainly immunoglobulin (Ig)M are a constant feature. ¾¾ Stage II disease involves invasion of central nervous system. With the invasion of central nervous system, which occurs after several months, the 'sleeping sickness' starts. This is marked by increasing headache, mental dullness, apathy, and day time sleepiness. The patient falls into profound coma followed by death from asthenia. Clinical staging of human African trypanosomiasis (HAT) ••Stage I: Characterized by hematogenous and lymphatic dissemination of the disease II: Characterized by centeral nervous system involvement ••Stage 42 Textbook of Medical Parasitology Fig. 5.3: Life cycle of Trypanosoma brucei ¾¾ Histopathology shows chronic meningoencephalitis. The meninges are heavily infiltrated with lymphocytes, plasma cells, and morula cells, which are atypical plasma cells containing mulberry-shaped masses of IgA. Brain vessels show perivascular cuffing. This is followed by infiltration of the brain and spinal cord, neuronal degeneration, and microglial proliferation. ¾¾ Abnormalities in cerebrospinal fluid include raised intracranial pressure, pleocytosis, and raised total protein concentrations. Trypanosoma Brucei Rhodesiense (East African Trypanosomiasis) ¾¾ It is found in Eastern and Central Africa (Uganda, Tanzania, Zambia, and Mozambique) (Fig. 5.2). ¾¾ Stephans and Fanthan discovered T. brucei rhodsiense in 1910 from the blood of a patient in Rhodesia suffering from sleeping sickness. ¾¾ The principal vector is G. morisitans, G. palpalis, and G. Swynnertoni, which live in the open savannah countries. Hemoflagellates ¾¾ Although the disease is usually transmitted by the vector from mantoman, the disease is actually a zoonosis, with the reservoir being wild game animals like bush buck, antelope and domestic animals like cattle. ¾¾ Its morphology, habitat, and life cycle is similar to T. brucei gambiense (Fig. 5.3). ¾¾ The difference between T. brucei gambiense and T. brucei rhodesiense are detailed in Table 5.2. Pathogenesis and Clinical Feature T. brucei rhodesiense causes East African sleeping sickness (Table 5.2). ¾¾ East African trypanosomiasis is more acute than the Gambian form and appears after an incubation period of 4 weeks. ¾¾ It may end fatally within an year of onset, before the involvement of central nervous system develops. ¾¾ Pathological features are similar in both diseases with some variations— € Edema, myocarditis, and weakness are more prominent in East African sickness. € Lymphadenitis is less prominent. € Febrile paroxysms are more frequent and severe. € There is a larger quantity of parasite in the peripheral blood. € Central nervous system involvement occurs early. Mania and delusions may occur but the marked somnolence, which occurs in T. brucei gambiense infection is lacking. Table 5.2: Differences Between West African and East African Trypanosomiasis Characteristics West African East African Organism T. brucei gambiense T. brucei rhodesiense Distribution West and Central Africa East and Central Africa Vector Tsetse fly (Glossina palpalis group) Tsetse fly (Glossina morsilans group) Reservoir Mainly humans Wild and domestic animals Virulence Less More Course of disease Chronic (late central nervous system invasion); months to years Acute (early central nervous system invasion); less than 9 months Parasitemia Low High and appears early Lymphadenopathy Early, prominent Less common Isolastion in rodents No Yes Mortality Low High 43 Parasites causing myocarditis •¾Trypanosoma brucei rhodesiense •¾Trypanosoma cruzi •¾Toxoplasma gondii •¾Echinococcus granulosus •¾Trichinella spiralis Laboratory Diagnosis The diagnosis of both types of African trypanosomiasis is similar (Flowchart 5.1). Nonspecific Findings ¾¾ Anemia and monocytosis. ¾¾ Raised erythrocyte sedimentation rate due to rise in gamma globulin levels. ¾¾ Reversal of albumin:globulin ratio. ¾¾ Increased cerebrospinal fluid (CSF) pressure and raised cell count and proteins in CSF. Specific Findings Definitive diagnosis of sleeping sickness is established by the demonstration of trypanosomes in peripheral blood, bone marrow, lymphnode, CSF, and chancre fluid. Microscopy ¾¾ Wet mount preparation of lymphnode aspirates and chancre fluid are used as a rapid method for demonstration of trypanosomes. These specimen are also examined for parasites after fixing and staining with Giemsa stain. ¾¾ Examination of Giemsastained thick peripheral blood smears reveals the presence of the trypomastigotes (Fig. 5.4). ¾¾ If parasitemia is low, then examination of concentrated blood smear is a highly sensitive method. Different concentration techniques employed are buffy coat examination, differential centrifugation, membrane filtration, and ion exanchange column chromatography. ¾¾ Examination of wet mount and stained smear of the CSF may also show trypanosomes (Flowchart 5.1). Culture The organisms are difficult to grow, hence culture is not routinely used for primary isolation of the parasite. However, it can be cultivated in Weinman's or Tobie's medium. 44 Textbook of Medical Parasitology Molecular Diagnosis Polymerase chain reaction (PCR) assays for detecting African trypanosomes in humans have been developed, but none is commercially available. Imaging Computed tomography (CT) scan of the brain shows cerebral edema and magnetic resonance imaging (MRI) shows white matter enhancement in patients with late stage central nervous systems involvement (Flowchart 5.1). •¾For Fig. 5.4: Trypanosoma rhodesiense, blood smear Giemsa stain, magn X 1100 Animal Inoculation Inoculation of specimens from suspected cases to white rat or white mice is a highly sensitive procedure for detection T. brucei rhodesiense infection. Serodiagnosis Antibody detection Almost all patients with African tryponosomiasis have very high levels of total serum IgM antibodies and later, CSF IgM antibodies. Various serological methods have been developed to detect these antibodies and are as follows: ¾¾ Indirect hemagglutination (IHA) ¾¾ Indirect immunofluroscence (IIF) ¾¾ Enzymelinked immunosorbent assay (ELISA) ¾¾ Card agglutination trypanosomiasis test (CATT) ¾¾ Complement fixation test (CFT) Specific antibodies are detected by these tests in serum within 2–3 weeks of infection. Specific antibodies in CSF are demonstrated by IIF and ELISA. These serological tests are useful for field use and mass screening (Flowchart 5.1). Antigen detection differentiation between the 'human strains' and 'animal strains' of T. brucei, the blood incubation infectivity test (BIIT) had been widely used. •¾The strain is incubated with oxalated human blood and then inoculated into the multimammate rat or other susceptible rodents. •¾The infectivity of 'animal strains' will be neutralized by human blood, while 'human strains' retain infectivity after incubation with human blood. •¾In vitro culture systems are now employed instead of rodents for testing infectivity. •¾More recently their differentiation is based on isoenzymes, DNA, and RNA characteristics (Flowchart 5.1). Treatment ¾¾ In the initial stages, when central nervous system is not involved i.e. stage I, pentamidine is the drug of choice for gambiense HAT (human African trypanosomiasis) and suramin is the drug of choice for rhodesiense HAT. Dose € € Antigens from serum and CSF can be detected by ELISA. Pentamidine: Dose 3–4 mg/kg of body weight, intramuscularly daily for 7–10 days. Suramin: Dose 20 mg/kg of body weight in a course of 5 injections intravenously, at an interval of 5–7 Flowchart 5.1: Laboratory diagnosis of trypanosomiasis Hemoflagellates days. Suramin does not cross blood brain barrier but it is nephrotoxic. ¾¾ In patients with central nervous system involvement, melarsoprol (MelB) is the drug of choice, as it can cross the blood brain barrier. Dose: 2–3 mg/kg/per day (max. 40 mg) for 3–4 days (Table 5.3). Table 5.3: Treatment of Human African Trypanosomiasis Causative organism Clinical stage I (Normal CSF) II (Abnormal CSF) T. brucei gambiense (West African) Pentamidine Eflornithine T. brucei rhodesiense (East African) Suramin Melarsoprol Prophylaxis Control is based on early diagnosis and treatment of cases to reduce the reservoir of infection. ¾¾ Control of tsetse fly population (most important preventive measure) by wide spraying of insecticides, traps, and baits impregnated with insecticides. ¾¾ No vaccine is available. 45 reduviid bugs, amastigote forms are found in the midgut and metacyclic trypomastigote forms are present in hindgut and feces. ¾¾ In Obligate intracellular parasites •¾Trypanosoma cruzi •¾Leishmania spp. •¾Plasmodium spp. •¾Babesia spp. •¾Toxoplasma gondii •¾Microsporidia Morphology Amastigote Amastigotes are oval bodies measuring 2–4 µm in diameter having a nucleus and kinetoplast (Fig. 5.5A). ¾¾ Flagellum is absent. ¾¾ Morphologically, it resembles the amastigote of Leishmania spp., hence it is frequently called as leishmanial form. ¾¾ Multiplication of the parasite occurs in this stage. ¾¾ This form is found in muscles, nerve cells, and reticulo enodothelial systems. Trypanosoma Cruzi Trypomastigote T. cruzi is the causative organism of Chagas's disease or South American trypanosomiasis. Trypomastigotes are nonmultiplying forms found in the peripheral blood of man and other mammalian hosts (Fig. 5.5B). ¾¾ In the blood, they appear either as long, thin flagellates about (20 µm long) or short stumpy form (15 µm long). ¾¾ Posterior end is wedgeshaped. History and Distribution It is a zoonotic disease and is limited to South and Central America. ¾¾ Carlos Chagas, investigating malaria in Brazil in 1909, accidentally found this trypanosome in the intestine of a triatomine bug and then in the blood of a monkey bitten by the infected bugs. ¾¾ Chagas named the parasite T. cruzi after his mentor Oswaldo Cruz and the disease was named as Chagas' disease in his honor. A B Habitat ¾¾ In humans, T. cruzi exists in both amastigote and trypomastigote forms. € Amastigotes are the intracellular parasites. They are found in muscular tissue, nervous tissue, and reticuloendothelial system. € Trypomastigotes are found in the peripheral blood. C Fig. 5.5: Trypanosoma cruzi. A. Amastigote; B. Trypomastigote; C. Epimastigote 46 Textbook of Medical Parasitology ¾¾ In stained blood smears, they are shaped like alphabet 'C', 'U', or 'S', having a free flagellum of about onethird the length of the body. ¾¾ These forms do not multiply in humans and are taken up by the insect vectors. Epimastigote Epimastigote forms are found in the insect vector, the reduviid bug and in culture also (Fig. 5.5C). ¾ It has a kinetoplast adjacent to the nucleus. ¾¾ An undulating membrane runs along the anterior half of the parasite. ¾¾ Epimastigotes divide by binary fission in hindgut of the vector. Life Cycle T. cruzi passes its life cycle in 2 hosts (Fig. 5.6). Definitive host: Man Intermediate host (vector): Reduviid bug or triatomine bugs. Reservoir host: Armadillo, cat, dog, and pigs. Infective form: Metacyclic trypomastigotes forms are the infective forms found in feces of reduviid bugs. ¾ The parasite occurs in 3 different but overlapping infection cycles a sylvatic zoonosis in wild animals like armadillos and opposums, peridomestic cycle in dogs, cats, and other domestic animals, and domestic cycle in humans. Different vector species are active in these infection cycles. Fig. 5.6: Life cycle of Trypanosoma cruzi Hemoflagellates ¾¾ The vectors important in human infection are the reduviid bugs adapted to living in human habitations, mainly Triatoma infestans, Rhodnius prolixus, and Panstrongylus megistus. These are large (upto 3 cm long) night-biting bugs, which typically defecate while feeding. The feces of infected bugs contain the metacyclic trypomastigote. Mode of transmission: €€ Transmission of infection to man and other reservoir hosts takes place when mucus membranes, conjunctiva, or wound on the surface of the skin is contaminated by feces of the bug containing metacyclic trypomastigotes. €€ T. cruzi can also be transmitted by the blood transfusion, organ transplantation, and vertical transmission i.e. from mother to fetus or very rarely by ingestion of contaminated food or drink. Development in Man ¾¾ The metacyclic trypomastigotes introduced in human body by bite of Tsetse fly invade the reticulo endothilial system and spread to other tissues. ¾¾ After passing through promastigote and epimastigote forms, they again become trypomastigotes, which are released into the blood stream and are the infective stage for triatomine bug. No multiplication occurs in this stage. Multiplication takes place only intracellularly in the amastigote form and to some extent as promastigote or epimastigotes (Fig. 5.6). Development in Reduviid Bugs ¾¾ Bugs acquire infection by feeding on an infected mammalian host. ¾¾ Most triatomine bugs are nocturnal. ¾¾ The trypomastigotes are transformed into epimastigotes in the midgut, from where they migrate to the hindgut and multiply. ¾¾ These, in turn, develop into non-dividing metacyclic trypomastigotes (infective form), which are excreted in feces (stercorarian transmission). ¾¾ The development of T. cruzi in the vector takes 8–10 days, which contitutes the extrinsic incubation period. Pathogenecity and Clinical Features The incubation period of T. cruzi in man is 1–2 weeks. The disease manifests in acute and chronic form. Acute Chagas' Disease Acute phase occurs soon after infection and may last for 1–4 months. ¾¾ It is seen often in children under 2 years of age. ¾¾ First sign appears within a week after invasion of parasite. 47 ¾¾ 'Chagoma' is the typical subcutaneous lesion occuring at the site of inoculation. Inoculation of the parasite in conjunctiva causes unilateral, painless edema of perioccular tissues in the eye called as Romana's sign. This is a classical finding of the acute Chagas' disease. ¾¾ In few patients, there may be generalized infection with fever, lymphadenopathy, and hepatosplenomegaly. ¾¾ The patient may die of acute myocarditis and meningoencephalitis. ¾¾ Usually within 4–8 weeks, acute signs and symptoms resolve spontaneously and patients then enter the asymptomatic or indeterminate phase of chronic T. cruzi infection. Chronic Chagas' Disease The chronic form is found in adults and older children and becomes apparent years or even decades after the initial infection. ¾¾ In chronic phase, T. cruzi produces inflammatory response, cellular destruction, and fibrosis of muscles and nerves, that control tone of hollow organs like heart, esophagus, colon, etc. Thus, it can lead to cardiac myopathy and megaesophagus and megacolon (dilalation of esophagus and colon). Congenital Infection Congenital transmission is possible in both acute and chronic phase of the disease causing myocardial and neurological damage in the fetus. Laboratory Diagnosis Diagnosis is done by demonstration of T. cruzi in blood or tissues or by serology. Microscopy ¾¾ The diagnosis of acute Chagas' disease requires detection of parasites. ¾¾ Microscopic examination of fresh anticoagulated blood or the buffy coat is the simplest way to see motile organisms. ¾¾ In wet mount, trypomastigotes are faintly visible but their snake-like motion against RBC's makes their presence apparent. ¾¾ Trypomastigotes can also be seen in thick and thin peripheral blood smear, stained with Giemsa stain (Fig. 5.7). ¾¾ Microhematocrit containing acridine orange as a stain can also be used. ¾¾ When used by experienced personnel, all these methods yield positive results in a high proportion of cases of acute Chagas's disease. 48 Textbook of Medical Parasitology ¾¾ The reduviid bugs are reared in a trypanosomefree laboratory and starved for 2 weeks. They are then fed on patients blood. If trypomastigotes are ingested, they will multiply and develop into epimastigotes and trypomastigotes, which can be found in the feces of the bug 2 weeks later. Histopathology Biopsy examination of lymphnodes and skeletal muscles and aspirate from Chagoma may reveal amastigotes of T. cruzi. Serology Fig. 5.7: Trypanosoma cruzi, blood smear Giemsa stain, magn x 1100 Note: Serologic testing plays no role in diagnosing acute Chagas' disease. Protozoan parasites detected in peripheral blood film •¾Trypanosoma cruzi •¾Trypanosoma brucei rhodesiense •¾Trypanosoma brucei gambiense •¾Leishmania spp. •¾Plasmodium spp. •¾Babesia spp. Culture Novy, Neal, and Nicolle (NNN) medium or its modifications are used for growing T. cruzi. ¾¾ This medium is inoculated with blood and other specimens and incubated at 22°–24°C. ¾¾ The fluid from the culture is examined microscopically by 4th day and then every week for 6 weeks. ¾¾ Epimastigotes and trypomastigotes are found in the culture. ¾¾ Culture is more sensitive than smear microscopy. Animal Inoculation Guinea pig or mice inoculation may be done with blood, CSF, lymph node aspirate, or any other tissue material and the trypomastigote is looked for in its blood smears in a few days after successful inoculation. Xenodiagnosis This is the method of choice in suspected Chagas' disease, if other examinations are negative, especially during the early phase of the disease onset. Antigen detection T. cruzi antigen can be detected in urine and sera in patients with chronic Chagas' disease. ELISA has been developed for detection of antigens. Antibody detection Antibodies (IgG) against T. cruzi may be detected by the following tests – ¾¾ IHA ¾¾ CFT (MachadoGuerreiro test) ¾¾ ELISA ¾¾ IIF ¾ Direct agglutination test (DAT). It is a simple test being recommended for field use. ¾¾ Chagas' RadioImmune Precipitation Assay (RIPA) is a highly sensitive and specific confirmatory method for detecting antibodies of T. cruzi. The disadvantage of the antibody based tests is that they may be false positive with other disease like leishmaniasis and syphilis. Intradermal Test The antigen 'cruzin' is prepared from T. cruzi culture and used for the intradermal test. A delayed hypersensitivity reaction is seen. Molecular Diagnosis PCR is available that detects specific primers, which have been developed against T. cruzi kinetoplastic or nuclear DNA. The disadvantage of the test is that it is not commercially available. Other Tests ¾¾ Electrocardiography (ECG) and chest Xray are useful for diagnosis and prognosis of cardiomyopathy seen Hemoflagellates in chronic Chagas' disease. The combination of right bundle branch block (RBBB) and left anterior fascicular block is a typical feature of Chagas' heart disease. ¾¾ Endoscopy helps in visualization of megaesophagus in Chagas' disease. Treatment No effective specific treatment is available for treating Chagas' disease. Nifutrimox and benznidazole have been used with some success in both acute and chronic Chagas disease. These drugs kill only the extracellular trypanosomes but not the intracellular forms. Dose: Nifutrimox: 8–10 mg/kg for adults and 15 mg/kg for children. The drug should be given orally in 4 divided doses each day for 90–120 days. Benznidazole: 5–10 mg/day orally for 60 days. Prophylaxis ¾¾ Application of insecticide to

control the vector bug. ¾¾ Personal protection using insect repellant and mosquito net. ¾¾ Improvement in rural housing and environment to eliminate breeding places of bugs. Trypanosoma Rangeli T. rangeli was first described by Tejera in 1920 while examining the intestinal content of reduviid bug (R. prolixus). ¾¾ It is nonpathogenic. ¾¾ T. rangeli infections are encountered in most areas where T. cruzi infection also occurs (Mexico, Central America, and northern South America). ¾¾ Morphologically, it is similar to T. cruzi, except that it is slender and long (26–36 µm long) and has a smaller kinetoplast (Table 5.4). ¾¾ It is commonly found in dogs, cats, and humans. ¾¾ Infection is transmitted by both bite of triatomine bug and fecal contamination from reduviid bug. Table 5.4: Differences Between T. cruzi and T. rangeli Trypanosoma cruzi Trypanosoma rangeli Pathogenic Nonpathogenic 15–20 µm long 30 µm long, more slender and longer C or Ushaped Not C or Ushaped Kinetoplast: Large and terminal Kinteoplast: Small and subterminal Primary reservoirs: Opposums, dog, cats, and wild rodents Primary reservoir: Wild rodents 49 ¾¾ T. rangeli multiplies in human blood by binary fission. Intracellular stage is typically absent. ¾¾ T. rangeli can circulate in blood of infected animals for a long period, unlike T. cruzi. ¾¾ Although T. rangeli appears to be a normal commensal, they do reduce the life span of reduviid bug. ¾¾ Diagnostic methods are similar to that of T. cruzi. Key points of Trypanosomes •¾Trypanosomes follow one of the two developmental modes in vectors. In Salivaria: The trypanosomes migrate to mouth parts of vector tsetse fly, e.g. T. gambiense, T. rhodesiense. In Stercoraria: The trypanosomes migrate to hindgut of vector bug, e.g. T. cruzi. •¾T. brucei gambiense causes West African sleeping sickness manifested by fever, hepatosplenomegaly, and posterior cervical lymphadenopathy with chronic central nervous system invasion. •¾T. brucei rhodesiense causes East African sleeping sickness manifested by fever, early and acute central nervous system invasion, with loss of weight and myocarditis. •¾Diagnosis: By detection of trypanosomes in wet mount preparations of lymph node aspirates or blood or by serology and PCR. •¾Drug of choice: For stage I HAT by T. brucei gambiense is pentamidine and by T. brucei rhodesiense is suramin. In stage II, the drug of choice is melarsopro in both cases. •¾South American trypanosomiasis (Chagas' disease) is caused by T. cruzi. •¾It is transmitted by wound or conjunctival conta mination of feces of the reduviid bugs. •¾Clinical features: 'Chagoma' is the typical subcutaneous lesion commonly on face (Romana's sign) in Chagas' disease. Damage to nerve cells and muscles leads to megaesophagus, megacolon, and cardiac myopathy. •¾Diagnosis: By demonstration of T. cruzi in blood or tissue or by serology and xenodiagnosis. •¾Treatment: Nifutrimox and benznidazole. Leishmania General Characteristics The genus Leishmania is named after Sir William Leishman, who discovered the flagellate protozoa causing Kala-azar, the Indian visceral leishmaniasis. ¾¾ All members of the genus Leishmania are obligate intracellular parasites that pass their life cycle in 2 50 Textbook of Medical Parasitology hosts—the mammalian host and the insect vector, female sandfly. ¾¾ In humans and other mammalian hosts, they multiply within macrophages, in which they occur exclusively in the amastigote form, having an ovoid body containing a nucleus and kinetoplast. ¾¾ In the sandfly, they occur in the promastigote form, with a spindleshaped body and a single flagellum arising from anterior end. ¾¾ Leishmaniasis has an immense geographical distribution in the tropics and subtropics of the world, extending through most of the Central and South America, part of North America, central and Southeast Asia, India, China, the Mediterranean region, and Africa. ¾¾ The disease affects the low socioeconomic group of people. Overcrowding, poor ventilation, and collection of organic material inside house facilitate its transmission. ¾¾ Across the tropics, 3 different diseases are caused by various species of genus Leishmania. These are: € € € Visceral leishmaniasis: The species L. donovani complex infecting internal organs (liver, spleen, and bone marrow) of human is the causative parasite. Cutaneous leishmaniasis: The species L. tropica complex, L. aethiopica, L. major and L. mexicana complex are the causative parasite. Mucocutaneous leishmaniasis : It is caused by the L. braziliensis complex . Classification The genus Leishmania includes a number of different varieties and subspecies, which differ in several features such as antigenic structure, isoenzymes, and other bio chemical characteristics, growth properties, host specificity, etc. (Table 5.5). Leishmania species can also be classified on the basis of geographical distribution as given in Table 5.6. The various manifestations of leishmaniasis and Leishmania species causing them have been summarized in Flowchart 5.2. Table 5.5: Leishmania species Involved in Human Disease Species Disease Geographical distribution Leishmania donovani Visceral leishmaniasis Middle East, (Kalaazar or Africa, and Indian dumdum fever) Subcontinent Leishmania infantum Visceral leishmaniasis, cutaneous leishmaniasis Leishmania chagasi Vector Reservoir Transmission Phlebotomus argentipes, Phlebotomus orientalis Humans Anthroponotic, occasionally zoonotic Mediterranean Coast, Phlebotomus Dog, fox, jackal, and Middle East, and perniciousus, wolf Phlebotomus ariasi, China. Phlebotomus papatasi Zoonotic Visceral leishmaniasis Tropical South America Lutzomyra longipalpis Fox and wild canines Zoonotic Leishmania tropica Cutaneous Leishmaniasis (oriental sore, Baghdad boil) Middle East and Central Asia Phlebotomus sergenti Humans Anthroponotic Leishmania major Cutaneous leishmaniasis Africa, Indian Subcontinent, and Central Asia Phlebotomus papatasi, Phlebotomus duboscqi Gerbil Zoonotic Leishmania aethiopica Cutaneous and diffuse cutaneous leishmaniasis Ethiopia and Kenya Phlebotomus longipes Phlebotomus pedifer Hydraxes Zoonotic Leishmania braziliensis complex Mucocutaneous leishmaniasis (Espundia) Tropical South America Lutzomyra umbratilis Forest rodents and peridomestic animals Zoonotic Leishmania mexicana complex Mucocutaneous leishmaniasis (Chiclero's ulcer) Central America and Amazon basin Lutzomyra olmeca, Lutzomyra flairscutellata Forest rodents and marsupials Zoonotic Hemoflagellates 51 Table 5.6: Classification of Leishmania based on Geographical Distribution Old world leishmaniasis New world leishmaniasis Leishmania donovani Leishmania braziliensis complex Leishmania infantum Leishmania mexicana complex Leishmania tropica Leishmania chagasi Leishmania major Leishmania peruviana Leishmania aethiopica Note: The vector for old world leishmaniasis is sandfly of the genus Phlebotomus and for new world leishmaniasis is sandfly of the genera Lutzomyra and Psychodopygus. Old World Leishmaniasis Leishmania Donovani L. donovani causes visceral leishmaniasis or Kala-azar. It also causes the condition, Post Kala-azar Dermal Leishmaniasis (PKDL). History and Distribution Sir William Leishman in 1900, observed the parasite in spleen smears of a soldier who died of 'Dumdum fever' or Kalaazar contracted at Dum Dum, Calcutta. Leishman reported this finding from London in 1903. In the same year, Donovan also reported the same parasite in spleen smears of patients from Madras. The name Leishmania donovani was, therefore given to this parasite. The amastigote forms of the parasite as seen in smears from patients are called Leishman Donovan (LD) bodies. ¾¾ Visceral leishmaniasis or Kalaazar is a major public health problem in many parts of world. According to the World Health Organization (WHO), a total of 5,00,000 cases of visceral leishmaniasis occur every year. Of these new cases, 90% are found in the Indian subcontinent and Sudan and Brazil. Fig. 5.8: Geographical distribution of visceral leishmaniasis. Endemic areas shaded; dots indicate sporadic cases ¾¾ The disease occurs in endemic, epidemic, or sporadic forms. Major epidemics of the disease are currently found in India, Brazil, and Sudan (Fig. 5.8). ¾¾ The resurgence of Kalaazar in India, beginning in the mid1970s, assumed epidemic proportions in 1977 and involved over 1,10,000 cases in humans. Initially, the disease was confined to Bihar (Muzaffarpur, Samastipur, Vaishali, and Sitamarhi). Since then, the cases are increasing and involving newer areas. The epidemic extended to West Bengal and first outbreak occurred in 1980 in Malda district. ¾¾ At present, the disease has established its endemicity in 31 districts in Bihar, 11 districts in West Bengal, 5 districts in Jharkhand, and 3 districts in Uttar Pardesh. Sporadic cases have been reported from Tamil Nadu, Maharashtra, Karnataka, and Andhra Pradesh. Habitat The amastigote (LD body) of L. donovani is found in the reticuloendothelial system. They are found mostly within the macrophages in the spleen, liver, bone marrow and less often in other locations such as skin, intestinal mucosa, and mesenteric lymph nodes. Flowchart 5.2: Distribution and disease caused by Leishmania spp. 52 Textbook of Medical Parasitology Morphology ¾¾ A single nucleus is situated at the center. The kinetoplast The parasite exists in 2 forms (Fig. 5.9). ¾ Amastigote form: in humans and other mammals. ¾ Promastigote form: in the sandfly and in artificial culture. Amastigote The amastigote form (LD body) is an ovoid or rounded cell, about 2–4 µm in size (Fig. 5.9A). ¾¾ It is typically intracellular, being found inside macro phages, monocytes, neutrophils, or endothelial cells. ¾¾ They are also known as LD bodies. ¾¾ Smears stained with Leishman, Giemsa, or Wright's stain show a pale blue cytoplasm enclosed by a limiting membrane. ¾¾ The large oval nucleus is stained red. Lying at the right angles to nucleus, is the red or purplestained kinetoplast. ¾¾ In wellstained preparations, the kineloplast can be seen consisting of a parabasal body and a dotlike blepharoplast with a delicate thread connecting the two. The axoneme arising from the blepharoplast extends to the anterior tip of the cell. ¾¾ Alongside the kinetoplast a clear unstained vacuole can be seen. ¾¾ Flagellum is absent. Promastigote It is a flagellar stage and is present in insect vector, sandfly and in cultures. ¾¾ The promastigotes, which are intitially short, oval or pearshaped forms, subsequently become long spindle shaped cells, 15–25 µm in length and 1.5–3.5 µm in breadth (Fig. 5.9B). A B Fig. 5.9: Morphology of Leishmania donovani. A. Amastigote (LD body); B. Promastigote lies transversely near the anterior end. ¾¾ The flagellum is single, delicate, and measures 15–28 µm. ¾¾ Giemsa or Leishmanstained films show pale blue cytoplasm with a pink nucleus and bright red kinetoplast. ¾¾ A vacuole is present near the root of the flagellum. ¾¾ There is no undulating membrane. ¾¾ Promastigote forms, which develop in artificial cultures, have the same morphology as in the sandfly. Life Cycle L. donovani completes its life cycle in 2 hosts (Fig. 5.10). Definitive host: Man, dog, and other mammals. Vector: Female sandfly (Phlebotomus species). Infective form: Promastigote form present in midgut of female sandfly. Vector species responsible for transmission of Leishmania donovani Country India China, Bangladesh Sudan and Africa Mediterrean countries Middle East and Russia Centra Asia South America   Phlebotomus species P. argentipes P. chineses P. sergenti P. pernicious P. orientalis (Sudan) P. longicupis P. sergenti P. pernicious P. papatasii P. major P. tobbi P. perfulievi P. papatasii P. papatasii P. longipalpis P. intermudias P. lutzi Mode of transmission: € Humans acquire by bite of an infected female sandfly. € It can also be transmitted vertically from mother to fetus, by blood transfusion, and accidental inoculation in the laboratory. Incubation period: Usually 2–6 months, occasionally it may be as short as 10 days or as long as 2 years. ¾¾ The sandfly regurgitates the promastigotes in the wound caused by its proboscis. ¾¾ These are engulfed by the cells of reticuloendothelial system (macrophages, monocytes, and polymorpho Hemoflagellates 53 Fig. 5.10: Life cycle of Leishmania donovani nuclear leucocytes) and change into amastigote (LD body) within the cells . ¾¾ The amastigote multiplies by binary fission, producing numerous daughter cells that distend the macrophage and rupture it. The liberated daughter cells are in turn, phagocytosed by other macrophages and histiocytes. Small number of LD bodies can be found in peripheral blood inside neutrophils or monocytes (Fig. 5.10). ¾¾ When a vector sandfly feeds on an infected person, the amastigotes present in peripheral blood and tissue fluids enter the insect along with its blood meal. In the midgut (stomach) of the sandfly, the amastigote elongates and develops into the promastigote form (Fig. 5.10). ¾¾ The promastigote multiples by longitudinal binary fission and reaches enormous numbers. They may be seen as large rosettes with their flagella entangled. ¾¾ In the sandfly, they migrate from the midgut to the pharynx and hypostome, where they accumulate and block the passage. ¾¾ Such blocked sandflies have difficulty in sucking blood. When they bite a person and attempt to suck blood, plugs of adherent parasites may get dislodged from 54 Textbook of Medical Parasitology the pharynx and they are deposited in the punctured wound. It takes about 10 days for the promastigotes to reach adequate numbers after ingestion of the amastigotes, so as to block the buccal cavity and pharynx of the sandfly. This is, therefore, the duration of extrinsic incubation period. This period is also synchronous with the gonadotropic cycle of the vector, so that amastigotes ingested during a single blood meal, are ready to be transmitted when the sandfly takes the next blood meal after its eggs have been laid. Pathogenicity L. donovani causes visceral leishmaniasis or kala-azar. ¾¾ Kala-azar is a reticuloendotheliosis resulting from the invasion of reticuloendothelial system by L. donovani. ¾¾ The parasitized macrophages disseminate the infection to all parts of the body. ¾¾ In the spleen, liver, and bone marrow particularly, the amastigotes multiply enormously in the fixed macrophages to produce a 'blockade' of the reticuloendothelial system. This leads to a marked proliferation and destruction of reticuloendothelial tissue in these organs. ¾¾ Spleen: €€ The spleen is the most affected organ. It is grossly enlarged and the capsule is thickened due to perisplenitis. €€ Spleen is soft and friable and cuts easily due to absence of fibrosis. €€ The cut section is red or chocolate in color due to the dilated and engorged vascular spaces. €€ The trabeculae are thin and atrophic. €€ Microscopically, the reticulum cells are greatly increased in numbers and are loaded with LD bodies. €€ Lymphocytic infiltration is scanty, but plasma cells are numerous. ¾¾ Liver: €€ The liver is enlarged. €€ The Küpffer cells and vascular endothelial cells are heavily parasitized, but hepatocytes are not affected. €€ Liver function is, therefore, not seriously affected, although prothrombin production is commonly decreased. €€ The sinusoidal capillaries are dilated and engorged. €€ Some degree of fatty degeneration is seen. The cut surface may show a 'nutmeg' appearance. ¾¾ Bone marrow: €€ The bone marrow is heavily infiltrated with parasitized macrophages, which may crowd the hematopoietic tissues. ¾¾ Peripheral lymphnodes and lymphoid tissues of the nasopharynx and intestine are hypertrophic, although this is not seen in Indian cases. ¾¾ Severe anemia with hemoglobin levels of 5–10 g/dL may occur in Kala-azar, as a result of infiltration of the bone marrow as well as by the increased destruction of erythrocytes due to hypersplenism. Autoantibodies to red cells may contribute to hemolysis. Ecological types The epidemiology and clinical features of visceral leish maniasis and the ecology of the parasite are very different in different geographical areas. The different clinical syndromes have, therefore been considered to be distinct entities and the parasite causing them have been given separate species or sub-species status, as listed below. ••Indian visceral leishmaniasis: Caused by L. donovani producing the anthroponotic disease Kala-azar and its sequel post Kala-azar dermal leishmaniasis (PKDL). The disease is not zoonotic; human beings being the only host and reservoir. Vector is the sandfly, P. argentipes. ••Mediterranean leishmaniasis: Middle eastern leishmaniasis caused by L. donovani infantum affecting mostly young children. It is a zoonotic disease; the reservoir being dog and wild canines such as foxes, jackals, and wolves. Vectors are P. pernicious and P. papatasii. ••East African leishmaniasis: Caused by L. archibaldi. The disease is zoonotic, found mainly in rural areas. ••South American leishmaniasis: Caused by L. donavani chagasis (L. chagasi). The disease is zoonotic. Foxes and wild canines are reservoirs. Dogs act as link between the reservoir hosts and humans. The main vector is the sandfly, Lutzomyra longipalpis. ••China: The disease resembles the mediterranean type (L. infantum) in the north-west and Indian type L. donovani in the East. ••American (New World) visceral leishmaniasis: Caused by L. chagasi. It is present is most parts of Latin America and resembles the disease caused by L. infantum. The main vector is L. longipalpis. ¾¾ Leucopenia with marked neutropenia and thrombo cytopenia are frequently seen. Antibodies against WBCs and platelets suggest an autoimmune basis for the pancytopenia observed in Kala-azar. Causes of anemia in Kala-azar ••Splenic sequestration of RBCs ••Decreased erythropoeisis due to replacement of bone marrow with parasitized macrophages ••Autoimmune hemolysis ••Hemorrhage Hemoflagellates 55 Table 5.7: Differences Between PKDL of India and East Africa Fig. 5.11: Kalaazar Spleen showing a greatly enlarged organ Characteristics India East Africa Incidence 5% 50% Time interval between visceral leishmaniasis and PKDL Occurs after visceral leishmaniasis. May take 3–5 years Occurs during visceral leishmaniasis Age group affected Any age Mostly children Appearance of rash Rashes appear after visceral leishmaniasis Rashes may appear during visceral leishmaniasis Spontaneous cure Not seen Seen Duration of treatment with sodium stibogluconate 60–120 days 60 days Clinical Features of Kala-Azar ¾¾ The onset is typically insidious. The clinical illness begins with fever, which may be continuous, remittent, or irregular. ¾¾ Splenomegaly starts early and is progressive and massive (Fig. 5.11). ¾¾ Hepatomegaly and lymphadenopathy also occur but are not so prominent. ¾¾ Skin becomes dry, rough, and darkly pigmented (hence, the name Kala-azar). ¾¾ The hair become thin and brittle. ¾¾ Cachexia with marked anemia, emaciation, and loss of weight is seen. ¾¾ Epistaxis and bleeding from gums are common. ¾¾ Most untreated patients die in about 2 years, due to some intercurrent disease such as dysentery, diarrhea, and tuberculosis. Post Kala-azar Dermal Leishmaniasis About 3–10% cases of patients of visceral leishmaniasis in endemic areas develop PKDL, about an year or 2 after recovery from the systemic illness. ¾¾ PKDL is seen mainly in India and East Africa and not seen elsewhere. The Indian and African diseases differ in several aspects; important features of PKDL in these two regions are listed in Table 5.7. ¾¾ PKDL is a nonulcerative lesion of skin. The lesions are of 3 types. € Depigmented macules: These commonly appear on the trunk and extremities and resemble tuberculoid leprosy. Erythematous patches: These are distributed on the face in a 'butterfly distribution'. € Nodular lesion: Both of the above mentioned lesions may develop into painless yellowish pink nonulcerating granulomatous nodules. ¾¾ The parasite can be demonstrated in the lesions. € Immunity ¾¾ The most important immunological feature in Kalaazar is the marked suppression of cellmediated immunity to leishmanial antigens. This makes unrestricted intracellular multiplication of the parasite possible. Cellular responses to tuberculin and other antigens are also suppressed and may be regained some 6 weeks after recovery from the disease. ¾¾ In contrast, there is an overproduction of immuno globulins, both specific antileishmanial antibodies as well as nonspecific polyclonal IgG and IgM. Circulating immune complexes are demonstrable in serum. Laboratory Diagnosis Laboratory diagnosis of Kalaazar depends upon direct and indirect evidences (Flowchart 5.3). Direct Evidence Microscopy ¾¾ Demonstration of amastigotes in smears of tissue aspirates is the gold standard for diagnosis of visceral leishmaniasis. 56 Textbook of Medical Parasitology Flowchart 5.3: Laboratory diagnosis of Kala-azar ¾¾ For microscopic demonstration of the parasite, the materials collected are: €€ Peripheral blood €€ Bone marrow €€ Splenic aspirate €€ Enlarged lymph node. ¾¾ The smears are stained by Leishman, Giemsa, or Wright's stains and examined under oil immersion objective. ¾¾ Amastigote parasite can be seen within the macrophages, often in large numbers. A few extracellular forms can also be seen. ¾¾ Peripheral blood smear: €€ Peripheral blood contains the amastigotes present inside circulating monocytes and less often in neutrophils, but the numbers are so scanty that a direct blood smear may not show them. €€ Chances of detecting them are somewhat improved by examination of a thick blood film. €€ It is best to examine buffy coat smear, although even these are not often found positive. €€ Buffy coat smears show a diurnal periodicity, more smears being positive when collected during the day than at night. ¾¾ Bone marrow aspirate: €€ Bone marrow aspirate is the most common diagnostic specimen collected. €€ Generally, the sternal marrow is aspirated by puncturing the sternum at the level of the 2nd or 3rd intercostal space, using a sternal puncture needle. €€ This consists of a short stout needle with a stylet. It has a movable guard, which is fixed at 1–2 cm from the tip, depending on the thickness of the chest wall over the sternum. €€ After disinfecting and anesthetizing the skin, the needle is introduced into the sternal marrow and about 0.5 mL of marrow fluid is aspirated using a syringe. The puncture wound is sealed with celloidin or tincture benzoin. Bone marrow samples can also be obtained by puncturing the iliac crest. ¾¾ Splenic aspirates: €€ Splenic aspirates are richer in parasites and therefore, are more valuable for diagnosis. €€ But, the procedure can sometimes cause dangerous bleeding and therefore, should be done carefully and only when a marrow examination is inconclusive. ¾¾ Lymphnode aspirates: €€ Lymphnode apsirates are not useful in the diagnosis of Indian Kala-azar, although it is employed in visceral leishmaniasis in some other countries. ¾¾ Comparison of aspiration biopsies: Although splenic aspiration is the most sensitive method (98% positive), bone marrow puncture (50–85%, positive) is a safer procedure when compared to spleen puncture, as there is risk of hemorrhage in splenic puncture particularly in patients with advanced stage of disease with soft enlarged spleen. Splenic aspiration is contraindicated in patients with prolonged prothrombin time or if platelet count is less than 40,000/mm3. Liver biopsy is also not a safe procedure and carries a risk of hemorrhage. Lymph node aspiration is positive in 65% of cases of African Kala-azar, but not useful in cases of Indian Kala-azar. Hemoflagellates A 57 B Fig. 5.12: Leishmania donovani. A. Culture form (Giemsa stain, magn. X 1100); B. Liver smear (Giemsa stain, magn. X 1100) Culture Different tissue materials or blood are cultured on NNN medium. This is a rabbit blood agar slope consisting of 2 parts of salt agar and 1 part of defibrinated rabbit blood. The material is inoculated into the water of condensation and culture is incubated at 22°–24°C for 1–4 weeks. At the end of each week, a drop of culture fluid is examined for promastigotes under high power objective or phase contrast illumination (Fig. 5.12). Other biphasic medium, like Schneider's drosophila tissue culture medium with added fetal calf serum can also be used. Animal inoculation Animal inoculation is not used for routine diagnosis. ¾¾ When necessary, Chinese golden hamster is the animal employed. ¾¾ The material is inoculated intraperitoneally or intradermally into the skin of nose and feet ¾¾ The inoculated animals are kept at 23°–26°C. ¾¾ In positive cases, the amastigote can be demonstrated in smears taken from ulcers or nodules developing at the sites of inoculation or from the spleen (Fig. 5.13). ¾¾ Animal inoculation is a very sensitive method, but takes several weeks to become positive. ¾¾ Detection of antibodies: CFT was the first serological test used to detect serum antibodies in visceral leishmaniasis. The antigen originally used, was prepared from human tubercle bacillus by Witebsky, Kliengenstein, and Kühn (hence, called WKK antigen). CFT using WKK antigen becomes positive early in the disease, within weeks of infection. Positive reaction also occurs in other conditions, including tuberculosis, leprosy, and tropical eosinophilia. € Specific leishmanial antigens prepared from cultures have been used in a number of tests to demonstrate specific antibodies. These tests include: ˆ Indirect immunofluroscent antibody test (IFAT) ˆ Counter immunoelectrophoresis (CIEP) ˆ ELISA and DOTELISA ˆ DAT Indirect Evidences Serodiagnosis ¾¾ Detection of antigen: The concentration of antigen in the serum or other body fluids is very low. ELISA and PCR have been developed for detection of leishmanial antigen. Fig. 5.13: LD body in spleen smear of experimentally infected animal (Giemsa stain) 58 Textbook of Medical Parasitology ˆˆ A specific rapid immunchromatographic dipstick (ICT) method for antibody has been developed using a recombinant leishmanial antigen rk 39 consisting of 39 amino acids conserved in kinesin region of L. infantum. The sensitivity of the test is 98% and specificity is 90%. Skin test ¾¾ Leishmanin skin test (Montenegro test): €€ €€ €€ Note: The direct agglutination test for antileishmanial antibody has been found to be highly specific and sensitive for diagnosis of Kala-azar. However, rk 39 antibody test is more useful and sensitive and recommended by National Vector-borne Disease Control Programme (NVDCP) in India. €€ Molecular diagnosis €€ A number of molecular diagnosis methods have been developed, which help in species identification of Leish mania. The methods include Western blot and PCR. The use of PCR is confined to specialized laboratories and is yet to be used for routine diagnosis of visceral leishmaniasis in endemic areas. Non-specific serum tests These tests are based on the greatly increased globulin content of serum in the disease. ¾¾ The 2 tests widely used are €€ Napier's aldehyde or formogel test €€ Chopra's antimony test. ¾¾ Napier's aldehyde test: 1 mL of clear serum from the patient is taken in a small test tube, a drop of formalin (40% formaldehyde) is added, shaken, and kept in a rack at room temperature. €€ A control tube with normal serum is also set up. €€ A positive reaction is jellification and opacification of the test serum, resembling the coagulated white of egg appearing within 3–30 minutes. €€ About 85% of patients with disease of 4 months or more give positive reaction. €€ Aldehyde test is always negative in cutaneous leishmaniasis. €€ The test merely indicates greatly increased serum gamma globulin and thus, is non-specific. ¾¾ Chopra's antimony test: It is done by taking 0.2 mL of serum diluted 1:10 with distilled water in a Dreyer's tube and overlaying with few drops of 4% solution of urea stibamine. Formation of floculant precipitate indicates positive test. €€ The reaction is said to be more sensitive than the aldehyde test. ¾¾ Both the tests give false positive reactions in several other disease such as multiple myeloma, cirrhosis of liver, tuberculosis, leprosy, schistosomiasis, African trypanosomiasis, etc., where hypergammaglobulinemia exists. €€ It is delayed hypersensitivity test. This was first discovered by Montenegro in South America and hence, named after him. 0.1 mL of killed promastigote suspension (106 washed promastigotes/mL) is injected intradermally on the dorsoventral aspect of forearm. Positive result is indicated by an induration and erythema of 5 mm or more after 48–72 hours. Positive result indicates prior exposure to leishmanial parasite. In active Kala-azar, this test is negative and becomes positive usually 6–8 weeks after cure from the disease. Blood picture blood count shows normocytic normo chromic anemia and thrombocytopenia. ¾¾ Leucocyte count reveals leucopenia accompanied by a relative increase of lymphocytes and monocytes. Eosinophil granulocytes are absent. During the course of disease, there is a progressive diminution of leucocyte count falling to 1,000/mm3 of blood or even below that. ¾¾ The ratio of leucocyte to erythrocyte is greatly altered and may be about 1:200 to 1:100 (normal 1:750). ¾¾ Serum shows hypergammaglobulinemia and a reversal of the albumin: globulin ratio. ¾¾ Liver function tests show mild elevations. ¾¾ Complete Diagnosis of PKDL ¾¾ The nodular lesions are biopsied and amastigote forms are demonstrated in stained sections. ¾¾ The biopsy material can be cultured or animal inoculation can be done. ¾¾ Immunodiagnosis has no role in the diagnosis of PKDL. Treatment Kala-azar responds to treatment better than other forms of visceral leishmaniasis. The standard treatment consists pentavalent antimonial compound, which is the drug of choice in most of the endemic regions of the world, but there is resistance to antimony in Bihar in India, where amphotericin-B-deoxycholate or miltefosine is preferred. Pentavalent Antimonial Compound Two pentavalent antimonial (Sbv) preparations are available: ¾¾ Sodium stibogluconate (100 mg of Sbv/mL) ¾¾ Meglumine antimonate (85 mg of Sbv/mL). Dosage: The daily dose is 20 mg/kg by rapid intravenous (IV) infusion or intramuscular (IM) injection for 20–30 days. Hemoflagellates Cure rates exceed 90% in most of the old world, except in Bihar (India) due to resistance. Amphotericin B ¾¾ Amphotericin B is currently used as a first-line drug in Bihar. In other parts of the world, it is used when initial antimonial treatment fails. ¾¾ Dosage: 0.75–1.0 mg/kg on alternate days for a total of 15 infusions. 59 Leishmania aethiopica these species cause old world cutaneous leishmaniasis. The disease is also known as oriental sore, Delhi boil, Bagdad boil, or Aleppo button. €€ ¾¾ All History and Distribution ¾¾ Liposomal amphotericin B: It has been developed and Cunnigham (1885) first observed the parasite in the tissues of a Delhi boil in Calcutta. ¾¾ Russian military surgeon, Borovsky (1891) gave an accurate description of its morphology and Luhe (1906) gave the name L. tropica. ¾¾ L. tropica and L. major are found in Middle-East, India, Afganistan, eastern Mediterranean countries, and North Africa. ¾¾ L. aethiopica occurs in Ethiopia and Kenya. ¾¾ In India, cutaneous leishmaniasis is restricted to the dry western half of the Indo-gangetic plains including dry areas bordering Pakistan, extending from Amritsar to Kutch and Gujrat plains. To the East, the cases have been reported from Delhi and Varanasi in Uttar Pradesh. Paromomycin Habitat Paromomycin is an intramuscular aminoglycoside antibiotic with antileishmanial activity. Dosage: It is given in a dose of 11 mg/kg daily for 21 days. L. tropica causing cutanaeous leishmaniasis (old world cutaneous leishmaniasis) are essentially the parasite of skin. The amastigote forms occur in the reticuloendothelial cells of the skin, whereas promastigote forms are seen in sandfly vector. Note: Fever with chills is almost seen in all patients, using amphotericin-B infusions. used extensively to treat visceral leishmaniasis in all parts of the world. It is the only drug approved by the US Food and Drug Administration (FDA) for the treatment of visceral leishmaniasis; dose being 3 mg/kg daily. By using liposomal amphotericin-B, higher doses can be given, improving the cure, without toxicity. Miltefosine Miltefosine is the first oral drug, approved for the treatment of leishmaniasis. Dosage: 50 mg daily for 28 days for patients weighing less than 25 kg, and twice daily for patients weighing more than 25 kg. Treatment of PKDL is same as that for visceral leishmaniasis. Prophylaxis ¾¾ Early detection and treatment of all cases. ¾¾ Integrated insecticidal spraying to reduce sandfly population. ¾¾ Destruction of animal reservoir host in cases of zoontic Kala-azar. ¾¾ Personal prophylaxis by using anti-sandfly measures like, using thick clothes, bed nets, window mesh, or insect repellants and keeping the environment clean. ¾¾ No vaccine is available against Kala-azar. Leishmania Tropica Complex It includes 3 species: €€ Leishmania tropica €€ Leishmania major Morphology Morphology of L. tropica complex is indistinguishable from that of L. donovani. Life Cycle The life cycle of L. tropica is similar to that of L. donovani except Vectors: The vectors of L. tropica complex are Phlebotomus sandflies. The following species of sandflies act as vector. €€ P. sergenti €€ P. pappatasi €€ P. causasiasus €€ P. intermedius Mode of transmission: €€ The most common mode of infection is through bite of sandflies. €€ Infection may also sometimes occur by direct contact. € € Infection may be transmitted from man-to-man or animal-to-man by direct inoculation of amastigotes €€ Infection may also occur by autoinoculation. ¾¾ The amastigotes are present in the skin, within large mononuclear cells, neutrophils, inside capillary endothelial cells, and also free in the tissues. 60 Textbook of Medical Parasitology ¾¾ They are ingested by sandflies feeding near the skin lesions. ¾¾ In the midgut of the sandfly, the amastigotes develop into promastigotes, which replicate profusely. ¾¾ These are in turn transmitted to the skin of persons bitten by sandflies in the skin, the promastigotes are phagocytosed by mononuclear cells, in which they become amastigotes and multiply. ¾¾ However, they remain confined to the skin, without being transported to the internal organs, as is the case in visceral leishmaniasis. Incubation period: Incubation period varies from 2–8 months. Pathology Amastigote forms are found in histiocytes and endothelial cells. There is an inflammatory granulomatous reaction with infiltration of lymphocyte and plasma cells. Early lesions are papular, followed by ulceration necrosis. Papule and ulcer are the main pathological lesions. They heal over months to years, leaving scars. Clinical Features L. tropica causes Old World Cutaneous leishmaniasis. ¾¾ Features of the disease vary with epidemiological pattern from region-to-region. ¾¾ Three distinct patterns of old world cutaneous leishmaniasis have been recognized. ¾¾ The anthroponotic urban type causing painless dry ulcerating lesions, leading to disfiguring scars, caused by the species L. tropica. €€ This is prevalent from the Middle East to northwestern India. The most important vector is P. sargenti. €€ It is seen mainly in children in endemic areas and is called as oriental sore or Delhi boil. €€ It begins as a raised papule, which grows into a nodule that ulcerates over some weeks. €€ Lesions may be single or multiple and vary in size from 0.5 to more than 3 cm. Lymphatic spread and lymph gland involvement may be palpable and may precede the appearance of the skin lesion. €€ The margins of the ulcer are raised and indurated. €€ The ulcer is usually painless unless secondary bacterial infection occurs. €€ There may be satellite lesions, especially in L. major and L. tropica infections. €€ The dry ulcers usually heal spontaneously in about an year. ¾¾ The zoonotic rural type causing moist ulcers which are inflamed, often multiple, caused by L. major. €€ The incubation period is usually less than 4 months. Lesions due to L. major heal more rapidly than L. tropica €€ This is seen in the lowland zones of Asia, Middle East, and Africa. €€ Gerbils, rats, and other rodents are the reservoirs. €€ P. papatasi is the most important vector. ¾¾ The non-ulcerative and often diffuse lesions caused by L. aethiopica and seen in the highlands of Ethiopia and Kenya are known as diffuse cutaneous leishmaniasis. €€ P. longipes is the usual vector. €€ It is a rare form of disease, where nodular lesions although restricted to skin are widely distributed. €€ It is characterized by low humoral as well as cellmediated immunity. €€ The lesions last for years or even for entire age. €€ It is difficult to treat. €€ Leishmaniasis recidivans is a type of lesion seen in persons with a high degree of cell-mediated immunity to the parasite. The lesions are chronic with alternating periods of activity and healing, characterized by a central scar with peripheral activity. The lesions resemble those of lupus or tuberculoid leprosy. Parasites are very scanty in the lesions. The Leishmanin test is strongly positive. Chemotherapy is not very useful. Better results follow local application of heat. Laboratory Diagnosis Microscopy ¾¾ Smear is made from the material obtained from the indurated edge of nodule or sore and stained by Giemsa or Leishman stain. ¾¾ Amastigotes are found in large numbers inside the macrophages. ¾¾ Definitive diagnosis is made by demonstration of amastigote in the smear collected from the lesion. Culture Promastigote forms can be isolated by culture of the aspirate material in NNN medium. Skin Test Leishmanin skin test is helpful. Positive leishmanin test in children under 10 years of age from endemic areas is highly suggestive of the disease. The skin test is negative in diffuse cutaneous leishmaniasis. Serology These are of limited value as the patient shows no detectable levels of circulating antibodies. Hemoflagellates Treatment The specific treatment of cutaneous leishmaniasis is same as visceral leishmaniasis. ¾¾ Antimony-resistant diffuse cutaneous leishmaniasis can be treated with pentamidine. ¾¾ Topical treatment consists of a paste of 10% charcoal in sulphuric acid or liquid nitrogen. Prophylaxis ¾¾ Control of sandfly population by insecticides and sanitation measures. ¾¾ Personal protection by use of protective clothing and use of insect repellants. ¾¾ Elimination of mammalian reservoir. New World Leishmaniasis L. braziliensis complex and L. mexicana complex History and Distribution Lindenberg and paranhos (1909) first described amastigotes in the ulcers of skin in a man in Brazil. Vianna (1911) named the species as L. braziliensis. ¾¾ L. braziliensis complex and L. mexicana complex cause new world leishmaniasis in Central and South America. Habitat These occur as intracellular parasite. The amastigote form is seen inside the macrophages of skin and mucous membrane of the nose and buccal cavity. The promastigote form occurs in vector species Lutzomyra. Morphology Morphology of amastigote and promastigote forms of both the parasites is same as that of the other 2 species of Leishmania. 61 ¾¾ Direct transmission and autoinfection also occurs manto-man. Clinical Features L. mexicana complex leads to cutaneous leishmaniasis which closely resembles the old world cutaneous leishmaniasis. However a specific lesion of caused by L. mexicana is chiclero ulcer which is characterized by ulcerations in pinna. ¾¾ Chiclero ulcer is also called as self healing sore of Mexico. L. brazilensis complex causes both mucocutaneous leish maniasis and cutaneous leishmaniasis. ¾¾ L. braziliensis causes the most severe and destructive form of cutaneous lesion. ¾¾ It involves the nose, mouth, and larynx. ¾¾ The patient experiences a nodule at the site of sandfly bite with symptoms consistent with oriental sore. ¾¾ Subsequent mucocutaneous involvement leads to nodules inside the nose, perforation of the nasal septum, and enlargement of the nose and lips (espundia). ¾¾ If the larynx is involved, the voice changes as well. ¾¾ Ulcerated lesions may lead to scarring and tissue destruction that can be disfiguring. ¾¾ The disease occurs predominantly in Bolivia, Brazil, and Peru. Pian Bois It is also known as 'forest yaws'. ¾¾ It is caused by L. brazilensis gyanensis and is characterized by appearance of single or multiple painless dry persistent ulcers appear all. Laboratory Diagnosis Microscopy Amastigotes are demonstrated in smears taken from lesions of skin and mucous membrane. L mexicana amastigotes are larger than those of L braziliensis and their kinetoplast is more centrally placed. Biopsy Life Cycle Amastigotes can also be demonstrated from slit-skin biopsy. The life cycle of Leishmania species causing the new world cutaneous and mucocutaneous leishmaniasis is similar to that of L. donovani except: ¾¾ Amastigotes are found in the reticuloendothelial cells and lymphoid tissues of skin, but not in the internal organs. ¾¾ The infection is transmitted to man from animals by bite of sandfly vectors of genus Lutzomiya. ¾¾ Sylvatic rodents and domestic animals are the common sources and reservoir of infection. Culture Culturing material obtained from ulcers in NNN medium demonstrates promastigotes. L. mexicana grows well in comparison to L. brazilensis, which is a grows slowly. Serology Antibodies can be detected in serum by IFA test, which is positive in 89–95% of cases. ELISA is also a sensitive method to detect antibody; being positive in 85% of cases. 62 Textbook of Medical Parasitology Skin Test Prophylaxis Leishmanin test is positive in cutaneous and mucocutaneous leishmaniasis. ¾ Treatment ¾ Treatment with a pentavalent antimonial compound is moderately effective for mild mucocutaneous leishmaniasis. ¾ AmphotericinB is the best alternative drug currently available. ¾ In case of respiratory complications, glucocorticoids can be used. ¾ ¾ Due to sylvatic and rural nature of the disease, control is often difficult. Use of insect repellants, spraying of insecticides, and screening are advisable. Forest workers should use protective clothing and other protective measures. A recently developed polyvalent vaccine using 5 Leish mania strains has been reported to be successful in reducing the incidence of cutaneous leishmaniasis in Brazil. Key points of Leishmania •¾Visceral leishmaniasis (Kalaazar) is caused by L. donovani and L. infantum. •¾Vector of Kalaazar is sandfly (argentipes). •¾Amastigote forms (LD body) is found in macrophages and monocytes in human. •¾Promastigote forms with a single flagellum is found in vector sandfly and artificial culture. •¾Clinical features: Kalaazar: Fever, hepatosplenomegaly, marked anemia, darkly pigmented skin, weight loss, cachexia, etc. •¾PKDL: Seen after 1–2 years of treatment in 3–10% cases and is a nonulcerative lesion of skin. •¾Diagnosis: By demonstrations of LD bodies in peripheral blood, bone marrow aspirate, splenic aspirate, and lymph node aspirate; culture done in NNN medium; aldehyde test; detection of specific antigen and antibody by IIF, ELISA, DAT and rapid rk 39 antibody detection test. •¾Blood picture: Anemia, thrombocytopenia, leucopenia with relative lymphocytosis, and hypergammaglobulinemia. •¾Treatment: Sodium stibogluconate amphotericinB, and oral miltefosine. •¾Old world cutaneous lesishmaniasis (oriental sore) is caused by L. tropica and the vectors are P. surgenti and P. pappatasi. •¾New world mucocutaneous and cutaneous leishmaniasis (espundia) are caused by L. brazilensis and L. mexicana. Vector is sandfly of genus Lutzomyra. Review Questions 1. Describe briefly the life cycle and laboratory diagnosis of: (a) Trypanosoma brucei gambiense (b) Trypanosoma cruzi (c) Leishmania donovani 2. Write short notes on: (a) Sleeping sickness (c) Antegenic variations of Trypanosoma brucei gambiense (e) Trypanosoma rangeli (g) Post kalaazar dermal leishmaniasis (i) Diffuse cutaneous leishmaniasis (b) (d) (f) (h) Chagas disease Morphological stages of hemoflagellates Kalaazar Cutaneous leishmaniasis 3. Differentiate between: (a) East African trypanosomiasis and West African trypanosomiasis (b) Trypanosoma cruzi and Trypanosoma rangeli Malaria and Babesia Protozoan parasites characterized by the production of spore-like oocysts containing sporozoites were known as sporozoa. ¾¾ They live intracellularly, at least during part of their life cycle. ¾¾ At some stage in their life cycle, they possess a structure called the apical complex, by means of which they attach to and penetrate host cells. ¾¾ These protozoa are therefore grouped under the Phylum Apicomplexa. ¾¾ The medically important parasites in this group are the malaria parasites, Coccidia, and Babesia. ¾¾ The Phylum Apicomplexa includes 2 classes viz. haematozoa and coccidia and 3 orders—eimeriida, haemosporida, and piroplasmida (Table 6.1). Note: Many minute intracellular protozoa formerly grouped as sporozoa have been reclassified because of some structural differences. These are now called microspora. They infect a Table 6.1: Phylum Apicomplexa (Sporozoa) Class Order Genera Haematozoa Haemosporida Piroplasmida Plasmodium Babesia Coccidia Eimeriida Toxoplasma Cyclospora Cryptosporidium Isospora Sarcocystis 6 large spectrum of hosts including vertebrates and invertebrates. Infection is mostly asymptomatic, but clinical illness is often seen in the immunodeficient. Classification Malaria parasite belongs to Phylum: Apicomplexa Class: Sporozoa Order: Haemosporida Genus: Plasmodium. ¾¾ The genus Plamodium is divided into 2 subgenera, P. vivax, P. malariae and P. ovale belong to the subgenus Plasmodium while P. falciparum is allocated to subgenus Laverania because it differs in a number of aspects from the other 3 species. ¾¾ P. vivax, P. malariae, and P. ovale are closely related to other primate malaria parasites. P. falciparum on the other hand, is more related to bird malaria parasites and appears to be a recent parasite of humans, in evolutionary terms. Perhaps for this reason, falciparum infection causes the severest form of malaria and is responsible for nearly all fatal cases. ¾¾ P. knowelsi, a parasite of long–tailed Macaque monkeys may also affect man. Causative Agents of Human Malaria ¾¾ Plamodium vivax: Benign Tertian Malaria ¾¾ Plasmodium falciparum: Malignant Tertian Malaria 64 Textbook of Medical Parasitology Fig. 6.1: Global distribution of malaria ¾¾ Plasmodium malariae: Benign Quartan Malaria ¾¾ Plasmodium ovale: Benign Tertian Malaria. Malaria Parasite History and Distribution Malaria has been known from antiquity. Seasonal intermittent fevers with chills and shivering, recorded in the religious and medical texts of ancient Indian, Chinese, and Assyrian civilizations, are believed to have been malaria (Fig. 6.1). ¾¾ The name malaria (mal: bad, aria: air) was given in the 18th century in Italy, as it was believed to be caused by foul emanations from marshy soil. ¾¾ The specific agent of malaria was discovered in RBCs of a patient in 1880 by Alphonse Lavaran, a French army surgeon in Algeria. ¾¾ In 1886, Golgi in Italy described the asexual development of the parasite in RBCs (erythrocytic schizogony), which therefore came to be called as Golgi cycle. ¾¾ Romanowsky in Russia in 1891, developed a method of staining malarial parasites in blood films. ¾¾ Three different species of malaria parasite infecting man, P. vivax, P. malariae, and P. falciparum were described in Italy between 1886 and 1890. The fourth species, P. ovale was identified only in 1922. ¾¾ The mode of transmission of the disease was established in 1897, when Ronald Ross in Secunderabad, India identified the developing stages of malaria parasites in mosquitoes. This led to various measures for the control and possible eradication of malaria by mosquito control. Both Ross (1902) and Laveran (1907) won the Nobel prize for their discoveries in malaria. ¾¾ Malaria affects mainly poor, underserved, and marginalized population in rural remote areas as well as in Urban areas. An epidemic can develop when there are changes in environmental, economic, and social conditions such as migrations and heavy rains following draughts. ¾¾ The relative prevalence of the 4 species of malaria parasites varies in different geographical regions (Fig. 6.1). € P. vivax is the most widely distributed, being most common in Asia, North Africa, and Central and South America. € P. falciparum, the predominant species in Africa, Papua New Guinea, and Haiti, is rapidly spreading in South-east Asia and India. € P. malariae is present in most places but is rare, except in Africa. € P. ovale is virtually confined to West Africa where it ranks second after P. falciparum (Fig. 6.1). ¾¾ Malaria may occur in endemic as well as epidemic patterns. It is described as endemic, when it occurs constantly in an area over a period of several successive years and as epidemic, when periodic or occasional sharp rises occur in its incidence. The World Health Organization (WHO) has recommended the classification of endemicity depending on the spleen or parasite rate in a statistically significant sample in the populations of children (2–9 years) and adults. According to this: € Hypoendemic (transmission is low): Spleen or parasite rate 75% ¾¾ In India, malaria continues to be a major public health threat. In India, about 27% population lives in high transmission (>1 case/1,000 population) and about 58% in low transmission (0–1 case)/1,000 population) area. Although total malaria cases has declined as compared to previous years, the proportion of P. falciparum malaria has increased. Vectors Human malaria is transmitted by over 60 species of female Anopheles mosquito. ¾¾ The male mosquito feeds exclusively on fruits and juices, but the female needs at least 2 blood meals, before the first batch of eggs can be laid. ¾¾ Out of 45 species of Anopheles mosquito in India, only few are regarded as the vectors of malaria. These are An culicifacies, An fluvatilis, An stephansi, An minimus, An phillippenensis, An sundaicus, etc. Malaria and Babesia Life Cycle € Malaria parasite passes its life cycle in 2 hosts. Definitive host: Female Anopheles mosquito. Intermediate host: Man. ¾ The life cycle of malarial parasite comprises of 2 stages— an asexual phase occurring in humans, which act as the intermediate host and a sexual phase occurring in mosquito, which serves as a definitive host for the parasite (Fig. 6.2). Asexual phase: € In this stage, the malaria parasite multiplies by division or splitting a process designated to as Schizogony (from schizo: to split, and gone: generation). € Because this asexual phase occurs in man, it is also called the vertebrate, intrinsic, or endogenous phase. € In humans, schizogony occurs in 2 locations—in the red blood cell (erythrocytic schizogony) and in the liver cells (exoerythrocytic schizogony or the tissue phase). € 65 Because schizogony in the liver is an essential step before the parasites can invade erythrocytes, it is called pre-erythrocytic schizogony. The products of schizogony, whether erythrocytic or exoerythrocytic, are called merozoites (meros: a part, zoon: animal). Sexual phase: The sexual phase takes place in the female Anopheles mosquito, even though the sexual forms of the parasite (gametocytes) originate in human red blood cells. € Maturation and fertilization take place in the mosquito, giving rise to a large number of sporozoites (from sporos: seed). Hence this phase of sexual multiplication is called sporogony. It is also called the invertebrate, extrinsic, or exogenous phase. ¾¾ There is, thus an alternation of generations in the life cycle of malaria parasites—asexual and sexual generations, alternatively. € Fig. 6.2: Life cycle of the Plasmodium vivax 66 Textbook of Medical Parasitology ¾¾ There also occurs an alternation of hosts, as the asexual phase takes place in humans followed by the sexual phase in the mosquito. Therefore, the complete life cycle of the malaria parasite comprises an alternation of generations with an alternation of hosts. ¾¾ The ¾ Human Cycle (Schizogony) Human infection comes through the bite of the infective female Anopheles mosquito (Fig. 6.2). ¾¾ The sporozoites, which are infective forms of the parasite are present in the salivary gland of the mosquito. ¾¾ They are injected into blood capillaries when the mosquito feeds on blood after piercing the skin. ¾¾ Usually, 10–15 sporozoites are injected at a time, but occasionally, many hundreds may be introduced. ¾¾ The sporozoites pass into the blood stream, where many are destroyed by the phagocytes, but some reach the liver and enter the parenchymal cells (hepatocytes). Pre-erythrocytic (Tissue) Stage or Exoerythrocytic Stage Within an hour of being injected into the body by the mosquito, the sporozoites reach the liver and enter the hepatocytes to initiate the stage of pre-erythrocytic schizogony or merogony. ¾¾ The sporozoites, which are elongated spindle-shaped bodies, become rounded inside the liver cells. ¾¾ They enlarge in size and undergo repeated nuclear division to form several daughter nuclei; each of which is surrounded by cytoplasm. ¾¾ This stage of the parasite is called the pre-erythrocytic or exoerythrocytic schizont or meront. ¾¾ The hepatocyte is distended by the enlarging schizont and the liver cell nucleus is pushed to the periphery. ¾¾ Mature liver stage schizonts are spherical (45–60 µm), multinucleate, and contain 2000–50,000 uninucleate merozoites. ¾¾ Unlike erythrocytic schizogony, there is no pigment in liver schizonts. These normally rupture in 6–15 days and release thousands of merozoites into the blood stream. ¾¾ The merozoites infect the erythrocytes by a process of invagination. ¾ ¾ interval between the entry of the sporozoites into the body and the first appearance of the parasites in blood is called the prepatent period. The duration of the pre-erythrocytic phase in the liver, the size of the mature schizont, and the number of merozoites produced vary with the species of the parasite (Table 6.2). Latent stage: In P. vivax and P. ovale, two kinds of sporozoites are seen, some of which multiply inside hepatic cells to form schizonts and others persist and remain dormant (resting phase). The resting forms are called hypnozoites (hypnos: sleep). From time to time, some are activated to becomes schizonts and release merozoites, which go on infecting RBCs producing clinical relapse. In P. falciparum and P. malariae, initial tissue phase disappears completely, and no hypnozoites are found. However, small number of erythrocytic parasites persist in the blood stream and in due course of time, they multiply to reach significant numbers resulting in clinical disease (short-term relapse or recrudescence). Erythrocytic Stage The merozoites released by pre-erythrocytic schizonts invade the red blood cells. ¾ The receptor for merozoites is glycophorin, which is a major glycoprotein on the red cells. The differences in the glycophorins of red cells of different species may account for the species specificity of malaria parasites. ¾ Merozoites are pear-shaped bodies, about 1.5 µm in length, possessing an apical complex (rhoptery). They attach to the erythrocytes by their apex and then the merozoites lie within an intraerythrocytic parasitophorus vacuole formed by red cell membrane by a process of invagination. ¾ In the erythrocyte, the merozoite loses its internal organelles and appears as a rounded body having a vacuole in the center with the cytoptasm pushed to the periphery and the nucleus at one pole. These young parasites are, therefore called the ring forms or young trophozoites. Table 6.2: Features of Pre-erythrocytic Schizogony in Human Malaria Parasites P. vivax P. falciparum P. malariae P. ovale Pre-erythrocytic stage (days) 8 6 15 9 Diameter of pre-erythrocytic schizont (µm) 45 60 55 60 No. of merozoites in pre-erythrocytic schizont 10,000 30,000 15,000 15,000 Malaria and Babesia ¾¾ The parasite feeds on the hemoglobin of the erythrocyte. It does not metabolize hemoglobin completely and therefore, leaves behind a hematin-globin pigment called the malaria pigment or haemozoin pigment, as residue. The appearance of malaria pigments varies in different species as follows: ••P vivax: Numerous fine golden-brown dust-like particles ••P. falciparum: Few 1–3 solid blocks of black pigment ••P. malariae: Numerous coarse dark brown particles ••P ovale: Numererous blackish brown particles. ¾¾ The malaria pigment released when the parasitized cells rupture is taken up by reticuloendothelial cells. Such pigment-laden cells in the internal organs provide histological evidence of previous malaria infection. ¾¾ As the ring form develops, it enlarges in size becoming irregular in shape and shows amoeboid motility. This is called the amoeboid form or late trophozoite form. ¾¾ When the amoeboid form reaches a certain stage of development, its nucleus starts dividing by mitosis followed by a division of cytoplasm to become mature schizonts or meronts. ¾¾ A mature schizont contains 8–32 merozoites and hemozoin. The mature schizont bursts releasing the merozoites into the circulation. ¾¾ The merozoites invade fresh erythrocytes within which they go through the same process of development. This cycle of erythrocytic Schizogony or merogony is repeated sequentially, leading to progressive increase in the parasitemia, till it is arrested by the development of host immune response. ¾¾ The rupture of the mature schizont releases large quantities of pyrogens. This is responsible for the febrile paroxysms characterizing malaria. ¾¾ The interval between the entry of sporozoites into the host and the earliest manifestation of clinical illness is the incubation period. This is different from prepatent period, which is the time taken from entry of the sporozoites to the first appearance of malaria parasite in peripheral blood. ¾¾ In P. falciparum, erythrocytic schizogony always takes place inside the capillaries and vascular beds of internal organs. Therefore, in P. falciparum infections, schizonts, and merozoites are usually not seen in the peripheral blood. ¾¾ The erythrocytic stages of all the 4 species of Plasmodium is shown in Figure 6.3. 67 Gametogony After a few erythrocytic cycles, some of the merozoites that infect RBC's do not proceed to become trophozoites or schizonts but instead, develop into sexually differentiated forms, the gametocytes. ¾¾ They grow in size till they almost fill the RBC, but the nucleus remains undivided. ¾¾ Development of gametocytes generally takes place within the internal organs and only the mature forms appear in circulation. ¾¾ The mature gametocytes are round in shape, except in P. falciparum, in which they are crescent-shaped. ¾¾ In all species, the female gametocyte is larger (macrogametocyte) and has cytoplasm staining dark blue with a compact nucleus staining deep red. In the smaller male gametocyte (microgametocyte), the cytoplasm stains pale blue or pink and the nucleus is larger, pail stained and diffuse. Pigment granules are prominent. ¾¾ Female gametocytes are generally more numerous than the male. ¾¾ Gametocyte appear in circulation 4–5 days after the first appearance of asexual form in case of P. vivax and 10–12 days in P. falciparum. ¾¾ A person with gametocytes in blood is a carrier or reservoir. ¾¾ The gametocytes do not cause any clinical illness in the host, but are essential for transmission of the infection. ¾¾ A gametocyte concentration of 12 or more per cumm of blood in the human host is necessary for mosquitoes to become infected. The Mosquito Cycle (Sporogony) When a female Anopheles mosquito ingests parasitized erythrocytes along with its blood meal, the asexual forms of malaria parasite are digested, but the gametocytes are set free in the midgut (stomach) of mosquito and undergo further development. ¾¾ The nuclear material and cytoplasm of the male gametocytes divides to produce 8 microgametes with long, actively motile, whip-like filaments. (exflagellating male gametocytes) (Fig. 6.4). ¾¾ At 25° C, the exflagellatation is complete in 15 minutes for P. vivax and P. ovale and 15–30 minutes for P. falciparum. ¾¾ The female gametocyte does not divide but undergoes a process of maturation to become the female gamete or macrogamete. It is fertilized by one of the microgametes to produce the zygote (Fig. 6.4). ¾¾ Fertilization occurs in half to two hours after the blood meal. The zygote, which is initially a motionless 68 Textbook of Medical Parasitology Fig. 6.3: Malaria parasites—Erythrocytic stages of the four species (Giemsa stain. Magn × 2000) round body, gradually elongates and within 18–24 hours, becomes a vermicular motile form with an apical complex anteriorly. This is called the ookinete ('travelling vermicule'). ¾¾ It penetrates the epithelial lining of the mosquito stomach wall and comes to lie just beneath the basement membrane. ¾¾ It becomes rounded into a sphere with an elastic membrane. This stage is called the oocyst, which is yet another multiplicatory phase, within which numerous sporozoites are formed. ¾¾ The mature oocyst, which may be about 500 µm in size, bulges into body cavity of mosquito and when it ruptures, the sporozoites enter into the hemocele or Malaria and Babesia 69 ¾¾ The degree of parasitization is not generally heavy, each Fig. 6.4: Schematic diagram showing formation of microgamete and macrogamete body cavity, from where some find their way to the salivary glands. ¾¾ The mosquito is now infective and when it feeds on humans, the sporozoites are injected into skin capillaries to initiate human infection. ¾¾ The time taken for completion of sporogony in the mosquito is about 1–4 weeks (exitrinsic incubation period), depending on the environmental temperature and the species. Types of Malarial Parasites Plasmodium Vivax P. vivax has the widest geographical distribution, extending through the tropics, subtropics and temperate regions. It is believed to account for 80% of all malaria infections. It is the most common species of malaria parasite in Asia and America, but is much less common in Africa. It causes benign tertian malaria with frequent relapses. ¾¾ The sporozoites of P. vivax are narrow and slightly curved. On entering the liver cells, the sporozoites initiate 2 types of infection. Some develop promptly into exoerythrocytic schizonts, while others persist in the dormant state for varying periods as hypnozoites. There may be 2 distinct types of sporozoites, the tachysporozoites (tachy: fast), which develops into the primary exoerythrocytic schizont and the bradysporozoite (brady: slow) which becomes the hypnozoite. ¾¾ The pre-erythrocytic schizogony lasts for 8 days and the average number of merozoites per tissue schizont is 10,000. ¾¾ Merozoites of P. vivax preferentially infect reticulocytes and young erythrocytes. ¾¾ All stages of erythrocytic schizogony can be seen in peripheral smears (Fig. 6.5). infected red cell usually having only one trophozoite and not more than 2–5% of the red cells being affected. Reticulocytes are preferentially infected. ¾¾ The trophozoite is actively motile, as indicted by its name vivax. The ring form is well-defined, with a prominent central vacuole. One side of the ring is thicker and the other side thin. Nucleus is situated on the thin side of the ring. The ring is about 2.5–3 µm in diameter, about a third of the size of an erythrocyte. The cytoplasm is blue and the nucleus red in stained films. The ring develops rapidly to the amoeboid form and accumulates malarial pigment (Figs 6.6 and 6.7). ¾¾ The infected erythrocytes are enlarged and show red granules known as Schuffner's dots on the surface. They become irregular in shape, lose their red color, and present a washed out appearance. A few of the parasitized erythrocytes retreat into the blood spaces of the internal organs. ¾¾ The schizont appears in about 36–40 hours. It occupies virtually the whole of the enlarged red cell. The schizont matures in the next 6–8 hours, with the development of merozoites, each with its central nucleus and surrounding cytoplasm. The pigment granules agglomerate into a few dark brown collections at the center, and with the merozoites around it, this stage presents a rosette appearance. There are about 12–24 (usually 16) merozoites per schizont. ¾¾ Erythrocytic schizogony takes approximately 48 hours. The red cell, which now measures about 10 µm in diameter is heavily stippled and often distorted. It bursts to liberate the merozoites and pigment. The pigment is phagocytosed by reticuloendothelial cells. ¾¾ The merozoites measure about 1.5 µm and have no pigment. ¾ Gametocytes appear early, usually within 4 days after the trophozoites first appear. Both male and female gametocytes are large, nearly filling the enlarged red cell. The macrogametocyte has dense cytoplasm staining deep blue and a small compact nucleus. The microgametocyte has pale-staining cytoplasm and a large diffuse nucleus. Pigment granules are prominent in the gametocytes. Plasmodium Falciparum The name falciparum comes from the characteristic sickle shape of the gametocytes of this species (falx: sickle, parere: to bring forth). This is the highly pathogenic of all the plasmodia and hence, the name malignant tertian or pernicious malaria for its infection. 70 Textbook of Medical Parasitology Fig. 6.5: Plasmodium vivax (Giemsa stain, magn x 2000) Fig. 6.6: Malarial parasite in blood film – Ring stage of P. vivax. Courtesy: Harsh Mohan, Textbook of Pathology, 6th ed. New Delhi: Jaypee Brothers, 2013(R), p. 189 Fig. 6.7: Malarial parasite in blood film – Amoeboid form of P. vivax. Courtesy: Harsh Mohan, Textbook of Pathology, 6th ed. New Delhi: Jaypee Brothers, 2013(R), p. 189 Malaria and Babesia ¾¾ The disease has a high rate of complications and unless treated, is often fatal. The species is responsible for almost all deaths caused by malaria. ¾¾ Schizogony: The sporozoites are sickle-shaped. The tissue phase consists of only a single cycle of preerythrocytic schizogony. No hypnozoites occur. The mature liver schizont releases about 30,000 merozoites. ¾¾ They attack both young and mature erythrocytes and so the population of cells affected is very large. Infected erythrocytes present a brassy coloration. ¾¾ Ring form: The early ring form in the erythrocyte is very delicate and tiny, measuring only a sixth(1/6) of the red cell diameter. Rings are often seen attached along the margin of the red cell, the so-called form appliqué or accole. Binucleate rings (double chromatin) are common resembling stereo headphones in appearance. Several rings may be seen within a single erythrocyte. In course of time, the rings become larger, about a third of the size of the red cell and may have 1 or 2 grains of pigment in its cytoplasm (Figs 6.8 and 6.9). ¾¾ The subsequent stages of the asexual cycle—late trophozoite, early and mature schizonts—are not ordinarily seen in peripheral blood, except in very severe or pernicious malaria. The presence of P. falciparum schizonts in peripheral smears indicates a grave prognosis. Pathogenesis of Malignant Malaria The trophozoites usually disappear from peripheral circulation after about 24 hours. By then, membrane protuberances appear in erythrocyte's surface, 12–15 hours after cell invasion. These knobs extrude a high molecular weight, antigenically variant strain-specific erythrocyte membrane adhesive protein, Pf MP1, that mediates attachment to receptors on venular capillary endothelium, an event termed 'cytoadherence'. Several vascular receptors have been identified of which intracellular adhesion molecule (ICAM-1) is probably most important in the brain, chondroitin sulfate B in the placenta, and CD36 in most other organs. The infected erythrocytes stick inside and eventually block capillaries and venules. At the same stage, these P. falciparum infected RBCs adhere to uninfected RBCs to form rosettes. The process of cytoadherence, resetting, and agglutination are central to pathogenesis of falciparum malaria. They result in sequestration of RBCs containing mature form of the parasite in vital organs like brain, kidney, heart, lungs, spleen, intestine, bone marrow, and placenta, where they interfere with microcirculatory flow and metabolism and are responsible for many of the serious complications of falciparum malaria, such as cerebral malaria. 71 ¾¾ The mature schizont is smaller than in any other species and has 8–24 (usually 16) merozoites. The erythrocytic schizogony takes about 48 hours or less, so that the periodicity of febrile paroxysms is 36–48 hours. ¾¾ Very high intensity of parasitization is seen in falciparum malaria. In very severe infections, the rate of parasitized cells may even be up to 50%. ¾¾ The infected erythrocytes are of normal size. They show a few (6–12) coarse brick-red dots which are called Maurer's clefts. Some red cells show basophilic stippling. ¾¾ Gametogony: It begins after several generations of schizogony. Gametocytes are seen in circulation about 10 days after the ring stage first appears. The early gametocytes seldom appear in peripheral circulation. The mature gametocytes, which are seen in peripheral smears are curved oblong structures, described as crescentic, sickle, sausage, or banana-shaped. They are usually referred to as crescents (Fig. 6.10). ¾¾ The male gametocytes are broad and sausageshaped or kidney-shaped, with blunt rounded ends as compared to the female gametocytes, which are thinner and more typically crescentic, with sharply rounded or pointed ends. The mature gametocyte is longer than the diameter of the red cell and so produces gross distortion and sometimes even apparent disappearance of the infected red cell. The red cell is often seen as a rim on the concave side of the gametocyte. The cytoplasm in the female gametocyte is deep blue, while in the male it is pale blue or pink. The nucleus is deep red and compact in the female, with the pigment granules closely aggregated around it, while in the male, it is pink, large and diffuse, with the pigment granules scattered in the cytoplasm. ¾¾ Falciparum crescents can survive in circulation for up to 60 days, much longer than in other species. Gametocytes are most numerous in the blood of young children, 9 months to 2 years old. They, therefore serve as the most effective source of infection to mosquitoes. Plasmodium Malariae This was the species of malaria parasite first discovered by Laveran in 1880 and the name malariae is the one given by him. It causes quartan malaria, in which febrile paroxysms occur every fourth day, with 72 hours' interval between the bouts. ¾¾ The disease is generally mild, but is notorious for its long persistence in circulation in undetectable levels, for 50 years or more. Recrudescence may be provoked by splenectomy or immunosuppression. ¾¾ The development of the parasite, in man and mosquito is much slower than with other species. Chimpanzees 72 Textbook of Medical Parasitology Fig. 6.8: Plasmodium falciparum (Giemsa stain, magn x 2000) Fig. 6.9: Malarial parasite in blood film – Ring stage of P. falciparum. Courtesy: Harsh Mohan, Textbook of Pathology, 6th ed. New Delhi: Jaypee Brothers, 2013(R), p. 189 Fig. 6.10: Malarial parasite in blood film – Gametocytes of P. falciparum. Courtesy: Harsh Mohan, Textbook of Pathology, 6th ed. New Delhi: Jaypee Brothers, 2013(R), p. 189 Malaria and Babesia may be naturally infected with P. malariae and may constitute a natural reservoir for quartan malaria. ¾¾ P. malariae occurs in tropical Africa, Sri Lanka, Burma, and parts of India, but its distribution is patchy. ¾¾ The sporozoites are relatively thick. Pre-erythrocytic schizogony takes about 15 days, much longer than in other species. Each schizont releases about 15,000 merozoites. Hypnozoites do not occur. The long latency of the infection is believed to be due to persistence of small numbers of erythrocytic forms in some internal organs. ¾¾ P. malariae preferentially infects older erythrocytes and the degree of parasitization is low. ¾¾ The ring forms resembles those of P. vivax, although thicker and more intensely-stained. The old trophozoites are sometimes seen stretched across the erythrocyte as a broad band. These band forms are a unique feature of P. malariae. Numerous large pigment granules are seen (Fig. 6.11). ¾¾ The schizonts appear in about 50 hours and mature during the next 18 hours. The mature schizont has an average of 8 merozoites, which usually present a rosette appearance. ¾¾ The infected erythrocytes may be of the normal size or slightly smaller. Fine stippling, called Ziemann's stippling, may be seen with special stains. The degree of parasitization is lowest in P. malariae. ¾¾ Erythrocytic schizogony takes 72 hours. ¾¾ The gametocytes develop in the internal organs and appear in the peripheral circulation when fully grown. Gametocytes occupy nearly the entire red cell. The male has pale blue cytoplasm with a large diffuse nucleus, 73 while the female has deep blue cytoplasm and a small compact nucleus. Plasmodium Ovale This parasite produces a tertian fever resembling vivax malaria, but with milder symptoms, prolonged latency and fewer relapses. ¾¾ It is the rarest of all plasmodia infecting humans and is seen mostly in tropical Africa, particularly along the West Coast. ¾¾ The pre-erythrocytic stage extends for 9 days. Hepatocytes containing schizonts usually have enlarged nuclei. The mature liver schizont releases about 15,000 merozoites. Hypnozoites are present. ¾¾ The trophozoites resemble those in vivax malaria, but are usually more compact, with less amoeboid appearance. Schuffner's dots appear earlier and are more abundant and prominent than in vivax infection (Fig. 6.12). ¾¾ The infected erythrocytes are slightly enlarged. In thin films, many of them present an oval shape with fimbriated

margins. This oval appearance of the infected erythrocyte is the reason for the name ovale given to this species. ¾¾ The schizonts resemble those of P. malariae, except that the pigment is darker and the erythrocyte is usually oval, with prominent Schuffner's dots. Mixed Infections In endemic areas it is not uncommon to find mixed infections with 2 or more species of malaria parasites in the same individual. Fig. 6.11: Plasmodium malariae stages of erythrocytic schizogony (Giemsa stain, magn x 2000) 74 Textbook of Medical Parasitology Fig. 6.12: Plasmodium ovale stages of erythrocytic schizogony (Giemsa stain, magn x 2000) infection with P. vivax and P. falciparum is the most common combination with a tendency for one or the other to predominate ¾¾ The clinical picture may be atypical with bouts of fever occurring daily ¾¾ Diagnosis may be made by demonstrating the characteristic parasitic forms in thin blood smears. The characteristics of the 4 species of plasmodia infecting man are listed in Table 6.3. ¾¾ Mixed Pathogenesis All clinical manifestation in malaria are due to products of erythrocytic schizogony and the host's reaction to them. ¾¾ The disease process in malaria occurs due to the local or systemic response of the host to parasite antigens and tissue hypoxia caused by reduced oxygen delivery because of obstruction of blood flow by the parasitized erythrocytes. ¾¾ Liver is enlarged and congested. Kupffer cells are increased and filled with parasites. Hemozoin pigments are also found in the parenchymal cells (Fig. 6.13). Fig. 6.13: Major pathological changes in organs in malaria Malaria and Babesia 75 Table 6.3: Comparison of the Characteristics of Plasmodia Causing Human Malaria P. vivax P. falciparum P. malariae P. ovale Hypnozoites Yes No No Yes Erythrocyte preference Reticulocytes Young erythrocytes, but can infect all stages Old erythrocytes Reticulocytes Stages found in peripheral blood Rings, trophozoites, schizonts, gametocytes Only rings and gametocytes As in vivax As in vivax Ring stage Large, 2.5 µm, usually single, prominent chromatin Delicate, small, 1.5 µm, double chromatin, and multiple rings common, Accole forms found. Similar to vivax, but thicker Similar to vivax, more compact Late trophozoite Large irregular, actively amoeboid, prominent vacuole Compact, seldom seen in blood smear Band form characteristic Compact, coarse pigment Schizont Large filling red cell Small, compact, seldom seen in blood smear Medium size Medium size Number of merozoites 12–24 in irregular grapelike cluster 8–24 grape-like cluster 6–12 in daisy-head or rosette pattern 6–12 irregularly arranged Microgametocyte Spherical, compact, pale blue cytoplasm, diffuse nucleus Sausage or bananashaped pale blue or pink cytoplasm, large diffuse nucleus As in vivax As in vivax Macrogametocyte Large, spherical, deep blue cytoplasm, compact nucleus Crescentic, deep blue cytoplasm, compact nucleus As in vivax As in vivax Infected erythrocyte Enlarged, pale, with Schuffner's dots Normal size, Maurer's clefts, sometimes basophilic stippling Normal, occasionally Ziemann's stippling Enlarged, oval fimbriated, prominent Schuffner's dots Duration of schizogony (days) 2 2 3 2 Prepatent period (days) 8 5 13 9 Average incubation period (days) 14 12 30 14 Appearance of gametocyte after parasite patency (days) 4–5 10–12 11–14 5–6 Duration of sporogony in mosquito (25oC) (days) 9–10 10–12 25–28 14–16 Average duration of untreated infection (years) 4 2 40 4 Parenchymal cells show fatty degeneration, atrophy, and centrilobular necrosis. ¾¾ Spleen is soft, moderately enlarged, and congested in acute infection. In chronic cases, spleen is hard with a thick capsule and slate grey or dark brown or even black in color due to dilated sinusoids, pigment accumulation, and fibrosis (Fig. 6.13). ¾¾ Kidneys are enlarged and congested. Glomeruli frequently contain malarial pigments and tubules may contain hemoglobin casts (Fig. 6.13). brain in P. falciparum infection is congested. Capillaries of the brain are plugged with parasitized RBCs. The cut surface of the brain shows slate grey cortex with multiple punctiform hemorrhage in subcortical white matter. ¾¾ Anemia is caused by destruction of large number of red cells by complement-mediated and autoimmune hemolysis. Spleen also plays an active role by destroying a large number of unparasitized erythrocytes. There is also decreased erythropoiesis in bore marrow due to ¾¾ The 76 Textbook of Medical Parasitology tumor necrosis factor (TNF) toxicity and failure of the host to recycle the iron bound in hemozoin pigments. ¾¾ Cytokines such as TNF, interleukin (IL)-1, and interferon (IFN)-gamma play a pivotal role in the pathogenesis of end organ disease of malaria. Causes of anemia in malaria ••Destruction of large number of RBCs by complement mediated and autoimmune hemolysis ••Suppression of erythropoesis in the bone marrow ••Increased clearance of both parasitied and non parasitized RBCs by the spleen. ••Failure of the host to recycle the iron bound in hemozoin pigment ••Antimalarial therapy in G6PD deficient patients. Clinical Features Benign Malaria The typical picture of malaria consists of periodic bouts of fever with rigor, followed by anemia and splenomegaly. Severe headache, nausea, and vomiting are common. ¾¾ The febrile paroxysm comprises of 3 successive stages— cold stage, hot stage, and sweating stage. €€ In the cold stage, that lasts for 15–60 minutes, the patient experiences intense cold and uncontrollable shivering. €€ This is followed by the hot stage, lasting for 2–6 hours, when the patient feels intensely hot. The temperature mounts to 41°C or higher. €€ Afterwards comes the sweating stage, when the patient is drenched in profuse sweat. The temperature drops rapidly and the patient usually falls into deep sleep, to wake up refreshed. Incubation period It is the interval between the infective mosquito bite and the first appearance of clinical symptoms. The duration of incubation period varies with the species of the parasite. The average incubation periods of different species of Plasmodium are as follows – ••P. vivax—14 (8–31) days ••P. falciparum—12 (8–14) days ••P. ovale—14 (8–31) days ••P. malariae—28 (18–40) days The incubation period is to be distinguished from the pre-patent period, which is the interval between the entry of the parasites into the host and the time when they first become detectable in blood. ¾¾ The paroxysm usually begins in the early afternoon and lasts for 8–12 hours. The febrile paroxysm synchronises with the erythrocytic schizogony. ¾¾ The periodicity is approximately 48 hours in tertian malaria (in P. vivax, P. falciparum, and P. ovale) and 72 hours in quartan malaria (in P. malariae). ¾¾ Quotidian periodicity, with fever occurring at 24 hour intervals may be due to 2 broods of tertian parasites maturing on successive days or due to mixed infection. ¾¾ Regular peridiocity is seldom seen in primary attack, but is established usually only after a few days of continuous, remittent, or intermittent fever. True rigor is typically present in vivax malaria and is less common in falciparum infection. ¾¾ There can be both hypoglycemia or hyperglycemia in malaria. ¾¾ Sometimes, there may be hyperkalemia due to red cell lysis and fall in blood pH. ¾¾ Infection with P. vivax usually follows a chronic course with periodic relapses, whereas P. ovale malaria is generally mild. Although P. malariae malaria is less severe, but it may lead to renal complications. ¾¾ Other features of benign malaria are anemia, spleno megaly, and hepatomegaly. Malignant Tertian Malaria The most serious and fatal type of malaria is malignant tertian malaria caused by P. falciparum. When not treated promptly and adequately, dangerous complications develop. The term pernicious malaria has been applied to a complex of life-threatening complications that sometimes supervene in acute falciparum malaria. These may present in various forms, the most important of which are the cerebral, algid, and septicemic varieties. ¾¾ Cerebral Malaria: It is the most common cause of death in malignant malaria. €€ Even with treatment, death occurs in 15% of children and 20% of adults who develop cerebral malaria. €€ It is manifested by headache, hyperpyrexia, coma or confusion, and paralysis. €€ This occurs particularly when non-immune persons have remained untreated or inadequately treated for 7–10 days after development of the primary fever. €€ Late stage schizonts of P. falciparum secrete a protein on the surface of RBCs to form knob-like deformities. This knob produces specific adhesive proteins, which promote aggregation of infected RBCs to other non-infected RBCs and capillary endothelial cells. These sequestrated RBCs cause Malaria and Babesia capillary plugging of cerebral microvasculature, which results in anoxia, ischemia, and hemorrhage in brain. ¾¾ Blackwater fever: A syndrome called blackwater fever (malarial hemoglobilnuria) is sometimes seen in falciparum malaria, particularly in patients, who have experienced repeated infections and inadequate treatment with quinine. € Patients with G6PD deficiency may develop this condition after taking oxidant drugs, even in the absence of malaria. € Clinical manifestation include bilious vomiting and prostration, with passage of dark red or blackish urine (black water). € The pathogenesis is believed to be massive intravascular hemolysis caused by anti-erythrocyte antibodies, leading to massive absorption of hemoglobin by the renal tubules (hemoglobinuric nephrosis) producing black water fever. Complications of black water fever include renal failure, acute liver failure, and circulatory collapse. ¾¾ Algid Malaria: This syndrome is characterized by peripheral circulatory failure, rapid thready pulse with low blood pressure, and cold clamy skin. There may be severe abdominal pain, vomiting, diarrhea, and profound shock. ¾¾ Septicemic malaria: It is characterized by high continuous fever with dissemination of the parasite to various organs, leading to multiorgan failure. Death occurs in 80% of the cases. Complications of falciparum malaria •¾Cerebral Malaria •¾Algid Malaria •¾Septicemic Malaria •¾Blackwater fever •¾Pulmonary edema •¾Acute renal failure •¾Hypoglycemia (

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