Sandra Jones, Ph.D.
Department of Biological Sciences Norfolk, VA 23529-0266
Ph: (757) 683-6903
E-mail: sjones@odu.edu

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Dear Editor,

We are submitting the manuscript "*Plasmodium falciparum* and malaria" for publication in *Current Pathogenesis*. This manuscript discusses present issues that are caused by *P. falciparum* and new approaches that are being tested to eradicate malaria associated with this pathogen. Malaria is currently a prevalent topic because it is one of the leading causes of death in many countries especially in Africa.

The discussion of a new vaccination and the prevalence of drug resistance is crucial to eradicating Malaria. The growing incidence of drug resistance could be detrimental to the progress that has been made in the treatment of malaria. This manuscript discusses the pathogenesis of Malaria and the different types of vaccinations undergoing trials. Included in the manuscript are preventative plans for drug resistance.

Thank you for your consideration of this manuscript.

Sincerely,

Sandra Jones
Associate Professor
Department of Biological Sciences Old Dominion University

Response to Reviewers

Reviewer's comments:

1. *Paragraph starting line 103 was disorganized.*
    a. This paragraph was split into two paragraphs. The first discusses the five morphological stages of *P. falciparum*, and the second discusses the different genes that are expressed in each morphological stage. The last sentence of the original paragraph was also moved into the first section to help with ease of reading.

2. *Reviewer questioned only 247 cases that were mentioned in the first paragraph.*
    a. This typo was corrected to 247 million cases.

3. *Include more details about the trafficking of host proteins as well as the RTS, S vaccine.*
    a. Information was added about the trafficking of host proteins to the paragraph that was referenced in the feedback. An additional paragraph was added about the RTS,S vaccine which is the only vaccination against malaria that is currently being implemented. Two studies were used to discuss the efficacy of the vaccination.

4. *Figures about pathogenesis, including genetic regulation or rosettes would be more helpful.*
    a. The original figures were removed and replaced with figures that demonstrated the genetics. One figure shows how PCR is used to identify the morphological stages of the gametocytes in a patient's blood. The second figure is a western blot which demonstrated that PfHP1 preferentially binds to H3K9me3 to silence var genes.

5. *P. falciparum should always be italicized and should not be abbreviated at the beginning of a sentence.*
    a. This issue was corrected throughout the manuscript.

6. *530 reviewer 1 suggested that the manuscript could be more concise.*
    a. Some sentences in the manuscript were combined to make the information less repetitive.

7. *530 reviewer one suggested removing definitive terms.*
    a. Terms such as "always" and "never" were removed from the manuscript.

8. *530 reviewer 1 suggested that the control be mentioned when discussing the efficacy of vaccines.*
    a. The percentage given for vaccine efficacy is calculated by including unvaccinated individuals, so I found it unnecessary to include information about the control group.

9. *530 reviewer 1 suggested removing the last sentence of the first paragraph in the pathogenesis section.*
    a. This sentence remains in the manuscript because it is important to understand the modes of transmission of *P. falciparum*. This parasite can be transmitted from the vector to the host and also from the host to the vector. This concept plays a role in the mechanism of many vaccines.

10. *530 reviewer 2 suggested that Plasmodium falciparum should be abbreviated where possible.*
    a. These changes were made throughout the manuscript.

11. *530 reviewer 2 suggested that the last paragraph of the pathogenesis section should be worded differently.*
    a. This paragraph was reconstructed to describe the end result of malarial pathogenesis and the symptoms of the comorbidities were moved to a different section of the manuscript.

Plasmodium falciparum and Malaria

UIN 01133723
Old Dominion University
April 6, 2023

Abstract

Plasmodium falciparum is a parasite that causes most cases of severe malaria and uncomplicated malaria. The epicenter of malaria is Sub-Saharan Africa where an average of two hundred forty-seven million people are diagnosed each year, and about one million of those patients die from the disease. Despite the development of antimalarial drug treatments, malaria remains one of the leading causes of death in many African and Asian countries, meaning that a new method must be developed to combat this disease. Many databases were used for research in the writing of this paper such as Nature and PubMed. A majority of information in this paper has been from the last eight years, with the exception of some background information from previous years. Twenty-one articles have been compiled to formulate a comprehensive review of *Plasmodium falciparum* and malaria. In recent years efforts have been put forth to limit the morbidity and mortality rates of malaria; however, many limitations have occurred such as drug resistance which has slowed the progress of finding a cure to the disease. Researchers are developing new vaccines, both monoclonal antibody vaccines and blood stage vaccines, in an attempt to eliminate the disease as a whole. Unfortunately, malaria is currently a dominating disease, but with new vaccinations being developed there is hope for a cure. Until vaccines are regularly used in a clinical setting, the implementation of standard protocols for antimalarial drug therapies will lead to a decrease in drug resistance and allow for more efficient treatment of malaria.

Lifestyle and Biology

Plasmodium falciparum is a protozoan parasite that is associated with severe malaria (1, 2). Sub-Saharan Africa is the epicenter of malaria where, for the past four years, about two hundred forty-seven million cases occur each year and about one million deaths (2-4). Cerebral and placental malaria are the most severe and complicated cases of malaria which have incredibly high mortality and morbidity rates (5, 6). Cerebral malaria is an unrousable coma that can only be attributed to a malarial infection and no other comorbidities which usually occurs because of the lack of hemoglobin left in the blood (5, 6). Placental malaria could lead to the death of both the mother and the fetus, making the mortality rate extremely high (7).

Plasmodium falciparum is an obligate intracellular microbe that infects human erythrocytes, changing their structure and function (2, 3). The parasite goes through five morphological distinct phases, with merozoite being the final stage when the parasite is fully mature and released from the cell (2, 8). These five stages take about ten days to come to full completion (9). Once the parasite is released, its only purpose is to infect erythrocytes since it is not viable without a host cell (2, 3). The merozoite is an ovoid cell with an apical end that specializes in puncturing human erythrocytes (3). When merozoites come in contact with human red blood cells, they reorient to a position in which the apical end is in contact with the erythrocyte (2). The apical end of the mature *P. falciparum* is home to all of its organelles including dense granules, rhoptries, and micronemes (2, 3). These organelles are all secretory enzymes that utilize the lysosomal pathway (2, 3). As the enzymes are released, the membrane of the erythrocyte thickens and envelopes the ovoid cell, making the junction between the parasite and the host cell much more stable and changing the composition of the host membrane (2, 3). This shift in composition also alters the function of the membrane allowing the parasite to control the trafficking of proteins with minimal disruption (3).

Pathogenesis

Malaria occurs when *P. falciparum* infects human erythrocytes and causes them to bind to the endothelial linings of blood vessels (3, 4). *Plasmodium falciparum* is a parasite that travels within a mosquito vector and can be transferred bidirectionally between human blood and the mosquito (1, 10). The parasite only can be transferred from the human host to the mosquito vector in cases of symptomatic malaria (10). The parasite is passed from the host to the parasite or from the parasite to the host during a blood meal (6).

Once the parasite has entered the blood stream of the host, it binds to the human erythrocyte and orients so that the apex of the ovoid parasite is in direct contact with the human erythrocyte in order to facilitate the transfer of the parasitic organelles to the host cell (2, 4). The organelles that must be transferred are the secretory organelles including dense granules, rhoptries, and micronemes (2, 4). The lack of organelles in the host cell can lead to challenges for the parasite to live and develop inside of the host cell (2, 3). Once the parasite has been enveloped by the host cell it resides within the parasitophorous vacuole where it changes the composition and structure of the erythrocyte's plasma membrane which impacts permeability and rigidity (2, 3). *Plasmodium falciparum* only remains in the peripheral blood for eighteen to twenty-four

hours (5). The life cycle of the mature parasite only lasts for about eighteen hours, meaning that it must act fast to infect the host and reproduce (5).

Plasmodium falciparum gains entry to the human erythrocyte by controlling the trafficking of the host proteins (3, 11). Erythrocytes contain no organelles; therefore, the host proteins are directed through the Golgi apparatus and endoplasmic reticulum of the parasite (3). Host cell cytosol uptake is required for growth and development of the parasite (11). This process leads hemoglobin to the food vacuole where it is broken down into amino acids which are used for parasitic growth (3). Vacuolar protein sorting-associated protein 45 is the main protein associated with host cell cytosol uptake by directing the cytosol and hemoglobin to the food vacuole where it can be properly broken down (3, 11). Without the breakdown of hemoglobin, *P. falciparum* is unable to effectively reproduce and develop into a chronic disease which causes maximum damage (3).

While in the human erythrocyte, the parasite undergoes five morphologically distinct stages of development (8, 10). Stage I gametocytes circulate in the peripheral blood along with stage V gametocytes; however, parasites in stage II, stage III, and stage IV are sequestered in the microvasculature of tissues (5, 8). The sequestration of gametocytes in stage II through stage IV of development helps them to evade the immune system so that they can fully develop without disruption, while stage I and stage V gametocytes must circulate in the peripheral blood in order to continue reproduction (5, 8). Stage I parasites have just been released and infected by host cell, not yet having the opportunity to sequester into tissue (5, 8). Stage V parasite must circulate in the peripheral blood to release the new, immature parasite that they have produced (5, 8). When *P. falciparum* reaches full maturation, the infected erythrocyte bursts and releases hemozoin which is malaria pigment along with the new generation of immature gametocytes to infect human erythrocytes (5).

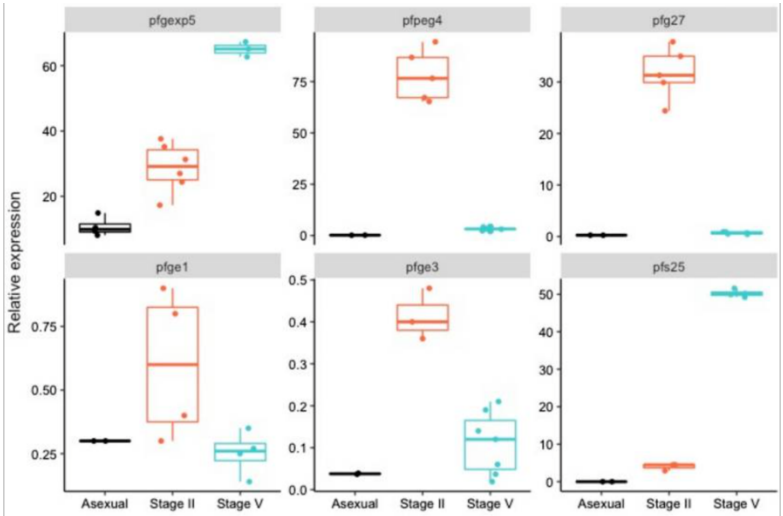

Figure 1: PCR is used to identify the genes in a sample of blood obtained from a patient with malaria. This information can then be used to identify the morphological stages of the gametocytes present (8).

By the use of PCR, researchers have identified that different genes are encoded at different stages of the gametocyte development process (5, 8). Because the gametocytes are sequestered in tissues between stage II and stage IV, the genes are rarely identified in patients' blood (3, 8). By the use PCR, researchers have been able to identify the genes that are encoded in stage I gametocytes and stage V gametocytes(3, 8). The primary genes that encode proteins in stage I gametocytes of *P. falciparum* are pfge1, pfge2 and pfg27; likewise, the genes identified by PCR in stage V are pfs25, pfs28 and pfs47 (8). PfGEZXP5 is the earliest detectable protein that is encoded by *P. falciparum* and is used for early diagnosis of malaria (8).

Plasmodium falciparum erythrocyte membrane protein 1 is the major virulence factor used in malarial pathogenesis because it aids the parasite in evading the immune system and leads to sequestration in the microvasculature of tissues (3, 12). This protein is a product of the sixty polymorphic *var* genes, which gives it the variability which enables the parasite to evade the immune system (3, 4). The PfEMP1 protein attaches to knobs on the host's plasma membrane which are created when *P. falciparum* releases secretory proteins (3, 11). When different genes are expressed, the parasite can evade detection by the immune system which is made capable by the numerous members of the *var* gene family. (3, 11) The *var* promoter is activated or silenced by epigenetic changes such as the position of the *var* locus and changes in the structure of the chromatin which is PfSIR2-dependent (11, 13). One *var* gene is expressed at a time on the outside of the cell but can easily be replaced in order to evade the immune system by the transcription of one of the three upstream sequences(11). *UpsA*- and *upsB*- subtypes of the *var* gene are located below the telomeres, while the *upsC*- are located within the clusters of the chromosomes (11). *UpsC*- is involved in silencing when working in tandem with *var* introns, but it also works as a promoter in coordination with *upsB*-(11). Silencing occurs in the presence of H3K9me3 (13). Methylation leads to genes not being expressed which has been proven by other methylation complexes in *P. falciparum*, but H3K9me3 is the most prominent in *var* genes (13).

Plasmodium falciparum erythrocyte membrane protein 1 is responsible for the formation of rosettes along with the binding of infected erythrocytes to the lining of the endothelium (3, 11). Rosetting is the process of infected erythorcytes clustering together which can be detrimental to the patient outcome and cause malarial anemia (4, 6). *Plasmodium falciparum* erythrocyte membrane protein 1 is a ligand that is recognized by many different surface receptors such as CD36m intracellular adhesion molecule 1, endothelial protein C receptor, gC1qR, and oncofetal chondroitin sulfate (4, 11). Because so many different receptors can recognize this ligand, the infectivity of the *P. falciparum* parasite increases (6, 11).

Plasmodium falciparum erythrocyte membrane protein 1 also binds to soluble plasma factors including IgM and α2-macroglobulin; however, the protein binds to the Fcμ region of IgM making this binding "non-immune" as shown in the figure below (4, 12). The binding of PfEMP1 to plasma factors has been proven to increase rosetting in patients with malaria making their cases more severe (12). The levels of IgM and α2-macroglobulin are much higher in patients with severe malaria when compared to patient with uncomplicate malaria (12). The binding of *P. falciparum* erythrocyte membrane protein 1 with non-immune IgM negatively correlates with the levels of

hemoglobin in the patient, meaning that the patient has a more difficult time up taking oxygen which can be detrimental (4).

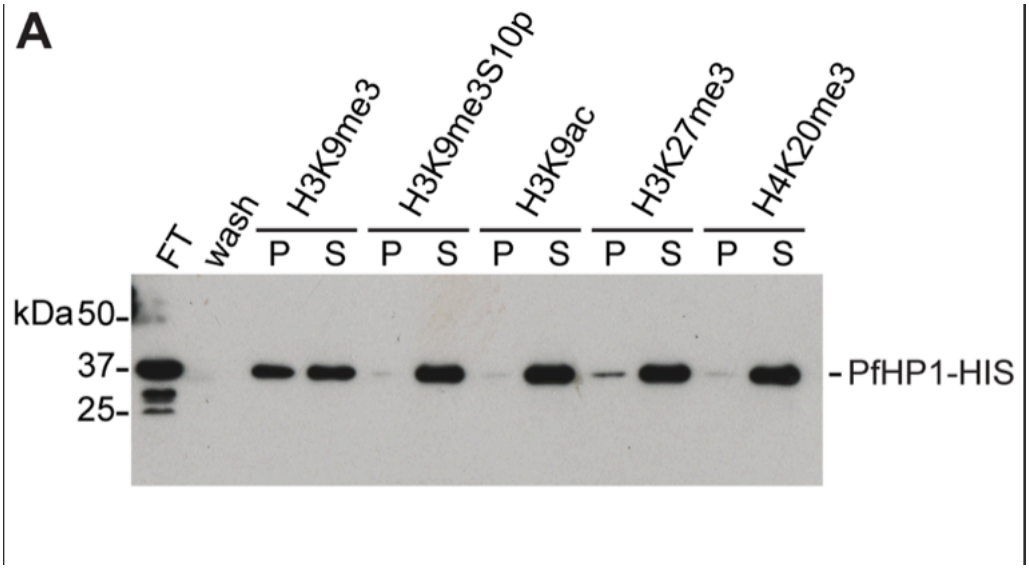

Figure 2: Plasmodium falciparum heterochromatin protein 1 preferentially binds to H3K9me3 over other methylated complexes. The result of this is var gene silencing (13).

Plasmodium falciparum heterochromatin protein 1 is another major virulence factor that aids the parasite in evading the immune system (13). The main function of this protein is to silence that *var* genes in order to add antigenic variation as well as the ability to invade the host cell (13, 14). This protein also allows the protein to switch between reproducing and invading other host cells which is crucial to maintaining a disease state (14). When replication and host cell invasion become imbalanced, the parasite is no longer able to maintain a disease state within the host (13, 14). The binding site of *P. falciparum* heterochromatin protein 1 is H3K9me3 which is known to silence *var* genes, leading to variation which allows the parasite to evade the immune system (13, 14).

Plasmodium falciparum export protein 60 is also an important virulence factor used by the parasite to bind to Maurer's clefts (15). The binding to Maurer's clefts is crucial for the virus to traffic the host proteins, allowing the protein to thrive within the cell and develop (3, 15). The Maurer's clefts are formed by the parasite when they entire into the host cell using the host's cytoplasm and they are where *P. falciparum* erythrocyte membrane protein 1 is displayed (11, 15). Without binding of *Plasmodium falciparum* export protein 60 to Maurer's clefts, *P. falciparum* erythrocyte membrane protein 1 cannot bind which would eliminate the antigenic variation of the parasite and abolish its ability to evade the immune system (12, 15). Without *Plasmodium falciparum* export protein 60, infected erythrocytes would not be able to form rosettes or bind to the endothelium of blood vessels, essentially making the disease ineffective (3, 15)

Anemia is one of the most common side effects of malaria due to the degradation of hemoglobin in the food vacuole of *P. falciparum* (3, 5). Due to the lack of hemoglobin,

patients with malaria are unable to up take a sustainable amount of oxygen (5). Anemia is one of the most detrimental comorbidities of malaria (5).

Ability to Control and Eradicate Disease

Malaria has both high morbidity and mortality rates in countries in the eastern hemisphere (16, 17). The death rates can be as high as twenty percent among children, although this ranges depending on location (6). In short, malaria is so detrimental to the human body because it ultimately leads to hypoxia which inhibits all bodily functions (4). Currently therapeutic treatments are being used in patients with malaria; however, researchers are studying to prevent the disease process before it begins for a long-term solution (18, 19). In countries where malaria has high morbidity rates, citizens are using tools such as bug nets treated with insecticides to prevent contact with mosquitos in general to lower the risk of infection (16). Indoor residual spraying is also performed in many of these countries to prevent mosquitos from residing in the homes (16). Pregnant women are also preemptively being treated to prevent infection before birth to increase the rate of survival in both the mother and the child (16).

Drug therapies mainly utilize artemisinin-based combination drugs as antimalarials in cases of uncomplicated malaria (16, 17). In Madagascar, artesunate-amodiaquine and artemether-lumefantrine are the most commonly used drug therapy treatments, both of which are artemisinin-based combination treatments which attack the food vacuole as their mechanism of action (3, 17). These drugs have above ninety-five percent effectivity, qualifying them as viable treatments by the CDC which states that drugs must be above ninety percent effective to be used (17). In the Comoros, Artemether-lumefantrine is the most common antimalarial drug with artemisinin-piperaquine as a close second (16). These drugs were followed in a forty-two day trial where researchers discovered that they were about ninety-eight percent effective in treating uncomplicated malaria (16). These drugs had minimal side effects, not including the normal side effects of the malarial disease process which include fever, headache, and chills. The drugs were proven to clear parasitemia within three days on average (16, 17).

Because the use of antimalarials is becoming so common and not done in the most effective way, malaria is still far from being eradicated (18). Some vaccines are in phase three of clinical trials including monoclonal antibody vaccines and blood stage vaccines (18, 20). Researchers propose that preventing the infection of the human host by P. falciparum will be much more effective than treating the disease after the infection has occurred (18-20).

The RTS,S vaccine is the first vaccination that has been implemented in endemic areas (21, 22). This vaccine is being used to combat malaria in young children ranging from five to seventeen months old (21, 22). After stage three trials were conducted, researchers concluded that this vaccine was about thirty percent effective in protecting against severe malaria (21). In some cases, this vaccination has prevented an infection with *P. falciparum*, but in other cases it has only lessened the severity of the disease (21, 22). The efficacy of the vaccination decline as the age of the child who received the vaccination increased (21).

The goal of the blood stage vaccine is to target the gene that codes for knob-associated histidine-rich protein which would eliminate the formation of knobs on the

plasma membrane of the host cell (19). The lack of knobs would prevent the display of *P. falciparum* erythrocyte membrane protein 1 (2, 19). Without this protein, the parasite will be unable to bind to other erythrocytes which would prevent rosetting (3, 4). *Plasmodium falciparum* would also be more harshly attacked by the immune system because it would be unable to evade these attacks (4). Without these features, the infection of a human host with *P. falciparum* would be infective causing a decrease in morbidity and mortality rates (3, 4).

Monoclonal antibody vaccines are also being tested for their effectivity against Malaria (23). CIS43LS is an antibody that is currently being tested in phase two clinical trials against malaria (18, 23). Researchers have found that the effectivity of the vaccine is dose dependent due to the short half-life of CIS43L (18). Of all the participants that underwent a controlled infection with *P. falciparum*, none of them had the parasite detected within their bloodstream by PCR after twenty-one days meaning that the vaccine was effective (18, 23).

Future Challenges and Opportunities

One of the major challenges with eradicating malaria is a lack of understanding of how disease transmission works (2, 8). With qRT-PCR, researchers are beginning to sequence malarial DNA and eventually develop a model of transmission which will lead to knowledge of the epidemiological process (8, 10). These PCR assays can detect early gametocytes enabling the treatment of malaria before severe symptoms set in (8, 10). Understanding how gametocytes differentiate could give the information needed to lower transmission by preventing mature parasites from developing (2, 8).

The sequestration of mature parasites in tissues is a major issue in treating malaria because it is no longer in the peripheral blood and is able to evade the immune system (3, 17). Currently, antimalarial drugs can eliminate parasitemia; however, drug resistance is becoming a major issue in regions where malaria has a high morbidity and mortality rate including Madagascar and Sub-Saharan Africa (10, 17). Drug resistances is threatening to reverse all of the progress made toward eradication of malaria (10, 16). To prevent drug resistance, organizations such as the CDC could determine the most effective dose of existing treatment and implement a standard for the treatment of malaria (8, 16). An aggressive fight against malaria leading to the eradication of the disease before it becomes fully resistant to existing treatments would be the most optimal plan (16).

Conclusion

In conclusion, much progress has been made over the years in pursuit to eradicate malaria, although it is still one of the major causes of death in eastern countries (3). A more aggressive approach must be taken in the treatment of malaria in order to completely eradicate the disease before drug resistance destroys all the progress that has been made (3, 16). The development of vaccinations is a huge step in the right direction for an expedited eradication of malaria (18, 19).

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