**MALARIA- a brief review**

**Introduction**

Malaria is a protozoan disease of humans. Malarial parasite belongs to the genus *Plasmodium.*

*P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* are five species of Plasmodium which exhibit different clinical pictures.

**History**

History of malaria dates back to 1700 BC in  China when *“Nei Ching – the Canon of Medicine”,* described splenomegaly and repeated paroxysm of fever which had a tendency to cause epidemics. Hippocrates described the various malaria fevers of human by 400 BC.

Malaria was linked with poisonous vapors of swamps or stagnant water on the ground from time immemorial. The term malaria (from the Italian mala *“bad”* and aria *“air”*) was given by the Italians to define the fevers associated with exposure to marsh air or miasma

The first medicine used for the treatment of malaria was discovered by the Chinese during the second century BC. *52 Remedies*, a medical treatise, documented the use of the Qinghao plant (Artemisia annua) to prevent malaria. However, the anti fever qualities of the plant were described in medical text in 340 CE

**Early seventeenth century:**

Quinine, is a constituent of the bark of the cinchona (quina-quina) tree, was used to treat malaria from as early as the 1600s, when it was referred to as the "Jesuits' bark," "cardinal's bark," or "sacred bark." The bark had been identified when it cured the Countess of Chinchon, the wife of the Viceroy of Peru, who had contracted the malarial fever. The tree was named Cinchona after the countess, and the bark was called Peruvian bark.

**Discovering the malarial parasite (1880):**

In 1880 **Charles Louis Alphonse Laveran,** a French army surgeon, discovered the malarial parasites in an unstained preparation of blood smear.

Six years later, **Camillo Golgi** demonstrated the multiplication of asexual blood forms.

Ross demonstrated the life cycle of the parasite in the stomach of mosquitoes and was awarded the Nobel Prize in 1902 for his contribution.

**Malaria nomenclature:**

In 1890, *Plasmodium vivax* and *Plasmodium malariae* named by Giovanni Batista Grassi and Raimondo Filetti.These were the first species of *Plasmodium* to be named. In 1897, William H. Welch renamed Oscillaria malariae, *P. falciparum*, which is the cause of tertian periodicity fever. In 1922, John William Watson Stephens named and described *Plasmodium ovale*.

**Hellemond *et al.* (2009)** reported that **Robert Knowles and Biraj Mohan Das Gupta** in 1931 discovered *Plasmodium knowlesi* in a long-tailed macaque (a type of monkey). The first case of malaria in human caused by *P. knowlesi* was documented in 1965.

**Vector:**

*Anopheles culicifacies* is the principal vector of rural malaria, but is considered as insignificant in the NorthEast region of India. *An. stephensi* is the primary urban vector. *An. fluviatilis* is a vector in the hills and foothills. *An. minimus*, *An. nivipes*, *An. Philippinensis* and *An. baimaii* are vectors in northeast India whereas *An. sun-daicus* is restricted to Andaman and Car Nicobar islands. *An. annularis* and *An. varuna* are secondary vectors with wide distribution.

**Geographical distribution:**

India has a very unique epidemiology of malaria where the rate of disease transmission varies from region to region. For example, Northeast or eastern parts of the country are highly endemic to malaria. On the other hand, Central India is mesoendemic, while many northern parts of the country are either non-endemic or have low malaria endemicity. For this reason, the antimalarial drug policy of the country varies from region to region

*P. falciparum* and *P. vivax* are the two major human species in India.

**Seasonal Variation:**

Malarial infection is maximum in India during the months of June to September i.e. during the rainy season. Incidence of *vivax* malaria is highest in the early part of the rainy season, however the epidemiological picture is different in *falciparum* malaria; cases show a delayed peak and continues at a relatively higher level beyond September when the breeding of mosquito is infrequent.

**Human host:**

Falciparum malaria is more severe in non-immune adults and rarely affects the malnourished children and characteristically. *Falciparum* malaria infection is more severe in pregnancy. Persons with the sickle cell trait (heterozygotes for the abnormal hemoglobin gene HbS) are protected against *P. falciparum* malaria.

**Lifecycle:**

The life cycle of *Plasmodium* shows an alternation of generation accompanied by alternation of the host. i.e. mosquito and human cycle.

(1) Human cycle: This cycle has three distinct phages –

(i) Pre-erythrocytic Schizogony

(ii) Erythrocytic Schizogony

(iii) Gametogony

**Human cycle:**

Human cycle starts with the introduction of sporozoites by the bite of an infected anopheline mosquito.

(i) **Pre-erythrocytic Schizogony:**

The sporozoites inoculated by the mosquito, travel through the bloodstream to the liver where they invade hepatocytes and mature to tissue schizonts called merozoites. This cycle lasts from an average of 5.5 days (*P. falciparum)* to 15 days (*P. malariae)*.

(ii) **Erythrocytic schizogony:**

The Merozoites released from the liver are capable of invading human red blood cells and establish the asexual cycle of replication. The parasite passes through the stages of trophozoite, schizont and merozoite. These asexual forms of parasites can be demonstrated in the thick smear of peripheral blood  3 to 4 days after the completion of pre-erythrocytic schizogony. The cycle of erythrocytic schizogony lasts 48 to 72 hrs, in Plasmodium vivax, ovale and falciparum it is 48 hrs whereas in P. malariae it is 72hrs

(iii) **Gametogony:**

After the parasites have undergone erythrocytic schizogony**, some  of the merozoites give rise to sexual forms called gametocytes and develop in the red blood cells of the capillaries of internal organs.** Only mature gametocytes are found in peripheral blood. The maturation is complete in 96 hours. Gametocytes do not cause febrile illness in the human host rather they harbor the parasites in the blood for continuation of species and are called as carriers.

**Latent stage:**

The initial tissue phase disappears completely in P.falciparum whereas it persists as dormant form in P. vivax and P.ovale, which are known as hypnozoite. These hypnozoites are responsible for relapses in vivax,ovale.

**Mosquito cycle:**

When a female anopheles mosquito bites an infected person, it ingests the sexual and asexual forms of the parasite but only the mature sexual forms are capable of development and rest die immediately.

**Clinical features:**

Malaria is a pyrexial illness. The clinical course is variable.The typical clinical picture consists of:

 (a) Prodromal period

 (b) Acute febrile phase

 (c) Uncomplicated or complicated malaria and

 (d) Chronic complications of malaria.

**Incubation period:**

The duration of the incubation period is usually between 10-12 days.

**Prodromal symptoms:**

It  includes malaise, anorexia, lassitude, body ache, headache and chills and appears 2-4 days before the onset of fever. Fever is classically intermittent with characteristic periodicity.

**Cold Stage:**

The patient feels sick with chills and appears pale with cold extremities and tachycardia and this stage lasts for 20 minutes to 1 hour.

**Hot Stage:**

The temperature rises to 39 degree Celsius–41 degree celsius, headaches persist, skin becomes dry, hot and flushed. This stage lasts for 1-4 hours.

**Sweating:**

In this stage there is profuse sweating with rapid decline in the temperature to normal or sub-normal level. Skin becomes cold and moist and BP is relatively low.

**Uncomplicated *falciparum* malaria:**

In uncomplicated *falciparum* malaria, various combinations of anemia, splenomegaly and hepatomegaly may occur besides prodromal symptoms. Abdominal discomfort, acute abdominal pain, constipation or diarrhea and dry cough have also been reported. Splenomegaly is one of the most important sign of malaria and usually palpable within 10 days. Tender hepatomegaly usually occurs during the acute attack. Mild icterus due to haemolysis can also occur.

Individuals who have acquired immunity or resistance to local parasite strain, as a result of previous infection; the effect of further infection are considerably modified.

**Complicated malaria or severe malaria:**

World Health Organisation has defined severe malaria as one or more of the following criteria + the presence of asexual parasitaemia.

**i.Cerebral malaria/unarousable coma:** Not attributable to any other cause in a patient with *falciparum* malaria. Coma should persist for at least 30 minutes after a generalized convulsion.

**ii.Severe anemia:** Normocytic normochromic anemia with haematocrit < 15% or hemoglobin <5 gm/dl in children and < 7 g/dL in adults in the presence of parasitaemia > 1,000/microl.

**iii. Renal failure:**Urine output of < 400 ml/24 hrs in adults and < 12 ml/kg body weight in children. No improvement with rehydration and a serum creatinine level > 3 mg/dl.

**iv. Pulmonary oedema/adult respiratory distress syndrome (ARDS)**

**v. Hypoglycaemia:** Plasma glucose level of < 40 mg/dl (2.2mmol/l).

**vi. Hypotension/shock:** Systolic BP< 50 mmHg in children aged 1-5 years or < 80 mmHg in adults, with cold, clammy skin, or core/skin temperature difference of ≥ 10°C.

**vii. Bleeding/Disseminated Intravascular Coagulation (DIC):** Significant bleeding from gums, nose, and GIT and evidence of DIC.

**viii. Convulsion:** Repeated generalized convulsions > 2 within 24 hrs, despite cooling.

**ix. Acidosis/Acidaemia:** Arterial pH < 7.25 or plasma bicarbonate level of < 15 mmol/l. Venous lactate level of > 15 mol/l. Manifests as labored, deep breathing.

**x. Macroscopic haemoglobinuria:** Black, brown, or red urine not associated with effect of oxidant drugs and red blood cell enzyme defects (such as G6PD deficiency).

**Additional criteria of severe malaria:**

**i. Impairment of consciousness** less marked than unarousable coma.

**ii. Prostration and extreme weakness –** Patients cannot sit or walk with no obvious neurological explanation.

**iii. Hyperparasitaemia:** Very high parasite densities are associated with increased risk of severe disease but is affected by the immune status (more than 5% parasitemia in non-immune is serious, but may be well tolerated in semi-immune children)>500,000 per µL

**iv. Jaundice:** Detected clinically by S. bilirubin concentration > 3 mg/dl.

**v. Hyperpyrexia:** Rectal temperature above 40° C (104° F) in adults and children.

**Pathophysiology:**

The pathophysiology of *falciparum* malaria results from breakdown of erythrocytes, the liberation of parasite and erythrocyte materials into the circulation and the host reaction to these events. *P. falciparum* malaria infected erythrocytes are sequestered into the microcirculation of the vital organs interfering with microcirculatory flow and host tissue metabolism.

**(a) Cytoadherence:**

Cytoadherence begins in the first half (12 hours) of the parasite’s 48 hours asexual life cycle. Parasitized RBC expresses *P.falciparum* membrane protein-1 or *Pf* EMP-1 in its surface.

**(b) Sequestration:**

In this process erythrocytes containing mature forms of *P. falciparum* adhere to the microvascular endothelium and then disappear from circulation. Once infected RBC adhere, they do not enter the circulation again and remain stuck until they rupture at schizogony. Sequestration occurs predominantly in the venules of vital organs, being greatest in the white matter of brain, heart, eyes, liver, kidney, intestine and least in the skin.

**(c) Rosetting:**

Erythrocytes containing mature parasites adhere to the uninfected erythrocytes. This process leads to formation of rosettes, which starts slightly after the beginning of cytoadherence.

Cytoadherence and rosetting leads to microcirculatory obstruction in *falciparum* malaria, which leads to anaerobic glycolysis, lactic acidosis and cellular dysfunction.

**(d) Vascular endothelial ligand:**

Several sticky proteins present on the surface of vascular endothelium bind to the  parasitized RBC. These sticky proteins include – ICAM-1, thrombospondin, VCAM etc.

**(e) Cytokine imbalance:**

Malarial parasites induce the release of cytokines. Malaria antigen related IgE complexes also activate cytokines release. But compared with bacterial endotoxin malarial parasites are not very toxic.

Cytokines up-regulate the expression of vascular ligands thus promote cytoadherence. They may also be important mediators for parasite killing, by activating leukocytes and possibly other cells to release toxic oxygen species, nitric oxide, lipid peroxidase. Cytokines are responsible for many of the signs and symptoms, particularly fever and malaise in malaria.

**Immunity:**

Genetic factors like sickle-cell trait (carriers of Hb-S), glucose-6-phosphate dehydrogenase (G6PD) deficiency and Duffy blood group negativity and thalassemia have protective effects against *P. falciparum* malaria and P. vivax infection, respectively.

**Acquired immunity:**

Infants are protected during their first months of life through transfer of maternal antibodies and also by fetal Hb.

Protection is more rapidly acquired in high endemic areas and results in reduced mortality or severe clinical disease already by the age of 5 years. In the absence of continual exposure, the immunity against clinical disease may be relatively short lived.

**Diagnosis of malaria:**

The gold standard for diagnosis of malaria is microscopy. There are various staining methods for malarial parasites.

**Giemsa staining (WHO)**

Giemsa is a classical stain used for microscopy. It consists of Giemsa powder, glycerol and methyl alcohol (methanol). A 5% Giemsa for 20-30 minutes is used for both thin and thick smears. The sensitivity of a thick smear is 15-20 times higher than a thin film but does not allow for species determination. In the thin smear the parasites are seen within the RBC and species identification is done.

**Jaswant Singh Battacharya (JSB) stain**

This is the standard method used by the laboratories under the National Malaria Eradication Programme in India.

**Preparation of the stain**

**JSB I stain:**

Methylene blue is dissolved in 500 ml of distilled water and 3 ml of 1% sulphuric acid is gradually added, followed by 0.5 g of potassium dichromate  when a purple precipitate forms. 3.5 g of disodium hydrogen phosphate dihydrate is next added and when the precipitate has dissolved, the solution is boiled for 1 hour. The stain is used immediately.

**JSB II stain:** 1 g eosin is dissolved in 500 ml water.

**Fluorescent staining techniques:**

Blood smears can be stained by fluorescent dyes, particularly with acridine orange. The fluorescent dye has affinity to the nucleic acid in the parasite. It  is 81%-100% sensitive and 86%-100% specific.

**Quantitative buffy coat:**

Quantitative Buffy Coat (QBC)  micro capillary tubes stained with acridine orange are observed under the fluorescence microscope near the buffy coat region where parasites are concentrated. The sensitivity of this method is comparable with Giemsa but species identification is not possible.

**Serology (Antigen detection tests):**

The antigens of malarial parasites can be rapidly identified by using rapid immunochromatographic techniques.

These commercially available kits are based on the detection of the histidine-rich protein 2 (HRP-II) , lactate dehydrogenase of *P. falciparum*.

**Molecular methods:**

At present,PCR is the most sensitive method for detection of malarial parasites.

Themajor advantage of this method is in detecting mixed infections or differentiating between infecting species when microscopic examination is inconclusive.

**Malaria case definition (CDC,NNDSS, 2014)**

**Clinical description:**

The patient initially presents with fever, chills, sweats, headaches, muscle pains, nausea and vomiting with physical findings of elevated temperature, perspiration, tiredness and in severe cases confusion, coma, neurological focal signs, severe anemia, respiratory difficulties may increase the suspicion index for malaria.

**Laboratory criteria for diagnosis:**

Detection of circulating malaria-specific antigens using rapid diagnostic test (RDT)

OR

Detection of species specific parasite DNA in a sample of peripheral blood using a Polymerase Chain Reaction (PCR) test.

OR

Detection of malaria parasites in thick or thin peripheral blood films, determining the species by morphological criteria, and calculating the percentage of red blood cells infected by asexual malaria parasites (parasitemia).

**WHO criteria for complicated malaria.**

**Cerebral malaria/unarousable coma**

**Severe anemia**

**Renal failure**

**Pulmonary oedema/adult respiratory distress syndrome (ARDS)**

**Hypoglycaemia**

**Hypotension/shock**

**Bleeding/Disseminated intravascular coagulation**

**Convulsion**

**Acidosis/Acidaemia**

**Macroscopic haemoglobinuria**

**Additional criteria of severe malaria:**

**Impairment of consciousness**

**Prostration and extreme weakness**

**Hyperparasitaemia**

**Jaundice**

**Hyperpyrexia**

**Treatment modalities:**

**Chloroquine:**

It is the most commonly used medication to cure uncomplicated cases of malaria.

Chloroquine is found in highest concentration in the food vacuole of *Plasmodium*. The alkaline nature of chloroquine raises the pH of the vacuole. The rise in pH inhibits the digestion of the amino acids which the parasite acquires from degrading the hemoglobin of the host’s red blood cells. Thus, chloroquine prevents *Plasmodium* from producing protein and creating energy that is needed for survival.

**Quinine sulphate:**

Quinine is a cinchona alkaloid that belongs to the aryl amino alcohol group of drugs. Quinine has rapid schizonticidal action against intra-erythrocytic malaria parasites. It is also gametocytocidal for *Plasmodium vivax* and *Plasmodium malariae*, but not for *Plasmodium falciparum*.

Quinine inhibits nucleic acids, protein synthesis, and glycolysis of *P. falciparum*. It also inhibits the parasite’s mechanism of detoxifying heme by binding to the chemical used to convert hemozoin.

**Primaquine:**

Primaquine acts by producing a reactive oxygen species or by disrupting the electron transport of *Plasmodium.* Primaquine may change the DNA of the parasite.

**Amodiaquine:**

Amodiaquine is a 4-aminoquinoline which is  similar in structure and mechanism of action to Chloroquine.

It is more effective in parasite clearance as compared to chloroquine in uncomplicated malaria.

**Mefloquine:**

Mefloquine was developed during the Vietnam War. It was used to protect the American troops against multi-drug resistant *P. falciparum*.

**I**t is a very potent blood schizonticide with a long half-life. It is thought to act by forming toxic heme complexes that damage parasitic food vacuoles.

**Halofantrine:**

Halofantrine is a phenanthrene methanol. It acts as a blood schizonticide effective against all *Plasmodium* parasites. Its mechanism of action is similar to other antimalarials.

**Sulphadoxine pyrimethamine:**

Sulfadoxine-pyrimethamine (SP) has been widely used as first-line therapy for uncomplicated *P. falciparum* malaria throughout sub-Saharan Africa, because of its affordability and ease of administration.

**Proguanil:**

Proguanil developed in 1945 is a biguanide.

Its action is primarily mediated through conversion to the active metabolite cycloguanil pamoate. This inhibits the malarial dihydrofolate reductase enzyme. It acts on the primary tissue stages of *P. falciparum*, *P. vivax* and *P. ovale*. There are very few side effects to Proguanil, with slight hair loss and mouth ulcers.