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College of medicine

Department of microbiology

Review article in

Vaccination Development in Malaria

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بسم الله الرحمن الرحيم

(يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ
اللَّهُ لَكُمْ ۖ وَإِذَا قِيلَ انشُزُوا فَانشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ
وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ).

صدق الله العلي العظيم

من سورة المجادلة الآية ١١

الاهداء

إلى صاحب السيرة العطرة، والفكر المستنير

الذي كان له الفضل الأول في بلوغي التعليم العالي

(والدي الحبيب)، أطال الله في عمره

إلى من وضعتني على طريق الحياة، وجعلتني رابط الجأش،

وراعتني حتى صرت كبير

(أمي الغالية)، حفظها الله من كل سوء .

.إلى إخوتي؛ من كان لهم بالغ الأثر في كثير من العقبات والصعاب

إلى جميع أساتذتي الكرام؛ ممن لم يتوانوا في مد يد العون لي

اهدي لكم مشروعي البسيط ومن الله التوفيق

الشكر والتقدير

بسم الله الرحمن الرحيم، والحمد لله رب العالمين الذي وفقنا وأعاننا على إنهاء هذا البحث والخروج به بهذه الصورة المتكاملة، فبالأمس القريب بدأنا مسيرتنا التعليمية ونحن نتحسس الطريق برهبة وارتباك، فرأينا (الطب) هدفا ساميا وحبا وغاية تستحق السير لأجلها، وإن بحثنا يحمل في طياته طموح شباب يحلمون أن تكون أمتهم العربية كالشامة بين الأمم. وانطلاقًا من مبدأ أنه لا يشكر الله من لا يشكر الناس، فإنني اتوجه بالشكر الجزيل للأستاذ المساعد الدكتورة (رواء عبد الخالق) التي رافقتني في مسيرتي لإنجاز هذا البحث وكان لها بصمات واضحة من خلال توجيهاتها وانتقاداتها البناءة والدعم الأكاديمي.

كما اشكر عائلتي التي صبرت وتحملت معي ورفدتني بالكثير من الدعم على جميع الأصعدة، واشكر الأصدقاء والأحباب وكل من قدم لي الدعم العلمي أو المعنوي، وأخيرا نتوجه بشكر خاص للأستاذ (إسماعيل إبراهيم لطيف) عميد الكلية المحترم لمساهمته الفعالة في تسهيل المشروع راجيا من الله التوفيق والسداد.

Introduction

Malaria remains a major public health concern, affecting 3.3 billion people in 97 countries and resulting in an estimated 200 million infections and 600,000 deaths each year (1). Malaria is derived from the Italian 'mal'aria,' which means 'poor air,' due to the disease's early link with marshy places. Charles Louis Alphonse Laveran, a French army surgeon, identified parasites in the blood of a malaria patient near the end of the nineteenth century, and Dr. Ronald Ross, a British medical officer in Hyderabad, India, discovered that mosquitoes transmitted malaria. Human malaria may only be spread by Anopheles mosquitoes, according to Italian professor Giovanni Battista Grassi (2). Malaria is caused by protozoan parasites of the genus Plasmodium, also known as the "King of Diseases." *Plasmodium falciparum* causes the most serious and occasionally fatal form of malaria. *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and occasionally *Plasmodium knowlesi,* the other human malaria species, can cause acute, severe sickness, although mortality rates are low. Malaria is the most common infectious disease in tropical and subtropical areas, and it remains a major worldwide health issue, with over 40 percent of the world's population living in places where malaria is a danger (3).

Over 500 million people are believed to be infected with malaria each year, resulting in 1-2 million fatalities, 90% of whom are children in Sub-Saharan Africa (3). In 1900, malaria posed a threat to more than 77 percent of the world's population in 140 countries, with more than 25 percent living in hyper- or holoendemic zones. In the twentieth century, malaria death rates altered substantially. This review will demonstrate the malaria disease and its pathogenesis, clinical features, management and the recent advances in the development of vaccine.

Taxonomy

Kingdom Protista

Subkingdom Protozoa

Phylum *Apicomplexa*

Class *Sporozoasida*

Order *Eucoccidiorida*

Family *Plasmodiidae*

Genus *Plasmodium*

Species *P. falciparum, P. malariae, P. ovale, P. vivax.*

Plasmodium parasites are thought to be found in about 250 species of mammals, birds, and reptiles. Plasmodium has been found in around 30 species of nonhuman primates, including apes, gibbons, and New and Old World monkeys. All types of malaria in primates are thought to be spread by *Anopheles* mosquitoes. *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium vivax* are the four restricted or adapted species traditionally identified as causing human malaria. Several simian species, including *P. cynomolgi cynomolgi, P. cynomolgi bastianelli, P. simiovale, P. brasilianum, P. schwetzi, P. inui, and P. knowlesi,* have emerged as a major cause of human malaria in Southeast Asia, particularly Malaysian Borneo, since 2004 (4).

Transmission

Malaria is spread among people by mosquitoes belonging to the genus *Anopheles*. The female mosquito is infected by gametocytes, the sexual stages of the malaria parasite, when they take a blood meal from an infected person. Male and female gametocytes then fuse to form zygotes (ookinetes), which embed in the gut wall as oocysts and then undergo further development in the insect for 6–12 days. The mature oocysts

rupture to liberate motile sporozoites, which migrate to the mosquito salivary glands to await inoculation into humans through the bite of the mosquito carrying them. The intensity of malaria transmission in an area is the rate at which people are inoculated with malaria parasites by infected mosquitoes. It is expressed as the annual entomological inoculation rate (EIR), which is the number of infectious mosquito bites received by an individual in 1 year. The EIR determines to a large extent the epidemiology of malaria and the pattern of clinical disease in an area (5).

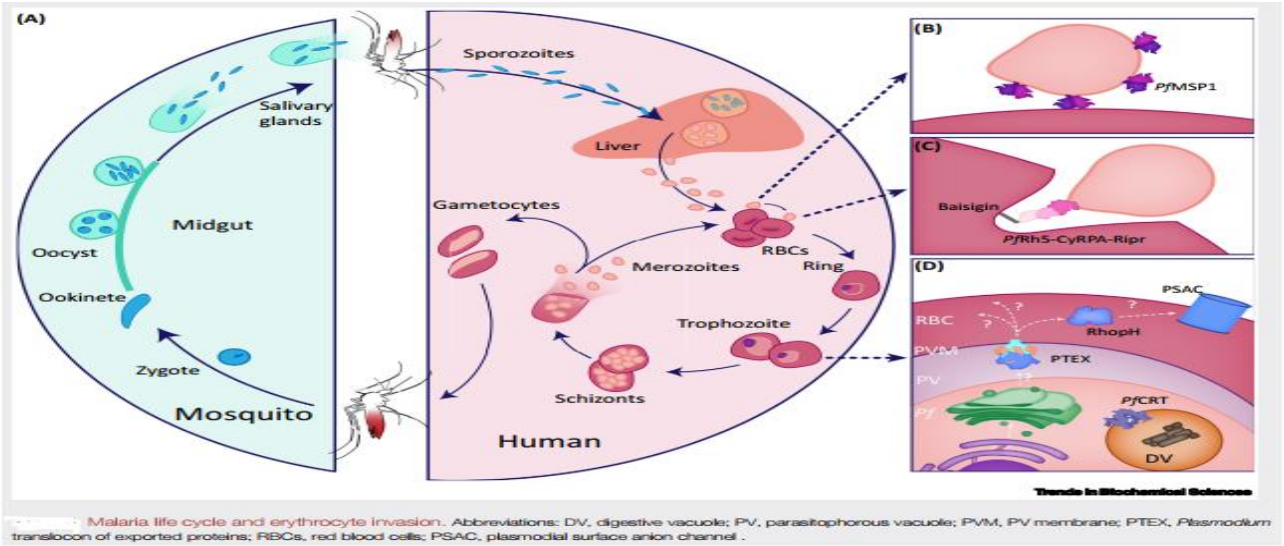
Transfusion-transmitted (TT) malaria is an alternative infection route that has gained little attention from authorities, despite presenting real danger to patients. Subjects requiring blood transfusion are a vulnerable population in debilitating conditions. Although cases of TT malaria are assumed based on different criteria, transmission can only be proven definitely by genotyping. However, most cases in the literature are "presumed" cases, where genotyping was not done or reported (6).

Life cycle

Plasmodium has sexual phase in the mosquito and alternates between vertebrate and mosquito hosts. As a prelude to a blood meal, the female mosquito injects the transmissive form, the sporozoite, into the skin of a vertebrate using anticoagulant saliva. Sporozoites infect the liver (mammals) or spleen (humans), as well as endothelial cells and macrophages, after entering the bloodstream or lymphatics (birds and lizards). They become intracellular and multiply to generate hundreds of invasive merozoites in this environment (the pre-erythrocytic or exo-erythrocytic phase). These enter the bloodstream and infiltrate erythrocytes (7). The parasite feeds on its host cell inside an erythrocyte, then multiplies to generate more merozoites, which exit and invade new erythrocytes, a cycle that is repeated many times (the asexual blood cycle) to substantially

increase numbers. The time from invasion to escape varies by species, with merozoites released synchronously with fever peaks. Asexual blood stages include in sequence, the merozoite, ring, trophozoite and schizont stages.

The parasite eventually enters sexual phase, where it develops into either a female gametocyte (macrogametocyte) or a male gametocyte (microgametocyte) inside its host cell (8). Gametocytes are now carried into the gut of a feeding female mosquito, where both types of gametocytes escape from their host cells, allowing the life cycle to continue. Male gametocytes split quickly into a variety of motile flagellated microgametes, each of which can fertilize a female macrogamete to produce a zygote. The parasite then transforms into a motile ookinete, entering the stomach wall of the mosquito and encysting as a rounded oocyst. Within this, the parasite replicates asexually, forming hundreds of motile sporozoites (sporogony). Mature sporozoites pass through the oocytst wall into the insect's blood cavity (haemocoel) and then to the salivary glands, penetrating their walls to reach the mosquito's stored saliva in preparation for transmission to a vertebrate during a subsequent blood meal (9). As shown in figure 1



Malaria life cycle and erythrocyte invasion. Abbreviations: DV, digestive vacuole; PV, parasitophorous vacuole; PVM, PV membrane; PTEX, *Plasmodium* translocon of exported proteins; RBCs, red blood cells; PSAC, plasmodial surface anion channel .

Figure 1. Life cycle of malaria (10)

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Morphology

Merozoites

It was established that merozoites typically possess a cluster of specialized secretory structures (rhoptries and micronemes) and cytoskeletal elements (polar rings) at their anterior end, and are bounded by a triple membranous layer (pellicle) consisting of the plasma membrane and two underlying membranes (inner membrane complex) to which a set of longitudinal microtubules is attached. Also discovered were a single mitochondrion and a structure initially named a spherical body, now known as the apicomplast, although this organelle only came into its own as an important apicomplexan feature much later (11). As shown in figure 2.

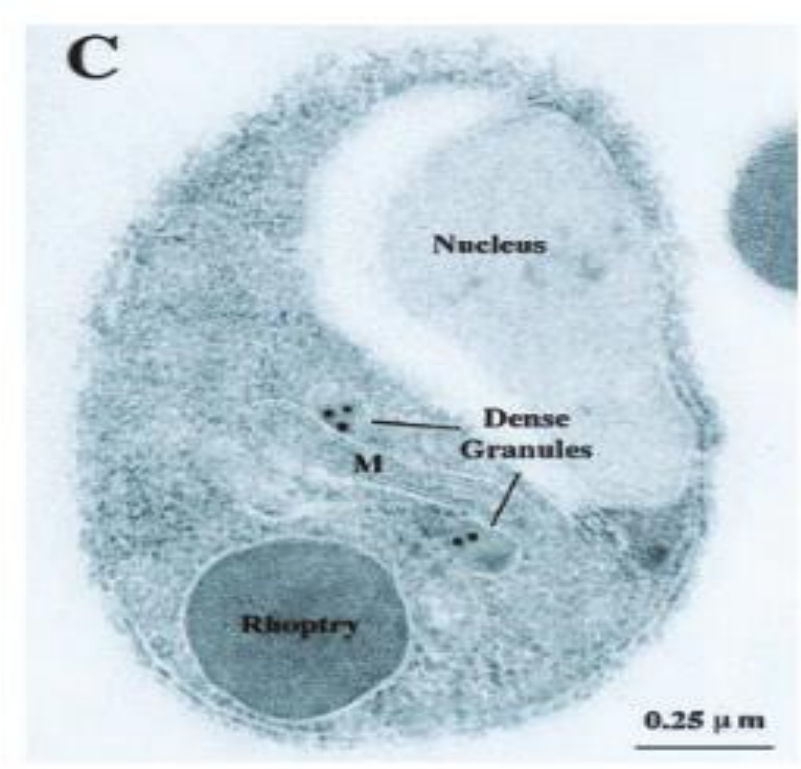

Figure 2. A Merozoite (12)

Ring and trophozoite stages

The merozoite loses its invasive organelles after entering. It becomes disc- or cup-like with a narrower center that appears as a hole under a light microscope or in some sections – hence the name 'ring' stage, As shown in in figure 3. It now starts ingesting erythrocyte cytoplasm and proteolytically destroying haemoglobin within tiny vacuoles. The parasite changes into a plumper trophozoite and eats more voraciously as the feeding progresses. In food vacuoles, a breakdown product of haemoglobin (haem) is transformed to an insoluble malaria pigment (haemozoin) (13).

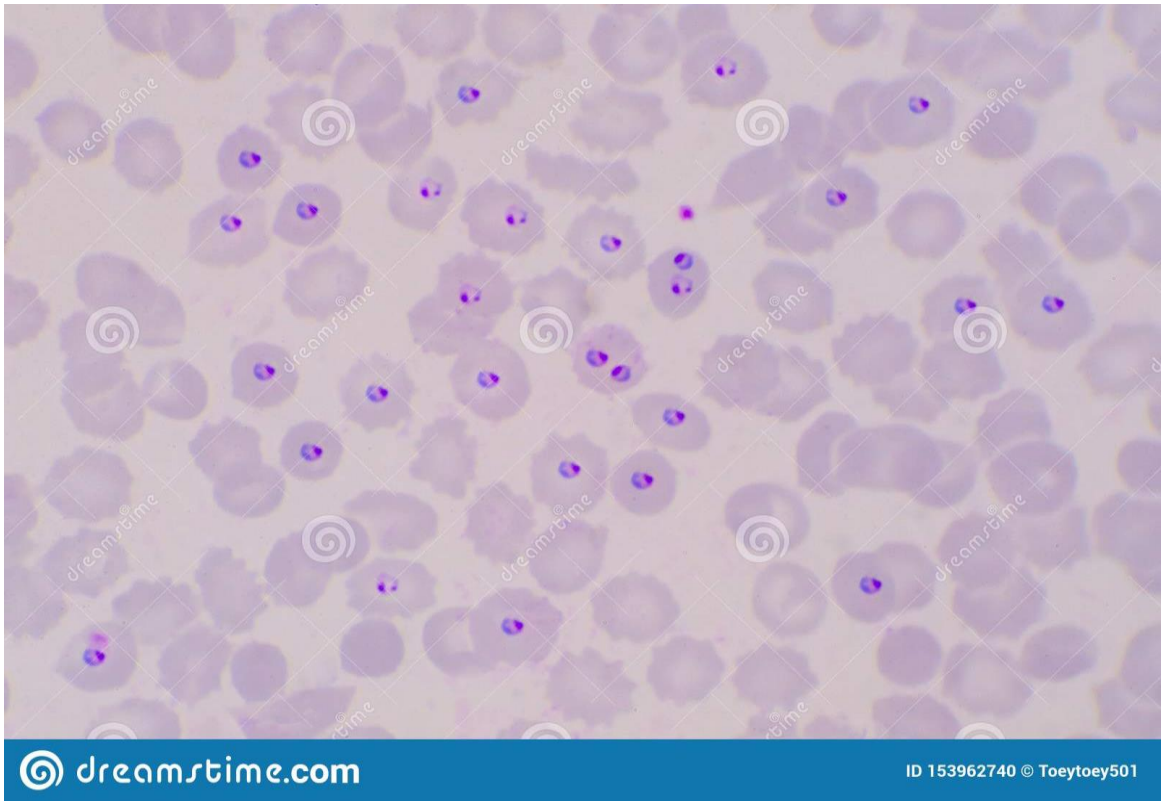

Figure 3. Ring stage (14)

Schizont stage

After a number of rounds of deoxyribonucleic acid (DNA) synthesis and mitosis, the parasite's nucleus divides, with the ultimate number varying by species (*P. falciparum* normally concludes with roughly 16, the

result of four rounds of DNA synthesis and mitosis). Each nucleus enters a merozoite bud, where it is surrounded by other organelles, As shown in in figure 4. The adult merozoites separate from the parent schizont, leaving behind a little amount of cytoplasm, known as the residual body, which contains the haemozoin. Finally, exonemes (specialized secretory vesicles) release protease (15).

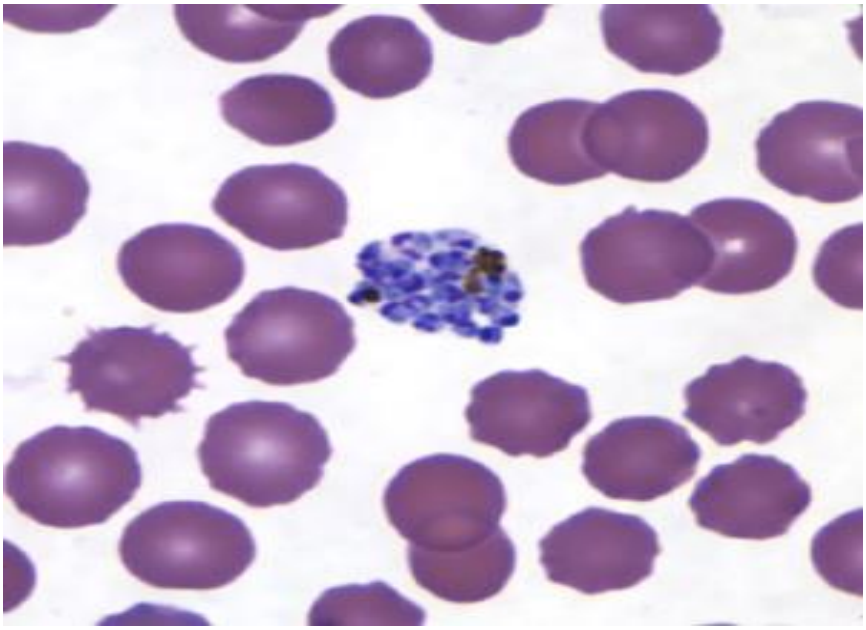

Figure 4. Schizonts (16)

Sexual stages

Gametocytes: it happens at or before the merozoite stage. Although their chromosomal contents are identical, parasites evolve into male or female lines, each with their own set of structure and chemistry. Male and female mature gametocytes in *P. falciparum* are long and curved. In terms of cellular detail, the two genders differ. Both include many long microtubules that define their shape during development, which varies by species but is mostly spheroidal. A three-membrane pellicle surrounds all adult gametocytes, which contain disclike secretory vesicles (osmiophilic bodies) that are used to exit the host cell (17,18). As shown in figure 5.

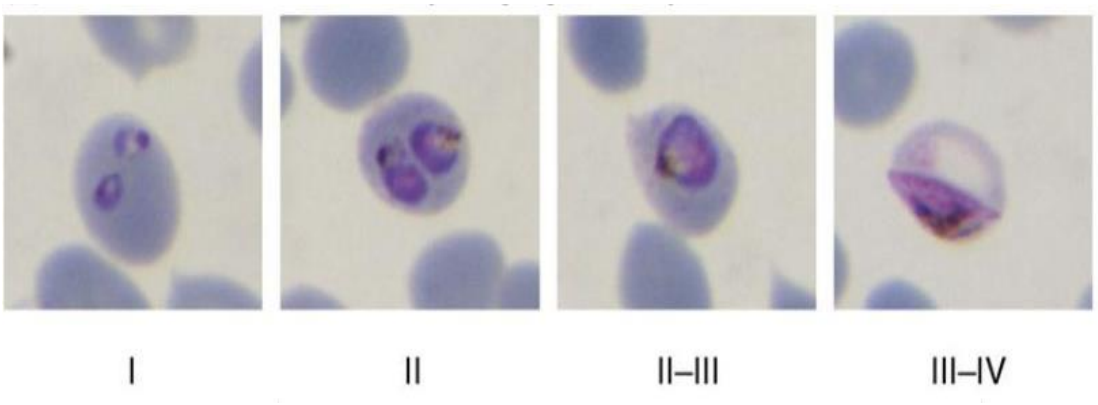

Figure 5. Gametocytes formation (19)

Gametes: When gametocytes are ingested by the mosquito, they escape from their surrounding erythrocyte membranes, triggered by the temperature drop and by chemicals in the insect. The male gametocyte divides rapidly into several gametes which sprout long motile flagella (exflagellation), enabling them to make contact with female gametes to fertilize them (20). As shown in figure 6.

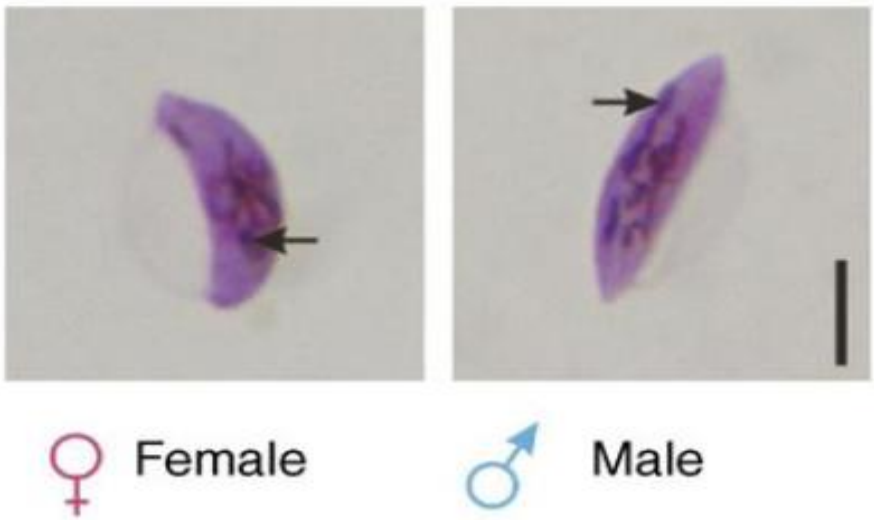

Figure 6. Gametes (21)

Pathogenesis

In recent decades, *P. vivax* has usually been considered a benign infection; reports of severe vivax malaria have been confined to case reports and small case series. A major feature that distinguishes *P. vivax*

from *P. falciparum* is its lower pyrogenic threshold (the level of parasitemia associated with fever). Organ-specific studies have also shown that the inflammatory response during *P. vivax* infection is greater than that seen in *P. falciparum* infections with a similar or greater parasite biomass. Cytokine production during *P. vivax* infections is higher than that in P. falciparum infections of similar parasite biomass. Although the inflammatory correlates of the lower pyrogenic threshold have been described, the underlying mechanism(s) have not (22).

Because all stages of *P. vivax* are visible in peripheral blood, *P. vivax* is not thought to sequester or cause end-organ dysfunction in the same manner as P. falciparum. However, based upon observations of a smaller proportion of circulating schizonts in relation to trophozoites. the deformability of vivax-infected RBCs is increased. This property might enable *P. vivax* to avoid destruction during passage through the 2 mm slits between endothelial cells of the splenic sinusoids. The rheological mechanisms underlying this are unknown. Although reduced RBC deformability is thought to contribute to impaired organ perfusion in falciparum malaria, increased deformability of *P. vivax*-infected RBCs makes this unlikely in vivax malaria. However, such deformability is accompanied by increased fragility of both infected and non-infected RBCs (23). *P. ovali* is similar to *P. vivax* in terms of pathogenesis.

Plasmodium falciparum is the most common cause of severe malaria due to its propensity to produce infected red blood cell (RBC) cytoadherence to the vascular endothelium, resulting in end-organ failure. Acute kidney injury (AKI) can be caused by other plasmodium species (24).

P. falciparum erythrocyte membrane protein 1 (PfEMP1), which serves as a critical ligand for cytoadherence, is transported to the RBC

membrane by parasites growing within infected RBCs. PfEMP1 is found on RBC protrusions, or 'knobs,' which provide attachment points to the endothelium. PfEMP1 is strain-specific, with antigenic variation for immune evasion and differential endothelium receptor binding provided by the var gene family. On most vascular beds, CD36 is a constitutively expressed endothelial receptor. Cytoadhesion causes parasitized RBCs to be sequestered in capillaries and postcapillary venules, resulting in heterogeneous microcirculation obstruction and tissue hypoxia (25).

There is endothelial and astroglial activation in the brain of adults with cerebral malaria, as well as varied inflammatory responses and a minor functional alteration in the blood–brain barrier. Breakdown of the endothelial barrier is seen in children with well characterized retinopathy-positive cerebral malaria, particularly in areas of sequestration. Adults and children have different patterns of histopathological alteration in the brain in cerebral malaria, with adults having less inflammatory cellular infiltrates and edema (26).

Despite the fact that cytokines and/or chemokines are obviously implicated in the pathophysiology of malarial fever and may be linked to illness severity and/or cerebral malaria, there is no evidence that they are the cause of coma. Recent research has focused on the function of cytokines and chemokines in the pathophysiology of acute kidney injury (AKI) in severe malaria. AKI and the need for renal replacement therapy were both linked to plasma-soluble urokinase-type plasminogen activator receptor, a sign of immunological activity. TNFa, but not interleukin 6 or the IL6:IL10 ratio, has previously been linked to AKI, suggesting that TNFa may cause localized renal tubular cell death Injuries (27,28).

The unique ability of *P. falciparum* to bind to endothelium produces the clinicopathological syndrome of Cerebral Malaria in both children and

adults. In highly endemic settings, a low level of infection (<1% parasitemia) can result in clinical signs of CM and be life-threatening. The clinical manifestations of CM may start with a typical malaria presentation and quickly (over minutes to hours) degenerate to a comatose state. After exclusion of other possibly causes of coma (e.g., postictal state, hypoglycemia, meningitis, bacterial sepsis, head trauma/cerebral bleed, etc.). The pathobiology of CM is not completely understood but a large body of evidence from both clinical and pathological studies has implicated a series of events and pathways at work within the disease landscape (29).

P. malariae shows an affinity for older cells, parasitizing about 0.2% of the victim's total erythrocyte population. Following incorporation into erythrocytes, early trophozoites begin to accumulate hemozoin and the pink-staining Ziemann's dots. The cytoplasm of the trophozoite is compact, often appearing as a band across the infected cell. Morphologically, mature trophozoites resemble macrogametocytes and are, therefore, difficult to distinguish. No change in diameter is evident in the infected erythrocyte, probably due to the parasite's affinity for older erythrocytes. The number of merozoites, following schizogony, varies from 6 to 12 (average, 8). Hemozoin usually accumulates as a dense mass in the center of the schizont (30).

Clinical features

Malaria infection in a naive person almost always results in a feverish illness. Rigors, headaches, nausea, and muscle pains are common symptoms that accompany the condition. If the symptoms are treated with the right medications at this point, they will go away in a few days, however with a lot of weariness. Complete treatment will eradicate *P. falciparum* infection, and any recurrence of symptoms indicates incomplete treatment, medication resistance, or a new infection. Unless the

dormant liver-resident hypnozoite stage is eliminated by a protracted therapy with an 8-aminoquinolone medication, future infections of *P. vivax* and *P. ovale* may recur at intervals as a result of reactivation of the dormant liver-resident hypnozoite stage (31). The initial infection is not managed in a proportion of untreated or partially treated patients, and the illness advances to severe or complex malaria, which can lead to death. The severity of malaria varies depending on age and transmission level (reflecting the immune status of populations). The three syndromes of severe anemia, cerebral malaria, and respiratory distress account for the majority of malaria deaths in Africa, and they can occur independently or in combination (32).

Epidemiology

Malaria is one of the most important public health problem in term of morbidity and mortality, causing more than 200 million cases and 655.000 deaths every year. According to the World Health Organization (WHO) Malaria Report 2011, a total of 106 countries in the world are at risk of transmission of malaria infection. A total of 216 million estimated malaria cases occurred in 2010, 81% of which were reported in the African Region, followed by South East Asia (13%) and Eastern Mediterranean Region (5%). The total number of malaria deaths was estimated to be 655.000 in 2010; 91% of whom occurred in the African Region, 6% in South-East Asia and 3% in Eastern Mediterranean Region (33).

Indigenous malaria cases have been reported mainly from two countries from the Middle East Region, namely Yemen and Saudi Arabia while the remaining countries have mostly reported imported malaria cases during the last couple of years (34).

According to the Ministry of Health, Iraq has persevered in its long battle with this disease. For example, in the 1960s Iraq faced a very bad outbreak where the number of malaria reported cases increased from 1533 in 1962 to 11,878 in 1965. In 1995 another outbreak peaked at 39,000 cases, especially in the three mountainous areas in the north Iraq due to challenges caused by the security situation and poor inter-sectoral coordination. More notably so, there have been no indigenous reported malaria cases in the country in 2009, 2010 and 2011 to date. Currently, Iraq has developed a national strategy of malaria for 2016–2020 (35).

Diagnosis

Malaria is diagnosed using a combination of clinical observations, case history, and diagnostic procedures, the most common of which is a microscopic blood examination. The best time to draw blood is when the patient's temperature is increasing, as this is when the most parasites are likely to be identified. In routine diagnosis, thick blood films are employed, and as few as one parasite per 200 lL blood can be recognized. Rapid diagnostic 'dipstick' tests are simple to use and do not require trained staff or specific equipment to identify malaria antigens in a finger prick of blood in a matter of minutes (36).

The quantitative buffy coat (QBC) technique has been shown to be a rapid and sensitive test for diagnosing malaria in numerous laboratories settings. While it enhances sensitivity for *P. falciparum*, it reduces sensitivity for non-falciparum species and decreases specificity due to staining of leukocyte DNA. Recently, it has been shown that acridine orange is the preferred diagnostic method (over light microscopy and immunochromatographic tests) in the context of epidemiologic studies in asymptomatic populations in endemic areas, probably because of increased sensitivity at low parasitemia. Diagnosis of malaria using serological

methods is usually based on the detection of antibodies against asexual blood stage malaria parasites. Immunofluorescence antibody testing (IFA) has been a reliable serologic test for malaria in recent decades (37).

Treatment

Combination therapy is becoming more popular. Artemisinin, derived from the plant Artemisia annua, is a highly effective antimalarial, and it is used in mainly pairwise combinations with other drugs such as Fansidar and mefloquine, the latter an important and still highly efficacious drug against which resistance is on the rise, particularly in Southeast Asia. Drug resistance is a critical concern in malaria prevention, especially because there are currently no clinically approved malaria vaccines, despite the fact that several are being developed and tested (38).

Vaccination development against malaria

There is currently no malaria vaccine that has been approved by the FDA. A malaria vaccine, according to some scientists, is required to eradicate malaria. The World Health Organization (WHO) has set strategic goals to license malaria vaccines targeting *Plasmodium falciparum* and *Plasmodium vivax* that have at least 75% protective efficacy against clinical malaria and reduce transmission to enable eradication. RTS,S/AS01, the most advanced candidate vaccine to date, completed phase 3 testing in seven Sub-Saharan African nations and achieved four-dose efficacy against clinical malaria of 27% over 38 months in children aged 6–12 weeks and 39% over 48 months in those aged 5–17 months (39).

The revised Malaria Vaccine Technology Roadmap to 2030 now calls for a next-generation vaccine to achieve 75% efficacy over 2 years against *P. falciparum* and/ or *P. vivax* (in an era of renewed global interest toward malaria elimination and eradication), while also retaining its

original 2015 “landmark” goal of a first-generation vaccine with protective efficacy of >50% lasting more than 1 year. Achieving this next-generation vaccine goal will necessitate building on the success of current pre-erythrocytic subunit and whole sporozoite-based vaccines, as well as new strategies to add blood-stage or transmission-blocking immunity (40).

The first attempts to produce a successful malaria vaccine date back to the 1930s, with a focus on parasites that had been inactivated or destroyed and had failed to immunize. The addition of adjuvant systems demonstrated the immunogenicity of malaria vaccine candidates in animal models, including a study by Jules Freund that showed partial protection in ducklings immunized with formalin-inactivated parasites with what is now known as Freund's complete adjuvant, which is made up of heat-killed Mycobacterium tuberculosis suspended in a water-in-oil emulsion (41).

Because malaria lacks a biological correlate to protection, vaccine research has taken an inordinate amount of time. Before phase 2 field testing in the target population of children in malaria endemic areas to determine vaccine efficacy, a series of steps were required, including initial development of a candidate vaccine antigenic product in the laboratory, testing for safety and proof of concept in animal models, age de-escalation phase 1 testing in adults and then in children for safety and reactogenicity, and age de-escalation phase 1 testing in adults and then in children for safety and reactogenicity. These several processes are a time-consuming procedure that necessitates large investment for a lengthy product development timetable, and the process is fraught with the possibility of a poor outcome (42).

Pre-erythrocytic vaccinations target Plasmodium's sporozoite and liver stages, which are clinically inactive. These vaccines are designed to kill parasites at the early stages of infection and, if very effective (i.e.,

establish sterilizing immunity), to prevent sickness and disrupt transmission. In the 1970s, whole sporozoite vaccinations were found to protect humans from *Plasmodium falciparum*, and modern whole organism vaccine techniques include irradiated parasites, genetically engineered parasites, and infection in combination with chemoprophylaxis, according to session chair Fidel Zavala (43).

Antigenic polymorphism of both merozoite and infected erythrocyte surface proteins, redundancy in the merozoite invasion pathways, including both sialic acid-dependent and sialic acid-independent pathways, and difficulties expressing conformationally correct proteins were all discussed by Dr. Carole Long of the NIAID. High antibody doses are needed to prevent merozoite invasion in vivo, according to studies thus far, and existing tests have failed to predict protection in humans. Merozoite protein trials have been unsuccessful, although continuous efforts are being made to increase the antigens' immunogenicity and functional activity. New antigens, as well as new antigen combinations, may have additive or synergistic activity, making them a research priority for a number of organizations (44).

Dr. Prakash Srinivasan of John Hopkins University (Baltimore, USA) described how the AMA1-RON2L complex is being developed as another merozoite invasion vaccine. Despite field investigations showed that apical membrane antigen 1 (AMA1) vaccinations gave action against homologous parasites, AMA1-only immunizations have failed to protect against homologous CHMI. To commence invasion, AMA1 forms a complex with rhoptry neck protein 2 (RON2) at the intersection between merozoite and erythrocyte surfaces, and recent studies have demonstrated that antibodies produced against the AMA1-RON2 peptide complex, rather

than AMA1 alone, protect mice from virulent *P. yoelii* infection (45). figure 7 show the possible targets of vaccines.

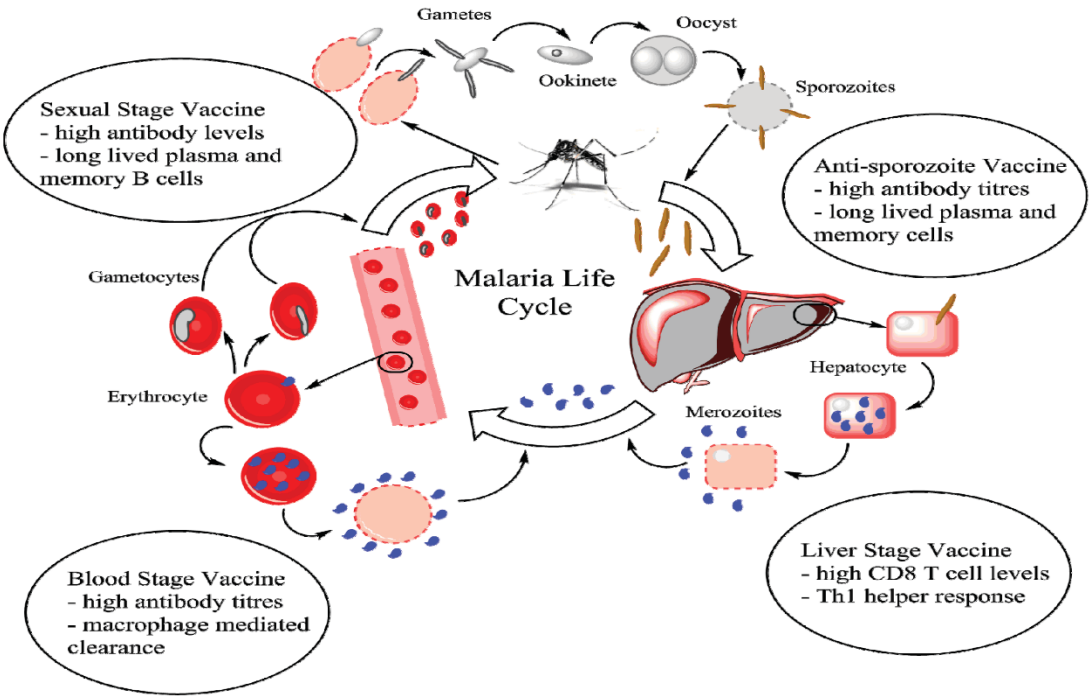

Figure 7. Malaria vaccines (43)

In comparison to other major global infectious diseases such as HIV and tuberculosis, malaria vaccine research is aided by the availability of a clinical challenge model and a high attack rate in endemic areas, allowing definitive assessment of vaccines that prevent infection through human experimentation. There is agreement that pre-erythrocytic vaccines should be tested in sporozoite challenge studies, and there is a growing consensus that some evidence of effect, such as sterile protection or at least a reduced parasite growth rate, is required as a pre-condition for moving asexual stage vaccines into costly large-scale clinical trials, as other measures, such as growth-inhibitory assays, have yet to deliver on their promise as surrogate markers of protection (46).

Combinations of antigens expressed at several life-cycle phases could be a promising method. Assuming we can overcome the issue of immune

response longevity, the pros of this approach are that adding a blood-stage component to an anti-sporozoite target would reduce the risk of morbidity and mortality while transmission declines, as well as provide immune protection against epidemics of severe disease should transmission rebound due to other intervention failures (47).

Recent scientific advances have given rise to the need for safer formulations increasing antigen efficacy. “Nanovaccinology” has emerged during the last few years, which will surely come to play an important role in malaria vaccine development. Using nanoparticles has enabled antigen stability, immunogenicity, selective administration and slow release to become improved. Such characteristics have facilitated developing different vaccines from nanoparticles which have been approved for human use, varying in composition, form, surface properties and size (1–1000 nm) similar to cell components, enabling them to enter cells via mechanisms such as pinocytosis (48).

A promising alternative delivery system for subunitbased vaccines has been developed recently. The technique is known as Self-Assembling Protein Nanoparticles (SAPNs) and involves the expression of a peptide/ protein containing a target antigen covalently linked to an adjuvant sequence (flagellin-derived) and, in some cases, a universal epitope such as the Pan-DR T-helper epitope (PADRE) sequence. This peptide/protein can self-assemble in specific conditions, thus forming ~ 20–50 nm nanoparticles and, when formulated or emulsified with an adjuvant such as GLA-SE or Army Liposome Formulation (ALF), has managed to produce a protection-inducing response against several diseases (49).

Prevention

Vector management is so effective at avoiding infection and limiting disease transmission, it is an important part of malaria control and elimination methods. Insecticide-treated nets (ITNs) and indoor residual (IRS) spraying are the two main therapies.

The use of drugs, either alone or in combination, to prevent malaria infections and their effects is known as preventive chemotherapy. Chemoprophylaxis, intermittent preventive treatment for infants and pregnant women (IPTi and IPTp), seasonal malaria chemoprevention (SMC), and mass medication administration are all part of it (MDA). These safe and cost-effective solutions are meant to supplement ongoing malaria control efforts, such as vector control, rapid identification of suspected malaria, and antimalarial treatment of confirmed cases (50).

Conclusion

Malaria is very serious and global health issue and the need for preventive and prophylactic vaccine is huge. No available vaccine meet the criteria of the WHO. The (WHO) has set strategic goals to license malaria vaccines targeting *Plasmodium falciparum* and *Plasmodium vivax* that have at least 75% protective efficacy against clinical malaria. There is a road map to develop effective vaccine by 2030.

References

1. WHO (2015). The World Malaria Report http://wwwwhoint/malaria/publications/world-malaria-report-2015/report/en/.
2. Tuteja R. Malaria− an overview. The FEBS journal. 2007 Sep;274(18):4670-9.
3. MMV website. Curing malaria together. [Accessed October 16,2008]. Available at: http://www.mmv.org
4. Ramasamy R. Zoonotic malaria–global overview and research and policy needs. Frontiers in public health. 2014 Aug 18;2:123.

5. Guidelines for the Treatment of Malaria. 3rd edition. Geneva: World Health Organization; 2015. Annex 2, Malaria transmission and antimalarial medicines. Available from: https://www.ncbi.nlm.nih.gov/books/NBK294437 .
6. Alho RM, Machado KV, Val FF, Fraiji NA, Alexandre MA, Melo GC, Recht J, Siqueira AM, Monteiro WM, Lacerda MV. Alternative transmission routes in the malaria elimination era: an overview of transfusion-transmitted malaria in the Americas. Malaria journal. 2017 Dec;16(1):1-4.
7. Cox-Singh J, Singh B. Knowlesi malaria: newly emergent and of public health importance?. Trends in parasitology. 2008 Sep 1;24(9):406-10.
8. Bannister LH, Sherman IW, plasmodium, In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. 2009.
9. Nureye D, Assefa S. Old and recent advances in life cycle, pathogenesis, diagnosis, prevention, and treatment of malaria including perspectives in Ethiopia. The Scientific World Journal. 2020 Feb 14;2020.
10. Anton L, Cobb DW, Ho CM. Structural parasitology of the malaria parasite Plasmodium falciparum. Trends in biochemical sciences. 2021 Dec 7.
11. Bannister LH, Mitchell GH. The malaria merozoite, forty years on. Parasitology. 2009 Oct;136(12):1435-44.
12. Barale JC, Blisnick T, Fujioka H, Alzari PM, Aikawa M, Braun-Breton C, Langsley G. Plasmodium falciparum subtilisin-like protease 2, a merozoite candidate for the merozoite surface protein 1–42 maturase. Proceedings of the National Academy of Sciences. 1999 May 25;96(11):6445-50.
13. Goldberg DE, Hemoglobin degradation. Current Topics in Microbiology and Immunology, 2005: 295: 275–291.
14. https://www.dreamstime.com/plasmodium-blood-parasite-ring-form-stage-infected-redblood-cells-malaria-disease-plasmodium-blood-parasite-ring-form-stage-image153962740
15. Yeoh S, O'Donnell RA, Koussis K, Dluzewski AR, Ansell KH, Osborne SA, Hackett F, Withers-Martinez C, Mitchell GH, Bannister LH, Bryans JS. Subcellular discharge of a serine protease mediates release of invasive malaria parasites from host erythrocytes. Cell. 2007 Dec 14;131(6):1072-83.
16. https://mcdinternational.org/trainings/malaria/english/dpdx5/html/frames/M-R/Malaria/vivax/body_malariadfvivschiz
17. Alano P. Plasmodium falciparum gametocytes: still many secrets of a hidden life. Molecular microbiology. 2007 Oct;66(2):291-302.
18. Khan SM, Franke-Fayard B, Mair GR, Lasonder E, Janse CJ, Mann M, Waters AP. Proteome analysis of separated male and female gametocytes reveals novel sex-specific Plasmodium biology. Cell. 2005 Jun 3;121(5):675-87.
19. WHO, the safety and effectiveness of single dose primiquine as a *P. falciparum* gametocytocide, 2012.
20. Billker O, Lindo V, Panico M, Etienne AE, Paxton T, Dell A, Rogers M, Sinden RE, Morris HR. Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. Nature. 1998 Mar;392(6673):289-92.
21. Anstey NM, Douglas NM, Poespoprodjo JR, Price RN. Plasmodium vivax: clinical spectrum, risk factors and pathogenesis. Advances in parasitology. 2012 Jan 1;80:151-201.
22. Hemmer CJ, Holst FG, Kern P, Chiwakata CB, Dietrich M, Reisinger EC. Stronger host response per parasitized erythrocyte in Plasmodium vivax or ovale than in Plasmodium falciparum malaria. Tropical Medicine & International Health. 2006 Jun;11(6):817-23.

23. Anstey NM, Russell B, Yeo TW, Price RN. The pathophysiology of vivax malaria. Trends in parasitology. 2009 May 1;25(5):220-7.
24. Plewes K, Turner GD, Dondorp AM. Pathophysiology, clinical presentation, and treatment of coma and acute kidney injury complicating falciparum malaria. Current opinion in infectious diseases. 2018 Feb;31(1):69.
25. Medana IM, Day NP, Sachanonta N, Mai NT, Dondorp AM, Pongponratn E, Hien TT, White NJ, Turner GD. Coma in fatal adult human malaria is not caused by cerebral oedema. Malaria journal. 2011 Dec;10(1):1-4.
26. Plewes K, Royakkers AA, Hanson J, Hasan MM, Alam S, Ghose A, Maude RJ, Stassen PM, Charunwatthana P, Lee SJ, Turner GD. Correlation of biomarkers for parasite burden and immune activation with acute kidney injury in severe falciparum malaria. Malaria Journal. 2014 Dec;13(1):1-0.
27. Dunst J, Kamena F, Matuschewski K. Cytokines and chemokines in cerebral malaria pathogenesis. Frontiers in cellular and infection microbiology. 2017 Jul 20;7:324.
28. Milner DA. Malaria pathogenesis. Cold Spring Harbor perspectives in medicine. 2018 Jan 1;8(1):a025569.
29. Collins WE, Jeffery GM. A retrospective examination of sporozoite-and trophozoite-induced infections with Plasmodium falciparum: development of parasitologic and clinical immunity during primary infection. The American journal of tropical medicine and hygiene. 1999 Jul 1;61(1 Suppl):4-19.
30. Bogitsh BJ, Carter CE, Oeltmann TN. Chapter 7—Blood and tissue protistans II: human malaria. Human parasitology. 2019;5:111-33.
31. Taylor TE, Molyneux ME. The pathogenesis of pediatric cerebral malaria: eye exams, autopsies, and neuroimaging. Annals of the New York Academy of Sciences. 2015 Apr;1342(1):44-52.
32. Autino B, Noris A, Russo R, Castelli F. Epidemiology of malaria in endemic areas. Mediterranean journal of hematology and infectious diseases. 2012;4(1).
33. Al-Awadhi M, Ahmad S, Iqbal J. Current status and the epidemiology of malaria in the Middle East Region and beyond. Microorganisms. 2021 Feb;9(2):338.
34. World Health Organization. Malaria Terminology. Geneva: World Health Organization; 2016. Available from: http://www.who.int. [Last accessed on 2019 Nov 07].
35. Bell D, Wongsrichanalai C, Barnwell JW. Ensuring quality and access for malaria diagnosis: how can it be achieved?. Nature Reviews Microbiology. 2006 Sep;4(9):682-95.
36. Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: a brief review. The Korean journal of parasitology. 2009 Jun;47(2):93.
37. Wiseman V, Kim M, Mutabingwa TK, Whitty CJ. Cost-effectiveness study of three antimalarial drug combinations in Tanzania. PLoS Medicine. 2006 Oct 10;3(10):e373.
38. Malar. Vaccine Funders Group. 2013. *Malaria Vaccine Technology Roadmap*. Geneva: World Health Organ. http://www.who.int/immunization/topics/malaria/vaccine_roadmap/TRM_update_nov13.pdf?ua=1
39. Draper SJ, Sack BK, King CR, Nielsen CM, Rayner JC, Higgins MK, Long CA, Seder RA. Malaria vaccines: recent advances and new horizons. Cell host & microbe. 2018 Jul 11;24(1):43-56.
40. Freund J, Sommer HE, Walter AW. Immunization against malaria: vaccination of ducks with killed parasites incorporated with adjuvants. Science. 1945 Aug 24;102(2643):200-2.

41. Laurens MB. The promise of a malaria vaccine—are we closer?. Annual review of microbiology. 2018 Sep 8;72:273-92.
42. Coelho CH, Doritchamou JY, Zaidi I, Duffy PE. Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium.
43. Satchwell TJ. Erythrocyte invasion receptors for Plasmodium falciparum: new and old. Transfusion medicine. 2016 Apr;26(2):77-88.
44. Srinivasan P, Baldeviano GC, Miura K, Diouf A, Ventocilla JA, Leiva KP, Lugo-Roman L, Lucas C, Orr-Gonzalez S, Zhu D, Villasante E. A malaria vaccine protects Aotus monkeys against virulent Plasmodium falciparum infection. npj Vaccines. 2017 May 22;2(1):1-0.
45. Powles L, Xiang S, Selomulya C, Plebanski M. The Use of Synthetic Carriers in Malaria Vaccine Design. Vaccines [Internet] 2015;3(4):894–929.
46. Schwartz L, Brown GV, Genton B, Moorthy VS. A review of malaria vaccine clinical projects based on the WHO rainbow table. Malaria journal. 2012 Dec;11(1):1-22.
47. Theisen M, Roeffen W, Singh SK, Andersen G, Amoah L, van de Vegte-Bolmer M, Arens T, Tiendrebeogo RW, Jones S, Bousema T, Adu B. A multi-stage malaria vaccine candidate targeting both transmission and asexual parasite life-cycle stages. Vaccine. 2014 May 7;32(22):2623-30.
48. Mamo T, Poland GA. Nanovaccinology: the next generation of vaccines meets 21st century materials science and engineering. Vaccine. 2012 Oct 19;30(47):6609-11.
49. Kaba SA, Karch CP, Seth L, Ferlez KM, Storme CK, Pesavento DM, Laughlin PY, Bergmann-Leitner ES, Burkhard P, Lanar DE. Self-assembling protein nanoparticles with built-in flagellin domains increases protective efficacy of a Plasmodium falciparum based vaccine. Vaccine. 2018 Feb 1;36(6):906-14.
50. WHO, visit: https://www.who.int/news-room/fact-sheets/detail/malaria.