A Pathophysiological, Clinical, and Epidemiological View of Malaria

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A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2023

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial
fulfillment of the requirements for graduation from the
Honors Program of Liberty University.

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Abstract

Malaria is a parasitic disease that is common among all ages in tropical and subtropical countries. Annually, there are an estimated 3.3 billion people in 97 countries at risk of contracting malaria. Malaria has been a major global health problem throughout history and is a leading cause of death and disease for many within tropical regions. In the past decade, efforts, such advances in medicine and insecticide techniques, have reduced the prevalence of malaria by 50% which suggests that the elimination of this disease is possible. With the prevalence of malaria and recent reduction, it is vital to aid in the research for new eradication methods and new treatments of malaria to continue to reduce and eliminate this disease.

A Pathophysiological, Clinical, and Epidemiological View of Malaria

The Current State of Malaria

Malaria is the cause of death for over 500,000 people each year and is especially prevalent in developing countries, specifically Sub-Saharan Africa (Garrido-Cardenas et al., 2018). Malaria is a parasitic disease caused by six Plasmodium species which are spread through infected Anopheles mosquitoes (World Health Organization, 2021). Anopheles are ideal vectors as they replicate close to humans and attack during daylight. Although most people experience uncomplicated malaria, complicated malaria is a life-threatening disease manifesting as cerebral conditions, respiratory issues, anemia, kidney injury, and more. As malaria is responsible for over a half million deaths each year, there is a need for change. Currently, there are tactics to keep malaria under control through the use of medications, insecticide sprays and nets, vaccines, diagnostic techniques, and genetic technology. Although rates of transmission have significantly decreased in the past century, there are still challenges in patient treatment and elimination of the mosquito vector. In developing countries, poverty, weak health systems, drug resistance, and insecticide resistance make it difficult to eliminate malaria. In order to understand the present state of malaria, it is vital to look at transmission, physiological importance, epidemiology, management methods, and the current challenges surrounding the disease, so that the future state of malaria improves.

Lifecycle of Parasite

In the parasite-human life cycle, the plasmodial species undergo at least 10 morphological states, replicate to over 10,000 cells, and have a varying total population from one to more than a million organisms (Milner, 2018). Although the exact number is unknown, only certain morphological states lead to clinical manifestations of malaria. For most humans, malaria

produces mild to no symptoms. The symptoms that do occur, such as a fever, are the result of any parasite combined with the human physiological response. The initial physiological response is due to the immune system response. As the disease progresses, there are recurring fevers during the blood stage. Regardless of manifestation, the parasite goes through the same life cycle and morphological stages which means the human clinical manifestation must be an exaggeration of the immune cells reacting to the parasite; this explains why malaria can be more serious for certain people with lack of immunity.

Lifecycle of Parasite: Mosquito Stage

To understand the biology and disease of malaria, it is crucial to understand the causative agent, *Plasmodium* (Milner, 2018). There are five plasmodial species that can cause malaria in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium knowlesi,* and *Plasmodium malaria*. To begin, the malaria life cycle involves two hosts and three stages. The mosquito stage involves the infection of a female *Anopheles* mosquito (Centers for Disease Control and Prevention, 2020). The mosquito stage begins when the gametocyte is ingested during feeding by the female *Anopheles* mosquito. Once the gametocyte is ingested, the gametocytes replicate and begin the sporogonic cycle within the mosquito (Beier, 1998). While the gametocyte is ingested in the *Anopheles* mosquito, the male gametocytes, known as microgametes, penetrate the female gametocytes, macrogametes; the penetration generates zygotes. The zygotes develop and invade the midgut of the wall of the *Anopheles* mosquito; in this location, the zygotes develop into oocysts. The oocysts grow, rupture, and release sporozoites. The sporozoites travel to the salivary glands of the mosquito. As the *Anopheles* mosquito probes for a blood meal from a human, the mosquito salivates. The protein anophelin, secreted in the mosquito's saliva, binds to the enzyme thrombin acting as an anti-coagulant; this

prevents blood from clotting and allows the mosquito to access its meal. As the mosquito takes a meal, the sporozoites, within the saliva, are injected into the human; this process allows the sporozoite life cycle to continue.

Lifecycle of Parasite: Human Stages

Once the saliva and sporozoite are injected into the human, the sporozoites are deposited in the human's dermis (Tuteja, 2007). The sporozoites must cross through dermal fibroblasts and endothelial cells to enter the bloodstream. Once in the bloodstream, the sporozoites travel to the liver where they infect liver cells and replicate in the liver; this is known as the liver stage. Next, the sporozoites invade hepatocytes and undergo asexual replication known as exo-erythrocytic schizogony. The mechanism of the invasion of the hepatocyte is not well understood. It is known that thrombospondin domains on the sporozoite are responsible for hepatocyte invasion; these domains bind to heparan sulfate proteoglycans on the hepatocytes. The replication of the sporozoites produces the multi-nucleated cell known as schizonts. The schizonts give rise to merozoites inside the hepatocyte. Upon schizont rupture, merozoites are released to infected red blood cells (RBCs), beginning the human blood stage. The clinical manifestations of malaria are a product of the blood stage. In the blood stage, the merozoites infect RBCs as a trophozoite is maturing into a schizont through multiple rounds of nuclear division. Then, the mature schizont ruptures releasing more merozoites; the rupture occurs after the lysis of the RBCs so that the merozoites can invade more uninfected RBCs. This release aligns with the increase in body temperature as the disease progresses. The contents released during RBC lysis stimulate the production of cytokines which are responsible for clinical manifestations of malaria. This cycle of invasion, replication, and release continues. For the cycle to continue, a small portion of the merozoites in the RBCs differentiate into microgametocytes or macrogametocytes which are

essential for transmitting infection into new female *Anopheles*. It can take up to two weeks before gametocytes are produced in this process; the timing is variable depending on the species of *Plasmodium*. Once a gametocyte is ingested by a female *Anopheles*, the Anopheles can take another blood meal on a human allowing the cycle to repeat.

Uncomplicated Malaria

The most common and less severe version of malaria is known as uncomplicated malaria (Milner, 2018). Uncomplicated malaria is defined as symptoms such as fever, chills, nausea, headaches, muscle pains, and vomiting, but the clinical signs do not indicate severity or vital organ dysfunction. A severe infection usually presents with cognitive dysfunction, severe anemia, and respiratory dysfunction; a severe infection is mostly due to the type of *Plasmodium* species and immune response. The symptoms of the malaria infection begin when the first liver schizont ruptures and the merozoites are released to infect RBCs. Although this is the first opportunity for symptoms to present, this event is silent in most people who will become ill. During this initial infection, cytokines such as tumor necrosis factor (TNF-α) are responsible for fever. Uncomplicated malaria is usually treated during each symptomatic episode with antimalarial medication. The malaria parasites can be cleared from the body once the antimalaria medication is used. The types of plasmodial species found to cause uncomplicated malaria are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium knowlesi, and Plasmodium malariae.*

Basics of Immunity

The innate and adaptive immune systems are both important in the control of malaria (Lopez et al., 2017). The innate immune response is triggered during the initial infection where innate immune cells respond. The innate immune cells include neutrophils, natural killer cells,

macrophages, dendritic cells, eosinophils, basophils, and mast cells. This is followed by an adaptive immune system response which triggers B and T cells. B cells mediate antibody-mediated immune responses, and T cells mediate cell-mediated immune responses. T cells are activated by dendritic cells (DCs). DCs are antigen presenting cells that can enhance immune responses by presenting antigens on its surface; an antigen is a foreign substance that induces an immune response. DCs are the link between the innate and adaptive immune systems. Pathogens can be recognized by DCs through pattern recognition receptors (PRRs) which detect pathogen-associated molecular patterns (PAMPs). Hemozoin, a PAMP, is a byproduct of the digestion of hemoglobin by the parasite during the infection of RBCs. As a PAMP, hemozoin binds DNA from host cell phagolysosomes and cytosol. Hemozoin is important as heme is converted to hemozoin through hemoglobin hydrolysis. These molecular components are vital as the parasite feeds off of hemoglobin in the RBC and heme is toxic to the parasite.

As immune responses induced by natural infection are stage and genotype-specific, the malaria life cycle in relation to the immune system can be quite complex (Riley & Stewart, 2013). RBCs lack major histocompatibility complexes (MHCs) antigens on their surface, so RBCs cannot present foreign peptides on their surface making them resistant to the normal targeting by cytotoxic T cells. Cytotoxic T cells, also known as $CD8^+$ T cells, are a type of immune cell that can kill foreign cells, cancer cells, and cells infected with a virus; they contain a CD8 receptor that can recognize the altered antigen that the MHC Class I molecule presents. Before the RBC stages of malaria, cytotoxic T cells can migrate from the skin and liver draining lymph nodes to the liver to recognize and kill infected hepatocytes; this process is assisted by the $CD4^+$ T cells (Kumar et al., 2019). Since RBCs cannot use MHCs, DCs are used to present the antigen to naïve $CD4^+$ T cells. After the DC encounters the parasite and its recognition of the

antigen via PRRs, DCs undergo maturation and migrate to the T cell zone of secondary lymphoid organs. If the DC migrated from a peripheral tissue site, the captured antigen is transferred to lymphoid-resident DCs for presentation to naïve T cells. On the other hand, parasitized RBCs may be captured by macrophages or DCs that line lymphoid organs; this process would occur during the blood infection stage. Once DCs recognize the parasite molecule, IL-6 production is stimulated to promote expression of the T helper (Th) 1 and T follicular helper (Tfh) cells. Th1 cells are important as they produce pro-inflammatory cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor (TNF); these cytokines stimulate the production of reactive oxygen species and promote capturing and destruction of parasitized RBCs. Tfh cells are essential as they help activate B cells so that the B cells can secrete antibodies and macrophages to kill microbes; they also help activate cytotoxic T cells to kill infected target cells.

Plasmodium Falciparum

Plasmodium falciparum is known to produce high levels of blood-stage parasites and to change the surface of the RBCs (Milner, 2018). *P. falciparum* creates an adhesive phenotype known as a "sticky cell." The sticky cell causes RBC sequestration inside small to medium-sized vessels; this removes the parasite from circulation for half of the asexual cycle. Sequestration leads to difficulty of parasite clearance in the spleen. This unique mechanism of the sticky cell results in blood cells clumping which allows the species to over-replicate and restrict blood flow to the human host. The clinical symptoms are mostly due to the results of the asexual stages and sticky cell phenotype. For example, due to the restricted blood flow, there is an association between infected RBCs clumping and severe anemia which is a symptom of severe malaria. Vascular dysfunction can also lead to blockage in the vascular system and tissue hypoxia.

Plasmodium Vivax

Plasmodium vivax is the most common species causing clinical manifestation outside of the African region (Moreno-Pérez, 2013). The unique feature of this species is that *P. vivax* prefers immature RBCs, known as reticulocytes, which are produced in the bone marrow and sent into the bloodstream. Reticulocytes lead to a lower level of parasites in the bloodstream, and it primarily uses the Duffy antigen receptor for chemokines (DARC) for invasion (Milner, 2018). Duffy antigens are expressed on most RBC surfaces including mature RBCs. Recent studies have suggested that *P. vivax's* inability to infect mature RBCs, even though they express the DARC, could be due to an interaction with other membrane receptors. More studies need to be conducted, but it is likely that the DARC is not the only component required for *P. vivax* invasion.

Furthermore, *P. vivax*, as well as *P. ovale,* have a dormant liver stage known as the hypnozoite (Menkin-Smith & Winders, 2022). Hypnozoites in the liver could remain dormant for weeks to months to years; this causes relapse of infection when the hypnozoite is released into the bloodstream. Due to this unique stage, *P. vivax* is difficult to eradicate.

Plasmodium Ovale

In *Plasmodium ovale,* there are two distinct species*: P. ovale curtisi and P. ovale wallikeri* (Milner, 2018). Some P. *ovale* become dormant in the liver, while others remain active. These species only differ due to *P. ovale wallikeri* having a shorter latency period and different genetic makeup, making it hard to distinguish between the two. *P. ovale* is found only in tropical western Africa. The *P. ovale* is distinguishable in its presentation on a blood smear. This species presents as a comet form of the trophozoite and the finger-like projections on the RBC membrane. The comet form is seen as an enlarged cell with thin hair-like projections called

fimbriae. These unique presentations on the blood smear allow diagnosis of P. *ovale.* Furthermore, in *P. ovale* the Duffy antigen is present, but does not appear to be a controlling factor for infections.

Plasmodium Malariae

Plasmodium malariae is the least harmful species and has several distinct clinical features (Milner, 2018). The unique clinical manifestations include a fever every 72 hours due to a longer parasite life cycle and a lower level of infection; the fever cycle is known as a quartan fever. The longer life cycle and level of infection lead to a less severe immune response. Those who have more severe symptoms may be coinfected with a second plasmodial species.

Unlike other species, *P. malariae* can be maintained at a low level of infection (Collins & Jeffery, 2007). This is due to a lower number of merozoites produced per erythrocytic cycle, the extended fever cycle, a preference for older erythrocytes, and a combination of these factors for the earlier development of immunity by the human host. Although *P. malariae* is considered to be the least harmful species of *Plasmodium*, recent studies show that there was a correlation between *P. malariae* and renal disease. One study suggested that immune complexes may cause structural glomerular damage leading to renal diseases and nephrotic syndrome.

Plasmodium Knowlesi

Plasmodium knowlesi is found in the Malaysian, Indonesian, and Southeast Asian populations; it is limited to these regions. *P. knowlesi* is unique because this species prefers younger blood cells, but, over time, adapts to infect more mature RBCs (Milner, 2018). Similarly, P. *knowlesi,* like *P. vivax,* requires the Duffy receptor for invasion of RBCs (Anstey et al., 2021). Also, recent studies have predicted that the presence of monkeys indicate higher risk for *P. knowlesi* as the parasite's natural host is the long-tailed macaque monkey. Research has

shown that monkey to human transmission is the main way of transmission as no human-to-human transmission has been reported.

Interestingly, the erythrocytic phase of *P. knowlesi* lasts approximately 24 hours (Anstey, 2021). This is the shortest duration of the erythrocytic phase in comparison to all *Plasmodium* species. Due to the duration of this phase, patients experience daily fever spikes; this fever cycle is known as quotidian.

Treatment

Patients with malaria may be treated in an inpatient or outpatient setting with anti-malarial medications (Pasvol, 2006). The most common antimalarials are known as chloroquine and artemisinin. Quinine, a chloroquine derivative, is given by slow intravenous infusion for a minimum of five days to kill the *Plasmodium* species. Quinine is able to kill *Plasmodium* by interfering with the parasite's ability to break down and digest hemoglobin (Achan et al., 2011). Furthermore, in the species of *P. vivax* and *P. malariae*, quinine can act as a gametocytocidal drug used to prevent transmission of the infection to the Anopheles. When taking quinine, a patient may experience the side effects of blurred vision, nausea, change in color vision, and spinning sensations.

Due to growing resistance in chloroquine derivatives, artemisinin is being used more often and can be used for all severity types due to its higher rate of clearance (Pasvol, 2006). Artemisinin is thought to clear the parasite by a two-step mechanism (Meshnick, 1998). The first step is activated by a heme-iron molecule catalyzing the cleavage of endoperoxide within the parasite; endoperoxide is a compound that binds to malaria infected blood cells. Next, a free radical intermediate kills the parasite via alkylation and releases toxins that affect essential

malarial proteins. When taking artemisinin, patients may experience nausea, vomiting, and dizziness.

Severe Malaria

The rare and more severe type of malaria is known as severe malaria (White, 2018). Severe malaria is characterized by the infection compromising organ function or abnormalities in a patient's blood and immune cells. This type of malaria is usually caused by *P. falciparum*, but can also be caused by *P. knowlesi* and *P. vivax.* Severe malaria typically occurs due to delayed treatment of uncomplicated malaria leading to more severe symptoms (Balaji et al., 2020). The life-threatening types and symptoms of severe malaria are placental malaria, cerebral malaria, severe anemia, respiratory distress, kidney injury, and hemodynamic shock.

Immune Response

Severe malaria has been associated with higher levels of inflammatory cytokines including TNF-α, interleukin-1 (IL-1), and interleukin-6 (IL-6) compared to uncomplicated malaria; these cytokines are present in uncomplicated malaria at much lower levels (Leão et al., 2020). There is a link between higher levels of inflammatory cytokines and severe malaria. Although TNF-α, is closely associated with reduced parasitic load, a recent study has shown there is an association between TNF-α levels and cerebral malaria. A study showed that there was an overproduction of TNF-α in the late phase of malaria leads to severe cerebral malaria. Although the overproduction of TNF-α is associated with severe malaria, researchers suggest the elevated levels of TNF-α is an attempt to control infection and potentially contribute to a reduction of death.

Also, interferon-γ (IFN-γ) has a role in both protection and pathogenesis. IFN-γ is key to controlling infection in the liver and blood stages of the parasite life cycle (Artavanis-Tsakonas

et al., 2003). In the role of protection, macrophages can be activated by Th-1 derived IFN-γ leading to anti-parasitic effects. Interestingly, new research has found IFN-γ can also increase the severity of severe malaria; research has shown a connection between increased IFN-γ levels and severe malaria. The harmful effects of IFN-γ are believed to be due to its ability to activate macrophages which result in the production of endogenous pyrogens; a pyrogen is an infectious organism producing toxins or cytokines. The production of pyrogens is believed to lead to an inflammatory cascade producing cerebral malaria.

Interestingly, it has been found that adults living in endemic areas develop mechanisms to limit the inflammatory response (Artavanis-Tsakonas et al., 2003). The acquisition of clinical immunity aligns with the development of a diverse pool of antibodies. Malaria-specific antibodies contain antiparasitic effector functions; these functions include inhibition of the parasite invading the RBC and antibody dependent cytotoxicity. There are also cell mediated immune effector functions which are enhanced phagocytosis, killing of parasitized erythrocytes, and inhibition of parasite growth due to increased levels of $CD8^+$ T cells. Since children, below the age of five, have not developed specific immunity to malaria, this age group is the most vulnerable for severe malaria. Compared to adults, children have not developed the mechanisms above that can lead to immunity.

Placental Malaria

Placental malaria is characterized by the accumulation of plasmodium-infected RBCs in the placental intervillous space (Milner, 2018). *Plasmodium falciparum* causes a unique change in the setting of pregnancy because the parasite can sequestrate important placental molecules, meaning it attaches infected erythrocytes to the endothelium. Chondroitin sulfate, a building block for cartilage, is one example of the complications that sequestration causes. In this, *P.*

falciparum proteins bind to chondroitin sulfate removing them from circulation. This ultimately leads to the accumulation of phagocytic cells in the placental space to phagocytose infected RBCs (Balaji et al., 2020). This results in an increased thickness of the trophoblast membrane, which is the layer of tissue that supplies the embryo with nourishment and forms a part of the placenta. Due to the increased thickness, there is a reduction of nutrient and oxygen transport across the placenta. The accumulation of phagocytes, leading to increased thickness of the trophoblast, create complications that affect the development of the fetus and birth outcomes. Some of the complications that may occur are anemia, miscarriage, low birth weight, and congenital malaria (Milner, 2018). Not only is *P. falciparum* dangerous for the infant, but *P. falciparum* also affects the mother; postpartum hemorrhage is a major cause of death for the mother during placental malaria (Balaji et al., 2020). Maternal anemia increases the risk of postpartum hemorrhage since anemia reduces the oxygen capacity of the blood and anemic women have a lower threshold for blood loss.

Cerebral Malaria

Cerebral malaria is the leading cause of death in severe malaria patients (Balaji et al., 2020). This type of malaria is defined as a serious neurological complication resulting from *P. falciparum* binding to the endothelium. Cerebral malaria often manifests as a typical malaria case in the beginning but then results in a coma. This is characterized by symptoms of brain swelling, changes in retinal function, multi-organ failure, and intracranial hypertension. Often, the symptom of brain swelling leads to a coma. One diagnostic tool to confirm cerebral malaria, specifically during an autopsy, is the presence of *P. falciparum* in 20% or more of the brain capillaries (Milner, 2018). An examination of the retina is also a useful tool in bedside diagnosis; retinal white spots and vessel discoloration are physical signs of cerebral malaria (Brejt &

Golightly, 2019). Interestingly, high levels of histidine-rich protein 2 levels seen in a collection of cerebrospinal fluid, and lower average voltage or lack of reactivity seen in electroencephalography (EEG) can be used as predictors of mortality in patients with cerebral malaria.

Severe Anemia

Severe anemia is a life-threatening symptom of severe malaria in which hemoglobin levels fall below 6 g/dl meaning rapid lysis of the RBCs occurs (White, 2018). When this occurs, a patient may experience symptoms of fatigue, shortness of breath, and the feeling of being cold. There are two mechanisms in how patients develop severe anemia: intravascular hemolysis and dysregulated erythropoiesis. Intravascular hemolysis alludes to the fissure of RBCs within the blood vessels leading to the depletion of RBCs in the blood (Balaji et al., 2020). In dysregulated erythropoiesis, there is interference with erythropoietic cells, found in bone marrow, which causes a reduction in RBC production leading to anemia. Along with antimalarial drugs, a blood transfusion can be a lifesaving treatment (Milner, 2018).

Respiratory Distress

Respiratory distress in patients with severe malaria presents with metabolic acidosis reflecting tissue hypoxia (Cowman et al., 2016). The *Plasmodium* parasite produces *Plasmodium* lactate dehydrogenase which creates lactic acid leading to a decreased pH level (Milner, 2018). Due to the metabolic acidosis mechanism, respiratory distress is a common symptom further contributing to pH imbalance. If respiratory distress occurs in a patient with severe malaria, this distress is an impending sign of death (White, 2018). In extreme cases, patients can develop Malaria-associated acute respiratory distress syndrome (MA-ARDS) which is often lethal (Vandermosten et al., 2018). MA-ARDS is characterized by extreme pulmonary inflammation

and the breakdown of the alveolar-capillary membrane. Although the pathogenesis of MA-ARDS is not understood, a potential cause could be due to the effects of sequestration of parasitized erythrocytes (Taylor et al., 2012). The sequestration of RBCs can lead to anemia which can impair oxygen delivery to the tissues causing respiratory distress.

Kidney Injury

Malaria is associated with kidney diseases specifically in the glomeruli, tubules, and interstitial region (Silva et al., 2017). The affected RBCs and capillary endothelium lead to RBC clumping which impairs circulation leading to kidney injuries such as hemodynamic instability. Hemodynamic instability is when there is abnormal blood pressure causing inadequate flow to organs, specifically the kidneys in this case. Hemodynamic instability can lead to acute tubular necrosis where damage occurs to the tubule cells of the kidneys causing acute kidney failure. Normally, the tubule cells help the kidneys filter blood.

In addition, glomerulonephritis, inflammation of glomeruli, can occur (Silva et al., 2017). Glomerulonephritis is a disorder of the glomeruli, or blood vessels in the kidney. The glomeruli are important as they remove excess fluid and waste from the bloodstream in the form of urine. As RBCs travel through the glomeruli, clumping of RBCs can be seen; this affects the filtration of the kidney. Due to decreased filtration, the kidneys slowly lose their ability to excrete waste, and urine is not properly filtered. The inability to filtrate urine causes inflammation and fatigue in the body. To treat glomerulonephritis along with a malaria diagnosis, antimalarials, hydroelectric corrections, fluid replacement, and dialysis are treatments used.

Hemodynamic Shock

Hemodynamic Shock is described as circulatory failure leading to multiple organ failure, also known as sepsis (Plewes et al., 2019). In this condition, blood loss causes the circulatory system to be incapable of pumping blood to tissues. The lack of blood supply to the body causes organ dysfunction. Although not common initially, hemodynamic shock is observed in about 10% of children and adults with severe malaria. Although this is still being researched, hemodynamic shock is thought to be caused by gram-negative bacteria in the intestinal tract along with macrophage dysfunction. The loss of gut integrity seems to be a marker for hemodynamic shock in severe malaria patients. Another likely factor that causes hemodynamic shock is anemia (Ackerman, 2013). In anemia patients, there is a lack of functioning RBCs to provide oxygen to tissues in the body. This aligns with the lack of blood supply in hemodynamic shock, which ultimately results in organ dysfunction.

Treatment

When treating severe malaria, artemisinin derivatives are typically used compared to other antimalarial medications (Pasvol, 2006). Recent studies have shown that artemisinin has improved outcomes in severe malaria compared to quinine derivatives. Intravenous artemisinin has been shown to reduce death by 35% in severe malaria cases. Although this medication is the preferable treatment for those with severe malaria, it is not widely available yet and has a limited shelf life. The shelf life of artemisinin is two years compared to the five-year shelf life of chloroquine (Bate et al., 2009). The reduction in shelf life disrupts treatment availability and increases reluctance among global health workers to adopt a higher standard of care. The difference in shelf life is most likely due to the properties of artemisinin being naturally extracted from sweet wormwood, whereas chloroquine is a synthetic drug.

Diagnostic Techniques

It is important to diagnose malaria correctly and quickly (Tangpukdee et al., 2009). Due to the global impact of malaria, it is vital to develop effective diagnostic tools that are available for low-income and low-resourced countries. Diagnosis of malaria involves recognizing the malarial parasites found in the blood. Although this seems straightforward, many factors contribute to a diagnosis.

Clinical Diagnosis

Physicians clinically diagnose patients with malaria based on a combination of patient symptoms and laboratory tests (Tangpukdee et al., 2009). This can be challenging since the symptoms can vary widely and overlap with common viruses or infections. There is an algorithm for malaria diagnosis, but studies show that this specific algorithm resulted in 30% over-diagnosis. The algorithm consisted of clinical signs and symptoms such as fever, digestive issues, respiratory issues, fatigue, and skin problems. The algorithm is used if there are no diagnostic tools available, so a diagnosis must be made on symptoms and signs alone. Due to the non-specific signs and symptoms, diagnosis is strengthened with access to laboratory and microscopic tools.

Laboratory Diagnosis

Laboratory diagnosis is vital to malaria diagnosis since the signs and symptoms are diverse (Tangpukdee et al., 2009). An accurate diagnosis can decrease transmission and prevent malaria from becoming more severe. In the laboratory, malaria is often diagnosed through thin and thick peripheral blood smears, quantitative buffy coat (QBC), and rapid diagnostic tests.

Blood Smear

Malaria can be diagnosed through microscopic examination and identification of *Plasmodium* species in stained blood films (Tangpukdee et al., 2009). To start, a patient's finger is pricked, and a spot of blood is transferred to a glass slide. A thick blood film is prepared by stirring the blood spot in a circular motion and drying it without a fixative. The slide is then stained with diluted Giemsa for 20 minutes and rinsed with buffered water for 3 minutes. Once the slide is prepared, it is observed under a light microscope.

Furthermore, a thin blood film begins by placing a small drop of blood in the center of the smear slide (Tangpukdee et al., 2009). Another slide, known as the spreading slide, is held at a 45-degree angle allowing the drop to spread along the contact line of the two slides. The spreading slide is pushed toward the end of the smear slide creating a decrease in thickness known as the feathered edge. The blood smear slide is dried with absolute methanol as a fixative. This sample is also stained with diluted Giemsa for 20 minutes and dipped in buffered water. This slide is viewed under a light microscope.

The thick and thin blood smear techniques are used widely due to their accuracy and low cost making it accessible to most communities (Tangpukdee et al., 2009). The education and training required to identify the *Plasmodium* species in the blood is one disadvantage of this technique as each species has specific presentations. To start, *P. falciparum* can be identified due to high levels of parasitemia within the RBCs and the crescent-shaped gametocytes. Also, *P. vivax* appears with low-grade parasitemia and ring structures within the RBCs (Fisher, 2021). Furthermore, *P. ovale* is known for its young RBCs that appear bigger than a normal RBC. In addition, *P. malariae* is identified by its band-like trophozoites and merozoites surrounding a schizont with a pigmented center. Lastly, *P. knowlesi* can look similar to other species making it

difficult to diagnose, but this species is known for having a ring-like structure and schizonts containing abundant pigment. If there is a low level of parasites in the blood, it can be difficult to identify accurately under the microscope (Tangpukdee et al., 2009). Although there are disadvantages, the blood smear is used as a diagnostic tool for clinical diagnosis when supplies are available.

QBC

The quantitative buffy coat (QBC) technique was designed to improve the microscopic detection of parasites in blood including the *Plasmodium* species (Kochareka et al., 2012). In this method, the malaria parasite is diagnosed using micro-centrifugation, fluorescence, and density gradient of infected RBCs. The parasitized erythrocytes get concentrated in a layer that can be visualized under a microscope using fluorescent dyes (Ahmed & Samataray, 2014). To start the process of QBC, a patient's finger is pricked and collected in a hematocrit tube that contains the dye acridine orange and an anticoagulant (Tangpukdee et al., 2009). The tube is centrifuged at 12,000 g for 5 minutes and viewed under an epi-fluorescent microscope. Under the microscope, parasite nuclei are identified by a bright green color compared to the yellow color of the cytoplasm. This technique of QBC is more accurate compared to the blood smear because the QBC can detect low levels of parasitemia. One disadvantage of using QBC is that this technique is more expensive compared to other diagnostic tools.

Rapid Diagnostic Test

Due to a need for accurate, quick, and cost-effective diagnostic tests for malaria, the use and development of rapid diagnostic tests increased (Mouatcho & Goldring, 2013). Rapid Diagnostic Tests use a colored detecting antibody, a process known as immunochromatography,

that binds to a lysed parasite antigen and is carried by capillary action and arrested by a capture antibody; this results in a colored band on a test.

One disadvantage is that there is possibility for false positive and false negative tests (Mouatcho & Goldring, 2013). Researchers have seen that if a parasite lacks the *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2), this gene deletion can lead to false-negative results in RDTs. False negative RDTs can be explained either from an excess of antibodies or antigens which appear to be restricted to *Pf*HRP2-based RDTs. In addition, false positive tests can occur in patients with rheumatoid factor (RF); RF is a protein produced by the immune system that can attack healthy tissue in the body (Lee et al., 2014). Although the mechanism for the interaction between the RF and RDT is not fully known, one hypothesis is that there is a reaction between RD and specific antibodies on the malaria RDT strips. The specificity of the RDT was low with a rate of 52% revealing a high level of false positive and negative results. Although there are chances for false positives and false negatives, this test is a simple option for communities that do not have trained professionals to read blood smears and QBC techniques.

PCR

Polymerase chain reaction (PCR) is a molecular diagnostic test that detects DNA from *Plasmodium* (Poostchi et al., 2018). PCR can detect low parasite concentrations in the blood and can differentiate between species of *Plasmodium.* Although PCR is specific, PCR takes expensive technology and trained individuals to perform the test.

Future of Smart Phones

As technology continues to advance, the ideal solution for malaria diagnosis in low economical settings would be to use smartphones when and if technology allows for it (Poostchi et al., 2018). Researchers believe it is possible for smartphone cameras to have a magnifying

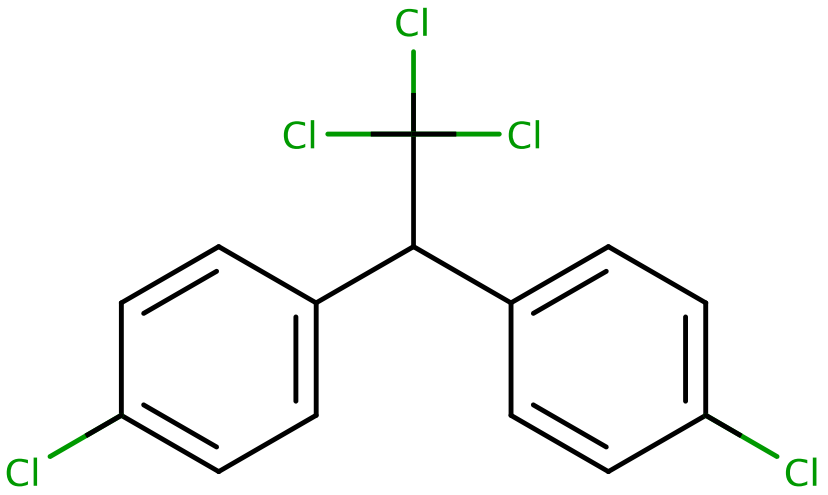
attachment that could analyze, image, and compute the parasitemia. Although technology is far from this advancement, this is the future of malaria detection techniques in low-resource countries

Epidemiology

Malaria is a severe public health disease that is the leading cause of death in developing countries (World Health Organization, 2021). In 2020, there were 241 million cases of malaria with an estimated 630,000 deaths. The majority of malaria cases develop in the Sub-Saharan African region. In this region, about 80% of all malaria-related deaths were children under the age of five.

Past

In 1900, 77% of the world's population was at risk of malaria with the most risk in Sub-Saharan Africa (World Health Organization, 2020). In the mid-twentieth century, malaria control interventions were developed. The insecticide dichlorodiphenyltrichloroethane (DDT) was used as an indoor residual spray as part of the Malaria Eradication Campaign; the structure of DDT is shown in Figure 1. This insecticide focused on reducing mosquito breeding sites reducing the population at risk. By 2004, the population percentage of those who are at risk dropped to about 40%. Although there was some success, malaria eradication was never attempted in Africa, even though malaria was more intense in this area. Most notably, the Nigerian Garki project attempted to use an insecticide in combination with antimalarials, but transmission continued; researchers concluded that the spray method would not be feasible for Africa.



Dichlorodiphenyltrichloroethane

Figure 1. Structure of Insecticide Dichlorodiphenyltrichloroethane (DDT).

Note: This figure was created.

Global Distribution

The African region carries the global malaria burden accounting for about 95% of all malaria cases globally and most related deaths (Cotter et al., 2013). The majority of North America, Europe, Australia, and Northern Asia are considered malaria-free (See Figure 2). Southern Asia and South America are controlling or eliminating malaria. Geographically, there has been remarkable success in the malaria burden, but Sub-Saharan Africa still struggles with controlling the disease. Sub-Saharan Africa is the most affected due to its weather conditions that allow transmission to occur year-round, low resources, and the socio-economic instability of the region.

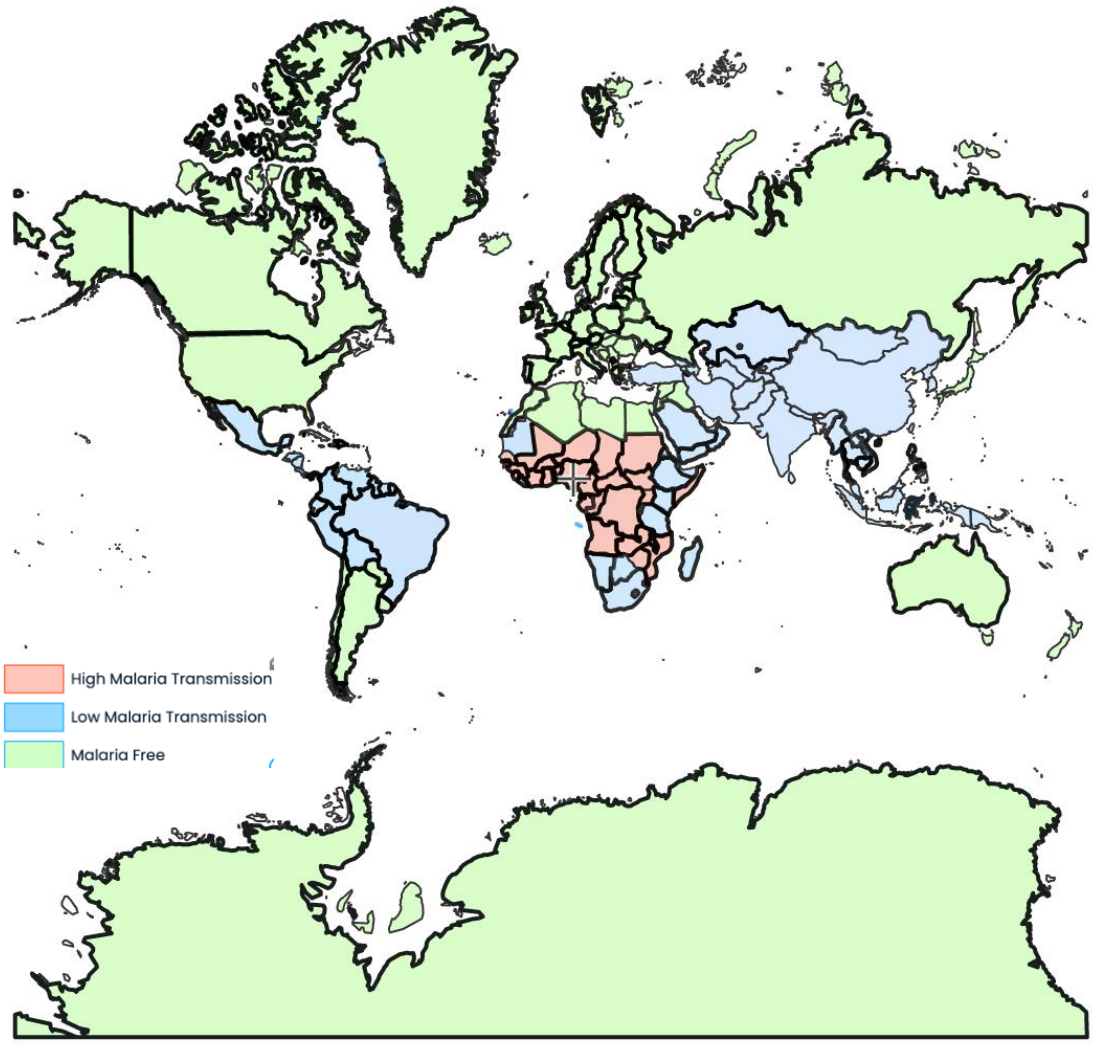


Figure 2. Global Distribution of Malaria Transmission. The geographical distribution of malaria was visually created on a map. As seen above, Africa has the highest malaria transmission rates. Southern Asia and South America have low transmission rates. Antarctica, the majority of North America, Australia, Europe, and Northern Asia are considered malaria-free. This image shows that the malaria burden is most prevalent in Africa.

Management Methods

The management techniques to combat malaria have been important in lowering the number of cases. Innovative methods could be the future of eradication worldwide. Advances in medications, indoor residual spraying, insecticide nets, a newly approved vaccine, and genetic technology have been shown to help reduce the transmission of malaria. Although there are challenges, these methods have and will continue to provide advancements in the combat against malaria.

Advancing Medications

Other than medications that treat clinical malaria as described above, new drugs have been needed as drug resistance is occurring (Enayati & Hemingway, 2010). One advancement is drugs that kill gametocytes. This type of drug destroys the gametocytes in the blood and interrupts transmission in the malaria-infected patient. Artemisinin, mentioned above, is effective against multiple blood stages and gametocytes making it effective for clearance.

Indoor Residual Spraying

Indoor Residual Spraying (IRS) is the application of insecticides to kill the resting adult female mosquitoes (Enayati & Hemingway, 2010).. The primary goal is to reduce the life span of female mosquitos so that transmission is lowered. The IRS insecticide can last up to 6 months, but most formulas last less than 4 months. To be more effective, research is needed to create new formulas that will last longer increasing efficiency and lowering transmission rates. In countries with resources to use other methods of eradication, IRS was abolished due to environmental and health concerns.

Insecticide-treated Nets

Insecticide-treated nets have become increasingly popular as this tool is simpler and less demanding than IRS (Enayati & Hemingway, 2010). New developments have allowed these nets to become more efficient and are now known as long-lasting insecticidal nets. The net forms a physical and chemical barrier against mosquitos and does not need retreatment. One downfall is that the insecticide-treated nets may be vulnerable to resistance in the future.

Vaccine

Since the 1960s, scientists have been conducting research to develop a sporozoite specific vaccine to be used in malaria cases (Zavala, 2022). Studies have identified the circumsporozoite protein (CSP) which is expressed on the surface of sporozoites of different *Plasmodium* species. CSP was used to develop the RTS,S vaccine. CSP contains tandem repeats; when binding antibodies to these repeats, the antibodies immobilize the sporozoites which prevents infection of hepatocytes. The RTS,S vaccine induces protective antibody responses against malaria sporozoites which depend on the neutralizing antibodies present at infection. Through years of vaccine trials, the results consistently have shown the immunization of children between the age of 6 to 12 weeks and 5 to 7 months old induces protective immunity which neutralizes the infection and reduces clinical symptoms. Later phases of the vaccine trials have shown that the vaccine is short-lived, and protection depends on sustained high levels of circulating antibodies.

The RTS,S vaccine is a positive first step toward reducing malaria, but there are problems to overcome (Zavala, 2022). The RTS,S vaccine is short-lived because neutralization is no longer effective after hepatocyte invasion and the CSP antigen will eventually no longer be expressed. With this, any recall response in the immune system that may occur after the primary infection would not affect the primary infection. Also, there has been limited research on the vaccination

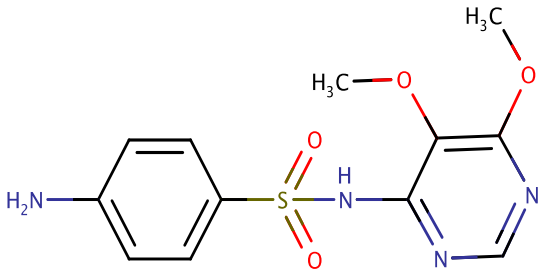
of adults. One study showed that in Gambia, 34% of immunized adults showed short-lived protection while others in Kenya showed no protection. Another issue is that the RTS,S does not interfere with the transmission stages of *Plasmodium* leading to transmission remaining unchanged.

One study focused on the antigenic domains expressed in nanoparticles using specifically the particle R21 (Zavala, 2022). The new vaccine was administered three times over the span of a year. This vaccine conferred 77% protection from severe malaria. More trials in different geographical locations are needed to support these results.

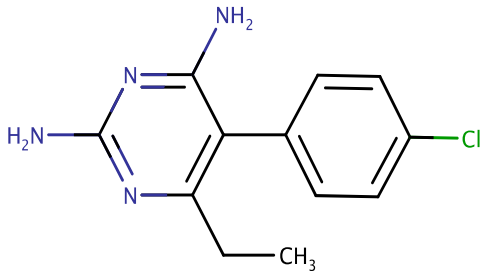
Advances in the anti-CSP protective antibody structure and specificity have been made (Zavala, 2022). Biophysical studies were able to characterize the properties of these antibodies. Crystallography studies defined the precise confirmation of CSP epitopes important for recognition. With these advances, structure-based vaccines could be developed in the future. Also, research has shown that combining an anti-sporozoite vaccine, such as RTS,S, with an anti-blood stage vaccine could be critical in the possibility of eradication. This advancement makes the RTS,S vaccine a powerful tool for future research.

Preventative Treatments

As malarial infection during infancy and pregnancy remains a significant problem, the World Health Organization recommends intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) in high-risk individuals in high-risk regions; the structures of these two chemicals used in IPTp are seen in Figure 3 (Gosling et al., 2010). The purpose of the IPTp is to reduce the risk of low birth weight and anemia by clearing placental parasitemia. This strategy is simple and effective in the areas that need it. If resistance occurs, the treatment may need to be adapted in the future.



Sulfadoxine



Pyrimethamine

Figure 3. Structures of Drugs Sulfadoxine and Pyrimethamine.

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CRISPR/CAS9

The CRISPR/CAS9 system with site-specific nucleases can perform a selective double-strand break in the genome which can be used for gene editing (Lee et al., 2019). The CRISPR/Cas systems evolved in prokaryotes as a mechanism against invaders and encode guide RNAs that program the Cas nuclease to perform exonuclease activity. There is great potential for this system to be used for the development of transgenic mosquitoes as it is highly adaptable to any genomic sequence (Enayati & Hemingway, 2010). The system could introduce artificial peptides to interfere with the parasite's development within a mosquito or enhance naturally occurring defense mechanisms.

Recently, researchers targeted an endonuclease gene that could reduce fertility in female anopheles leading to population suppression (North et al., 2020). The gene in question is called the second transgene target (DSX). In the absence of the DSX gene, females are sterile as they develop male and female characteristics lacking functional reproductive organs. Researchers concluded that, with additional research and population modeling, this modification in mosquitoes could be effective.

Zinc Finger Nuclease

Zinc finger nuclease (ZFN) is a site-specific genome editing technology using engineered nucleases (Nain et al., 2010). There are 90 zinc finger proteins that are considered essential for blood stage proliferation; zinc finger proteins bind three amino acid sequences on DNA. The goal of this technology would be to develop nucleases of specific sequences that typically occur only once in the genome. Future technology could allow for ZFN to introduce deletions in the genome ultimately eliminating the parasite from the infected cell; the ZFN would be delivered

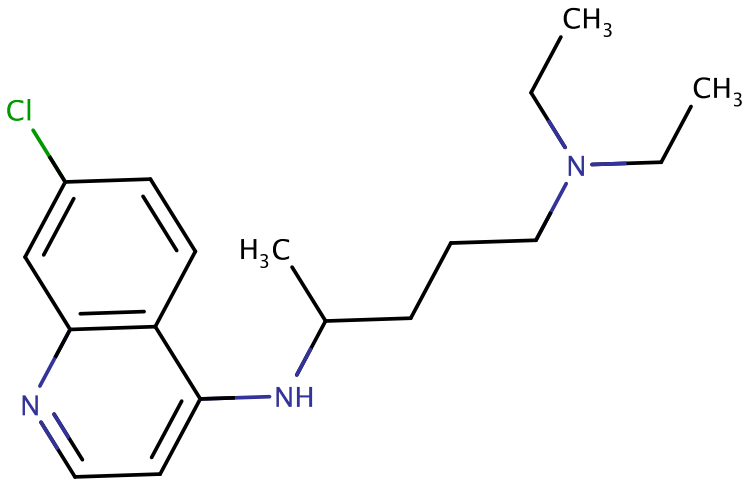
through cell-penetrating peptides (CPP). CPPs can deliver small molecules, proteins, and nucleic acids into the cytosol that is normally impermeable to the membrane. This would allow the CPP-mediated ZFN to be delivered to RBCs and interact with the DNA. Overall, ZFN technology is advancing and could help in the management of malaria through genetic modifications leading to parasite elimination in infected cells.

Challenges of Malaria Elimination

Malaria is a difficult disease to eliminate as the vector and parasite are adaptable and are financially costly. Although there have been positive strides toward elimination, drug resistance, insecticide resistance, financial challenges, and climate change has affected the successful eradication of malaria in underdeveloped countries. It is important to understand the challenges of elimination in order to research and propose solutions for ultimate elimination.

Drug Resistance

Chloroquine has been shown to be the most used antimalarial for decades, but due to drug resistance, chloroquine has lost its effectiveness (Enayati & Hemingway, 2010).The structure of chloroquine is shown in Figure 4. Chloroquine resistance is a challenge as many malaria affected countries are low on resources. Due to the resistance, artemisinin was introduced in combination with chloroquine with the goal of reducing resistance. Artemisinin derivatives are also being developed to potentially avoid potential resistance to this drug.



Chloroquine

Figure 4. Structure of Drug Chloroquine.

Note: This figure was created.

Insecticide Resistance

Although IRS and insecticide nets have been successful in reducing malaria transmission, there is potential for insecticide resistance in the future (Enayati & Hemingway, 2010). As seen in Figure 5, Glutathione S-transferases (GSTs) are often overexpressed in resistant mosquitoes which act as a detoxification route and reduce oxidative damage leading to resistance. Due to this potential, combinations of insecticides are being researched in order to decrease the potential for resistance in these tools.

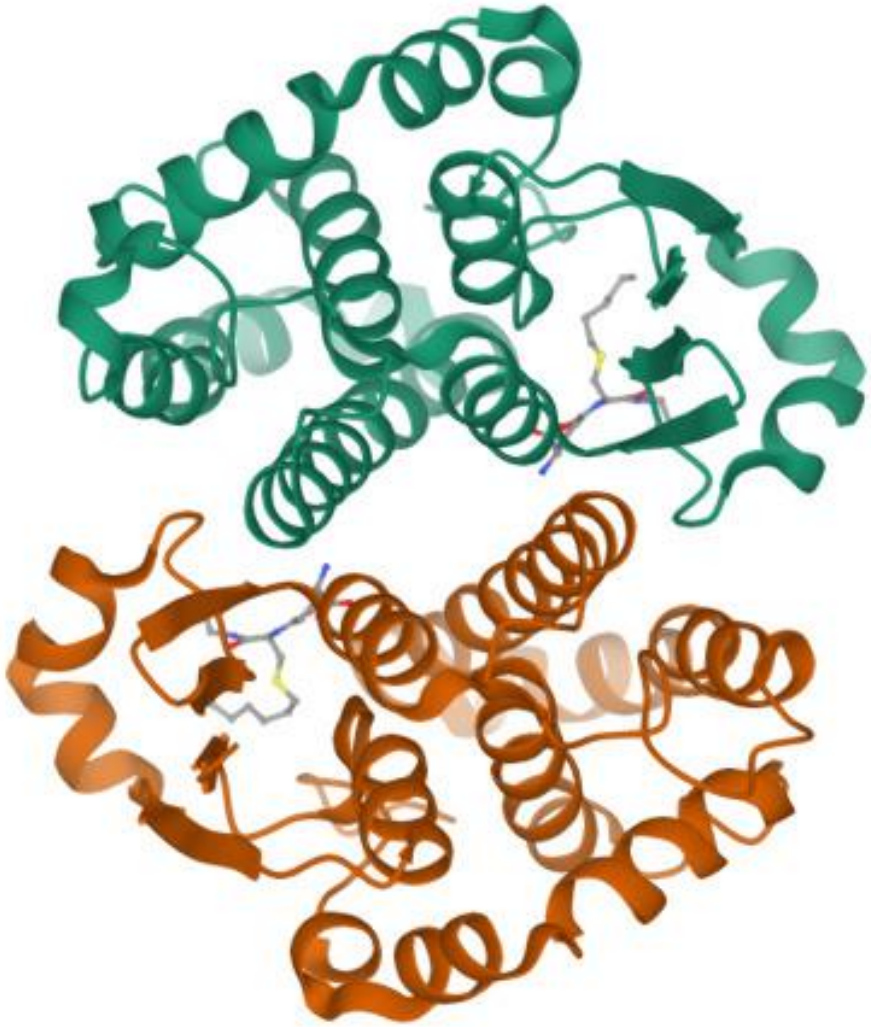


Figure 5. Crystal Structure of Enzyme Glutathione-S-transferase.

Note. This model was produced by authors K. Tars, U.M. Hellman, and B. Mannervik and published in the RCSB Protein Data Bank in 2006.

Insecticide resistance has been reported globally for all anopheles vectors (Dhiman, 2019). Over 60 countries have confirmed resistance to at least one class of insecticide. It is likely that this number is larger as many endemic countries do not have the means to monitor resistance. At this time, there is no alternative available meaning more research and technology must be conducted to introduce more effective tools.

Economic Challenges

Theoretically, if IRS and insecticide nets were executed with the utmost precision and numbers, elimination is possible. Although possible, it takes a united nation, increased finances, increased healthcare, and many personnel to achieve this goal. With increasing costs of supplies, increasing division, and deteriorating economies, this goal will not be met. The consequences of reaching for elimination would require support from local health systems which are often too handicapped in the populations that are affected most.

Covid-19

In 2019, the fight against malaria was underway through the new malaria vaccine and Phase IV Expanded Program on Immunization in Sub-Saharan Africa (Nghochuzie et al., 2020). As these efforts were at their peak, Covid-19 was introduced in late 2019 becoming a worldwide burden that halted the efforts against malaria. Antimalarials, such as artemisinin and chloroquine, were being used as a treatment for Covid-19. Also, companies such as SD Bioline shifted their focus of rapid diagnostic tests to Covid-19 from malaria. With lockdowns in many countries, there was a shortage of medical supplies and treatments for illness. Due to the shortage of medications and lack of access to healthcare, there were malarial outbreaks in the region leading to about 200,000 more malarial related deaths in 2020 compared to 2019. Overall, the focus to

combat malaria quickly changed to a focus on Covid-19 leading to disruption in advancements made.

Not only did Covid-19 affect the overall focus and resource distribution that was once used for malaria, but the clinical manifestation of Covid-19 also overlapped with malaria (Heuschen et al., 2021). The similarities in symptoms between both diseases made the diagnosis by symptoms alone difficult; in resource-low countries where malaria is most prevalent, this became a problem. Diagnosis based only on symptoms lead to misdiagnosis and harmful consequences. Untreated malaria can be fatal and misdiagnosed Covid-19 leads to higher transmission rates.

Climate Change

As climatologists have concluded that the planet is experiencing an increase in temperature due to greenhouse gases, many researchers predict that malaria transmission will increase in specific regions (Caminade et al., 2014). Results from research models propose that transmission could increase in climates such as the African Highlands, South America, and Southeast Asia. High temperatures allow Anopheles mosquitoes to thrive. In other climates, it is unlikely transmission will occur due to socioeconomic factors.

Benefits of Eradication

Lower Death Rate

The most obvious benefit of malaria eradication is permanently ending the historic toll of death due to malaria (Feachem et al., 2019). As human life is inherently valuable, it is important to lower disease and death caused by malaria. Also, eradication is the only way to overcome resistance challenges. Due to many cases and deaths, naturally, the burden of malaria flows to areas of social and economic distress.

Economic Growth

Malaria has put a toll on the economy of many developing countries (World Health Organization, 2020). There is a correlation between poverty and health; ill health reduces productivity leading to poverty. Although eradication would be costly, investment in eradication would contribute to the economic well-being of low-income nations. If eradication would occur, there would be potential for increased productivity and increased education that could lead to prosperity.

Resource Distribution

If eradication is achieved, the resources used for malaria could be devoted to other areas within healthcare (World Health Organization, 2020). As developing countries' health systems are generally poor, alleviating resources previously committed to malaria could have a positive impact on the health system leading to greater economic benefits as well. Eradication could lead to increased education, productivity, health, and economic status.

Conclusion

In conclusion, this research aims to provide an overview of the transmission cycles, human physiology, methods of management, and the challenges of malaria. Malaria is life threatening to many populations globally specifically in Sub-Saharan Africa. The Plasmodium species is responsible for the disease through infected Anopheles mosquitoes. The parasite enters the bloodstream where it infects and destroys RBCs leading to serious symptoms. For most developed parts of the world, malaria transmission rates are close to non-existent, but many still suffer from this disease. Populations living in underdeveloped countries experience high rates of uncomplicated and complicated malaria. Complicated malaria is more serious as it can manifest as cerebral conditions, respiratory issues, anemia, kidney injury, and affects pregnancy. The

future of malaria is bright as there are tactics to keep malaria under control using medications, genetic manipulations, diagnostic techniques, and tools. There is a need for the eradication of malaria worldwide, but there are struggles as socioeconomic status plays a role in the reality of eradication. Despite the challenges, malaria eradication is possible with continual efforts such as developing technology and treatments.

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