MALARIA

LYFE CYCL

Erythrocytic Stage

.The merozoites released by pre-erythrocytic schizonts invade the red blood cells

The receptor for merozoites is glycophorin, which is a major glycoprotein on the

red cell. The differences in the glycophorins of red cells of different species may

account for the species specificity of malaria parasites. Merozoites are pear-shaped bodies about 1.5 μm in length, possessing an apical complex(rhoptry). They attach to erythrocytes by their apex, which has certain organelles that secrete a substance producing a pit on the erythrocyte membrane. The merozoite then enters the erythrocyte by endocytosis and the red cell membrane seals itself parasitophorous vacuole) enclosing the merozoite. The process) to form a vacuole of entry into the red cell takes about 30 seconds. Once inside the red cell the merozoite rounds up and loses its internal organelles In the erythrocyte, the merozoite appears as a rounded body having a vacuole in the centre with the cytoplasm pushed to the periphery and the nucleus situated at one pole. When stained with Giemsa or other Romanowsky stains, the cytoplasm is stained blue and the nucleus red the central vacuole remaining unstained. This

.

gives the parasite an annular or signet ring appearance. These young parasites are

.therefore called the ring forms

The parasite feeds on the haemoglobin of the erythrocyte. It does not metabolise

:varies in the different species as follows

.P. vivax Numerous fine golden brown dust-like particles

.P. falciparum Few (one to three) solid blocks of black pigment

.P. malariae Numerous coarse dark brown particles

.P. ovale Numerous blackish brown particles

The malaria pigment released when the parasitised cells rupture is taken up by

reticuloendothelial cells. Such pigment laden cells in the internal organs provide

.histological evidence of previous malaria infection

As the ring form develops it enlarges in size becoming irregular in shape and

shows amoeboid motility .This is called the amoeboid form. Bits of membrane from

developing parasites accumulate on the inner surface of the erythrocyte and these

appear as stippling or clefts on the erythrocyte surface. When the amoeboid form

reaches a certain stage of development its nucleus starts dividing. The parasite within

the erythrocyte till the time its nucleus starts dividing is called the trophozoite(from

trophos—growth). The ring form is called the early trophozoiteand the amoeboid from

.the late trophozoite

From the time the nucleus starts dividing, the parasite within the erythrocyte

is called the schizontor meront(formerly also known as segmenter or rosette .(forms

At first only the nucleus divides into a variable number of small nuclei, the cytoplasm

remaining entire and undivided. This stage is called the early schizont. This continues

into the late schizontstage when each daughter nucleus becomes surrounded by

cytoplasm. The mature schizontis the fully grown form, in which a number of small

merozoites are seen, each having a nucleus with surrounding cytoplasm. The mature

schizont bursts releasing the merozoites into the circulation. The residual mass of

unutilised cytoplasm containing all the accumulated malarial pigment is also released

at the same time into the circulation. This is phagocytosed and can be seen as pigment

granules within polymorphs and macrophages. The merozoites invade fresh erythrocytes in 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 intensity of parasitaemia till it is arrested by the development of

.immune response in the host

The rupture of the mature schizont releases large quantities of pyrogens. This

is responsible for the febrile paroxysms characterising malaria. The interval between

the entry of the sporozoite into the host and the earliest manifestation of clinical

illness is the incubation period. This is different from the prepatent period, which

is the time taken from the entry of the sporozoite to the first appearance of malaria

.parasites in peripheral blood

The duration of the erythrocytic schizogony varies according to the species of

the parasite. An important feature determining the clinical manifestations of malaria

is the tendency for the erythrocytic schizogonic cycles to become synchronised, so

that all the mature schizonts in the body burst at the same time releasing merozoites

and other pyrogens into circulation, causing the febrile paroxysms. It has been

suggested that this schizogonic periodicity is related to the human circadian rhythm

of approximately 24 hours. Thus the schizogonic periodicity is about 48 hours in

,P. vivax, P. falciparumand P. ovalewhile in P.malariaeit is 72 hours. This in turn

is reflected in the periodicity of the bouts of fever in these different infections. Malarial

,periodicity has been recognised from early times and the colloquial terms tertian

quartan and quotidian had been applied to the different types of malaria as detailed

.below

Malaria Parasites 73

P.vivax: Benign tertianor BTmalaria. (Tertian, because the fever recurs after

intervals of 48 hours or every third day, according to the Greek or Roman system

of counting, which counts the first and last days also. Benign, because it is relatively

.(less dangerous than falciparum malaria which is called malignant tertian

P.falciparum: Malignant tertianor MTmalaria. (Also called subtertian. because the

cycles are often poorly synchronised and febrile paroxysms recur at intervals of less

than the expected 48 hours. It was also called pernicious malaria because of its lethal

.(nature, and aestivo-autumnal referring to its seasonal prevalence

P. malariae: Quartanmalaria. (Occurring every fourth day, as it has a cycle of 72

.(hours

P. ovale: Ovale tertian. (Because of its tertian periodicity and the irregular oval

.(shape of infected RBCs

Sometimes, especially in early P.vivaxinfections, there may be two independent

.broods of parasites with overlapping cycles so that there may be daily paroxysms

.This is called quotidianperiodicity

Gametogony

After a few cycles of erythrocytic schizogony, some merozoites that infect red cells

do not proceed to become schizonts, but instead develop into sexually differentiated

forms, the gametocytes. They grow in size till they almost fill the red cell, but the

nucleus remains undivided. Development of gametocytes generally takes place within

the internal organs such as spleen and bone marrow, and only the mature forms

appear in circulation. The mature gametocytes are round in shape, except in

P.falciparum, in which they are crescent-shaped. In all species, the female gametocyte

is larger (macrogametocyte) and has cytoplasm staining dark blue with a small compact

nucleus staining deep red. In the smaller male gametocyte (microgametocyte), the

cytoplasm stains pale blue or pink and the nucleus is larger, pale stained and .diffuse

Pigment granules are prominent. Female gametocytes are generally more numerous

.than the male

Gametocytes appear in circulation 4 to 5 days after the first appearance of asexual

form in the case of P.vivaxand 10 to 12 days in P.falciparum. A person with gametocytes

in circulation is a carrier or reservoir. Children are more effective carriers than .adults

.Gametocytes are more numerous in the early phase of infection

The gametocytes do not cause any clinical illness in the host, but are essential

for transmission of the infection. The gametocytes do not develop further or divide

in the vertebrate host and unless taken up by the vector mosquito, they die in a

few days. A gametocyte concentration of 12 or more per c.mm of blood in the human

.host is necessary for mosquitoes to become infected

The Mosquito Phase

When a female Anopheles mosquito ingests parasitised erythrocytes along with its

blood meal, the asexual forms of malaria parasites are digested, but the gametocytes

are set free in the stomach and undergo further development. Within 15 minutes

of entry into the stomach of the mosquito, the male gametocyte divides into 8 ,nuclei

from each of which protrudes a long, actively motile whip-like filament. These flagella

which are the male gametes (microgametes) lash about for sometime and then break

free. This process of formation of male gametes from the gametocyte is called

exflagellation. This can take place outside the body of the mosquito also and can be

.observed under the microscope

Exflagellation can be demonstrated by making a thick film of freshly drawn blood

containing mature gametocytes on a slide and placing it in a warm moist ,chamber

such as a Petri dish containing filter paper soaked in warm water. When examined

under the microscope after about 10 minutes, the male gametocyte can be seen to

shed its erythrocytic envelope and put forth up to eight slender active flagella

containing nuclear material from the original nucleus. Detaching from the cell ,body

the flagella lash about vigorously in the plasma. At 25°C, the exflagellation is complete

.in 15 minutes for P. vivaxand P. ovale,and 15 to 30 minutes for P.falciparum

The female gametocyte does not divide but undergoes a process of maturation

to become the female gamete or macrogamete. It is fertilised by one of the microgametes

to produce the zygote. Fertilisation occurs in half to two hours after the blood .meal

The zygote, which is initially a motionless round body elongates and within 18

.to 24 hours, becomes a vermicular motile form with an apical complex anteriorly

This is called the ookinete('travelling vermicule'). It penetrates the epithelial lining

of the mosquito stomach wall and comes to lie just beneath its basement .membrane

It becomes rounded into a sphere with an elastic membrane. This stage is called

the oocyst. There may be up to several hundred pigmented oocysts in the stomach

of a mosquito. It was the discovery by Ronald Ross, of pigmented oocysts in the

stomach walls of dissected mosquitoes that established the mosquito transmission

.of malaria

The oocyst matures, increasing in size, with the nucleus undergoing multiple

divisions. This sporogonyleads to the development within the oocyst of about a

thousand sporozoites, 10 to 15 μm in length, each with a central nucleus and an

anterior apical complex. The mature oocyst which may be about 500 μm in size bulges

into the body cavity of the mosquito, and when it ruptures the sporozoites enter

the haemocoele. The sporozoites reach the salivary glands situated in the thorax

of the mosquito, penetrate the acinar cells and enter the salivary ducts. The mosquito

is now infective and when it feeds on humans, the sporozoites are injected into the

skin capillaries to initiate human infection (Fig. 5.4). The time taken for completion

of sporogony in the mosquito is about 1 to 4 weeks; depending on the environmental

.temperature and the species

The characteristics of the four species of plasmodia infecting man are listed in

.Table 5.2

Plasmodium Vivax

,P. vivaxhas the widest geographical distribution, extending through the tropics

subtropics and temperate regions. It is believed to account for 80 per cent of all

malaria infections. It is the most common species of malaria parasite in Asia and

Table 5.2: 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 and thicker chromatin	Similar to vivax, but more compact ,chromatin multiple rings common	Similar ,to vivax Accole forms found
Late trophozoite characteristic	Large irregular, Band from Compact coarse	Compact, seldom seen in		

actively amoeboid, blood smear pigment

prominent vacuole

Schizont Large filling red cell Small, compact, seldom Medium size Medium size

seen in blood smear

Number of merozoites 12-24 in irregular 8-24 in grape-like 6-12 in daisy-head or 6-12 irregularly

grape-like cluster cluster rosette pattern arranged

...Contd

...Table 5.2: Contd

P.vivax P. falciparum P. malariae P. ovale

Microgametocyte Spherical, compact, Sausage or banana- As in vivax As in vivax

pale blue cytoplasm, shaped pale blue or pink

diffuse nucleus cytoplasm, large diffuse

nucleus

Macrogametocyte Large, spherical, deep Crescentic, deep blue As in vivax As in vivax

blue cytoplasm, compact cytoplasm, compact

nucleus nucleus

Infected erythrocyte Enlarged, pale, with Normal size, Maurer's Normal, occasionally Enlarged, oval

,Schuffner's dots clefts, sometimes Ziemann's stippling fimbriated

basophilic stippling prominent

Schuffner's dots

Duration of schizogony

days) 2 2 3 2)

Prepatent period (days) 8 5 1 3 9

Average incubation

period (days) 1 4 1 2 3 0 1 4

Appearance of gametocyte

after parasite patency

days) 4-5 10-12 11-14 5-6)

Duration of sporogony

in mosquito (25°C) (days) 9-10 10-12 25-28 14-16

Average duration of

untreated infection (years) 4 2 4 0 4

Malaria Parasites 77

FIGURE 5.4: Cycle of the malaria parasite (text shows development of Plasmodium (vivax in man and mosquito

America, but is much less common in Africa. It causes benign tertian malaria with

.frequent relapses

The sporozoites of P.vivaxare narrow and slightly curved. On entering the liver

cells, the sporozoites initiate two types of infection. Some develop promptly into

exoerythrocytic schizonts, while others persist in the dormant state for varying

periods as hypnozoites. There may be two distinct types of sporozoites, the

tachysporozoite (tachy-fast) which develops into the primary exoerythrocytic schizont

.and the bradysporozoite (brady—slow) which becomes the hypnozoite

P. vivaxshows strain differences with respect to the proportion of sporozoites

that develop into hypnozoites. Strains prevalent in the temperate zones (P.vivax hibernans) produce a high proportion of hypnozoites, thereby causing relapses after long periods of time. This feature may provide survival advantage to the parasite by avoiding possible cessation of its transmission, due to the absence of vector mosquitoes during overwintering or drought seasons. Tropical strains produce fewer hypnozoites. This apparently does not affect survival prospects of the parasite as .vector mosquitoes are constantly present in the tropics

The pre-erythrocytic schizogony lasts for 8 days and the average number of merozoites per tissue schizont is 10,000. Merozoites of P.vivaxpreferentially infect reticulocytes and young erythrocytes. All stages of erythrocytic schizogony can be seen in peripheral smears. The degree of parasitisation is not generally heavy , each infected red cell usually having only one trophozoite and not more than 2 to 5 per .cent of the red cells being affected. Reticulocytes are preferentially infected

The trophozoite is actively motile, as indicated 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 to 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. The infected erythrocytes are enlarged and show red granules known as Schuffner's dotson the surface. They

become irregular in shape, lose their red colour and present a washed out .appearance

A few of the parasitised erythrocytes retreat into the blood spaces of the internal

.organs

The schizont appears in about 36 to 40 hours. It occupies virtually the whole of

the enlarged red cell. The schizont matures in the next 6 to 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 ,centre

and with the merozoites around it, this stage presents a rosette appearance. There

are about 12 to 24 (usually 16) merozoites per schizont. 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. The merozoites

.measure about 1.5 µm and have no pigment

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

(FIGURE 5.5: Plasmodium vivax (Giemsa stain, magn x 2000

(FIGURE 5.6: Plasmodium falciparum (Giemsa stain, magn x 2000

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 (Fig. 5.5

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 most highly pathogenic

of all the plasmodia and hence the name malignant tertian or pernicious malaria

for its infection. 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

It is deeply entrenched in tropical Atrica and some parts of Asia. It is limited to

the tropical and subtropical regions because at temperatures below 20°C, its

development in the mosquito is greatly retarded. This is the species of the greatest

public health importance due to its increasing resistance to antimalarial drugs and

its spread to new areas. In India, it has been spreading widely, causing large epidemics

.in some places

The sporozoites are sickle-shaped. The tissue phase consists of only a single cycle

of pre-erythrocytic schizogony. No hypnozoites occur. The mature liver schizont

releases about 30,000 merozoites. They attack both young and mature erythrocytes

and so the population of cells affected is very large. Infected erythrocytes present

.a brassy colouration

The early ring form in the erythrocyte is very delicate and tiny, measuring only a sixth of the red cell diameter. Rings are often seen attached along the margin of the red cell, the so-called form appliqueor accole. Binucleate rings 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 one or two grains of pigment in its cytoplasm

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.falciparumschizonts in peripheral smears indicates a grave prognosis. The trophozoites usually disappear from peripheral circulation after about 24 hours. By then, a strain-specific high molecular weight antigen appears on the surface of the infected red cells, associated with knob-like projections on the erythrocyte membrane. Such red cells disappear from peripheral circulation and adhere to the walls of venules and capillaries in internal organs—brain, heart, ,kidney lungs, spleen, intestine, bone marrow, placenta. This cytoadherence causes sequestration of infected red cells in, these sites and is responsible for many of the serious complications .of falciparum malaria, such as cerebral malaria

The mature schizont is smaller than in any other species and has 8 to 24 (usually

merozoites. The erythrocytic schizogony takes about 48 hours or less, so that (١٦
the periodicity of febrile paroxysms is 36 to 48 hours. Very high intensity of parasitisation is seen in falciparum malaria. In very severe infections the rate of parasitised cells may even be up to 50 per cent. 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 (Fig. 5.6

Gametogony 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 variously described as crescentic, sickle, sausage or banana-shaped. They are usually referred to as .crescents

The male gametocytes are broad and sausage-shaped 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. 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. 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. The development of the parasite, in man and

mosquito is much slower than with other species. Chimpanzees may be naturally

infected with P.malariaeand may constitute a natural reservoir for quartan .malaria

P.brasilianum,a parasite of South American monkeys is virtually identical with

P.malariae. P.malariaeoccurs in tropical Africa, Sri Lanka, Burma and parts of ,India

.but its distribution is patchy

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. P.malariaepreferentially infects older erythrocytes and the degree of

.parasitisation is low

The ring forms resemble those of P.vivax, though thicker and more intensely

stained. The older trophozoites are sometimes seen stretched across the erythrocyte

as a broad band. These band formsare a unique feature of P. malariae. Numerous

.large pigment granules are seen

.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 (Fig. 5.7

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

.parasitisation is lowest in P. malariae. Erythrocytic schizogony takes 72 hours

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, 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. It is the rarest of all plasmodia infecting humans and is seen mostly in tropical Africa, particularly along the West .Coast

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

,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. 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 (Fig. 5.8

The schizonts resemble those of P. malariae, except that the pigment is darker .and the erythrocyte usually oval, with prominent Schuffner's dots

Mixed Infections

In endemic areas it is not uncommon to find mixed infections with two or more species of malaria parasites in the same individual. Mixed infection with P.vivaxand

P. falciparumis the most common combination with a tendency for one or the other

to predominate. The clinical picture may be atypical with bouts of fever occurring daily. Diagnosis may be made by demonstrating the characteristic parasitic forms .in thin blood smears

Culture of Malaria Parasites

Attempts to culture malaria parasites in vitro were started in 1912 by Bass and Johns

who obtained limited multiplication of human plasmodia. The breakthrough came

in 1976 with the discovery by Trager and Jensen of a simple method for the continuous

culture of P. falciparum. The technique has been extended to culture other species

.also

Malaria Parasites 83

(FIGURE 5.8: Plasmodium ovale (Giemsa stain, magn x 2000

The original method of Petri dish culture employed a candle-jar to provide an

atmosphere of 3 per cent CO2and 10 per cent O2and a relatively simple culture

medium supplemented with human, rabbit or calf serum to maintain infected

erythrocytes. Fresh red cells were added periodically for continuation of the growth

and multiplication of plasmodia. The continuous flow method devised by Trager

enables the prolonged maintenance of stock cultures. Computer-controlled culture

.systems introduced subsequently provide a steady abundant supply of parasites

Several culture lines have been established from blood of infected Aotus monkeyor

.directly from human patients

(FIGURE 5.7: Plasmodium malariae (Giemsa stain, magn x 2000

.Schizogony proceeds normally in culture. Gametocytes are formed infrequently

Pre-erythrocytic stages of some species have been obtained in tissue cultures. Plasmodia

.retain their infectivity in culture

Culture of plasmodia provides a source of the parasites for study of their antigenic

structure, for use in seroepidemiologic surveys, for drug sensitivity tests and for

.studies in immunoprophylaxis

Pathogenesis and Clinical Picture

The incubation period varies usually from 8 to 40 days, being shortest in P.falciparum

and longest in P. malariaeinfec.tions. The average incubation periods are 8-11 days

for falciparum, 10 to 12 days for vivax and ovale and 18 to 40 days for quartan

malaria. However, very much longer incubation periods, up to 9 months have been

.(recorded with some strains of P. vivax(P. vivax hibernans

The incubation period is to be distinguished from the prepatent period, which

is the interval between the entry of the parasites into the host and the time when

they first become detectable in blood. The minimum level of parasitaemia for their

microscopic detection is called the microscopic threshold. This is about 20 to 25 parasites

per cu. mm. Clinical disease develops only later, when after a number of further

cycles of multiplication, the level of parasitaemia rises high enough to cause ,fever

the so-called fever thresholdor pyrogenic density. The first clinical illness marking the

.end of the incubation period is called the primary attack

,The typical picture of malaria consists of periodic bouts of fever with rigor

followed by anaemia and splenomegaly. True rigor is typically present in vivax malaria

and is less common in falciparum infection. The febrile paroxysm comprises three

successive stages. In the cold stage, lasting for 15 to 60 minutes, the patient experiences

intense cold and uncontrollable shivering. This is followed by the hot stage, lasting

for 2 to 6 hours, when the patient feels intensely hot. The fever mounts to 41°C

or higher. Severe headache, nausea and vomiting are common. 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

.The paroxysm usually begins in the early afternoon and lasts for 8 to 12 hours

.The periodicity of the attack varies with the species of the infecting parasite

The periodicity is approximately 48 hours in tertian and 72 hours in quartan .malaria

Quotidian periodicity , with the fever occurring at 24 hour intervals may be due

to two broods of tertian parasites maturing on successive days, or due to mixed

infection. Regular periodicity is seldom seen in the primary attack, but is established

.usually only after a few days of continuous, remittent or intermittent fever

All clinical manifestations in malaria are due to the products of erythrocytic

schizogony and the host’s reactions to them. The exoerythrocytic liver cycle and

gametogony do not appear to contribute to clinical illness. The febrile paroxysms

follow the completion of erythrocytic schizogony, when the mature schizont ,ruptures

releasing red cell fragments, merozoites, malarial pigment and other parasitic .debris

Macrophages and polymorphs phagocytose these and release large quantities of

endogenous pyrogens, leading to elevation of temperature. Cytokines such as tumour

necrosis factor (TNF) and interleukin-l may play a pivotal role in the pathogenesis

.of malarial fever

Recrudescence and Relapse

After a number of paroxysms, the primary attack subsides with the development

of partial immunity in the host. This is followed by a period of latency during which

,there is no clinical illness or sometimes even parasitaemia. The parasites are not

however, eliminated at this stage, but persist in some erythrocytes, though the level

of parasitaemia is below the fever threshold, or sometimes even below the microscopic

threshold. Erythrocytic schizogony continues in the body at low levels and gradually

the numbers of parasites build up to cross the fever threshold. Fresh malarial attacks

then develop. These new malarial attacks that appear after a period of latency usually

within eight weeks after the culmination of the primary attack and resulting from

.persistence of the erythrocytic cycle of the parasites are called recrudescences

Recrudescence may be due to waning immunity of the host or possibly to antigenic

variations in the parasite. There may be several such recrudescences, which are ,generally milder than the primary attack. After a varying number of such attacks .the infection is eliminated in P. falciparumand P. malariaeinfections

In P. vivaxand P. ovaleinfections the parasites may survive for long periods in a dormant exoerythrocytic stage as hypnozoites in liver cells. Reactivation of hypnozoites leads to initiation of fresh erythrocytic cycles and new attacks of malarial fever. Such new attacks of malaria caused by the dormant exoerythrocytic forms being reactivated after long periods, usually from 24 weeks to 5 years after the .primary attack are called relapses

The term recurrence has been used to refer to both recrudescence and relapse and so carries no specific meaning. Several factors including stress, intercurrent infection, pregnancy and alcoholism have been proposed as precipitating causes for .recurrences

Malignant Tertian Malaria

The most serious and fatal type of malaria is malignant tertian (MT) 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 supervenes in acute falciparum malaria. These may present in various forms, the most important of which are the cerebral, algid and septicaemic varieties. These occur following heavy parasitisation of red cells. The

parasitised red cells become deformed, sticky and adhere on the capillary endothelium

in internal organs causing anoxic damage, oedema and inflammatory reaction. Cerebral

malaria is characterised by hyperpyrexia, coma and paralysis. Algid malaria resembles

surgical shock, with cold clammy skin, peripheral circulatory failure and profound

hypotension. Gastrointestinal symptoms such as vomiting, dysenteric or choleraic

diarrhoea may occur. Some cases develop severe hiccup, with profuse bilious ,vomiting

,a condition formerly called bilious remittent fever. In septicaemic malaria

characterised by a high degree of prostration, there is high continuous fever with

involvement of various organs. Acute renal failure and acute pulmonary oedema

.are other serious complications

Blackwater Fever

A syndrome called blackwater fever(malarial haemoglobinuria) 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

manifestations include bilious vomiting and prostration, with passage of dark red

or blackish urine (blackwater). The pathogenesis is believed to be massive intravascular

haemolysis caused by antierythrocyte autoantibodies, leading to haemoglobinaemia

.and haemoglobinuria

Anaemia

Anaemia occurs in all types of malaria, but is most pronounced in falciparum .infections

The type of anaemia is haemolytic, normocytic, normochromic. The degree of anaemia

.is greater than what could be explained by the destruction of parasitised red cells

In addition, there occurs increased destruction of red cells possibly by autoimmune

.mechanisms, and decreased erythropoiesis

Splenomegaly

The spleen is invariably affected, being always enlarged in malaria. The initial change

is congestion, leading to a soft enlargement. Later, it becomes dark due to

accumulated malarial pigment. Diffuse cellular hyperplasia, dilated sinusoids and

accumulation of macrophages accentuate the enlargement of spleen, which becomes

.hard due to fibrosis

Tropical Splenomegaly Syndrome

Tropical splenomegaly syndrome (TSS) also known as hyper-reactive malarial

splenomegaly (HMS) is a chronic benign condition seen in some adults in endemic

areas, mainly tropical Africa, New Guinea and Vietnam. This results from an abnormal

immunological response to malaria and is characterised by enormous ,splenomegaly

high titres of circulating antimalaria antibody and absence of malaria parasites in

peripheral blood smears. Hyperimmunoglobulinaemia (IgM, but not IgG), cryoglobulinaemia, reduced C3 and presence of rheumatoid factor without arthritis are

other features. A normocytic normochromic anaemia is present, not responding to

haematinics or anthelmentics. TSS differs from various other types of splenomegalies

seen in the tropics in its response to antimalarial treatment, and histological changes

in spleen (dilated sinusoids lined with reticulum cells showing ,erythrophagocytosis

lymphocytic infiltration of pulp) and liver (marked sinusoidal infiltration with

.(lymphocytes

Malaria Parasites 87

The liver is also congested, enlarged and pigmented. Numerous pigment-laden

Kupffer cells dot the liver. Changes are also seen in bone marrow, kidney and .adrenals

Cerebral Malaria

In cerebral malaria, lesions occur in the central nervous system. These consist of

congestion of the meninges and brain, occlusion of capillaries in brain, numerous

petechial perivascular haemorrhages, and necrotic lesions in mid zonal brain ,tissue

.with peripheral glial reaction (malarial granuloma) around occluded blood vessels

Merozoite-induced Malaria

Natural malaria is sporozoite-induced, the infection being transmitted by sporozoites

introduced through the bite of vector mosquitoes. Injection of merozoites can lead

to direct infection of red cells and erythrocytic schizogony with clinical illness. Such

.merozoite-induced malaria may occur in the following situations

Blood transfusion can accidentally transmit malaria if the donor is infected with

malaria. The parasites may remain viable in bank blood for 1 to 2 weeks. The incubation

period in transfusion malariadepends on the number of parasites introduced and the

.species. It varies from 10 days in P. falciparumto 40 days or longer in P. malariae

Malaria can also be transmitted by procedures other than transfusion when small

quantities of blood are conveyed from one person to another. Shared syringesamong

drug addicts may be responsible. Renal transplantationmay lead to malaria if the

.donor had parasitaemia

Therapeutic malariais a special type of merozoite-induced malaria which was used

.formerly as a treatment for late syphilis

Congenital malariaa natural form of merozoite-induced malaria where the parasite

.is transmitted transplacentally from the mother to the foetus

.Merozoite-induced malaria causes febrile paroxysms as in the natural disease

But it is self-limited and undergoes spontaneous cure due to the absence of any

.exoerythrocytic stage

Immunity

.Immunity in malaria may be classified into innate and acquired types

Innate Immunity

Only little is known about innate immunity in malaria, but a few naturally occurring

.examples illustrate its importance

The invasion of red cells by merozoites requires the presence of specific glycoprotein

receptors on the erythrocyte surface. It has been found that persons who lack the

Duffy blood group antigen (Fya Fyb) are refractory to infection by P. vivax. This

blood group antigen appears to be the receptor for the malarial parasite. Duffy blood

group is absent in the native population of West Africa. This may be one reason

.why vivax malaria is not prevalent there

P. falciparumdoes not multiply properly in sickle red cells containing the abnormal

haemoglobin S. Sickle cell trait is very common in Africa where falciparum infection

is hyperendemic. It has been proposed that the sickle cell trait, which is otherwise

undesirable has been conserved there because of the survival advantage it offers

in falciparum malaria. Haemoglobin F present in neonates protects them from .malaria

Innate immunity to malaria has also been related to the G6PDH deficiency found

in the Mediterranean coast, Africa, the Middle East and India. HLA-B53 is associated

.with protection from malaria

There is some evidence that severe malnutrition and iron deficiency may confer

some protection against malaria. It was observed that during severe famine in North

Africa malaria was rare, but on providing food and iron supplements, the patients

began to develop clinical malaria. Falciparum malaria is more severe in ,pregnancy

.particularly in primigravida, and may be enhanced by iron supplementation

.The spleen appears to play an important role in immunity against malaria

.Splenectomy enhances susceptibility to malaria

Acquired Immunity

Infection with malaria parasites induces specific immunity which can bring about

.clinical cure, but cannot lead to complete elimination of parasites from the body

It can prevent superinfection, but is not powerful enough defence against re-infection.

This state of resistance in an infected host, which is associated with continued

asymptomatic parasitic infection is called premunition. The host is resistant to fresh

infection (superinfection) as long as the pre-existing infection continues even though

in subclinical form. But once the infection is eradicated, the immunity does not persist

.(for long and is not capable of preventing subsequent infection (re-infection

Specific immunity is evident in endemic areas where infants below the age

of 3 months are protected by passive maternal antibodies. Young children are

highly susceptible to malaria. As they grow up they acquire immunity by subclinical

or clinical infections so that the incidence of malaria is low in older children and

.adults

The antigenic fractions of malaria parasites have been investigated in detail. The

four species of human parasites have both common as well as species-specific .antigens

Within each species, the different stages in the life-cycle have stage-specific .antigens

The practical importance of these studies is in the development of vaccines and for

serological diagnosis of malaria. Immunity appears to be strain-specific and one

infection may not be protective against infection by a different strain of the same

species of thc parasite. In endemic areas, repeated infections by multiple strains

.broadens the scope of immunity

Experimental studies on immunisation against malaria date back to early in the

th century. Injection of erythrocytic parasites with Freund's complete adjuvant٢٠

was shown to induce immunity in monkey malaria, but this was not practicable in

humans. It is only recently, after successful continuous culture of malaria ,parasites

the availability of monoclonal antibodies and the development of cloning techniques

Malaria Parasites 89

that significant progress was achieved in this field. Several possibilities are being

.tested for the immunoprophylaxis of malaria

Immunisation against the sporozoite antigens could check the first step in human

infection by blocking the invasion of liver cells. An antigenic surface component has

been identified on the sporozoites. This ‘circum-sporozoite protein’ has been cloned

and its immunodominant epitope identified. The epitope sequence consisting of a

small number of amino acids has been chemically synthesised. Its gene has been

introduced into the vaccinia virus and the recombinant virus has been shown to

.produce the sporozoite antigen

Several other antigens have been considered as potential vaccines, including the

merozoite surface protein-l, apical membrane antigen, erythrocyte binding ,antigen

a soluble antigen released during rupture of parasitised erythrocytes and a zygote

.antigen Pfs 25

A vaccine that has undergone several field trials is the spf 66 vaccinedeveloped

by Manuel Patarroyo in Columbia. This is a synthetic peptide containing the amino

acid sequences of three P.falciparummerozoite proteins linked together by a

tetrapeptide from the circumsporozoite protein. Field trials in South America and

Tanzania showed moderate protection, but in Gambia and Thailand it was much

.less effective

A method of blocking mosquito transmission has been proposed, by immunising

malaria patients or carriers with vaccine containing gamete or zygote antigens. When

mosquitoes feed on them, the antibodies sucked in along with gametocytes prevent

sporogony taking place. This method has been termed transmission blocking .immunity

An ideal malaria vaccine should be one inducing multistage, multivalent, multiimmune response. Nothing approaching this is available at present. Much work is

.being done in developing DNA vaccines to meet these requirements

Cell-mediated immunity is operative in malaria, but little is known about its scope

.and importance. Malaria does not appear to be aggravated by AIDS

Immunopathology

Malaria is known to produce some depression of the immune system. It has been

suggested that immune depression caused by endemic malaria is responsible for

the Burkitt's lymphoma seen in African children. While the Epstein-Barr virus causes

asymptomatic infection or infectious mononucleosis in immunocompetent ,persons

in African children whose immune system is severely compromised by recurrent

.malaria infection, the virus leads to lymphoma

Parasitised erythrocytes may undergo antigenic changes, which may lead to

autoimmune phenomena. Immune complexes occur in malaria. These may lead to

.nephropathies

Laboratory Diagnosis

The most important method for the diagnosis of malaria is the demonstration of

the parasite in blood. Clinical diagnosis of malaria can be made with considerable

confidence in residents of endemic areas and recent visitors, but confirmation requires

.the finding of parasites in blood smear

All asexual erythrocytic stages as well as gametocytes can be seen in peripheral

blood in infection with P.vivax, P. ovaleand P. malariaebut in P. ,falciparuminfection

only the ring form and gametocytes can be seen. Late trophozoites and schizont

stages of P.falciparumare usually confined to the internal organs and appear in

.peripheral blood only in severe or pernicious types of MT malaria

,The parasites are most abundant in peripheral blood late in the febrile paroxysm

a few hours after the peak of the fever. Therefore. blood smears ideally should

be collected at this period. In practice, it is advisable to obtain a blood smear when

the patient is first seen, and then a few hours after the height of the fever. In smears

taken between paroxysms the parasites may be scanty or absent. This is particularly

so in falciparum malaria. Repeated blood smears have to be examined before a

negative result is given. If finger prick smears cannot be obtained, blood sent in

.EDTA tubes may be used instead for making smears

Two types of blood films are prepared for examination—the thick and the thin

films. They can be made on separate slides, or more conveniently on the same .slide

After cleaning with ether or spirit and drying, the finger tip is pricked and gently

squeezed till a good drop of blood exudes. The drop of blood is touched with a

clean dry slide, near one end. The blood on the slide is spread with the corner of

another slide to produce a square or circular patch of moderate thickness. This is

the thick film. When correctly prepared the thick film will just allow printed letters

to be read through it. For preparing a thin film. collect a small drop of blood on

the slide, away from the thick film and separated from it by a line drawn with a glass marking pencil. The blood is spread evenly and thinly with the edge of a spreader slide. A properly made thin film will consist of an unbroken smear of a single layer .of red cells, ending in a tongue which stops a little short of the edge of the slide .The slide is kept flat protected from dust, to dry

Chinese workers recommend intradermal smears taken from multiple punctures ,on the upper forearm using a 25-gauge needle. The punctures should not bleed but a serosanguinous fluid can be expressed on to a slide by squeezing. This is claimed .to be more sensitive than peripheral blood smears

The thin film is fixed in methanol for 30 seconds. The thick film is not to be fixed as it is to be dehaemoglobinised. Diluted Giemsa stain is applied over both thick and thin films and allowed to stand for half to two hours. The slide is then washed and dried. A rapid method of staining, particularly useful in field work is the Field's stain. The rapid method commonly employed in India is the JSB stain .named after Jaswant Singh and Bhattacharji

The stained film is examined under the oil immersion microscope. The thick film is more sensitive, when examined by an experienced person, because it concentrates to 30 layers of blood cells in a small area. The dehaemoglobinised and stained ٢٠ thick film does not show any red cells, but only leucocytes and, when present, the parasites. But the parasites are often distorted in form, and as the diagnostic changes in blood cells such as enlargement and stippling cannot be made out, species

identification is difficult. In falciparum malaria, the presence of gametocyte crescents

makes species identification simple. It is recommended that 200 oil immersion fields

.(should be examined before a thick film is declared negative (see Fig. 5.2

When parasites are found, an approximate quantitative estimate may be given

.as follows

parasites per l00 thick films fields ١٠-١ +

parasites per 100 thick film fields ١٠٠-١١ ++

parasites per each thick fIlm field ١٠-١ +++

.More than 10 parasites per each thick film field ++++

The morphology of the parasites is preserved in thin films and so species

.identification is easy in them

Some other tests have been introduced to simplify malaria diagnosis. In the QBC

test (Beckton-Dickinson, USA), a small quantity of blood (50 to 110 µl) of blood is

spun in the QBC centrifuge. The parasites get concentrated near the tip of the RBC

column. Pre-coating of the tube with acridine orange induces a fluorescence on the

parasites which can then be readily visualised under the oil immersion .microscope

The QBC method is sensitive and specific and has been widely accepted as a rapid

test for malaria. Many other similar tests have been developed, but none can replace

the thick and thin smear which alone can reveal the parasite morphology clearly

enough for accurate identification of the species. A careful and patient smear

.examination still remains as the ‘gold standard’ in malaria diagnosis

Another useful approach is immunodiagnosis of malaria by detection of parasitespecific antigens using monoclonal antibodies The Para Sight–F test(BD) is a dipstick antigen capture test targeting the “histidine-rich protein–2” (HRP-2), specific

for P.falciparum.The test is sensitive, specific and rapid, results being ready in ten

minutes. It is of interest that the Para Sight-F test has enabled the diagnosis of malaria

in ancient Egyptian and Nubian mummies, demonstrating the remarkable stability

.of the HRP-2 antigen over thousands of years

.A dip-stick test targeting species specific lactic dehydrogenase is also available

It is useful not only in diagnosing malaria, but also in confirming cure after ,treatment

because the test will detect only live parasites, and so will be negative if the parasites

.have been killed by treatment. Dipstick tests are also available for vivax malaria

Molecular methods such as dot-blot assay, DNA probes and PCR amplification are

.not useful in routine diagnosis, but may be used in special situations

Serological tests are not employed for routine diagnosis. Several serological tests

have been described for detection of specific malaria antibodies. These include indirect

immunofluorescence test (IFAT), indirect haemagglutination (IHA), immunoprecipitation (gel diffusion), ELISA, RIA. IFAT uses erythrocytic schizonts on a slide

as antigen. It detects IgM, IgG and IgA antibodies. Antibodies appear within a few

days of clinical illness and persist for months or years. It is of some use for diagnosis

of cases, particularly in between recrudescences in nonendemic areas. IFAT can also

be used to detect parasitaemia. lHA uses antigen-coated erythrocytes. The technique

is simple and suitable for testing large numbers of sera. The test becomes positive

even before parasites appear in blood. However, false-positives are common. Gel

diffusion percipitation is simple and convenient for screening purposes, but its

sensitivity is low. ELISA is highly specific, but less sensitive. It is best suited for

screening large batches of sera. RIA is costly, complex and suited only for .research

Serological tests are more often employed for seroepidemiological surveys than for

diagnosis of individual cases as presence of antibody need not indicate active .infection

It is better to use homologous antigens for the test, but in their absence, related

.monkey malaria antigens can also be employed

Epidemiology

Malaria had been recorded in places as far north as Archangel, Russia and as far

south as Cordoba. Argentina, in places as low as the Dead Sea (400 metres below

sea level) and as high as Cochabamba, Bolivia (2800 metres above sea level). ,However

malaria is essentially a focal disease and its distribution is patchy in most parts

.(Fig. 5.9)

Till the 19th century, it was prevalent in Northern Europe, Russia and North

America, but it has disappeared from those areas and is now confined to the tropics

and subtropics. However, imported cases of malaria are not infrequent in nonendemic

areas, due to the entry of infected persons. Thousands of such cases are recorded

in the USA and Europe every year .In addition, a few instances of introduced malaria

occur in nonendemic areas through infected mosquitoes from endemic areas entering

as stowways in jet planes. These infect residents near airports and, if vector mosquitoes

.are present, can initiate small local outbreaks

The relative prevalence of the four species of malaria parasites varies in different

geographical regions. P. vivaxis the most widely distributed, being 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.malariaeis 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

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 terms stable and unstable malaria have been frequently employed to refer

respectively to endemicity without fluctuation, and to highly variable degrees of

.malaria transmission

:Endemic malaria has been classified into the following types

Hypoendemic: When transmission is low and malaria is not an important problem

.in the area

Mesoendemic: Intensity of transmission is moderate and varies depending on local

.circumstances

.Hyperendemic: When transmission is intense, but seasonal

.Holoendemic: When transmission of high intensity is constantly present

Malaria Parasites 93

FIGURE 5.9:Global distribution of malaria

The above classification is based on the results of malaria surveys. The basic

investigations in malaria surveys concern data regarding the human host, the vector

mosquito as well as environmental conditions. Two measurements made in the

population are the spleen rate, which is the proportion of children aged 2 to 10 years

in a population, with enlarged spleens, and the parasite rate, which is the proportion

of persons in the populati