Review Article

# Biology, Pathophysiology, Identification and Prevention of Malaria: A Review Paper on an Introductory Concepts and New Advances

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## Abstract

Malaria continues to be the main community health problem in numerous nations. Six species of Plasmodium are documented as the cause of human malaria infection. Among others, P. falciparum and P. vivax parasites produce an immense challenge in the public health. Anopheles funestus and Anopheles gambiae are the major transimmiter of the disease (malaria) from one person to another. The disease parasite has a complicated cycle of life that occurs in human and mosquitos. In general, malaria diagnosis is divided into parasitological and clinical diagnosis. Internationally, the death rate of malaria becomes reduced although few records from Ethiopia describe the presence of raised prevalence of malaria in certain areas. Apart from reduction in incidence and prevalence, transmission of malaria is continued throughout the globe. Hence, its control needs a combined approach comprising prevention. As a result of resistance development among commonly used insecticides and antimalarial drugs, 3 types of vaccines are under per-clinical and clinical studies. In addition to the old targets used to develop malaria vaccines and diagnostic tools, scientists are in search of new targets. However, the absence of clear knowlege on Plasmodium biology has created an obstacle in an endeavor to develop novel vaccines and other preventive techniques to combat malaria.

**Keywords:**

Malaria; Plasmodium; Vector control; Vaccine; Clinical trial

## Introduction

Malaria itself or a disease looks like malaria has been distinguished before 4, 000 years. Malaria is happened by the genus Plasmodium (mosquito-borne apicomplexan parasite).

At the time of bite by infected female Anopheline mosquitoes, this protozoal blood infection become conveying from one to the next person.

It is also defined as an illness brought by a parasite that lives some of the life in humans and some in mosquitoes.

Moreover, malaria case is noted as clinically compatible illness with a positive result of peripheral blood smear for Plasmodium [1].

This article aims to present introductory concepts, life cycle, cell biology, pathogenesis, diagnosis, control and preventive measures of malaria including past and current advances. It is therefore intended to serve as a reference material in resource limited nations.

This notion is also very important for undergraduate and postgraduate medical and health science students to access a little bit more information for their seminar papar regarding molecular biology, pathology, examination and prevention of malaria.

Malaria is a very old disease that could be occurred for the first time at ancient history of human beings. In 2700 BC, malaria symptoms were recorded in the Chinese Canon of Medicine. Egyptian papyri also mention malaria symptoms in around 1550 BC. In the 6th century BC, malaria-like fever affecting Mesopotamia was listed under Cuneiform tablets.



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Hippocrates was the first to describe malaria paroxysm as a disease in the century of 4th BC. In the 2nd century BC, Artemisia annua (the Qinghao plant) was described in the China medical treatise. The use of Peruvian bark of Cinchona tree (a source of quinine) to treat fever was recognized during early 17th Century. Even today, quinine is produced entirely from the natural sources due to the complexity of synthesising this molecule.

For the first time, humans may have acquired P. falciparum from gorillas. It is also believed that P. vivax is originated in African gorillas and chimpanzees but P. malariae species is very host specific to human beings of this world. Recently known parasite, knowlesi, might have instigated in an Asian long-tailed and pig-tailed macaque monkeys [2]. Such types of finding can provide information for efforts made on malaria eradication about potential zoonotic Plasmodium reservoirs and give clues regarding adaptive changes taken by ape Plasmodium to infect humans.

## Literature Review

### Malaria etiology and vectors

The causative agents for malaria infection are among the phyllum Apicomplexa, class sporozoa, order Haemosporidia, family Plasmodidae, and genus Plasmodium. Plasmodium is considered to be instigated from photosynthetic protozoa, which is known as dinoflagellate. Among >200 different Plasmodium species, around 14 species are pathogenic to humans. The remaining species affect animals, such as rodents, monkeys and reptiles. Six of the human pathogens, P. ovale sub-species (P. ovale curtisi and P. ovale wallikeri), P. falciparum, P. vivax and P. malariae are well known etiologic agents for human malaria. Infrequently, we could be naturally or accidentally infected by many simian species including P.knowlesi, P.cynomolgi, P. bastianelli, P. brasilianum, P.schwetzi and P.inui. Disease with knowlesi happens in individuals if an Anopheles mosquito previously diseased by a monkey malaria parasite bites humans. Incubation period (the time between the bite of mosquito and developing malaria symptoms) for falciparum, vivax and ovale, and malariae is 12, 14 and 30 days, respectively. But infections by P. malariae can exist in the blood for a very long period, maybe decades, without ever producing symptoms. Incubation period is different for different persons and depends on the amount of the parasite involved.

Falciparum and vivax create a huge challenge on public wellbeing. Falciparum is mostly common in African continent, and is responsible for the majority of deaths due to malaria. The development of Plasmodium species become slows as the temperature drops. When the temperature drops below 60 0F, P. vivax totally stops developing. P. falciparum can regrate to develop at a bit elevated temperatures. This effect elaborates why malaria parasites are present in temperate environments. Vivax has a wider geographic distribution since it can grow in its vector at lower temperatures. It can also stay alive at cooler climates and elevated altitudes. Despite it occurs in overall Africa, the risk of vivax infection is relatively low there due to lack of Duffy gene in most people of Africa. However, there is a supporting facts that vivax can be transmitted to negative Duffy blood group residents in Africa. P. ovale has an unusual distribution (present in West Africa, New Guinea and Philippines). Even if, malariae has been cleard off from temperate climates, it survives in the sub-region of Africa.

The genera Culex, Anopheles, Mansonia and Aedes mosquitoes may act as malaria vectors. Nonetheless, malaria is transmitted mainly *via* the bite of Anopheles mosquitoes, which comprise 537 known species and majority (87%) of them have been formally named. Nearly, 70 of these species are able to transmit Plasmodium parasite to human hosts and 41 of 70 are considered to be dominant vector species. A. gambiae and A. funestus are the most efficient vectors of malaria in the world. They are also the primary vectors of malaria in Africa. In Ethiopia, two primary vectors of Africa and A. pharoensis are recognized as the dominant malaria vectors. As the Plasmodum resides in red blood cells, malaria is also transmitted *via* donation of blood, transplantation of organ and sharing of needles or syringes contaminated by infected blood. A new born child could also acquire congenital malaria from her/his mother before/during birth. Moreover, transmission of malaria can largely be affected by global warming.

While some species grow in temperate climates and even continue to exist in the Arctic summer, majority of anopheline mosquitoes survive in tropical and subtropical regions. It was believed that anopheline mosquitoes are not breed on altitudes higher than 2,000 to 2,500 m. In this geographical boundary, there are a lot of malaria free places as its transmission is extremely reliant on the local environment and epidemiologic situations. Anopheline mosquitoes prefer comparatively clean water as their larval habitat (site for egg-laying and development of larvae) though species vary in the quantity of salinity and organic content and amount of sun exposure and temperature they prefer in their breeding sites. For example, city conditions can generate new spaces to mosquito larvae for development. Agricultural activities can also affect breeding site of mosquitoes. While the draining and drying of swamps removes the breeding areas of larvae, water-filled irrigation ditches could provide mosquitoes a new site for breeding. Egg, larva, pupa and adult (imago) are the four developmental phases of anopheline mosquitos. Adult males copulate to females in flight to provide adequate sperm for all subsequent egg-laying. To develop the first batch of their eggs, adult females require at least 2 blood meals but one blood meal is enough to develop each successive batch. As development of egg needs around 48 h, blood-seeking is recurring every two to three nights. Under most favorable conditions, the average lifetime of the female (adult) anopheline mosquito is equal to or more than three weeks. External factors including temperature, moisture and natural enemies could decrease its prolonged existence. Aadult males, in contrast, generally live a few days. If the mean ambient temperature goes beyond 35°C or humidity drops

below fifty percent, longevity is drastically decreased, directly affects malaria transmission. In most tropical regions, cases of malaria become increased at the time of rainy season as the rainfall expands breeding grounds. The adult male anopheline feeds on nectar, while the adult female feeds primarily upon blood of warm-blooded animals, predominantly mammals. Some female anopheline mosquitoes that have a preference towards humans are termed anthropophagic (anthropophilic). Others who choose animals, such as cattle, are expressed as zoophagic (zoophilic). The interval over which a mosquito is attracted to its favorite source of blood usually ranges 7-20 m. Many Anopheles mosquitoes are either nocturnal (active at night) or crepuscular (active at dusk or dawn). Some are endophagic (feed indoors) while others are exophagic (feed outdoors). After blood feeding, some of them wishs to rest indoors (endophilic) while others intended to rest outdoors (exophilic).

## Malaria morbidity and mortality

Approximately 229, 000,000 cases of malaria, most (94%) from the World Health Organization (WHO) African Region, are taken place globally in 2019. The disease was caused 409,000 deaths worldwide and most (94%) of which are also from the African Region. Most cases of malaria in Africa are resulted from P. falciparum. In 2019, global case incidence and mortality rate of malaria was reduced by 57 and 10%, respectively. Malaria continues to strike hardest against children and pregnant women in Africa. Children aged <5 years are the most exposed group affected by malaria, accounted 67% of global malaria deaths in 2019. In the USA, roughly 1,500-2,000 cases of malaria in recent travelers are reported every year. Pregnant mothers have high vulnerability to falciparum malaria. P. falciparum malria contributes 8 to 14 percent low birth weight in malaria-endemic areas, which in turn minimize the likelihood of a baby's survival. In addition to health and social related impacts, there is a huge financial burden of malaria in terms of lost working days. It was estimated that malaria deducts 1.3 percent from the economic development and 40% from public health costs of some countries in Africa. Malaria also affects third world countries in different aspects including tourism sectors.

Malaria is the most common infectious disease in Ethiopia. About 30, 485, 416 Ethiopians are living at high risk places to infection by malaria. In 2019, 213 deaths and 904, 496 confirmed cases due to malaria were reported by Ministry of Health (FMoH) from Ethiopia. In spite of reduced malaria incidence and mortality rate in Ethiopia since 2010, high occurrence of malaria was reported from certain areas in contrast to increased family level coverage of preventive measures. This rise may be related with households having weak socio-economic status. Ethiopia was attained only 50% of malaria reduction target of the millennium development goal. So, the country should reinforce its preventive and therapeutic strategies of malaria to accomplish the sustainable development targets.

## Malaria parasite life cycle

All types of malaria parasite have a similar and complex life cycle. The life cycle of every Plasmodium species infecting humans is distinguished by an exogenous sexual phase (sporogony), in which replication takes place in many Anopheles mosquito species, and an endogenous asexual phase (schizogony), which occurs in the vertebrate hosts. The sexual cycle is taken place in the gut and abdominal wall of some species of female mosquito, whereas the asexual cycle that causes the disease symptoms is taken place in the liver and RBcs of the humans. The life cycle within the mosquito takes approximately 8 to 35 days, after which the parasite is infective. When the mosquito bites the skin, the sporozoite (motile infectious form of the parasite) will be injected in to human's dermis and then searchs a blood vessel to feed from it. The insect discharges different vasodilators to raise the possibility of finding a vessel. It also salivates into our blood to avoid blood clotting. The destiny of these sporozoites is not clearly illustrated; however they can take one to two hour to exit from the dermis. The trap-like protein of the sporozoites plays a role to exit the dermis (using gliding motility) and enters to the blood-stream. Those sporozoites remained in the skin could be killed and drained by the lymphatics, where a host immune response is activated. After 30 to 60 minutes of the injection, the thread-like shaped sporozoites will be transported to the liver through the vascular system. One single sporozoite in one hepatocyte multiplies into tens of thousands of exoerythrocytic merozoites [Figure 1].

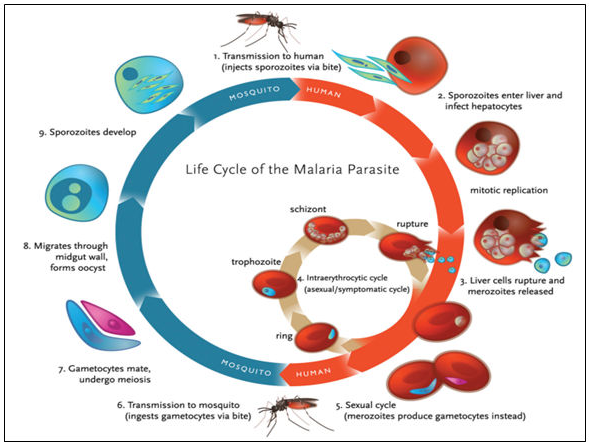


**Figure 1:** The life cycle of malaria parasites.

Within 7 to 12 days, the sporozoites develop into schizonts and then grow up to thirty thousand merozoites, which burst the liver cells. Alternatively, some of the sporozoite of vivax and ovale species turn into hypnozoites (dormant form) in the liver for months/years and can cause relapsed malaria. Unusually, the reappearance of falciparum malaria was observed in patient's years after departure of an endemic area. This indicates that P. falciparum has a dormant stage although occurs occassionally. Then, the asexual erythrocytic cycle begins and the merozoites start invading red blood cell to consume hemoglobin for their growth. The parasites then

multiply 10 times every 2 days, destroying RBCs and infecting new cells throughout the body. Inside the host red blood cell, the Plasmodium continues its maturity from the early ring stage to late trophozoite. Then, following mitotic divisions, the trophozoite undergoes to the schizont stage, which consists 6-32 merozoites depending on the Plasmodium species.

The period from acquiring infection through mosquito bite and the first appearance of the trophozoites in RBCs is called "prepatent period". This constant time is the characteristic of every species. It lasts 9 days in falciparum, 11 up to 13 days in vivax, 10 up to 14 days in ovale, 15 days in malariae and 9 up to 12 days in knowlesi. When the blood schizont bursts, the discharged merozoites maintain the life cycle through invading the neighbor red blood cells until it is brought under control. The rupture of schizonts is accompanied by the manifestation of the malaria febrile paroxysm typically lasting 8-12 h ("Golgi cycle") and characterized by 3 stages. The first stage (cold stage) is manifested by the quick rise of the temperature together with chills (sensation of the extreme cold). The patient desires to cover with the blankets. The second stage (hot stage) is with the temperature peak (may rises to 41°C), skin vasodilatation, myalgia and very severe headache. Patients feel too burning hot and cast their clothes. During the third stage (sweating stage), the patients have profuse sweating and their fever become drops. Then after, the patients may go to sleep due to tiredness. The typical (classical) symptoms which are stated above may not be appeared in some patients. Cyclical fevers are classically occurs soon before or during lysis of RBC (schizonts rupture). This happens every 48 h in tertian malaria (vivax, ovale and falciparum), and every 72 h in malariae infection (quartan malaria). At the time of this repetitive cycle, some merozoites differentiate into male and female sexual stages, which are called erythrocytic gametocytes (the only stages transmitted to the mosquito vector) with one nucleus and then cleared by drugs or the immune system, or awaiting the arrival of a blood-seeking Anopheles mosquito.

The time required for the maturation of gametocytes (do not cause disease) are prominently different among different Plasmodium species. P. falciparum gametocytes require 8 up to10 days for development into 5 morphologically different phases or stages (I-V) but vivax gametocytes take 48 h for maturity and disappear from blood within three days of sexual phase. In falciparum, the first identifiable stages of gametocytes are round compact forms having hemozoin. This stage (stage I) and the subsequent growth steps (stage 2-4) are principally absent from the vascular system, but sequestered in deep tissue in which they grow into mature sausage-shaped stage 5 gametocytes and reappeared in the blood and infective for mosquitoes. In diffrent to falciparum, matured vivax gametocytes are large and round, filling up almost the whole stippled red blood cell with a prominent nucleus. Because of their rapid maturation than falciparum, vivax gametocytes become exist in vascular system within a week subsequent to inoculation by mosquito and prior to parasite detection by light microscopy. This creates a major challenge in strategies of vivax elimination, as infected persons may be infectious prior to parasite detection using microscopy.

When a mosquito takes up erythrocytic gametocytes at the time of blood meal, the gametocytes migrate to the mosquito gut. At the midgut of mosquito, matured gametocytes egress from the host cell and differentiate into male and female gametes. The triggering factors for this differentiation are a fall in temperature, raise in pH and increase in xanthurenic acid concentration. Afterward, undergo fertilization (gametogenesis)-the flagellated forms of microgametes/male gametocytes formed by exflagellation penetrate/fertilize the macrogametes/female gametocytes to form a diploid zygote. The zygote develops into motile ookinetes, which penetrate the mosquito midgut and develop into round oocysts. The oocyst development is the longest developmental phase (takes three up to thirty days) and the only extracellular portion of the Plasmodium life cycle. The falciparum oocysts mature over a period of 11 to 16 days before releasing the infectious sporozoites. The sporozoites vigorously get away from the oocyst and only 25 percent of those released from oocyst travel through the hemocoelomic fluid to the acinal cells of salivary glands, where after about a day of residence, they became highly infective. They are permanently programmed for their trip in the vertebrate host because they totally lost their capability to re-infect salivary glands. The chance of a mosquito for acquiring an infection at the time of blood meal is depend on various human, parasite and mosquito factors. The maturity of gametocytes in humans is fundamental to the continuation of malaria transmission and represents a potential bottleneck in the parasite's life cycle. Understanding the biology of gametocyte maturity and the human infectious reservoir at both the individual and population level is therefore essential to ablate disease transmission nonetheless, it is remained ambiguous.

## Malaria biology and pathology

The biology of malaria parasite is believed to be analogous to other eukaryotes because members of the genus Plasmodium are eukaryotic micro-organisms. Nonetheless, the overall biology and thier pathology are not evidently well-known at molecular and cellular levels. Plasmodium species have 14 chromosomes (vary in length from 500 kilobases to 3.5 megabases), one mitochondrion, and one apicoplast organelle similar to chloroplast but not photosynthetic. All protists of the Apicomplexa including Plasmodium are characterized by a set of apical organelles (rhoptries, dense granules and micronemes) because of the localization of those organelles at one end of the parasite. These organelles have been involved in the host cell invasion. In Plasmodium, there are 3 invasive forms involving the apical organelles: sporozoite, merozoite, and ookinete.

First of all, the sporozoites injected into the host skin moves to the blood stream and rapidly access the liver through traversal proteins by a process called traversal. This process involves crossing of the sinusoidal barrier including fenestrated endothelial cells and macrophage-like Kupffer

cells. Proteins required for traversal include sporozoite microneme protein essential for traversal (SPECT), SPECT2 (also known as perforin-like protein 1, PLP1), cell traversal protein for ookinetes and sporozoites (CelTOS), phospholipase (PL), and gamete egress and sporozoite traversal protein (GEST). The role of these proteins in cell traversal is not clearly recognized, except a suggestion that the membrane attack complex/perforin-like (MAC/PF) domain of SPECT2 plays a role in punching holes in membranes. The sporozoites pass through the cells by making a transient vacuole. The sporozoite initiate the exo-erythrocytic cycle in the parenchymal cells of the liver. The co-receptors [thrombospondin (TSP) domains on the circumsporozoite protein (CSP) and thrombospondin-related adhesive protein (TRAP)] on the sporozoites that mediate invasion attach distinctively to the glycoaminoglycan chains of the heparan sulfate proteoglycan (HSP) proteins on hepatic cells in the region in apposition to the sinusoidal endothelium and Kuppfer cells. At least 2 (CD81 and CD68) receptors are required for entry and invasion of falciparum in hepatic cells. After passing space of Disse, the sporozoites travel *via* many hepatic cells and enter into a final invasion (develops into merozoites) in the liver. Lastely, they form a parasitophorous vacuolar membrane (PVM). PVM is then lysed by proteolytic enzymes of the Plasmodium and the merozoites migrate from the infected hepatic cell to the vascular system.

Inside the circulation, the merozoites are rapidly and specifically enter the RBCs (where they grow by consuming hemoglobin), thus implying ligand-receptor interactions. The initial interaction is most likely an arbitrary collision and involves reversible interactions among proteins on the surface of the merozoites and the host RBC. Merozoite surface protein 1 (MSP1) connected to the Plasmodium membrane *via* a glycosylphosphatidylinositol (GPI) anchor binds to proteins found on the surface of RBC. MSP1 is one of the best characterized MSPs and undergoes primary and secondary proteolytic processes that are coincident with merozoite maturation and invasion, respectively. About 8 or more additional surface bound GPI anchored merozoite proteins that interact with a single red blood cell are reviewed somewhere else. A newely discovered protein found on the outer part of erthyrocytes (codded as CD55) acts as an essential entry point of Plasmodium into the interior part of RBC. This protein opens the door to develop novel compounds to manage malaria. Following RBC binding, the merozoites reorient themselves using a transmembrane protein (apical membrane antigen-1 (AMA1)) so that the apical end of the merozoite will move close to the erythrocyte membrane with a temporary deformation of RBC. As the parasite invades RBC, the contents of the apical organelles will be expelled out. With the initial contact between the parasite and RBC, the micronemes become expelled. Microneme discharge is coupled with an increase in cytoplasmic calcium concentration and possibly involves the signal pathways including phospholipase C, inositoltriphosphate and calcium dependent protein kinases. Immediately after the expelling of micronemes, the rhoptries will be discharged and the release of their contents associates with the formation of the parasitophorous vacuole. After the Plasmodium completed its entry to RBC, the contents of dense granules will be released from the parasite. Subtilisin-like proteases (SUB), involved in the secondary proteolytic process of MSP1, are found in dense granules.

Following reorientation, a junction has been created between merozoite and the host cell by microneme proteins, which recognizes and binds to receptors present in the host cell. Proteins exist within the micromenes include: erythrocyte binding antigens (EBAs) of falciparum, duffy binding proteins (DBP) of vivax and knowlesi, sporozoite surface protein-2 (SSP-2)/TRAP, and circumsporozoite and TRAP-related protein (CTRP) of the ookinete. DBP and EBAs (EBA175, Ebl1 and EBA140) are able to recognize and binds to the Duffy antigen receptor and glycophorin (at sialic acid residue) A, B and C receptors in the host, respectively. DBP and EBAs are part of adhesin families found in Plasmodium usually named as eyrthrocyte-binding like (EBL) proteins. Another adhesins participated in binding merozoites to RBCs are the Rh protein families in the neck of the rhopthry. These diverse adhesins bind to different RBC receptors and offer redundancy for merozoite to establish a junction with RBC. Proteins of the neck of the rhoptry, in particular RON2, have been inserted into the RBC membrane and bind to AMA1 to make the tight junction.

Next, the contact region becomes free of the erythrocytic membrane proteins and the merozoite enzymes (serine proteases) results in a localized distraction of the sub membrane cytoskeleton and lipid architecture of the erythrocyte. The junction formation (between the merozoite and RBC) triggers rhoptry bulb discharge, availing lipids and proteins needed for the development of PVM. So, an initial PVM would be produced in the junction region. The junction becomes converted to a ring-like structure and the merozoite starts to travel through this opening (the expanding parasitophorous vacuole). Subsequent to entry, both the parasitophorous vacuole and the RBC membrane will be closed. Note that ookinetes have no rhoptries and do not make a PVM inside the mosquito midgut epithelial cells. The ookinetes travel quickly through the epithelial cells and bring vast damage as they head towards the basal lamina. Iin the same way, the sporozoites could enter and egress hepatic cells without undergoing exoerythrocytic schizogony [Figure 2].

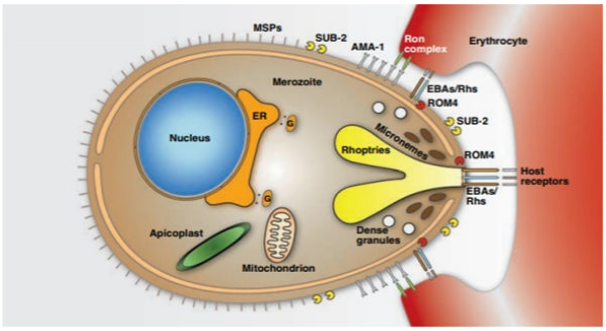


**Figure 2:** Invasion of RBC by merozoite of P. falciparum.

The invasive stages of all apicomplexan parasites including Plasmodium are motile forms that move slowly along the substratum using gliding motility. At the time of invasion, the malaria parasite literally moves slowly into the RBC through the moving junction. During gliding motility, the micronemes should be incessantly discharged as the organism is passing and incessant developments of new junctions occur between the zoite and substratum. The force necessary for the invasion and gliding motility is based upon the actin-myosin cyto-skeletal elements. The Apicomplexa parasites have a unique myosin that anchored into the Inner Membrane Complex (IMC) lying beneath the plasma membrane (PM). Actin interacts with myosin as part of the glidesome. Different adhesions, which make up the Moving Junction Complex (MJC), are then coupled to the glidesome. The myosin pushes the actin filaments towards the back of the zoite. Since myosin is fixed to the IMC, it will not go. As a result, the transmembrane adhesins become pulled through the fluid lipid bilayer of the PM. So, the complex formed by the interaction among adhesins and actin filaments is transported to the back of the host cell to generate forward movement of the Plasmodium. While the adhesins get in touch to the posterior end of the Plasmodium they are proteolyitcally sliced and shed from the the zoite surface and a trail of adhesive molecules are remained at the back of the moving zoite on the substratum.

Within the erthrocyte, the Plasmodium makes changes on the host cell. The major changes occurred are knob formation, cytoadherence and rosetting. Erythrocyte membrane protein-2 (PfEMP2) and Knob-associated histidine rich protein (KAHRP) are among the proteins which restructure the red blood cell sub-membrane cyto-skeleton and induce the formation of knob. These two proteins are localized to the cytoplasmic side of the host membrane. PfEMP1 (polymorphic protein) are attached to the knobs by KAHRP and is protrude to the erthrocyte surface and function as a ligand. Additional ligands used for cytoadherence are illestrated.

PfEMP1, a var gene family, has a long N-terminal (extracellular domain), a transmebrane region and an intracellular domain (C-terminal). The intracellular domain anchor PfEMP1 to the RBC submembrane cytoskeleton and may also interact to the basic KAHRP, spectrin and actin. The N-terminal, characterized by one to five copies of DBL domains with rosetting domain, is followed by a cysteine-rich interdomain region (CIDR) having CD36 binding domain. The RBCs infected with matured Plasmodium cytoadhere to the capillary and post-capillary venular endothelium in the deep small circulatory vessels. The cytoadherence results in sequestration of the Plasmodium in many organs of the body including heart, lung, brain, liver, kidney, intestines, subcutaneous tissues and placenta. This phase of pathogesis is correlated solely to falciparum malaria. However, it is also observed in reticulocytes invaded by vivax malaria. Inorder to cytoadhere to the endothelium, PfEMP-1 (antigenic variant molecule) emerges on the infected red blood cell (IRBC) surface approximately sixteen hours following invasion. PfEMP1 can bind to a lot of adhesion receptors exposed on the surface of endothelial cells. From these receptors, ICAM-1 is the main receptor used for sequestration. It also acts as a rolling receptor. Supplementary to this, CD36 provides stationary and stable adherence under flow [Figure 3].

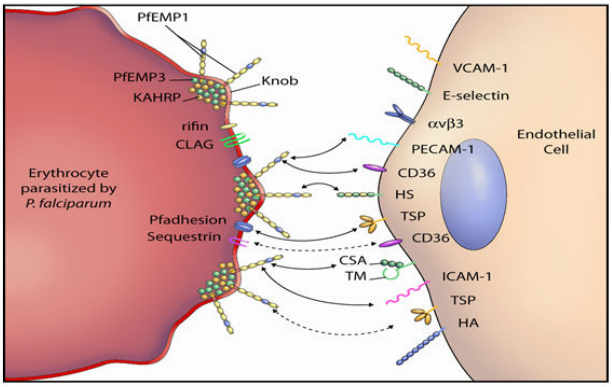


**Figure 3:** Cytoadherence of P. falciparum.

Through Variant Two CSA Antigen (VAR2CSA) and other adhesion molecules mediation parasite sequestration is also observed in pregnant women as IRBC attaches to the placental CSA antigen. So that placental malaria can cause miscarriage, preterm birth, intrauterine growth retardation (IUGR), low birth weight (LBW), fetal anemia, congenital malaria and perinatal death. Placental malaria is most common in falciparum and rare in vivax infection. Sequestration of the parasites in tissues offers the microaerophilic venous environment for Plasmodium that is better place for their growth. Adhesion to endothelium enables the malaria parasite to avoid clearance by the spleen and to conceal from the immune system. The infected erythrocytes can adhere to the non-infected erythrocytes to form rosetting of red cells. The infected erythrocytes are also adhering to other infected erythrocytes to form agglutination.

During the formation of rosette, PfEMP1 binds to heparin sulfate (HS), complement receptor 1 (CR1), and ABO blood types. The DBL (lectin-like) of PfEMP-1 has made tough adhesion with carbohydrate structures, primarily with blood type-A-antigen. This is the reason that individuals with non-O-blood types are more susceptable to severe malaria infection due to enhanced formation of rosette. Despite all malaria parasites are capable of forming rosettes, only falciparum are associated with life threatening malaria. Falciparum could attack all developmental ages of erythrocytes and produce extremely high parasitic loads. Vivax and ovale have a noticeable affinity for invasion of young erythrocytes, whereas malariae has a preference towards old red cells. As a result, malaria caused by vivax, ovale and malariae has low parasitemia level in the bloodstream of patients. If the above pathophysiologic process (cytoadherence, sequestration, rosetting, agglutination, altered deformability and fragility of parasitized RBC) become continued in the absence of proper patient manegment, there will be blockage of blood flow,

shortage of local oxygen supply, hinderance of mitochondrial ATP synthesis, and stimulation of cytokine secretion – all of these factors attributes to the progress of the disese into severe malaria.

Moreover, following subtilisin-like protease 1 (SUB1) processed MSP1 that interacts with the spectrin network of the erythrocyte cyto-skeleton, the host erythrocyte become ruptured to enable egress of the parasite from RBC. Concurrent to IRBC lysis, toxins such as products of RBC membrane, hemozoin pigment and GPI, will be released into the vascular system.

These products then activate endothelial cells and macrophages, which produce cytokines (to up-regulate the expression and re-localization of endothelial receptors) and mediators of inflammation [tumor necrosis factors (TNF), interferon (IF)-γ, interleukin (IL)-1, 6 and 8, and lymphotoxin as well as superoxide and nitric oxide]. Including fever, the systemic symptoms of malaria have been mainly ascribed to the produced cytokines and the released toxins.

Furthermore, the plasmodial DNA is also highly proinflammatory and can bring fever and cytokinemia. Following presented by hemozoin, the plasmodial DNA interacts with the Toll-like receptor 9 (TLR9) in the host cell and cause the secretion of proinflammatory cytokines.

These cytokines induce cyclooxygenouse (COX-2) upregulating prostaglandins, which initiate the generation of fever. Hemozoin is also associated with apoptosis induction in growing erythroid cells, thus cause anemia.

On the other hand, the Plasmodium emerged from the hepatic cell packed with vitamin A and use retinoic acid as a PM destabilizer to infect erthyrocytes, leads to hemolysis and anemia.

The clinical feature of life-threatening malaria (coma, severe anemia, metabolic acidosis, hypoglycemia, acute renal failure or acute pulmonary oedema), caused particularly by falciparum malaria, are directly associated with the stimulation of pro-inflammatory immune reactions.

The macrophage activation is a key incident of severe malaria pathogenesis in both humans and laboratory models of malaria.

Hyperactive immune response is one of the most important causing fector of cerebral malaria vasculopathy, and the fatal outcome is usually ascribed to the sequestration of stimulated macrophages, parasitized RBCs and platelets in cerebral vessels. Deitsch and Chetan suggested that one of the two specific combinations of binding domain cassettes, either DC8 or DC13, at N-terminal part of PfEMP1, binds to unclear host receptors are associated with both cerebral malaria and severe anemia [Figure 4].

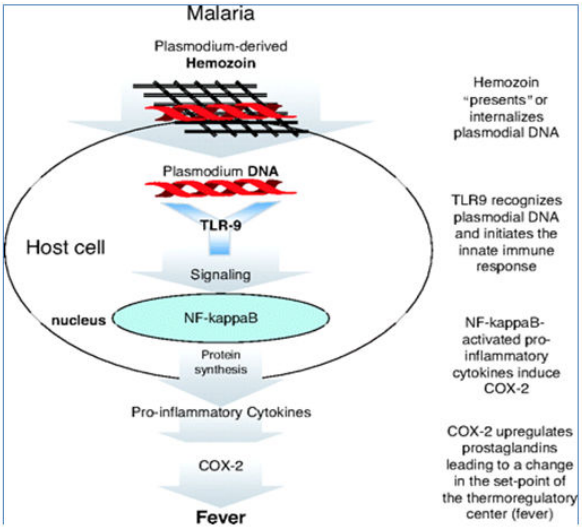


**Figure 4:** Induction of fever by malaria parasites.

## Malaria diagnosis

To end up with effective patient management, malaria should be diagnosed early and precisely. Misdiagnosis can result in mismanagement and leads to extra spread of infection in the community. Generally, we can categorize malaria diagnosis as parasitological based on parasite detection and clinical based on the signs and symptoms during physical diagnosis of patients. The first malaria symptoms (headache, lassitude, fatigue, abdominal discomfort and muscle and joint aches, usually followed by fever, chills, anorexia and vomiting) are often not specific and similar to several viral illnesses. Exceptionally, young children with malaria may manifest cough, lethargy and poor feeding status. The WHO recommends that malaria must be suspected in any patient presenting with a history of fever or temperature >37.5°C in the absence of other obvious causes. The WHO also commands that in stable malaria transmission areas or at high transmission period of seasonal malaria, it must be suspected in children with palmar pallor or less than 8 gram per deciliter of hemoglobin concentration. Ethiopian FMoH advocates that clinical malaria diagnosis should be done in a patient with fever or has fever history in the last 48 h and lives in malaria endemic areas or has travel history within the last 30 days to malaria endemic areas. Malaria is therefore frequently over diagnosed in endemic areas. Conversely, health care providers would be alert not to miss malaria case as very young children with malaria may present with low body temperature or hypothermia.

In all settings, every suspected malaria cases must be confirmed with a parasitological diagnosis. RDTs and Light Microscopys (LM) are the methods employed for routine parasitological malaria diagnosis. Parasite detection on giemsa-stained smears of the peripheral blood through LM is used as the gold standard method of malaria diagnosis. Using of thick blood films to detect the parasite by LM is very sensitive technique. Thin blood film is helpful for identifying Plasmodium species, and is less sensitive procedure. Since

knowlesi and malariae species have more or less the same morphology, LM alone is not enough to identify knowlesi species. All maturity stages next to the hepatic cycle can be observed in the peripheral blood during vivax, ovale and malariae infections. But, in case of falciparum infection only banana-like gametocytes and ring stages are commonly exist in the peripheral blood as that of matured Plasmodium become sequestered. Microscopy provides the merit to identify the existence of mixed malaria infections. LM could also be used to monitor treatment efficacy through successive assessment of parasitemia level until completely clearanced from our blood. Nonetheless, very low parasitemia level may not be identified in slide examination using LM. Moreover, it consumes much time and requires well educated (qualified) and skilled professionals for reading the smears to make correct and reproducible diagnosis.

RDTs (detect antigens or enzymatic activities related to Plasmodium) are used in regions where LM is not easly available. They are rapid (give results in 20 minutes), easy method and simple to carry out. The sample of blood taken from the patient is dropped in a test strip; dye-labeled antibodies combined to the Plasmodium antigen, the complex formed travel on a nitrocellulose strip and then detained by a capture antibody, forming a visible line. The appreance of specific bands on the window of the test card shows whether the person is infected and a control line provides a message on the integrity of antibody–dye conjugate. Due to its unique features (simplicity, easy to perform, easily transportable and non-requirement of electricity source); RDT is very useful in malaria endemic areas. Furthermore, in non-endemic district, it can act as a complementary test in case of LM inexperience.

There are two types of RDTs. Some detects only one and others detect more than one species of malaria parasities. P. falciparum histidine rich protein-2 (PfHRP2) and the two enzymes (plasmodial lactate dehydrogenase (pLDH) and aldolase) involved in the glycolytic pathways of Plasmodium are the most common antigens detected by RDTs. PfHRP2 is specific for falciparum malaria. But LDH is either specific to falciparum and vivax malaria or it can be common to all 6 Plasmodium species (variant pan specific). Rapid diagnostic tests have the capacity to measure Plasmodium antigens in spite of matured parasites are sequestered. Peripheral blood parasitemia counted using LM did not include parasites sequestered with IRBCs. Nonetheless, these parasites release PfHRP2 into the plasma and plasma concentration of PfHRP2 can offer best estimation regarding the total biomass of the parasite. When parasite recognition by LM is difficult because of its sequesteration and very low parasitic load in the peripheral blood, it is practicable to measure antigens using RDTs. RDTs detect only single species were entered in Ethiopia in 2005. Ethiopian FMoH was supplied multi-species RDTs to health posts after 2005. The drawbacks for RDTs are: (a) lack of antigen specificity in case of ovale, malariae, or knowlesi malaria; (b) they are not used to determine the level of parasite or to monitor the treament given that the antigens (in particular PfHRP2) can live in the blood following clearance of the parasite or the antigens can be released from gametocytes (usually not killed by the antimalarial drugs); (c) PfHRP2 persistence in blood following Plasmodium clearance can result in false positivity; (d) RDTs based on PfHRP2 antigen cannot be useful in Africa, India, Asia, middle east and Amazon regions since some isolates lacking this antigen have been found from these areas.

Polymerase chain reaction based examination, one of the parasitological diagnostic methods, is the most sensitive diagnostic tool used to detect low parasitemia levels, different species of Plasmodium and mixed infections. However, this is not an appropriate technique for routine purpose. Nowadays, a species specific loop-mediated isothermal amplification (LAMP) is become a widely acknowledged method for identification of knowlesi infections. Additionally, polymerase chain reaction technique is used as a research instrument in epidemiological investigations, clinical trials, and in identification of molecular biomarker for resistant antimalarial drugs. The 4th way of parasitological diagnosis is serology test (finding of antibodies secreted against Plasmodium) by either enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence. This test is not used detect recent exposure to malaria rather it measures past infection.

Rolling circle enhanced enzyme activity detection (REEAD) and micromagnetic resonance reflaxometric (MMR) tests are recently developed parasitological methods appropraite for utilization in field detection of malaria infected individuals for population screenings. They are very precise plus cost and time effective tools. REEAD enumerates the activity of Plasmodium specific topoisomerase-1 (pTOP-1) enzyme present in the IRBCs/saliva since this enzyme is not found in Plasmodium free human cells. This is the most sensitive malaria diagnostic method available. It is also applicable to monitor the progress of malaria therapy. MMR measures the existence of paramagnetic Fe3+ ions in the hemozoin of the RBCs invaded by malaria parasite. New diagnostic targets of malaria including proteins of extremely conserved genes are in searching by experts. According to the recent report, the smell of children infected with malaria is more attractive for disease carying A. gambiae mosquitoes. A raise in discharges of aldehyde from the children accounted for much of the changes in charisma. This discovery might be helpful for developing novel noninvasive diagnostic techniques because it allow diagnosing malaria parasite carriers by means of odors even though childrens don't experience sick and visit health institute.

## Discussion

### Vector Control

In our planet, more countries are going to be free from malaria. About 44 countries were reported less than ten thousand cases of malaria in 2016. Ethiopia has a plan to get rid of malaria by 2030. To achieve this plan, controlling

malaria vectors is an efficient method that should be implmented. The population of Anopheles mosquitoes could be significantlye decreased through indoor spraying of residual insecticides (IRSs) and utilization of insecticide treated bed nets (ITNs) at household level. IRS is commonly employed to control endophilic mosquitoes. Larviciding and avoidance of breeding sites are the best controlling system against exophagic/exophilic vectors. The insecticides mostly used within countries in Africa are organochlorines, organophosphates, pyrethroids (advised to use only in bed nets) and carbamates [3]. However, their effectiveness is at risk by resistance developing mosquitoes. Long lasting insecticidal nets (LLINs) continue to be effective even with resistance. The recent WHO recommendation for managing LLINs resistance is additive spraying, with non-pyrethroids employed in a rotational basis.

DDT (pesticide having a long history of extensive global use) is the most well-known organochlorine (chlorinated hydrocarbon) compound. Organochlorines are inexpensive and highly effective; however, they have dangerious outcomes on environmental and human well-being. Organochlorines are indeed extremely stable chemicals and can stay for a prolonged time in the environment. Due to their lipophilic nature, they become concentrated in the fat tissue and accumulated in humans and animals in the course of food chains. Inhibiting of sodium channels is belived to be the mechanism of action for DDT since insects with sodium channel gene mutations developed resistance to DDT and other insecticides with the same mode of action. Resistance to DDT is also coccurred by overproduction of detoxifying enzymes, for example cytochrome P450, through enhanced metabolism of insecticides.

Organophosphates and carbamates have similar mode of action. They inhibit acetylcholinesterase enzyme, which is responsible for the breakdown of acetylcholine (a neurotransmitter at the cholinergic nerve synapse). Differences in chemical structure among various organophosphates and carbamates have an effect on the degree and efficiency of inhibiting acetylcholinesterase. Secondary to widespread pyrethroid resistance, organophosphates and carbamates are imperative alternative of insecticides for IRS. Using of synthetic pyrethroids (one of the most recent groups of insecticides) has been drastically increased in the past twenty years. Despite there is a difference in chemical structure, synthetic pyrethroids and organochlorines are inhibitors of the insect's voltage-gated sodium channels in the axonal membranes. As a result, their toxic effects are similar. These compounds are also shared resistance mechanisms and cross-resistance among pyrethroids and DDT limits the selection of optional insecticides to handle resistance issues.

Now a day, many new forms of vector control methods are under optimization. Practicing of the attractive toxic sugar bait (ATSB) techniques are among the new approaches devised to kill mosquitoes which are in search of essential sugars from the outdoor environments. This system uses the smell of fruit/flower as bait, sugar solution as stimulant of feeding, and oral toxin as a slaying agent to kill the mosquitoes. Spraying swarms with aerosols is also a new method that brings a significant decline in the density of mosquitoes. Personal bite avoidance methods, such as repellant of insects and protective clothing, are also employed to decrease malaria transmitted through Anopheles mosquitoes. Chicken-specific compounds (non-host volatiles) such as isobutyl butyrate, naphthalene, trans-limonene oxide and hexadecane can give prevention against mosquito vectored diseases in combination with conventional preventive programmes. Most Anopheles mosquitoes exhibit more varied behavior, feed on humans and livestocks. Malaria vector species feeding on livestock can be targeted by treating the structure of the livestocks (example, cattle sheds by IRS). Direct cattle treatment by sponging, dipping, or spraying with insecticides is another paranoma to destroy malaria vectors and decrease malaria in the world. Administering systemic veterinary insecticides to attack malaria vectors during blood sucking is an additional choice. In this regard, ivermectin was fruitfully tested in cattle and shown to destroy malaria vectors and shorten the existence of the survivors.

The other remarkable means to hinder malaria transmission are disruption of steroid hormone signaling in malaria vectors, utilize transgenic mosquitoes, using paratransgenesis to deliver anti-Plasmodium effector molecules through genetically modified symbiotic microbes of the insect and/or transinfection of Anopheles mosquitoes by symbiotic fungi and bacteria (for example Wolbachia). The implementation of transgenic procedure will advance the sterile insect method of anopheles brought by radiation. Inimitably, polymorphisms (some genetic disorders) in human erthrocytes make the individuals resistant to malaria infections. The most commons among these abnormalities are polymorphic glycophorins, thalassaemia traits, sickle cell traits, spherocytosis, ovalocytosis, elliptocytosis, deficiency in glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase deficiency. The allele of c. located in the FAS promoter region was significantly associated with protection from severe malaria in childhood age. These preventive polymorphism studies can offer hints regarding naturally occurring host defense mechanisms, which might be used in discovery of novel therapeutic agents to fight against malaria.

Moreover, drugs have a key role in reducing malaria transmission. Sulfadoxine-pyrimethamine (SP) based Intermittent Preventive Therapy (IPT) is an additional protective approach in infants, preschool children and pregnant mothers in malaria endemic vicinity. Fansider (SP) combined with amodiaquine is also used at monthly intervals for seasonal malaria prophylaxis in children aged 3-59 months. This combination is now advisable to use in the Sahel region of sub-Saharan Africa in localities where malaria transmission is extreme and majority (more than 60%) of the clinical malaria cases occur within a limited time (less than 4 months). Repeated Mass Drug Administration (MDA) using ivermectin could be a very useful method to slow down transmission of malaria. Chemoprophylactic

agents [chloroquine, mefloquine, doxycycline or Malarone (atovaquone plus proguanil)] are generally used to avert malaria in travelers (those travel from malaria free regions to districts where malaria is there). The antibiotic drug cotrimoxazole prescribed to protect HIV/AIDS patients from Pneumocystis jirovecii pneumonia has been known by its reduction effect on malaria infections. So, it is now a promising candidate to be used as a chemoprophylactic drug in HIV negative children/pregnant mothers. Methylene blue, an old and parasite killing drug with eryhrocytic activity, has additional benefit secondary to its activity against matured female and male gametocytes of falciparum malaria. In spite of the absence of innovative compounds in the pipe line only for chemoprophylaxis purpose, DSM-265 displays its potential as a chemoprophylactic agent for travelers. As mentioned above, erthrocytic Plasmodium enclosed by PVM multiplies inside the RBCs and subsequent to every cycle of the intracellular growth first the PVM ruptured by the parasite SUB-1 and then the erthrocyte membrane lysed by serine repeat antigen protease-like protein-6 (SERA-6) to facitate merozoite egress to invade new erthrocytes. Chemicals which inhibit SUB1 and SERA6 could target successive interdependent paths in the egress process and so could be a novel group of compounds to avert Plasmodium proliferation and disease progression.

## Vaccines for Malaria

The spread and emergence of resistance against insecticidal compounds has limited the present malaria control activities. Thus, efficient and safe vaccines are necessary to attain the objectives of global malaria eradication agenda. The rationale for developing malaria vaccines is the occurrence that individuals residing in endemic regions developed clinical protective immunity even though the antigenic variations of the Plasmodium enable it to escape the defensive immune reaction of the host. The best malaria vaccine has need 3 important features: (a) multiple components that will provoke an efficient immune response to various stages of malaria infection (sporozoites, infected hepatocytes and asexual and sexual stages); (b) multiple epitopes that are limited to presentation by different major histocompatibility complex (MHC) molecules to conquer genetic multiplicity and antigenic variation; and (c) multi-immunogenicity inducing greater than single type of immune response, including cell-mediated and humoral components.

Untill now, 3 types of candidate vaccines have been deeply investigated. Pre-erythrocytic vaccines are the first vaccines developed to avert blood-stage infections. The second type or blood-stage vaccines are used to remove parasitaemia to avoid clinical disease. The last type (transmission-blocking) vaccines are invented to use for prevention of mosquito infections and break off transmission of malaria in populations. The liver stages of the Plasmodium and/or the sporozoites are the targets for pre-erythrocytic vaccines. These classes of vaccines should have the ability to bring an immune response mediated through either an antibody that protect invasion or through T-cells that affect the infected hepatocyte. However, one Plasmodium parasite may be enough for the proceeding of the infection into erthyrocytic stage since the immune system has inadequate amount of time for eradication of the parasite due to high replication rate of the sporozoites. A proficient hepatic stage vaccine should be 100 percent successful to guard us from malaria in the absence of the natural immunity. Vaccines included here are those contain whole killed sporozoites and those based on antigenic components of CSPs.

Sterile vaccination with whole irradiated sporozoites, injected through mosquito bite, was tested both in mice and in humans. Nevertheless, the sporozoites should be cryopreserved and then given through standard techniques in order to get a whole parasite vaccine.

RTS, S is a recombinant subunit vaccine comprised of a truncated CSP of P. falciparum directly combined to hepatitis B surface antigen (HBsAg). The fused protein will be coexpressed with free HBsAg in yeast cells. Since RTS, S antigen created on its own has incomplete immunogenicity it has been formulated with potent adjuvant system, AS02 and AS01. Through *in vitro* models, P. falciparum liver stage antigens (PfLSA1, PfLSA2 and PfLSA3) and P. vivax liver stage antigens (PvLSAs) are now identified as a new candidate of vaccines targeting infected liver cells.

Many blood stage vaccines (majority of them target antigens of merozoite) are under clinical studies. Targets eligible for asexual blood stage vaccine candidacy are AMA-1, EBA175, MSP1, MSP119, MSP2, MSP3 and SERA5. None of them brings defined clinical protection, most likely due to the high polymorphic characters of the vaccines structure [4]. However, efforts have been increasing to improve the effictivness either with new adjuvants through viral vector prime-boost strategies or by combining AMA-1 and MSP-1. Conversly, novel non-polymorphic P. falciparum ligands, CX3CL1 binding proteins (CBP1 and CBP2), are discovered, which opens a new perspectives for pioneering approaches in vaccine development. Through further investigation, innovative antigens [P. falciparum reticulocyte binding protein homolog-5 (PfRH-5) and rhoptry associated leucine zipper-like protein-1 (RALP-1)] with a potential to be candidates of blood stage vaccines are being revealed. PfRH-5 is in fact in first phase of human trials. Since MSP4 provokes a strong natural antibody response in malaria endemic regions, it has an opportunity to be incorporated into candidates of bood stage vaccines. Women with multiple pregnancies, who acquire antibodies against VAR2CSA, are definitely immuned from pregnancy-related malaria following the first pregnancy. Based on this result, candidate vaccines aimed to target VAR2CSA are in development process.

The targets for transmission blocking vaccines are surface proteins expressed by zygotes, ookinetes and gametocytes [5]. These vaccines are used to stop parasite growth in the mid gut of malaria vector using specific host antibodies, complement proteins, and cytokines. The candidates in this class contain P. falciparum gametocyte antigens (Pfs-48/45

and Pfs-230), P. falciparum ookinete antigens (Pfs-25 and Pfs-28) and the P. vivax homologues (Pvs-25 and Pvs-28). Additional more recently identified targets of interest comprise Pfs-47 (involoved in Plasmodium immune avoidance in the malaria vector) and PfHAP-2 (expressed by the male gametocyte and microgamete). Various vaccines grouped within the above 3 categories of malaria vaccines beyond the scope of this paper are described in detail (including their formulation).

## Conclusion

Malaria is continued to be an important cause of morbidity and mortality in endemic countries. Falciparum and vivax species of Plasmodium create a huge problem in the wellbeing of the society. Apart from reduction in old and new cases of malaria, the transmission is quite active throughout the world, where the disease presents. Hence, malaria control necessitates preventive measures primarily vector control. However, rising of resistance against chemopreventive agents is a big confronts to struggle against malaria. In spite of tremendous research in the past decades, there is no approved malaria vaccine until now. Achievement and resistance are generating malaria landscape that call for novel approaches. Therefore, our planet is in calling for immediate action to discover latest, safe and effective vaccines and insecticidal drugs.

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