UNIVERSITY OF CAPE COAST

EFFECT OF IRON FORTIFICATION ON IgG RESPONSES AGAINST MALARIA VACCINE CANDIDATE ANTIGENS AMONG GHANAIAN CHILDREN

GIDEON KWESI NAKOTEY

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BY

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Thesis submitted to the Department of Microbiology and Immunology of the School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast in partial fulfilment of the requirements for the award of Master of Philosophy degree in Infection and Immunity.

AUGUST 2024

DECLARATION

Candidate's Declaration

I affirm that this thesis is the outcome of my independent and original research and that no portion has been previously submitted to obtain another degree within this institution or elsewhere.

Candidate's Signature……Date……

Name: Gideon Kwesi Nakotey

Supervisor's Declaration

We hereby affirm that the preparation and presentation of the thesis under our supervision adhered to the standards on thesis supervision established by the University of Cape Coast.

Name: Rev. Dr. Benjamin Amoani

Supervisor's Signature……. Date……

Name: Mr. Kwabena Dankwa

Co-supervisor's Signature…… Date……

ABSTRACT

Malaria continues to pose a substantial worldwide health concern, with emphasis on its prevalence in sub-Saharan Africa. The WHO recommended adding iron supplements to children's diets in highly prevalent malaria regions. However, the effect of iron fortification on the immune response of *P. falciparum* remains debated. While some studies suggest that iron supplements protect against malaria, others indicate that it may worsen infant malaria infection. This study aimed to investigate the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children. The study employed 400 randomly selected archival samples of children aged 6 to 36 months collected between April and May 2010 in Wenchi and Tain Municipalities from a double-blinded cluster-randomized control trial. Participants with haemoglobin <7g/dL, on food supplements before the commencement of the study, and exhibited a severe form of anaemia were excluded. The measurement of immunoglobulin G levels to GLURP R0, GLURP R2, and MSP-3 recombinant antigens was conducted using indirect enzyme-linked immunosorbent assays (ELISA). The results showed no significant differences in IgG responses between patients with and without malaria infection in the iron group. However, the results showed significantly higher levels of IgG responses among malaria-positive individuals than malaria-negative individuals in the non-iron group. Also, the study recorded significantly higher IgG responses against GLURP R2 and MSP-3 antigens in the non-iron group compared to the iron group with malaria-positive status. Results also showed no significant differences in parasitaemia's impact on IgG antibodies. The findings of this investigation indicate that iron fortification substantially downregulates IgG responses against GLURP R2 and MSP-3 malaria vaccine candidate antigens among individuals with malaria, thus increasing malaria severity. Further research with a larger sample size is required to understand the intricate relationships between iron fortification, malaria, and immune responses.

KEY WORDS

Glutamate rich protein

Merozoite surface protein

Micronutrient power

Recombinant antigen

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DEDICATION

To my late grandmother, Esther Dede Ahesume

LIST OF ACRONYMS

ADCI - Antibody-Dependent Cellular Inhibition

cAMP – cyclic Adenosine Monophosphate

CSA - Chondroitin-4-sulfate

EBA – Erythrocyte Binding Antigen

ELAM -1 - Endothelial Leukocyte Adhesion Molecule -1

ELISA- Enzyme-Linked Immunosorbent Assay

EPAC – Exchange Protein Activated by cAMP

EPCR – Endothelial Protein C Receptor

FPIX - Ferriprotoporphyrin IX

GAP50 - Glideosome Associated Protein 50

GLURP - Glutamate Rich Protein

HPX2 – Heme Peroxidase - 2

HRP2 - Histidine-Rich Protein-2

ICAM1 – Intracellular Adhesion Molecule 1

ID – Iron Deficiency

IgG - Immunoglobin G

iRBC - Infected Red Blood Cells

JNK- Jun N-terminal Kinase

LDH - Lactate Dehydrogenase

MAVS - mitochondrial Anti-viral Signaling Protein

MDA5 - Melanoma Differentiated Associated Gene 5

MSP - Merozoite Surface Protein

NOX5 – NADPH Oxidase 5

PAMP – Pathogen Associated Molecular Pattern

PfEBA – *P. falciparum* Erythrocyte – Binding Antigen

PfEMP1 - *P. falciparum* Erythrocyte Membrane Protein 1

PfRHs – *P. falciparum* Reticulocyte -Binding Homologues

PRBCs - Parasitized Red Blood Cells

RIFIN – Repetitive Interspersed Family in *P. falciparum*

STEVOR – Subtelomeric Viable Open Reading Frame

SURFIN – Surface- associated Interspersed gene family

Th1,2,17- T helper type 1,2,17

VCAM – Vascular cell Adhesion Molecule

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CHAPTER ONE

INTRODUCTION

Malaria and iron deficiency anaemia are two of the leading killers of children worldwide, with the highest cases in the sub-Saharan region. Scholars have debated the effectiveness of administering iron supplements to children residing in regions with a high malaria prevalence over the years. Studies have surfaced claiming that iron supplementation may protect against malaria. At the same time, other studies have observed that iron supplements may worsen malaria infection in infants. Various candidate antigens for malaria vaccines are being studied. This study aimed to ascertain the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children.

1.0. Background of the study

Malaria is still persistent around the globe, predominantly in sub-Saharan Africa (van den Berg et al., 2019). In 2021 an increase in the incidence of malaria cases was observed in 84 countries, including French Guiana, known with notable prevalence of malaria infection. The projected number of cases in 2021 was 247 million, slightly higher than the 245 million reported in 2020. Notably, this increase was seen in the WHO (World Health Organization) African Area. According to predictions from the World Health Organization's Global Malaria Report in 2022, 13.4 million more cases were linked to the interruptions caused by the COVID-19 pandemic between 2019 and 2021 World Malaria Report, 2022).

According to existing scientific literature, it has been observed that the prevalence of malaria tends to be higher among individuals who are under the

age of five, pregnant women, non-immune individuals such as tourists, and immunocompromised individuals (WHO Factsheet, 2022). According to the Severe Malaria Observatory, Ghana is among the 15 nations with a significant malaria burden, accounting for 1.9% of global malaria deaths and 2.1% of global malaria cases (WHO, 2022b). Between 2017 and 2020, Ghana significantly improved in controlling malaria, although mortality remained unchanged at 0.39 per 1000 at-risk individuals. The frequency of cases showed a reduction of 19%, falling from 201 per 1000 individuals in the at-risk group to a total of 163 instances (https://www.severemalaria.org/countries/ghana)

Malaria is a disease that is linked to the eukaryotic pathogen *Plasmodium* species. This infection is mainly spread via the bites inflicted by female *Anopheles* mosquitoes that have been infected with the disease-causing agent. *Plasmodium* passes through a multistage life cycle marked by antigenically different stages in its vertebrate and invertebrate hosts. *P. falciparum* and *P. vivax* are the most dangerous of the five *Plasmodium* species known to cause malaria. *P. vivax* is predominantly found in nations outside sub-Saharan Africa, while *P. falciparum* is predominant on the African continent and most malaria-related fatalities worldwide are associated with it (Sonon, 2018). *P. falciparum* has an intricate life cycle, involving an arthropod host and a human host. In humans, it is observed that the life cycle commences upon the introduction of sporozoites into the dermis via the bite of a female *Anopheles* mosquito that is carrying the infection. The sporozoites enter circulation and infect hepatocytes, leading to the development of merozoites. These merozoites then enter red blood cells and mature into

blood-stage merozoites. The illness is perpetuated by recurrent red blood cell (RBC) infections and merozoite release, linked to malaria symptoms (Nureye & Assefa, 2020). During a blood infection, merozoites transform into female or male gametocytes, which are taken in by mosquitoes during a blood meal, which initiates the vector stage life cycle (Casares et al., 2010).

Parasitized erythrocytes of the malaria parasite feature many antigenic surface proteins that induce an immunological response (Courtin et al., 2009; Dodoo et al., 2008). Merozoite surface protein (MSP) and Glutamate rich protein (GLURP) antigens of the asexual malaria blood stage induce an immune response that protects against in vitro parasitemia; their efficacy is being evaluated in phase 2 and phase 3 vaccination studies in African preschoolers (Bergmann-Leitner, Duncan, & Angov, 2012).

People from malaria-endemic regions gain some protection from the malaria parasite with repeated exposure. During several years of exposure, individuals develop clinical immunity to the disease (Milet et al., 2016). The crucial function of IgG antibodies in protecting against malaria was shown by groundbreaking research that included the transfer of pure IgG from the serum of individuals with partial immunity to non-immune patients. This transfer led to the elimination of parasitemia, as Milet et al. proved in 2016. This protection shows the production of anti-*P.falciparum* antibodies to blood-stage antigens; however, the exact mechanism(s) involved are still unknown. It has been proposed that particular IgG can directly or indirectly inhibit parasite development. IgG1 and IgG3 are two kinds of IgG believed to be especially crucial for protection. The subclasses mentioned in the literature have been

observed to directly impact parasites by impeding their invasion or growth within erythrocytes.

Additionally, they can indirectly neutralize parasites through a mechanism that involves the collaboration between parasite-opsonizing antibodies and monocytes. This collaboration occurs via the attachment of antibodies to the Fcγ receptor IIA, which subsequently leads to the production of soluble factors that hinder parasite growth. Such factors include nitric oxide and TNF-alpha (Courtin et al., 2013; Sabbagh et al., 2018). The discovery that IgG antibodies can passively transfer immunity supports the notion that antibodies against the asexual blood stage antigens of *Plasmodium falciparum* are a significant component of acquired immunity.

According to research by Stevens et al. (2013), the frequency of anaemia among children under five was estimated to be 43% worldwide in 2011, with a higher rate of 71% seen in the central and western regions of Africa. Preschoolers in Ghana had a prevalence rate of 35.6% for anaemia, 21.5% for iron deficiency, and 12.2% for iron deficiency anaemia in 2017 (Ghana Micronutrient Survey, 2017). Anaemia in early life has been linked to diminished cognitive ability, developmental delays, and disability (Black et al., 2008). Like iron insufficiency, zinc deficiency is believed to affect approximately 293 million children under five (Suchdev, Jefferds, Ota, da Silva Lopes, & De-Regil, 2020). Despite their vital role in human health, the lack of essential micronutrients is a worldwide issue, especially among young children in impoverished nations (Horton, Shekar, McDonald, Mahal, & Brooks, 2009).

The use of multiple micronutrient powder (MNP), as advised by the World Health Organization (WHO), is to mitigate malnutrition among children and address nutritional needs during periods of health crises. This powder typically contains essential micronutrients such as iron, zinc, and vitamin A. According to Muthayya et al. (2013), the implementation of vitamin A and zinc supplementation in children and the supplementing meals with iron and iodine represent the most economically efficient approaches for mitigating the worldwide impact of micronutrient deficiencies.

According to Sazawal et al. (2006), children under two years in regions with a high incidence of anaemia are encouraged to take iron and folic acid supplements, per international guidelines in malaria-stricken areas. This suggestion has been met with some resistance. Supplemental iron has been shown to improve child development and decrease the incidence of severe anaemia (Sazawal et al., 2006). Recent initiatives to decrease malaria and anaemia by iron supplementation have only partially been successful, and malaria-specific immune responses to specific antigens have produced inconsistent outcomes in both malaria-endemic and non-endemic regions (Tielsch et al., 2006). The body's capacity to fight against infections is aided by sufficient iron, which boosts immune cell development and proliferation (Pandey et al., 2023). Iron shortage or overload can compromise the antibody response, as immune cells, such as B-cells, require sufficient iron for proper function. Lower B-cell activity due to iron deficiency or overload can reduce the immune response to infections and vaccines, increasing infection risk (Preston, Drakesmith, & Frost, 2021). Thus, this study sought to investigate

the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children.

1.1. Statement of the Problem

The global incidence of malaria is increasing. Based on the findings presented in the World Malaria Report of 2022, there was an observed increase in malaria incidence from 245 million in 2020 to 247 million in 2021 across 84 countries where malaria is prevalent. Notably, the WHO African Region saw the majority of these cases. Although malaria-related deaths have decreased significantly in recent years, a significant proportion of those who die are young African children below five years who lack sufficient immune protection (Sabbagh et al., 2018). According to UNICEF, one kid under five dies from malaria every 75 seconds. UNICEF data from 2019 show that 9 percent of all under five years of malaria fatalities occur in Ghana (Sanyang, 2019).

Iron supplementation's influence on antibody responses against malaria vaccine candidate antigens has not been thoroughly investigated, particularly in the Ghanaian population. According to the Severe Malaria Observation report, Ghana has made impressive efforts in malaria control, which could cause a decrease in malaria cases and deaths in 2021, among children under the age of 5 in particular (*https://www.severemalaria.org/pays /ghana*). Ghana participated in the ongoing phase three clinical study of the malaria vaccine for all children under five, as the WHO recommended in 2019.

According to a 2006 WHO statement, children diagnosed with anaemia who are susceptible to developing iron deficiency, particularly in regions where malaria is prevalent should receive iron supplements (drops, syrup, or pills). The consequences of iron fortification on children's immunity are still debatable. Studies have shown conflicting results on the relationship between iron supplementation in children and their susceptibility to malaria and anaemia. On the one hand, some studies have demonstrated that children receiving iron supplements have enhanced protection against malaria and anaemia (Tchum et al., 2021). Conversely, other studies have indicated that administering iron supplements to children increases their likelihood of contracting malaria (Sangare, van Eijk, Ter Kuile, Walson, & Stergachis, 2014)

Nevertheless, no study has examined the impact of iron supplementation on immunological responses against prospective malaria vaccine candidate antigens in the Ghanaian population. Hence, could iron supplementation affect immunological responses to possible malaria-candidate antigens? Could iron fortification enhance the effectiveness of prospective malaria vaccines? These questions necessitated this study to investigate the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children.

1.2. Research Hypothesis

Iron fortification could cause increased production of IgG responses against malaria vaccine candidate antigens among Ghanaian children.

1.3 Aim and Objectives

1.3.1 Aim

To study the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children

1.3.2 Specific Objectives

The study seeks to:

1. determine IgG levels against malaria vaccine candidate antigens among iron-fortified Ghanaian children with or without malaria infection
2. determine IgG levels against malaria vaccine candidate antigens among non-iron fortified Ghanaian children with or without malaria infection
3. evaluate the effect of iron fortification on IgG response against malaria vaccine candidate antigens among Ghanaian children with or without malaria infection
4. assess the association between the antibody levels against the malaria vaccine candidate antigens with age
5. assess the association between the antibody levels against the malaria vaccine candidate antigens and parasitaemia

1.4. Significance of the Study

Research conducted in Africa to determine the effectiveness of potential malaria vaccines has not shown promising results (Amoani et al., 2021; Baumann et al., 2012). The observed sub-optimal efficacies of malaria vaccinations are puzzling because the factors affecting immune responses to

these vaccines are poorly understood. Certainly, this study will determine if iron supplementation can enhance the efficacy of potential malaria vaccines. In addition, this study will compare the antibody expression levels of iron-fortified and non-iron-fortified infants against candidate antigens for the malaria vaccine. Other aims of the Global Technical Strategy for Malaria (GTS) include a 75% decrease in annual malaria deaths by 2025 and a 90% decrease by 2030 (World Malaria Report, 2020). The findings of this study will inform policymakers about the need to continue or discontinue iron fortification, depending on whether or not it improves immune responses in infants. Again, this study will help in understanding how malaria vaccine could be optimized among individuals with varying nutritional status. Overall, this study will help us get closer to achieving sustainable development goal (SDG) 3 to ensure that everyone enjoys a high health and well-being standard.

1.5. Delimitations

The research was carried out in the Tain and Wenchi districts within the "Kintampo North Municipality in the Bono Region", formerly Brong Ahafo Region. Children aged 6 to 36 months who had fever with an axillary temperature greater than 37.5°C or recent fever within 48 hours) had blood samples taken or those admitted to a healthcare facility were included in the study. Children who were severely anaemic (haemoglobin level 70 g/L), severe malnutrition (weight-for-length -3.0), iron supplementation during the previous six months, or a chronic condition were not included in this study.

1.6. Limitations

This study used a double-blinded cluster-randomized controlled trial design and archival samples from April to May 2010. It is empirical that this

study's methodological weaknesses are pointed out and how they could affect the results obtained.

Firstly, the study's use of archival materials obtained throughout a limited duration of just two months (April and May 2010) may not sufficiently encompass the range of diversity and issues inherent in the phenomena under investigation. The limited duration of data collection may introduce possible biases and restrict the applicability of the results to other periods. Thus, the generalizability of the study's results may be limited to the particular period and geographical location in which the data was gathered.

The use of archived samples from a specific period in the research led to a small sample size. The limited sample size may impact the study's statistical power, making it challenging to identify subtle but potentially significant effects and raising the probability of committing type II errors. Using archival data increases the likelihood of missing specific details because researchers may be unable to ensure the completeness and correctness of the data acquired during the initial experiment, which might reduce confidence in the results.

1.7. Definition of Terms

ELISA: A widely used laboratory technique to detect and quantify specific proteins, antibodies, antigens, and other molecules.

Coating: The process of immobilizing the target antigen onto the microplate

Blocking: The process of preventing nonspecific binding

Washing: The process of getting rid of unbound molecules or contaminants

1.8. Organization of the Study

There are a total of five chapters in this study. The opening chapter provides the background for the study. Chapter two, which is the literature review, looks at various scholarly articles on the following: a brief history of malaria; the parasite; global distribution of malaria; *Plasmodium falciparum* life cycle; pathogenesis; immune response against malaria; immune evasion; malaria transmission; iron fortification and malaria; iron fortification, IgG and malaria; malaria candidate antigens and malaria vaccine development. Chapter three discussed the methodological processes employed, considering study area and design, subject recruitment, sample and data collection, laboratory work, data analysis, and ethical clearance. Results obtained from the study were presented and discussed in Chapter Four. Chapter Five of the research study delved into the critical aspects of summarising the findings, drawing appropriate conclusions, and formulating valuable recommendations.

1.9. Chapter Summary

This chapter highlighted the background of the study, problem statement, research hypothesis, aims and objectives, significance of the study, delimitations and limitations of the study, contextual definition of terms used in the study and concluded with a detailed explanation of the organization of the entire study.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Sub-Saharan Africa continues to bear the burden of malaria, with death rates among African children under five years still high. Results from studies on iron supplementation and antibody responses to malaria-specific antigens have been inconsistent in both endemic and non-endemic regions. This study sought to investigate "the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children". The literature review follows the following pattern. It begins with a historical overview of malaria and examines the parasite and the global distribution of malaria. The chapter also reviews the life cycle of *Plasmodium falciparum* life cycle, its pathogenesis, immune response against malaria, and immune evasion. It also explores the issue of malaria transmission. Finally, the chapter highlights the effect of iron fortification on malaria vaccine candidate antigens and concluding with malaria vaccine development and a chapter summary.

2.1 Brief Historical Overview of Malaria

Malaria or "bad air" has existed for several number of years. About 2700 B.C., Nei Ching's book documented the symptoms of "bad air," attributing the fever to insect bites. The first documentation of patients' blood containing malaria parasite was reported by Charles Louis Alphonse Laveran, a French Nobel winner, in 1880, marking a significant milestone in understanding the disease. The parasites *P. vivax* and *P. malariae* were first identified in 1890, with further characterization of *P. falciparum* in 1897, *P. ovale* in 1931, and *P. knowlesi* in 1931. In 1886, Camillo Golgi, an Italian

scientist, described two distinct clinical manifestations of malaria. These manifestations were characterized by fever, occurring either daily (known as tertian periodicity) or every three days (known as quartan periodicity). In 1897, William Welch successfully identified the *P. falciparum* parasite as the etiological agent accountable for the manifestation of tertian malaria. In 1898 and 1899, a group of Italian researchers conducted a study that confirmed the transfer of human *P. falciparum* from infected individuals to healthy individuals. This study further supported Ronald's earlier assertion, made between 1897 and 1898, about mosquitoes serving as malaria transmission vectors (Cox, 2010). Currently, malaria remains a significant issue in the realm of public health.

2.2 Global Distribution of Malaria

Malaria cases globally grew from 245 million in 2020 to 247 million in 2021 across 84 countries where malaria is prevalent, including French Guiana. The bulk of this rise is seen in nations within the WHO African Region. The COVID-19 pandemic is believed to have resulted in an additional 13.4 million cases throughout the period spanning from 2019 to 2021 (WHO, 2022a). According to the data presented in the 2021 World Malaria Report, it is evident that a significant proportion of global malaria cases, amounting to 96%, were concentrated among 29 specific countries. Nigeria emerged as the primary contributor, accounting for almost 27% of the reported cases. Following closely, the Democratic Republic of the Congo, Uganda, and Mozambique contributed 12%, 5%, and 4% of the cases, respectively. Ghana is one of the nations that contributes to 2.1% of worldwide cases of malaria.

In 2020, it was estimated that approximately 625,000 individuals died due to malaria, representing a 10% increase compared to the previous year, 2019. The death toll, however, declined in 2021 to 619,000 individuals (WHO, 2023). Cumulatively, a total of 627,000 individuals died between the years 2019 and 2021, with these fatalities being ascribed to the disruptions engendered by the COVID-19 epidemic (WHO, 2023). Ninety-six percent of global malaria fatalities were attributed to twenty-nine nations. The findings revealed that a noteworthy portion of malaria-associated mortalities on a global scale during the year 2021 exhibited a concentrated distribution across four distinct nations, namely Nigeria with a prevalence of 31%, the Democratic Republic of the Congo with 13%, Niger with 4%, and the United Republic of Tanzania also with 4%. According to the Malaria World Report of 2022, Ghana accounts for 1.9% of the worldwide mortality attributed to malaria.

The percentage of malaria-related mortality among under five years children saw a significant decline from 87% in 2000 to 76% in 2015. Based on the findings of the World Malaria Report for the year 2021, a significant proportion of malaria-related fatalities, namely 80%, were recorded among Children who are below the age of five years. Health statistics around the globe reveal that the main contributing factor to child deaths is still malaria, resulting in the deaths of more than 500,000 children annually. Concerning the WHO's data on child mortality caused by malaria, it is worth noting that UNICEF has provided additional information stating that a child under five years succumbs to malaria every 75 seconds. Effective public health initiatives and economic development have successfully eliminated malaria in many

temperate locations, such as Western Europe and the United States (Tuteja, 2007)

Based on the statistics presented by UNICEF in 2019, it is evident that Ghana accounts for 9 percent of the overall malaria-related mortalities reported in sub-Saharan African infants under the age of five. The current state of affairs in sub-Saharan Africa contradicts the findings of Tuteja's study, mainly due to insufficient implementation of public health policies and the coexistence of many malaria species within the Sub-Saharan Africa area.

2.3 The Malaria Parasite

Malaria is attributed to the infection of five distinct *Plasmodium* parasites: *P. falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi*. *Plasmodium ovale curtisi* (Poc) and *Plasmodium ovale wallikeri* (Pow) are two types of *P. ovale* that have recently been confirmed (Rutledge et al., 2017; Sutherland et al., 2010). Moreover, there is evidence that *Plasmodium cynomolgi* may cause infections in humans (Ta Tang et al., 2014). *P. falciparum* and *P. vivax* are known to pose substantial risk to human health (Commons et al., 2019). However, there have been reports recently of *Plasmodium ovale* malaria cases with severe circumstances and even mortality (Lau et al., 2013; Yerlikaya, Campillo, & Gonzalez, 2018) and both severe acute kidney failure and severe anaemia are known to be perpetuated by *P. malariae* infection (Douglas et al., 2013; Langford et al., 2015). Additionally, *P. falciparum's* infected erythrocytes can block off small blood arteries, leading to cerebral malaria, a potentially fatal consequence, especially for African neonates. Malaria relapses are attributed to the presence of hypnozoites, quiescent forms of the *Plasmodium ovale,* and *Plasmodium vivax*

parasites. These hypnozoites can persist in the liver for long periods, ranging from weeks to years, until a new cycle of pre-erythrocytic schizogony starts (Sonon, 2018). According to Chora, Mota, and Prudêncio (2022), in the event of neglect, *P. malariae* has the potential to induce persistent blood-stage infections in humans that may persist for many decades without manifesting any symptoms.

In sub-Saharan Africa, the *P. falciparum* strain of malaria is by far the most common. *P. falciparum* accounts for most malaria-related fatalities worldwide, whereas *P. vivax* is the predominant malaria parasite in most countries outside the sub-Saharan African region (Sonon, 2018). In contrast, the latest report by Yusof et al. (2016) indicates that malaria is caused mostly by *P. knowlesi* in Malaysia. Arora, C Anbalagan, and Pannu (2021) concluded that if the World Health Organization's (WHO) aims of eradicating malaria are to be achieved, all *Plasmodium* species capable of infecting humans should be of concern, particularly the mode of transmission of malaria.

2.4 Malaria Transmission

The female *Anopheles* mosquito is known to be the carrier of the malaria parasite (Boissière et al., 2012; Dahalan, Churcher, Windbichler, & Lawniczak, 2019). Approximately 400 distinct species of *Anopheles* mosquito exist, but only thirty are known to be significant malaria vectors and active between dusk and dawn. (Sonon, 2018). Blood-sucking mosquitoes detect and locate their vertebrate hosts primarily by smell and human body emanations, such as breath and skin odours, and their constituents, such as lactic acid, ammonia, and carbon dioxide (CO2) gas are the most potent attractants that

the *Anopheles* takes advantage of during the deep sleep of the vertebrate host (Zwiebel & Takken, 2013; Matowo et al., 2013).

Mosquitoes of the genus Anopheles lay their eggs in stagnant water, where they develop into larvae and mature into adulthood. Blood feeding is necessary for female mosquitoes to obtain enough nutrition for egg development (Klug, Gautier, Calvo, Marois, & Blandin, 2023).

2.5 Plasmodium falciparum

According to Jensen, Adams, and Hviid (2020), the aetiology of the fatal manifestation of malaria is attributed to *P. falciparum*, which is responsible for all occurrences of the illness on a global scale. According to Lavstsen et al. (2012), infections caused by *P. falciparum* exhibit a diverse range of clinical outcomes, spanning from the presence of subclinical parasitemia to the manifestation of severe malaria symptoms that are associated with considerable fatality rates. "The expression of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) has been observed on the surface of erythrocytes that have been infected with the *P. falciparum* parasite". The attachment of *P. falciparum* to the vascular lining is linked with its virulence (Lavstsen et al., 2012).

2.5.1 Plasmodium falciparum Life Cycle

Malaria parasites undergo a specific developmental cycle that occurs in both invertebrate and vertebrate hosts, with each stage of this cycle necessitating the expression of highly specialized proteins. Intracellular and extracellular survival, cell invasion, and evasion of host immunological responses all depend on these proteins (Walker et al., 2014).

P. falciparum has an intricate developmental stage, with sequential multiple phases of development inside each host (Jensen, Adams, & Hviid, 2020). Several studies have shown that there are two distinct phases to the malaria parasite's life cycle: the asexual phase, which occurs entirely within the vertebrate host organism, and the sexual stage, which begins in the invertebrate host and ends in the midgut of a female mosquito (Chora, Mota, & Prudêncio, 2022; Florens et al., 2002; Tuteja, 2007).

According to Jensen et al. (2020), when a female *Anopheles* mosquito goes feeding, it introduces sporozoite stage parasites into the dermis of the human host, commencing the asexual phase of the parasite's reproductive cycle. According to Tang et al. (2014), the *Anopheles* mosquito introduces infected sporozoites into the human host via its saliva, which has anticoagulant properties, thereby facilitating successful blood feeding. A recent study using *Plasmodium yoelli*, a parasite that infects rodents as a model organism, casts doubt on the commonly held belief regarding disseminating injected sporozoites throughout the body. This study demonstrates that, contrary to previous assumptions, a significant proportion of infective sporozoites remain localized at the area of injection for a long time before gradually entering the bloodstream (Yamauchi, Coppi, Snounou, & Sinnis, 2007). Hepatocytes inside the liver are susceptible to infection by extracellular sporozoites, which rapidly traverse the peripheral circulation originating from the skin (Loubens et al., 2021). During the asymptomatic liver stage, the intrahepatic parasite undergoes multiplication and develops into a schizont, either pre- or extraerythrocytic. This schizont comprises a minimum of 30,000 daughter parasites (Jensen et al., 2020). The duration of the asymptomatic liver stage is

approximately one week. Previous research has provided evidence that the culmination of the developmental phases in the mammalian host requires the sporozoite to pass through numerous hepatocytes (Aly, Vaughan, & Kappe, 2009; Dundas, Shears, Sinnis, & Wright, 2019). However, the mechanisms involved in targeting and invading hepatocytes remain poorly understood.

2.5.2 Hepatic Phase

Hepatocyte invasion is made possible by the thrombospondin domains present on the circumsporozoite protein and the thrombospondin-related adhesive protein found on sporozoites. Heparan sulfate proteoglycans on hepatocytes are the target of these domains' binding (Morahan, Wang, & Coppel, 2009). After developing within the hepatocyte, a single sporozoite can produce thousands of merozoites, each capable of invading an RBC. Merosomes from hepatocytes are known to help the parasite evade successful infection establishment. Thus, the discharge of more merozoites into circulation initiates the erythrocytic phase of the cycle (Cowman, Healer, Marapana, & Marsh, 2016)

2.5.3 Erythrocytic Phase

After leaving the infected hepatocyte, the merozoites enter the bloodstream. The intraerythrocytic multiplication cycle starts when the merozoites quickly penetrate red blood cells (Jensen et al., 2020). According to Wright and Rayner (2014), merozoites penetrate erythrocytes through four complicated processes. The initial step involves the identification and reversible fusion of the merozoite with the erythrocyte membrane. Subsequent to this stage, a crucial event takes place involving the establishment of a connection at the apex of the merozoite, accompanied by a reorganization of

the membrane. This intricate process ultimately culminates in the assembly of the parasitophorous vacuole. This is facilitated by the release of chemicals from the rhoptry and microneme organelles. Following this, a notable phenomenon occurs involving the relocation of the junction and the inward folding of the erythrocyte membrane around the merozoite. This process leads to the elimination of the merozoite's surface coat. Finally, the parasitophorous vacuole and erythrocyte membranes are resealed once the invasion by the merozoite is completed.

Following erythrocyte penetration, the parasite enzymatically degrades host haemoglobin into its constituent essential amino acids. These amino acids are subsequently utilized by the parasite for its developmental and growth". Free heme Fe (II), a byproduct of the haemoglobin breakdown process, is also released. The free heme rapidly oxidizes from the Fe (II) state to the Fe(III) state (ferriprotoporphyrin IX, or FPIX) in this process (Ecker, Lehane, Clain, & Fidock, 2012). Regarding this same phenomenon, Van and colleagues stated that the parasites initiate their asexual replication process within the erythrocyte and undergo a series of distinct developmental stages giving rise to the trophozoite called the "ring form" due to its distinctive appearance. Trophozoite expansion is followed by metabolic activities, which include the proteolysis of haemoglobin into its amino acids, the consumption of host cytoplasm, and the glycolysis of significant quantities of glucose (Van Biljon et al., 2018), which is similar to the writing of Ecker and colleagues. The FPIX is a potentially toxic waste that is exceedingly insoluble and undegradable by the parasite and can damage the membrane of the parasite (Ecker et al., 2012). Thus, as a defensive mechanism, most of the heme released during

haemoglobin destruction is transformed into a malaria pigment called hemozoin. This crystalline material is retained in the parasitophorous vacuoles (Van Biljon et al., 2018).

Nuclear division that occurs repeatedly without cytokinesis marks the conclusion of this trophic stage, leading to the formation of schizonts. Each mature schizont may hold about twenty merozoites, which will be released following RBC lysis and enter other uninfected RBCs. This release occurs at the same time that the illness progresses and body temperature rises dramatically. Infections with *P. falciparum*, *P. ovale*, and *P. vivax* take approximately 48 hours, whereas infections with *P. malariae* take 72 hours to complete. A repetitive intraerythrocytic cycle characterized by the sequential processes of invasion, multiplication, release, and subsequent reinvasion persists unless interrupted by an immune response (Muhaimin et al., 2019). TNF and other cytokines are produced once an infected RBC is lysed, and these cytokines cause the illness's recognizable clinical signs.

Many distinct ligand-receptor communications have been implicated in erythrocyte invasion, and it has been shown that genetic loss of any of them causes a switch to alternative pathways (Sridhar, 2020). The successful completion of genomic sequencing of *P. falciparum* in 2002 suggests that several components involved in invasion are members of broader gene families. Merozoite surface proteins mediate the first recognition of erythrocytes. During invasion, the *P. falciparum* protein known as EBA175 binds to the main glycoprotein (glycophorin A) present in human erythrocytes. PfEMP1 plays a crucial function in *P. falciparum* pathogenesis and is expressed at the surface of the infected RBCs after invasion. It has been

discovered that a particular epigenetic imprint connected to repressed var genes benefits the parasite in pathogenesis and immune evasion (Beeson et al., 2016; Boyle et al., 2014; Chan, Fowkes, & Beeson, 2014).

Instead of completing the asexual multiplication cycle, some merozoites transform invasion, differentiating into either male or female gametocytes. The parasite life cycle is completed by sexual reproduction and further asexual multiplication stages carried out by a blood-feeding mosquito after the gametocytes do not differentiate but stay within the RBCs until they are taken up by a female *Anopheles* mosquito (Miller, Ackerman, Su, & Wellems, 2013).

2.5.4 Sexual Stage

A mosquito might potentially consume these gametocytes into its midgut when it feeds on blood from an infected individual, where exflagellation of microgametocytes results in microgametes while macrogametocytes create macrogametes. Fusing these gametes produces a zygote that changes into an ookinete, which becomes an oocyst by breaking through the wall of a midgut cell. Recent research by Nureye and Assefa (2020) has shown that the gamete surface antigen Pfs230 plays a crucial role in facilitating the attachment of human red blood cells (RBCs) to ex-flagellating male parasites. This attachment process leads to the formation of ex-flagellation centres, which serve as sites for the discharge of motile microgametes. Thus, this protein is crucial for forming the oocyst, a vital stage in malaria transmission. Numerous sporozoites are produced by sporogony inside the oocyst, and once the oocyst opens up, they go to the salivary glands, where they might spread to another host. Another study reports that

sporozoites first move into the hemolymph before moving into the salivary glands (Douglas, Amino, Sinnis, & Frischknecht, 2015). Nevertheless, the precise mechanism underlying the invasion of salivary glands by sporozoites remains elusive, despite the existence of several studies positing that this process occurs within a span of a few minutes (Klug et al., 2023). The sporozoites stay in the salivary glands for 10 to 18 days, and the mosquito continues to transmit it for another one to two months. In contrast, Klug et al. (2023) reported that sporozoites stay in the salivary gland for 10 to 12 days. The life cycle of Plasmodium is initiated again upon the biting of a vulnerable host by an infected insect leading to the development of the disease.

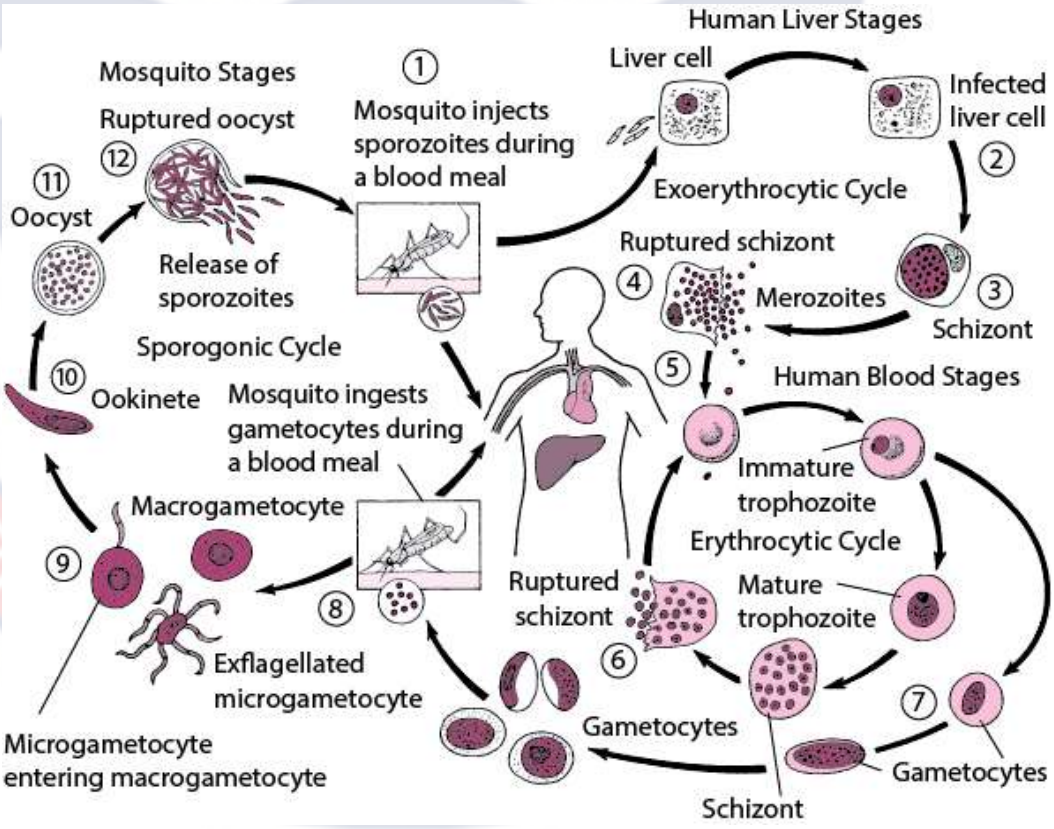

Figure 1: Step-by-step Diagrammatic Depiction of the P. falciparum Life Cycle

Source: Plasmodium life cycle - MSD Manual Professional Edition (msdmanuals.com)

2.6 Malaria Pathogenesis

"The most severe clinical illnesses caused by *P. falciparum* primarily affects children under five years, nonimmune individuals, and pregnant women. Human malaria pathogenesis is a complicated narrative. Fever, headache, vomiting, fatigue, and chills are some symptoms of uncomplicated malaria, defined as symptoms without clinical or laboratory indications indicating severe illness or damage to essential organs (WHO, 2015). Studies show that the rupture of infected RBCs leads to the release of potential malaria toxins, which activate macrophages and other peripheral blood mononuclear cells and induce cytokine production, resulting in clinical manifestations (Gazzinelli, Kalantari, Fitzgerald, & Golenbock, 2014)".

In contrast, cerebral malaria, pulmonary oedema, acute renal damage, severe anaemia, haemorrhage, acidosis, and hypoglycemia are some of the most severe *Plasmodium falciparum* malaria complications (Wilainam, Nintasen, & Viriyavejakul, 2015).

The pathogenesis of malaria is significantly influenced by the cytoadhesion and sequestration of parasitized red blood cells (PRBCs) within vital organs, as well as the activation of soluble cytokines. A study by Hanisch, Bangirana, Opoka, Park, and John (2015) reports that disease severity is usually related to the amount of pro- and anti-inflammatory cytokines, chemokines, growth factors, and effector molecules present in the individual. For example, while high levels of TNF-alpha were seen in complicated malaria, low levels of IL-10 have been recorded in uncomplicated malaria (Perera et al., 2013). The parasite employs various mechanisms, especially *P. falciparum*, leading to severe malaria.

Infected red blood cells do not persist in the bloodstream throughout *P. falciparum's* life cycle, unlike other human malarial species. After 24-32 hours, juvenile parasites undergo a developmental transition from the ring stage to the trophozoite stage, during which infected red blood cells adhere to endothelial cells in the microvasculature of various organs. This process, known as "sequestration," is thought to occur primarily to ensure these cells escape splenic destruction. Sequestration contributes to the severity of the illness by causing microcirculatory obstruction, inadequate tissue perfusion, and the stimulation of inflammatory cells (Autino, Corbett, Castelli, & Taramelli, 2012). The pathogenicity of *Plasmodium falciparum* is associated with its ability to generate proteins that facilitate the binding of infected RBCs to the vascular endothelium (Turner et al., 2013). As per a comprehensive review, it has been observed that proteins originating from the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family exhibit a distinct affinity towards various host receptors. Notably, these proteins demonstrate a specific binding capability to receptors such as ICAM1 (CD54), CD36, VCAM (CD106), ELAM-1 (CD62), CD31, and CSA. This binding interaction plays a crucial role in facilitating the process of adhesion. It is known that while underflow, CD36 provides steady and stable adhesion (Milner Jr, 2010).

Sequestration is also seen in cases of gestational malaria (Clark, 2019). PfEMP1 is the primary adhesion receptor that binds to the trophoblastic villous endothelium primarily via chondroitin-4-sulfate (CSA) and sugars such as glycosaminoglycans hyaluronic acid (Clark, 2019). As addressed by studies, malaria during pregnancy may cause severe symptoms. It can result in

the death of the foetus, particularly for first-time expectant mothers whose immunity against CSA-binding parasites is often insufficient (De Beaudrap et al., 2013; Fried & Duffy, 2017). Therefore, it is imperative that the promotion of appropriate diagnostic protocols, as stipulated by the World Health Organisation (WHO, 2022), be prioritized to achieve effective treatment and mitigate the incidence of malaria.

2.7 Diagnosis and Treatment of Malaria

The WHO advocates microscopy and rapid diagnostic tests (RDTs) as the preferred methods for diagnosing and confirming cases of malaria in individuals displaying symptoms suggestive of the disease (Monroe, Williams, Ogoma, Karema, & Okumu, 2022). The three major malaria antigens used in malaria rapid diagnostic tests (RDTs) consist of lactate dehydrogenase (LDH), histidine-rich protein-2 (HRP2), and aldolase. Aldolase is an antigen all Plasmodium species share, whereas histidine-rich protein-2 is a *Pf*-specific antigen. Fast diagnostic tests are crucial in controlling malaria by facilitating access to quick diagnoses and the proper treatment. RDTs provide an affordable, simple-to-use, and Field-ready option, particularly in environments where microscopy is not ideal (Plucinski et al., 2019). It is important to note that several techniques have been employed to diagnose malaria. PCR, flow cytometry, and ELISA are a few methods that have played critical roles in the clinical and research diagnosis and confirmation of malaria (Fitri et al., 2022). Effective diagnosis of malaria plays a pivotal role in treatment.

Uncomplicated *P. falciparum* malaria in both paediatric and adult populations is now managed with the administration of one of the following "artemisinin-based combination therapies (ACTs): artemether-lumefantrine

(AL), artesunate-amodiaquine (AS+AQ), artesunate-mefloquine (ASMQ), dihydroartemisinin-piperaquine (DHAP), or artesunate in combination with sulfadoxine-pyrimethamine (AS+SP)". However, it is not advised to use artesunate and sulfadoxine-pyrimethamine or artesunate and pyronaridine during the first trimester of pregnancy (WHO, 2022a). It must be noted that quinine is the choice of drug for first-trimester pregnant women (Dellicour et al., 2017). The parasitemia and morbidity of malaria in pregnant women and newborns have been considerably decreased by sulfadoxine-pyrimethamine (SP) intermittent preventative therapy (IPT) (Moya-Alvarez, Abellana, & Cot, 2014).

For at least 24 hours, or until oral treatments can be given, patients with severe malaria are given artesunate intravenously or intramuscularly. This includes adults, children, newborns, pregnant women across all trimesters, and nursing mothers (WHO, 2022a). This action aims to mitigate the impact of malaria infection among these groups of individuals.

2.8 Impact of Malaria on Child Health and Mortality

Malaria significantly affects children's health and mortality, notably within the sub-Saharan African region, which bears the highest burden of malaria cases and fatalities. Based on data provided by the Centres for Disease Control and Prevention (CDC), it was projected that approximately 627,000 individuals will die due to malaria in 2020 (Patel, Bagada, & Vadia, 2024). Most of these fatalities were seen among the younger demographic residing in sub-Saharan Africa. According to the study by Lu et al. (2022), malaria is a prominent contributor to child mortality worldwide, with children under five years particularly susceptible to its adverse effects. Approximately 75% of

those affected by malaria are young, resulting in an annual death toll of roughly 500,000. Nonetheless, substantial advancements have been made in the fight against malaria since the beginning of the 21st century, resulting in a noteworthy reduction in the worldwide annual mortality rate for all age groups, which has decreased from 900,000 to 630,000 fatalities per year (Cao et al., 2022).

Malaria can cause many symptoms, including fever, chills, headache, muscle pain, and exhaustion. Malaria, in its most severe form, can lead to organ failure and death. Children who survive a malaria infection can experience long-term health problems, such as anaemia, neurological damage, and developmental delays (Kerac et al., 2014).

The effects of malaria on child health and mortality extend beyond the disease's immediate symptoms and consequences. Malaria also has a significant economic impact, as it can cause families to incur high healthcare costs and miss work, reducing their income and perpetuating a cycle of poverty (Alonso et al., 2019)

The implementation of efficient strategies for controlling and preventing malaria may have a substantial influence on child health and death rates. The World Health Organization (WHO) advocates for a comprehensive approach to managing malaria, which encompasses many effective interventions such as using insecticide-treated bed nets, indoor residual spraying, timely diagnosis and treatment, and establishing efficient surveillance and monitoring systems.

"The World Health Organization's Global Technical Strategy for Malaria 2016–2030, offers a comprehensive technical framework designed to assist and direct malaria-endemic nations in their efforts to manage and eradicate malaria". According to the World Health Organization (2015), robust malaria monitoring systems have the potential to assist governments in formulating efficient health interventions and assessing the effectiveness of their malaria control initiatives.

A noteworthy scale-up of interventions to control malaria has saved millions of lives globally and cut malaria mortality by more than 60% since 2000 (Cohen, Okumu, & Moonen, 2022). However, the COVID-19 pandemic has severely impacted essential child health services across all levels of care, interrupting the fight against malaria (Rogerson et al., 2020).

It can be concluded that malaria has a profound and detrimental influence on the well-being and survival of children, especially within the sub-Saharan African region. Children under five years are considered very susceptible, and malaria infection may lead to enduring health complications and sustain cycles of poverty. Nevertheless, implementing efficient strategies for controlling and preventing malaria, such as using insecticide-treated nets, room spraying with insecticides, timely diagnosis and treatment, and robust monitoring systems, may considerably reduce the negative impacts of the illness on the health and death of children. The Global Technical Strategy developed by the World Health Organization (WHO) is an auspicious approach for decreasing malaria, especially among iron-deficient malaria-infected individuals.

2.9 Iron Deficiency Anaemia in Ghanaian Children

Iron deficiency anaemia (IDA) is a prevalent public health concern worldwide, with a particular emphasis on poor nations. In this regard, Ghana is not exempt from the challenges posed by this condition. Below are elements that contribute to the occurrence of the phenomenon, as well as its influence on the population.

2.10 Prevalence of Iron Deficiency Anaemia in Ghana

As per the findings of the Ghana Demographic and Health Survey (GDHS) conducted in 2014, the incidence of anaemia in Ghana was 66.8%, with 42.4% classified as mild, 23.8% as moderate, and 0.6% as severe (Ewusie, Ahiadeke, Beyene, & Hamid, 2014). Children under the age of five recorded an alarmingly high rate of iron deficiency anaemia (IDA) of 50.8%, while in women of reproductive age (15-49 years), it was 34.7%. However, the prevalence of IDA varied significantly by region, with the highest prevalence of 75.9% observed in the Upper East region and the lowest in the Greater Accra region at 23.8% (Andago, 2004). In a study conducted on preschoolers in Ghana, 35.6% were found to have anaemia, 21.5% to be iron deficient, and 12.2% to have iron deficiency anaemia (Senbeta, 2021)

2.11 The Causes of Iron Deficiency Anaemia in Ghana

Various factors, including poor diet, chronic blood loss, and poor absorption of iron by the body, are implicated as causes of iron deficiency anaemia (Elstrott et al., 2020). In Ghana, these factors are particularly prevalent due to poverty, insufficient accessibility to healthcare services, and inadequate education on proper nutrition.

2.12 Poor Diet

Inadequate intake of iron-rich foods is one of Ghana's leading causes of iron deficiency anaemia. Iron-rich foods, including red meat, chicken, fish, eggs, and dark leafy greens, are often unaffordable or inaccessible for many Ghanaians. The traditional Ghanaian diet is based mainly on starchy staples, such as yams, plantains, cassava, and maize, which are low in iron. As a result, most Ghanaians, particularly those living in rural areas, do not consume enough iron-rich foods to meet their daily requirements (Wiafe, Apprey, & Annan, 2021).

2.13 Chronic Blood Loss

Chronic blood loss is another leading cause of iron deficiency anaemia in Ghana. This can occur due to various factors, including malaria, hookworm infestations, menstruation, and childbirth. Malaria is endemic in many parts of Ghana, and repeated infections can cause chronic blood loss, which, over time, can lead to iron deficiency anaemia. Hookworm infestations are common in Ghana and can lead to chronic blood loss in infected individuals (Mwangi, Mzembe, Moya, & Verhoef, 2021).

2.14 Poor Absorption of Iron

The inadequate assimilation of iron inside the human body contributes to the high occurrence of iron deficiency anaemia in Ghana. Factors affecting iron absorption include infections, such as helminths and malaria, and diets low in vitamin C. The traditional Ghanaian diet is often deficient in vitamin C, which is necessary for iron absorption. Additionally, some conventional Ghanaian foods, such as cassava, contain high levels of phytates, which can inhibit the absorption of iron (Mantadakis, Chatzimichael, & Zikidou, 2020)

2.15 Impact of Iron Deficiency Anaemia on Child Development and Health

Iron is a fundamental mineral that plays a pivotal part in the growth and physiological processes of the human body. The production of haemoglobin, a crucial protein found in red blood cells that plays a pivotal role in the transportation of oxygen from the respiratory system to various bodily organs and tissues, is of utmost importance. Iron is further implicated in synthesizing myoglobin, a protein in muscle cells that facilitates the storage and conveyance of oxygen to the muscles. Insufficient iron levels in the body hinder the production of haemoglobin and myoglobin, resulting in anaemia, which is recognized as a variable that increases the risk of malaria (Miller, 2013).

Iron deficiency anaemia can affect children of all ages, but it is most common in infants, toddlers, and adolescents. In infants, it can occur if the mother has insufficient iron stores during pregnancy or if the infant is not receiving enough iron from breast milk or formula. In toddlers and adolescents, iron deficiency anaemia can result from a diet that lacks iron-rich foods, increased iron requirements during growth, or blood loss from injury or menstruation (Chouraqui, 2022).

One of the primary impacts of iron deficiency anaemia on child development is delayed growth and development. Iron is essential for developing tissues and organs, and a lack of iron can impair growth and development in children. Children with iron deficiency anaemia may have a shorter stature, smaller head circumference, and delayed motor and cognitive development than their peers (Cusick, Georgieff, & Rao, 2018). They may

also have a reduced appetite, which can further exacerbate the problem by limiting the intake of other essential nutrients (Oliveira, Rocha, & Fernandes, 2014).

Iron deficiency anaemia can also affect cognitive development in children. Numerous studies have demonstrated that children with insufficient levels of iron have poorer cognitive function, lower IQ scores, and reduced attention and memory than non-anaemic children. (Cusick et al., 2018);More, Shivkumar, Gangane, & Shende, 2013). This is because iron is essential for the development and function of the brain, and a lack of iron can impair the formation and maintenance of neural connections. The impact of iron deficiency anaemia on cognitive development is most significant during critical periods of brain development, such as infancy and early childhood, and can be irreversible if not treated promptly (Doom & Georgieff, 2014).

Iron deficiency anaemia can also impact the immune system of children. Iron is vital for producing and functioning immune cells, and a lack of iron can impair the immune response to infections (Hassan et al., 2016). Children with iron deficient anaemia have increased vulnerability to disease and develop severe or recurrent infections. They may also have a slower recovery time from conditions, which is associated with a notable rise in both morbidity and mortality rates (Das et al., 2014). Thus, strategies must be developed to address the problem of iron deficiency within the Ghanaian population.

2.16 Strategies for Addressing Iron Deficiency Anaemia in Ghana

One strategy for reducing IDA in Ghana is iron supplementation. Iron supplements are commonly used to treat IDA and have been effective in increasing haemoglobin levels in pregnant women and children (Li et al., 2020). In Ghana, the government has implemented a program to provide iron supplements to pregnant women and has also incorporated iron supplementation into the national child health policy (Olson, Gavin-Smith, Ferraboschi, & Kraemer, 2021).

Another strategy is the use of iron fortification. Fortification involves adding iron to commonly consumed foods, such as flour, to increase the iron content of the food. The government in Ghana has authorized the fortification of wheat flour with iron. It has also encouraged fortifying other staple foods, such as maize flour and rice, with iron (Kancherla et al., 2021).

Dietary diversification is another approach to reducing IDA. A varied diet with iron-rich foods, such as meat, fish, and leafy green vegetables, can help prevent IDA (Taneja, Rai, & Yadav, 2020). In Ghana, efforts have been made to promote dietary diversification, particularly among women and children. This includes educational campaigns to increase awareness about the importance of a balanced diet and programs encouraging cultivating and consuming nutrient-rich foods (Codjoe, Okutu, & Abu, 2016).

Biofortification is another strategy that has been explored in Ghana. Biofortification involves breeding crops to increase their nutrient and iron content. In Ghana, efforts have been made to develop and promote biofortified

crops, such as maize and sweet potatoes, which effectively increase iron intake and reduce IDA prevalence (Kotu et al., 2022).

2.17 Iron Fortification and Malaria

Anaemia is often caused by malaria, which is widespread in sub-Saharan Africa. Children with iron deficiency anaemia have a high chance of experiencing anorexia, slower growth rates, and decreased cognitive and motor development (Gwamaka et al., 2012).

In regions where anaemia is prevalent, it is suggested that children under two years take iron and folic acid supplements (Haidar, 2010). The WHO recommends iron supplements for pregnant women and persons aged below five years, especially those with highly endemic malaria. But in the past ten years, there has been a heated discussion about its safety, especially where infectious diseases like malaria are high (Mwangi, Prentice, & Verhoef, 2017).

A comprehensive randomized study done in Pemba, Zanzibar, a place known for its high prevalence of malaria, revealed that administering iron supplements to young infants was associated with increased susceptibility to malaria and a higher incidence of infection-related illnesses and mortality. However, food fortification could improve iron status (Aimone, Brown, Owusu-Agyei, Zlotkin, & Cole, 2017). This conclusion is consistent with a study conducted in Tanzania where they explored the potential correlation between iron levels and susceptibility to malaria. The study used cross-sectional and longitudinal analyses in a cohort of children who received thorough health monitoring and early treatment for malaria. Despite rigorous blood smear monitoring and rapid antimalarial treatment, the research

indicated that ID was frequent and that children with iron fortification had considerably higher malaria infection rates, morbidity, and death (Gwamaka et al., 2012).

Strikingly, a recent study also reported that children with HbAS genotypes had higher anaemia rates, while those with "HbAC and AS and blood" types A and O had reduced malaria protection from iron supplementation (Tchum et al., 2023).

Juxtaposing the myriad of shreds of evidence from the various reviews on iron fortification and malaria to a study by Prentice, Verhoef, and Cerami (2013) conducted in Ghana, The iron group exhibited a significantly decreased incidence of malaria compared to the no-iron group throughout the intervention period, as shown by the "intention-to-treat analysis (risk ratio, RR = 0.87; 95% CI = 0.78-0.96). Upon controlling for baseline iron deficiency and anaemia status on a global scale (RR = 0.87; 95% CI = 0.75-1.00) and during the 5-month intervention period (RR = 0.86; 95% CI = 0.74-1.00)", the observed disparities ceased to be statistically significant in subsequent analyses. Nevertheless, there remains a lack of evidence supporting the notion that iron intake is associated with an elevated risk of malaria. Zlotkin et al. (2013) argue that this study could be due to insufficient iron content in the MNP supplement. Other studies have reported similar findings (Prentice et al., 2013). To further substantiate the findings from Prentice and colleagues, Tchum et al. (2021) found out that when proper anti-malarial treatment and insecticide-treated bed nets were made available, long-term consumption of iron-fortified MNP among babies and young children residing in highly endemic malaria burden regions did not increase the prevalence of malaria. A

meta-analysis by Ojukwu, Okebe, Yahav, and Paul (2009) also firmly concluded that there was no increased mortality among children in malaria-endemic areas who were on iron supplements. The divergent perspectives about the relationship between iron fortification's influence on immune response and malaria have engendered ongoing scholarly debate.

2.18 Immune Response against Malaria

Infection with malaria triggers host reactions that are controlled by the innate and adaptive immune systems. The innate, cellular, and humoral activities all work together in mounting immunity against the malaria parasite (Uchechukwu, Priscella, Egondu, & Florence, 2017)

2.18.1 Innate Immunity

The initial line of defence against malaria is innate immunity, which detects and starts the body's defence against invading malaria parasites. Some immune cells that possess receptors for recognizing patterns (PRRs) such as dendritic cells and macrophages, may recognize conserved PAMPs on the surface of parasites. Important PRRs implicated in the identification of *Plasmodium*-associated pathways and the activation of the immune response are Toll-like receptors (TLRs) and NOD-like receptors (NLRs) (D. Li & Wu, 2021). According to Ortiz and Rodal (2023), when PAMPs are detected, phagocytic cells, such as macrophages, engage in a process known as phagocytosis to engulf the parasites. TNF-alpha, IL-6, and IL-12 are just a few of the pro-inflammatory cytokines released due to this engulfment, causing inflammation and causing other immune cells to become active, which helps mount defence against the malaria parasite. The complement system, a collection of soluble proteins, further aids the innate immune response to

malaria. When the complement system is activated, a series of complement proteins are released that either directly destroy the invading parasites or improve phagocytosis.

IFN-alpha and IFN-gamma are two interferons essential for controlling Plasmodium parasite multiplication and spread. These cytokines cause the adjacent cells to assume an antiviral state, rendering them immune to parasite invasion and slowing the infection's development (Uchechukwu et al., 2017). New research reveals that innate lymphoid cells (ILCs) may also play a role in the innate defence against malaria (Ivanova et al., 2019).

2.18.2 Humoral Immunity against Malaria

Humoral immunity, which concentrates on the extracellular phases of the parasite's life cycle, is essential in the fight against malaria.

After contact with Plasmodium parasites, B cells in the host's immune system activate and differentiate into plasma cells, secreting antibodies. These antibodies predominantly target sporozoites, merozoites, and gametocyte surface antigens at different parasite stages. Notably, given their significance in humoral immunity, antibodies against the merozoite surface proteins (MSPs) and circumsporozoite protein (CSP) have received substantial attention in terms of research (Akkaya, Kwak, & Pierce, 2020).

By neutralizing sporozoites in the skin, impeding their movement to the liver, and preventing their invasion of hepatocytes, some antibodies can stop the initial development of malaria infection (Cockburn & Seder, 2018). Additionally, antibodies that target merozoite surface proteins can hinder red blood cell invasion, preventing parasite multiplication and illness

development. IgG has been reported as the significant antibody playing these roles (Woehlbier, Epp, Hackett, Blackman, & Bujard, 2010). Studies have demonstrated that the frequency of exposure, the variety of parasite strains, and host genetic traits may all affect the length and effectiveness of antibody responses against malaria antigens (Dobaño et al., 2019; Elliott et al., 2014). It is essential to comprehend the durability of antibody responses for vaccine development and determining the likelihood of developing natural immunity.

The development of vaccines using humoral immunity is a potential method for reducing and eventually eliminating malaria. In preclinical and clinical studies, some potential vaccines targeting important antigens, including CSP and MSPs, have yielded encouraging results (Carvalho, Daniel-Ribeiro, & Goto, 2002; Wilder et al., 2022). Addressing antigen diversity and producing long-lasting antibody responses is necessary to develop an effective malaria vaccine.

2.18.3 Cellular Immunity against Malaria

Controlling the intracellular phases of malaria infection depends heavily on cellular immunity, particularly T-cell responses. To eliminate parasites and create immunological memory, cytokine signaling coordinates T cells' activation, differentiation, and control. Cellular immunity against malaria is greatly aided by T cells, especially CD4+ and CD8+ T cells. By producing cytokines, CD4+ T cells aid in directing the immune response, whereas CD8+ T cells are responsible for eradicating infected host cells. Antigen-presenting cells (APCs), such as dendritic cells, are essential for activating and developing these T lymphocytes (Gasteiger, Ataide, & Kastenmüller, 2016; Son & Sun, 2021).

According to Walther et al. (2006), the interaction of cytokines has a critical role in determining the type and magnitude of cellular immune responses in malaria. By boosting phagocytosis and activating macrophages, pro-inflammatory cytokines, including interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha), improve parasite clearance (Nasr, Allam, Hamid, & Al-Ghamdi, 2014). On the other hand, regulatory cytokines, such as interleukin-10 (IL-10), on the other hand, aid in regulating excessive inflammation and averting further infections (Neumann, Scheffold, & Rutz, 2019).

Th1, Th2, Th17, and Treg cells are a few examples of diverse subsets of CD4+ T cells, each having a distinctive effector function. Th1 cells are essential to eradicate intracellular parasites, while Th2 cells stimulate B cell responses that produce antibodies. Treg cells assist in regulating immune responses and averting immunopathology, while Th17 cells aid in attracting immune cells to the site of infection (Golubovskaya & Wu, 2016; Zhou, Chong, & Littman, 2009).

Malaria-induced cellular immunity can result in the development of immunological memory, in which T cells are primed and prepared to react more quickly and forcefully when exposed to the same parasite or antigen in the future. For the creation of vaccines and long-lasting protection, it is essential to comprehend the formation and maintenance of immunological memory (Coelho, Doritchamou, Zaidi, & Duffy, 2017; Crompton, Pierce, & Miller, 2010). Cellular immunity is a crucial target for creating vaccines because it prevents malaria. Subunit vaccines, viral vectors, and entire sporozoite-based methods are only a few vaccine possibilities that attempt to

stimulate T-cell responses. However, optimizing T cell immunogenicity and dealing with antigenic diversity continue to be difficult tasks (Bettencourt, 2020).

2.19 Human Immune Response to *P. falciparum*

Studies have established that the host has a very intricate and stage-specific immune response against *P. falciparum.* The immune response during the erythrocytic stage is characterized by a greater degree of immune assault than the pre-erythrocytic stage. Specifically, CD8+ T cells and antibodies play crucial roles in the immune response throughout the pre-erythrocytic and erythrocytic phases. The skin, which is the first point of contact, also has unique responses against the parasite (Duffy, Sahu, Akue, Milman, & Anderson, 2012).

2.19.1 The Skin

After sporozoite deposition in the dermis, the first phases are essential for developing malaria infection and activating defence mechanisms (Sinnis & Zavala, 2012).

The skin is the first crucial physical barrier and initial line of defence against many infections, and sporozoites remain in the epidermis for many hours after injection. The inhibitory effect of neutralising antibodies present in the skin on the movement of sporozoites within the dermis has been observed (Rénia & Goh, 2016). According to studies, only half of the sporozoites inoculated into the skin reach the hepatic phase (Mac-Daniel et al., 2014; Menard et al., 2013). Thus, this stage has been recommended to be vital in vaccine development (Sinnis & Zavala, 2012).

2.19.2 Hepatic Phase Immune Response

Hepatic infection and the immunological response are directed at both free sporozoites and hepatocytes that have been infected. The invasion of liver cells might be hindered by neutralizing the essential proteins for traversal and invasion of cells. This can be achieved using antibodies targeting free sporozoites and the circumsporozoite protein (CSP). While studies have reported that IgG is the primary antibody playing this neutralizing role, a survey conducted in 2019 in Australia said that IgM has a similar neutralizing potential (Boyle et al., 2019). Furthermore, antibodies are responsible for triggering the processes of phagocytosis, complement fixation, and cytotoxic lysis by NK and NKT cells. Alternatively, Kupffer and natural killer cells use an antibody-dependent cell-mediated mechanism to eliminate parasites. This process is initiated by the recognition of parasite antigens present on the outer membrane of infected hepatic cells (Burrack, Hart, & Hamilton, 2019).

Intrahepatic parasites are killed mainly by CD8+ T lymphocytes that produce interferon-gamma. Other cells, such as NK, NKT, and T cells, also eliminate intrahepatic parasites by releasing type I interferons and IFN gamma (Dinko & Pradel, 2016). This finding corroborates Cockburn et al. (2013) established that liver parasites are immediately killed by nitric oxide, produced when IFN-gamma activates the inducible nitric oxide enzyme.

Without Toll-like receptors, *P. falciparum* malaria parasites employ MDA5(melanoma differentiated associated gene 5) and MAVS (mitochondrial anti-viral signaling protein) to activate type I IFNs in innate immune cells contrary to viruses and bacteria. A class I IFN response was recently induced by the recognition of an exo-erythrocyte-form (EEF) RNA by MDA5 in

hepatocytes. Hepcidin, a hormone that regulates host iron, has been shown to inhibit sporozoite proliferation via unidentified processes (Zheng, Tan, & Xu, 2014).

According to Holz, Fernandez-Ruiz, and Heath (2016), the ability of immune cells to kill infected liver cells and prevent erythrocyte invasion could be harnessed in developing a potential vaccine.

2.19.3 Blood Stage Immune Response

The immune response against the blood-stage parasite is more sophisticated than that against the liver forms. Merozoite-specific antibody cell-dependent inhibition (ADCI) makes it easier for phagocytic cells to eliminate infected RBCs. They may also agglutinate released merozoites and increase merozoite phagocytosis. To destroy intra-erythrocytic parasites (schizonts), ADCI includes anti-merozoite cryophilic antibodies such as IgG1 or IgG3 attaching to merozoites and inducing phagocytes to produce cytokines like TNF-alpha, which results in merozoite killing (Burrack et al., 2019; Liehl et al., 2015).

It has been established that antibodies targeting parasite toxins such as malaria pigment, glycophosphatidylinositol (GPI), TatD-like DNase, and tyrosine-tRNA synthase could protect against sickness. Experimental proof of protection against *P. falciparum* was provided by Rénia and Goh (2016) using synthetic glycans that imitated GPI.

The generation of proinflammatory cytokines by CD4+ T helper cells, which stimulate macrophages, is also crucial in eliciting immune response. They also facilitate the activation of B cell clones. It was formerly believed

that CD8 T lymphocytes had very little part in immunity in the blood stage. However, recent research has shown that these cells may prevent blood-stage infection. Particularly crucial for avoiding persistent blood-stage infection in mice are IFN-secreting CD8 T cells (Rénia & Goh, 2016).

The immune response also involves other cells, such as NK and $\gamma\delta$T cells. NK cells generate the IFN-gamma, perforin, and granzyme necessary to destroy *P. falciparum*-infected RBCs (Chen et al., 2014).

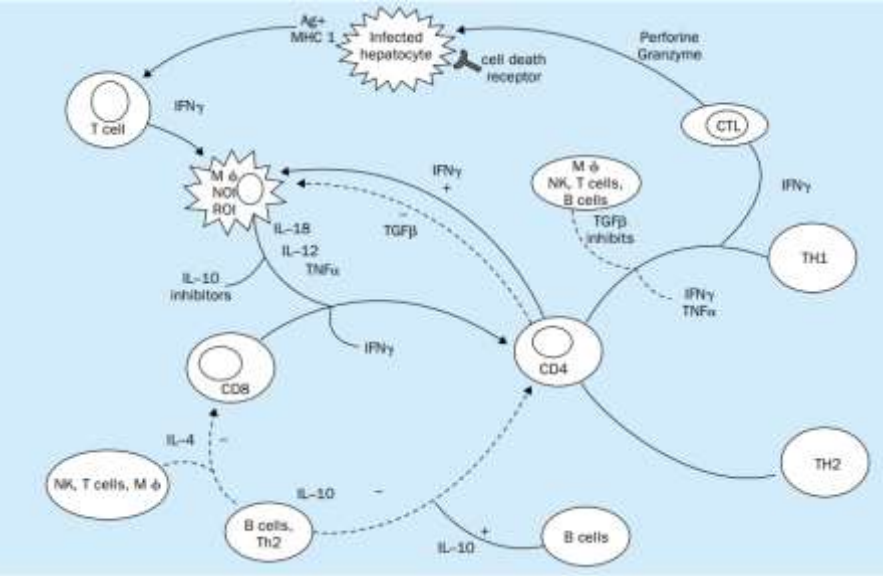

Figure 2: Blood Stage Immune Response

Source: Adopted from Chen et al, 2014

"Sporozoite antigens are presented to MHC I and displayed on the surface of infected hepatocytes. When this occurs, NK and CD4+ T cells are stimulated to produce interferon (IFN), or CTLs recognize it and destroy the infected cell. Naive CD4+ T cells differentiate into Th1 cells, which is crucial for eliminating the parasite. Cytokines are the primary activators of Th1 and Th2 subset development. Inhibitors of the synthesis of proinflammatory

cytokines, such as IL-4, TGF-β, and indirectly IL-10, may block the development of the Th1 response".

2.19.4 Immunity against Gametocytes

Gametocyte sequestration and maturation are prevented by antibodies, which kill gametocytes by complement-mediated lysis. The acquisition of antibodies by female *Anopheles* mosquitoes whilst feeding on the host's blood has been previously documented. This phenomenon leads to the activation of the complement system, which subsequently induces the death of gametocytes and hinders the fusion of gametes within the mosquitoes. Activation of macrophages leading to the killing of gametocytes has been linked to nitric oxide (Dennison, BenMarzouk-Hidalgo, & Dimopoulos, 2015). Despite the adoption of several mechanisms by the host, the malaria parasite continues to devise strategies to evade the host's immunological response.

2.20 Immune Evasion

Over the years, it has been established that parasites have evolved various escape mechanisms to prevent host immunity, and *P. falciparum* is no exception. *P. falciparum* shows immune evasion in both human hosts and the vector.

2.20.1 Hepatic Stage Immune Evasion

In order to transition into the erythrocytic stage, it is imperative for both free sporozoites and intrahepatic parasites to successfully traverse Kupffer cells (KCs) and endothelial cells (ECs) (Gomes, Bhardwaj, Rivera-Correa, Freire-De-Lima, & Morrot, 2016). CSP attachment to the surface proteins of KC serves as a mechanism to overcome the barrier posed by these

cells. This interaction leads to the generation of substantial quantities of intracellular cyclic adenosine monophosphate (cAMP) and exchange protein directly activated by cAMP (EPAC), which in turn inhibits the production of reactive oxygen species (ROS). Furthermore, it has been observed that this particular interaction can enhance the production of anti-inflammatory Th2 cytokines while simultaneously reducing the levels of pro-inflammatory Th1 cytokines (Ikarashi et al., 2013). In rare circumstances, the binding of sporozoites also causes KC apoptosis and decreases the production of MHC-I. Sporozoites can control KC processes, including lysosomal degradation prevention and host inflammatory response control (Gomes et al., 2016).

In addition to encouraging the growth of intrahepatic parasites, host heme oxygenase-1 (HO-1) regulates the host's inflammatory response. Additionally, sporozoites obstruct the mTOR pathway, contributing to intrahepatic parasite growth (Zheng et al., 2014).

2.20.2 Blood Stage Immune Evasion

The evasion mechanism employed by the malaria parasite to avoid detection and elimination by the human immune system relies on the existence of a diverse array of parasite proteins that are found on the surfaces of merozoites and infected red blood cells (iRBCs). These proteins exhibit significant genetic variability, which contributes to the capacity of the parasite to effectively escape immune attack. Polymorphic proteins can augment evasion mechanisms, thereby facilitating the establishment of chronic asymptomatic infections (Dinko & Pradel, 2016). The lack of major histocompatibility complex class I (MHC-I) expression on the surface of red blood cells (RBCs) aids the parasite's evasion of detection by CD8+ T cells.

Moreover, it has been observed that *P. falciparum* rosettes possess the ability to adhere to specific epitopes on red blood cells (RBCs) and thus evade immune responses (Gomes et al., 2016; Stanisic, Barry, & Good, 2013).

Expressing different antigenic surface proteins on infected red blood cells permits *P. falciparum* to elude the host immune response (Gomes et al., 2016). Several studies have shown that PfEMP1 is one of the most polymorphic proteins, encoded by approximately 60 copies of different genes (Claessens et al., 2014; Dinko & Pradel, 2016). According to Clayton, Dong, and Dimopoulos (2014), it contains multiple variable domains that establish its interaction with distinct ligands on endothelial cells. A unique var gene, var2csa, has been proven to enhance the cytoadherence of iRBC to syncytiotrophoblasts of the placenta, facilitating the establishment of infection (Dennison et al., 2015). It is common knowledge that the difficulty in developing an effective vaccine against *P. falciparum* is mainly due to its high antigenic variations.

Sequestration, mediated by the multigene families PfEMP-1, RIFIN, and STEVOR, is the second immune evasion strategy at this stage. These permit iRBC adhesion to vascular endothelium, preventing the splenic clearance of the parasite (Stanisic et al., 2013). For sequestration, endothelium receptors, including EPCR, CSA, CD36, and ICAMs, play significant roles. For Gomes et al. (2016), immune evasion by sequestration and the development of cerebral malaria, rosette formation, and adherence are essential. Though the mechanism is still unclear, study reports show that IgM is also involved in resetting, leading to sequestration and preventing splenic destruction (Belachew, 2018; Gomes et al., 2016).

The role of malaria pigment in erythrocytic immune evasion cannot be left out. Hemozoin from *P. falciparum* also impairs macrophages' ability to perform phagocytic tasks. Hemozoin-containing macrophages cannot phagocytose more iRBC, which lowers the formation of ROS. Interestingly, checkpoint inhibitor molecules are also activated by *P. falciparum* infection (Dennison et al., 2015).

Most *Plasmodium falciparum* parasites use sialic acid (SA) independent mechanisms to elude the immune response. They can avoid the host immune response by creating structures out of sialic acid that are nearly identical to those produced by the host (Ord et al., 2012). In addition, Stanisic et al. (2013) reported that *P. falciparum* can switch the immune response from one antigen to another.

2.20.3 Merozoites' Immune Evasion Mechanisms

Antigenic merozoite surface proteins (MSPs), PfAMA1, PfEBA, and PfRHs are employed as evasion strategies. Due to their high polymorphism, MSPs are essential for evasion because they block antibody activity. Evasion depends on the expression of RIFINs, STEVORs, and SURFINs. Free merozoites bind to factor H and factor H-like protein 1 to inhibit C3b and prevent lysis (Dennison et al., 2015; Dinko & Pradel, 2016).

Since merozoite invasion is a necessary component of the parasite life cycle, blocking it has become a desirable vaccine target. However, immunological evasion mechanisms used by merozoites during their invasion of red blood cells would have to be overcome to ensure the vaccine's effectiveness. Irrespective of the strategies used by parasites to circumvent the

immune response of their host, it has been shown that antibodies, such as IgG, play a crucial role in effectively eliminating parasites (Carithers, 2017).

2.21 Iron Fortification, IgG, and Malaria

The global recommendation of iron supplements for children living in malaria-endemic regions by the WHO continues to create scholarly debate among researchers, especially on its efficacy and level of protection. Another question has evolved: Could iron supplements affect the immune response among malaria patients? Interestingly, little research has been done in these areas. Zlotkin and colleagues conducted a trial test among preschool children in Ghana to answer this question. They concluded that iron fortification did not significantly affect the immune response (Zlotkin et al., 2013). What remains unanswered is the potential effect of iron supplements on IgG responses against malaria vaccine candidate antigens. What remains to be elucidated is the yet unexplored influence of iron fortification on the concentrations of IgG antibodies targeting malaria vaccine candidate antigens in children residing in regions with high malaria prevalence.

2.22 Malaria Candidate Antigens and IgG Response

The host's ability to fight off disease depends on antibodies. They bind to and destroy microbes, aid in eradicating bacteria, viruses, fungi, and parasites, and produce immune complexes that enhance antigen presentation, hasten parasite sequestration and absorption, clear toxins, eliminate infected cells, and reduce inflammation. Numerous effector activities of antibodies are dynamically controlled by a differential antibody constant domain mutation (Guzman, Alvarez, & Halstead, 2013). Several potential vaccine-candidate antigen genes have been identified and characterized. These antigens are

collectively referred to as malaria vaccine candidate antigens due to the exponential effects of antibodies, especially IgG, against them, resulting in parasite clearance. These malaria vaccine-candidate antigens are present in the three major phases of the *P. falciparum* life cycle (Chan et al., 2014).

The glurp gene, which produces the immunodominant glutamate-rich protein (GLURP), is one of these malaria-candidate antigens. An amino acid for GLURP was discovered using nucleotide sequencing, including two blocks of repetitive sequences (R1 and R2) and an amino-terminal nonrepetitive region (R0). The hydrophobic amino- and carboxy-terminal portions of the anticipated polypeptide, symbolic of secreted proteins, are compatible with the discovery of GLURP in *P. falciparum* (Pratt-Riccio et al., 2013). The asexual, hepatic, and gametocyte stages of the parasite were found to react with anti-GLURP antibodies, indicating that GLURP is produced throughout the whole life cycle of *P. falciparum* in the vertebrate host. According to investigations carried out in Liberian and Gambian adults, antibody responses against recombinant GLURP fragments have a high incidence. In contrast, children aged five to nine showed a negative association between the immunoglobulin G (IgG) response against GLURP and parasite density, whereas children aged two to four did not. While shortened recombinant GLURP fragment was discovered to exhibit the ability to stimulate lymphocytes derived from individuals with a history of malaria exposure, IgG antibodies to GLURP were related to lower morbidity in children 5-8 years old (Funwei Roland, Olusola Fiyinfoluwa, Orimadegun Adebola, Badejo Joseph, & Michael Obaro, 2023; Homoet, 2020).

Although many merozoite surface proteins (MSPs) have been discovered, it is still unclear what most of them do. A hypothesized mechanism involves the attachment of merozoites to red blood cells (RBCs) and the subsequent involvement of metabolic processes in the invasion, specifically related to the MSP-1 protein (Chandramohanadas et al., 2014). Cowman and colleagues established that in the late schizont stage, the protein is synthesized as a 185-210 kDa precursor, then processed to produce several polypeptides of different molecular weights. A 19kDa polypeptide, the sole one that enters the host cell, is created by further processing a 42 kDa polypeptide (MSP-1) maintained connected to the merozoite membrane (Cowman, Tonkin, Tham, & Duraisingh, 2017).

MSP-1 has received the greatest attention as a potential blood-stage malaria vaccine candidate antigen. MSP-1 19 and 42 were the main targets of several investigations that demonstrated the ability of antibodies against MSP-1 to prevent plasmodial proliferation and growth. Antibodies against MSP-3 have also been shown to provide an excellent defence. MSP-2 has gone through phase I clinical trials in both malaria-exposed and malaria-unexposed persons and has been extensively described (Versiani, Almeida, Mariuba, Orlandi, & Nogueira, 2013).

A recent study conducted a thorough examination of longitudinal trials and found a correlation between the presence of “IgG antibodies against potential vaccine candidate antigens, including merozoite surface protein 1 (MSP119), MSP3, and apical membrane antigen 1 (AMA-1), and the level of protection against clinical malaria” (Dobbs & Dent, 2016; Folegatti et al., 2017).

2.23 Malaria Vaccine Development

Plasmodium parasites still pose a serious threat to world health in areas where the illness is prevalent. Given the complicated life cycle of the parasite and its capacity to evade the host's immunity, producing a potent malaria vaccine remains a difficult task. Initial efforts to produce malaria mostly concentrated on whole-parasite vaccines employing weakened or dead parasites (Plowe, 2012). Although several of these vaccines demonstrated possibilities of offering some protection, their low effectiveness and safety concerns prompted the investigation of alternate strategies (Crompton et al., 2010; Vaughan & Kappe, 2012).

The circumsporozoite protein (CSP) derived from the *Plasmodium falciparum* parasite has been utilized as the fundamental component in the development of the subunit vaccine known as RTS, S. This vaccine has achieved the significant milestone of obtaining regulatory clearance, marking it as the first-ever approved vaccine for malaria (Parums, 2022). The vaccine showed modest effectiveness when administered with the AS01 adjuvant in preventing clinical malaria in infants and toddlers, but its efficacy declined with time and differed among various ages and geographic areas (Kaslow & Biernaux, 2015).

Molecular biology developments and genome studies have discovered novel vaccine-candidate antigens possible. A few of the potential candidate antigens being explored in preclinical and clinical studies are merozoite surface proteins (MSPs), apical membrane antigen 1 (AMA1), and the sexual-stage antigen Pfs25 (Draper et al., 2015). Combinations of various adjuvants and candidate antigens are being investigated to improve malaria vaccine

effectiveness. Whole sporozoite-based vaccines' preclinical trials have shown much promise. From animal studies and preliminary human investigations, genetically attenuated parasites, radiation-attenuated sporozoites, and "viral vectored vaccines" expressing sporozoite antigens all showed significant protective effectiveness (Wilson, Flanagan, Prakash, & Plebanski, 2019).

The complicated developmental process of malaria, genetic diversity among *Plasmodium* species, antigenic variety, and the parasite's capacity to avoid host immune response provide substantial obstacles to developing effective malaria vaccines. To improve vaccine immunogenicity and effectiveness, novel immunization techniques are being investigated, including prime-boost regimens and heterologous vaccination. Viral vectors and nanoparticles are two new delivery systems promising to produce robust immune responses (Ulmer & Geall, 2016). Another area that researchers are exploring is the use of transmission blockers that target the parasite's life cycle, thus reducing the transmission rate in the communities. The most advanced candidate for a transmission-blocking vaccine (TBV) currently under development is Pfs25 (Nikolaeva, Draper, & Biswas, 2015).

2.24 Chapter Summary

Malaria continues to burden countries within sub-Saharan Africa. Of the five plasmodium species, *P. vivax* and *P. falciparum* are the most predominant with P. falciparum being the most dangerous in Africa. Iron deficiency due to lack of vitamin C and malnutrition have proven to have a detrimental impact on individuals in malaria-endemic regions. Immune responses against the malaria parasite could be humoral or cellular. Various malaria vaccine candidate antigens are undergoing clinical trials toward the

development of an efficacious and safe vaccine against malaria. The development of an effective and safe malaria vaccine is hindered by various challenges, including antigenic variations exhibited by the malaria parasite and its ability to evade the immune responses of the host. Despite these challenges, the RST/AS01 vaccine has been approved by WHO for children below two years.

CHAPTER THREE

METHODOLOGY

3.0 Introduction

This study employed archival samples between April and May 2010 from a double-blinded cluster-randomized control trial by Zlotkin et al. (2013). The study was conducted in the Wenchi and Tain districts of the Bono Region, which has a high malaria transmission rate. Eligible children aged 6 to 35 months were randomly assigned to either the iron micronutrient powder (MNP) group or the MNP with no iron group for five months and monitored for one month after the iron fortification period. Each child received a net sprayed with insecticide. Blood samples were collected from children with fever or admitted to a healthcare facility to determine malaria status using RDT and microscopy. A data and safety monitoring board convened three times throughout the experiment to discuss progress. Thus, this study sought to evaluate the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children using indirect enzyme-linked immunosorbent assay (ELISA). The mode of sample randomization, laboratory work, data analysis, data management, and recombinant antigens are explained in detail in this chapter.

3.1 Study Design

This study was a double-blinded cluster-randomized control trial which employed archival plasma samples.

3.2 Study Area

The research was conducted in Wenchi and Tain, two contiguous districts in the Bono Region with a high malaria transmission rate (Addy-Tayie, 2019). According to the Ghana Health and Demographic Survey

(Stevens et al., 2013), 34% of Bono's 6- to 59-month-old infants were affected by severe-to-moderate anaemia in 2014. Participants in the experiment were infants and young children who could consume semi-solid foods and were identified at home (with or without breast milk). All participants were administered multiple micronutrient powders (MNP) daily for five months. The MNP was provided with or without adding iron (12.5 mg) to the supplementary meals. The research excluded children who had severe anaemia (haemoglobin level of 70.0 g/L), severe malnutrition (weight-for-length z-score of -3.0), had iron supplementation during the last six months, or had a chronic disease. The research was conducted during a period of increased rainfall, which coincided with the peak of malaria transmission.

3.3 Sample Size Determination

The sample size was calculated using the Yamane formula (Yamane, 1967).

N=1958 (Zlotkin et al., 2013)

"n=$N/1+N(e)^2$ where n is the sample size and 'e' is the margin error (0.05)"

n=1958/1+1958(0.05)^2

= 332

n (minimum number of participants) = 332

Even though a minimum number of 332 samples was required for this study, a total of 400 archival plasma samples were sorted for the study. This comprised 200 iron-fortified participants and another 200 non-iron-fortified children's plasma samples. Out of the 200 for both groups, half of the samples (100) were malaria-positive, and the other half were negative for malaria.

3.4 Participant Recruitment

Children between the ages of 6 and 35 months who met the eligibility criteria were recruited and then allocated at random in a 1:1 ratio to one of two groups: the iron MNP group or the MNP group without iron. This process took place between March and April 2010. Participants were clustered and randomized to prevent any cross-contamination resulting from meal sharing using a computer-generated model. (Senga, Harper, Koshy, Kazembe, & Brabin, 2011). A cluster comprises two or more families with at least one enrolled child in the same compound.

Participants were randomly assigned at the cluster level because they shared the same rural community residence with multiple related families. Participants' movements, inside and outside the study area, may be tracked more easily with this randomization technique. Each child who participated received a net sprayed with an insecticide (ITN). The caretakers and the research team were unaware of which sachets contained iron and which did not, as the only difference was a small 'A' or 'B' label. Microencapsulated ferrous fumarate (12.5 mg), ascorbic acid (30 mg), zinc (5 mg), and vitamin A (400 g) were all included in the iron-MNP dose (5 mg)(National Academies of Sciences & Medicine, 2017; Zlotkin et al., 2013). The group also received an MNP but without any iron. For five months, kids in the iron group ate semi-solid food with one sachet of MNP (equal to 12.5 mg iron) daily.

A field research assistant conducted weekly home visits to take morbidity data, and axillary temperature, evaluate compliance with ITN use and MNP intervention, and restock supplies. Unused and empty packets were saved by caregivers and tallied during check-ins. Multiplying the number of

packets by a factor of 100 and dividing them by the predicted amount utilized yielded the adherence rate. After the MNP was discontinued, the children were followed for another month.

3.4.1 Sample Sorting for ELISA: Randomization

Clinical demographic data such as age, sex, pathological number, the period sample was taken, haemoglobin level, axillary temperature >37.5°C, or reported fever within the past 48 hours, and parasite density count for positive samples were obtained from the laboratory information management system linked to patients' archived plasma samples of the study participants.

Archived samples that met the selection criteria were randomly selected to avoid selection bias. Randomization was performed using Microsoft Excel. The total number of archived plasma samples was 1958. Initially, only 872 samples that satisfied the selection criteria were selected, whereas 1086 samples did not meet the criteria and were therefore excluded. For the 872 samples that met the requirements, a new Excel column was created, and data was randomized using Excel. The data was then sorted from the smallest to the largest, and the first 400 samples for iron-fortified and non-fortified were selected using their pathological numbers. The initial samples of 400 from both groups were subsequently subjected to randomization and sorted using the same concept. This resulted in 200 samples from the fortified and non-iron-fortified groups along with their corresponding pathological numbers. These randomly generated pathological numbers were used to select 200 plasma samples from the previously chosen 400 samples for both iron and non-iron groups. After this stage, samples in each group were sorted into malaria negatives and positives (100 negatives and 100 positives). Positives

were further categorized into complicated (parasitemia ≥100,000) and uncomplicated (parasitemia ≤100,000) groups. This process aided the selection of plasma samples for the ELISA.

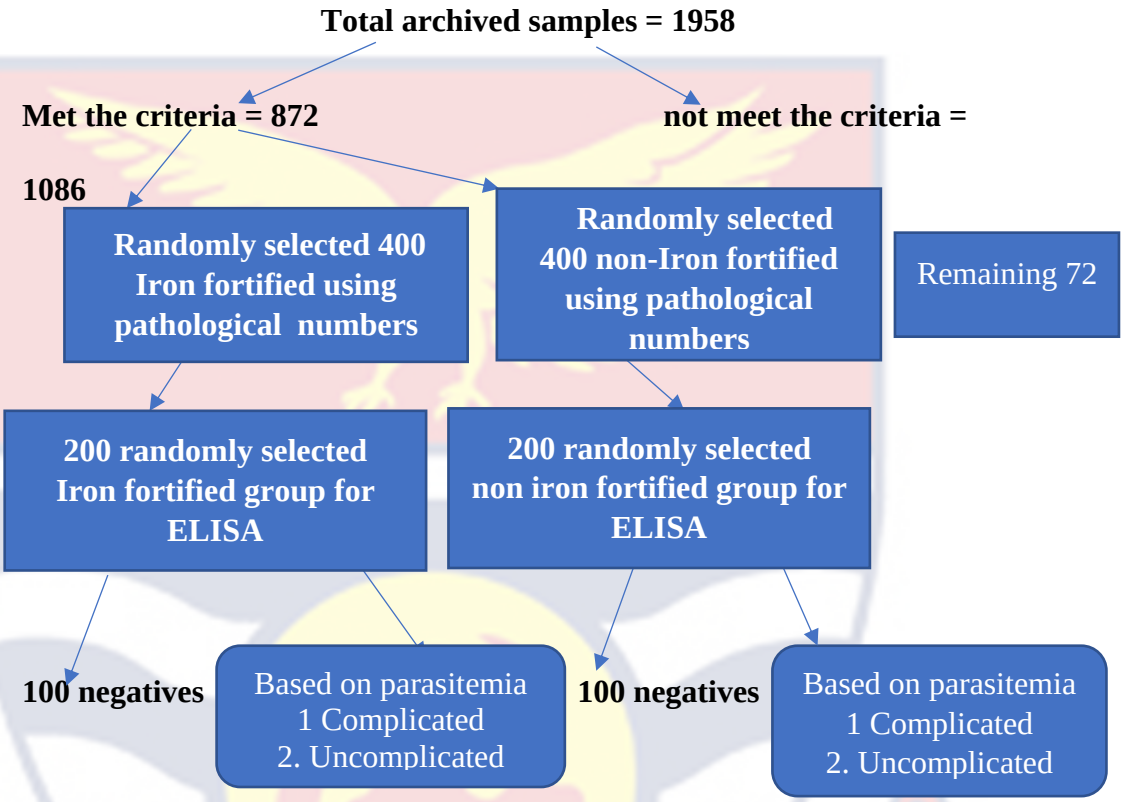

Figure 3: Diagrammatic Representation of Data Sorting for Plasma Sample Selection

3.5 Data Collection Instruments

This study employed structured questionnaire to obtain data from the study participants. Participants who attended a health facility were tracked using individual study identity cards that were given to guardians during enrollment.

3.6 Data Collection Procedure

Participants' visits to a healthcare facility were recorded using study identification cards (issued to caregivers at enrolment). Blood samples were collected from a cohort of children who presented with symptoms of fever

(axillary temperature >37.5°C or recent fever within 48 hours) or those admitted to a healthcare facility. These samples were collected to determine malaria status and subsequent treatment. The diagnostic test employed for this purpose was the Paracheck Pf Device, developed by Orchid Biomedical Systems. Additionally, microscopy was utilized to ascertain parasite speciation and count. The study included monitoring children for 14 days, during which blood samples were collected on days 7 and 14 to determine their malaria status and assess the effectiveness of therapy. This was achieved by the use of both fast diagnostic tests and microscopy. The blood smears were processed by the KHRC (Kintampo Health and Research Centre) laboratory. The thick and thin films were immersed in methanol and then subjected to staining using Giemsa. Each smear was evaluated by two distinct microscopists. Children with confirmed malaria, as determined by a quick diagnostic test, were given first-line antimalarial treatment consisting of artesunate and amodiaquine or artemether and lumefantrine.

3.6.1 Sample Collection for ELISA

A total of 450 µL of finger or heel pricked blood was collected from study participants 6-35 months old in an EDTA tube. The blood sample was centrifuged at 2000 revolutions per minute for 15 minutes, and the plasma was collected using a 5 ml Pasteur pipette into cryovial tubes and stored in a -20°C refrigerator for ELISA.

3.6.2 Measurement of Antibody Responses against Malaria Vaccine Antigens

“The measurement of IgG responses to GLURP R0, GLURP R2, and MSP-3 recombinant antigens was conducted through the utilization of indirect

enzyme-linked immunosorbent assays (ELISA). These assays were performed with slight adjustments per the guidelines provided by the manufacturer. The antigens were directly coated into individual wells of a 96-well microtiter ELISA plate (Maxisorp Nunc, Denmark). Coating buffer, specifically plain PBS with a pH of 7.04, was used for this purpose. The recombinants were coated at a concentration of 1.0 µg/mL, while the peptides were coated at a concentration of 5.0 µg/mL. The plates, which had been coated were subjected to an overnight incubation. This incubation took place within the controlled temperature range of 2 to 8 degrees Celsius, utilizing a refrigerator. The plates underwent a thorough four-cycle washing process, with a specialized washing buffer. This buffer, known as phosphate-buffered saline (PBS), was supplemented with 0.1% Tween-20, a nonionic detergent, and 0.5 M sodium chloride (NaCl). The plates underwent a drying process on tissue paper through a padding technique. Subsequently, they were subjected to blocking by the addition of 200 µL of a blocking solution composed of PBS with 5% skimmed milk and 0.1% Tween-20. This blocking step was carried out for one hour at ambient temperature. The plates again underwent an additional four cycles of washing followed by the drying process. The experimental procedure involved the addition of plasma samples, each measuring 100 µL, into individual wells. These samples were diluted at a ratio of 1:200 using a serum dilution buffer. The serum dilution buffer consisted of phosphate-buffered saline (PBS) supplemented with 2.5% milk powder, 0.1% Tween-20, and 0.02% sodium azide".

The plates containing the samples were incubated for another 2 hours at room temperature and washed four times. Then, 100 µL of human IgG was

added and incubated for one hour. After one hour, the plates were washed. The bound antibody was quantified by green colour formation with 100 µL per well of TMB Peroxidase Substrate System Solution A + solution B at a ratio of 1:1 and incubated in the dark for 30 minutes. Sulfuric acid (0.2M) (100 µL/well) was used to stop the reaction with a yellow colour indication. The experimental setup involved the utilization of a microplate absorbance reader to quantify the optical density (OD) at two specific wavelengths, namely 450 nm and 530 nm. To ensure accuracy and consistency, a reference wavelength of 620 nm was employed during the measurements. The conversion of optical densities to arbitrary units was performed using ADAMSEL version 1.1.

3.6.3 Recombinant Antigens

The various malaria vaccine candidate antigens used for this study are GLURP R0, GLURP R2, and MSP-3. While the GLURP R0 variant consists of the non-repetitive sequence conserved N- terminal domain, GLURP R2 contains the repeat carboxyl-terminal domain. However, they are both observed in *Escherichia coli*. In contrast, MSP-3 is the FVO strain expressed in *E. coli* (Opoku-Mensah, 2014). It is important to note that these recombinant candidate antigens are malaria blood-stage antigens (Hamre et al., 2020; Masson, Thibaudon, Bélec, & Crépeaux, 2017). These antigens play major roles in antibody-dependent cellular inhibition (ADCI) (Theisen et al., 2000).

3.6.4 Monitoring and Evaluation

A data and safety monitoring board convened three times throughout the experiment to discuss progress. After the initial enrolment period and the halfway point of the intervention was over, the results were collected and

summarized. Everyone involved in the trial agreed beforehand to call it quitting if the iron group experienced a statistically and clinically significant higher incidence of malaria or surrogate measures of clinical severity (such as deaths and hospitalizations) compared to the no-iron group.

3.7 Data Processing and Analysis

3.7.1 Data Processing

Data obtained from the study were first entered into Excel Software (XLSTAT Version 16). The data was cleaned to avoid issues of missing data and mismatch by carefully entering data obtained to match each variable. After that, data was password protected and later imported to SPSS (IBM Version 23) for data analysis.

3.7.2 Data Analysis

Normality tests were conducted using histogram, Kolmogorov-Smirnov, and Shapiro-Wilk tests. Non-parametric ANOVA (Kruskal Walis Test) was used to compare the effects of IgG levels against malaria vaccine candidate antigens among iron- and non-iron-fortified Ghanaian children with and without malaria. The study also employed linear regression analysis to investigate the potential relationship between antibody level and age. Mann - Whitney U test was used to compare the levels of IgG responses among the groups, and Chi-square was used to generate p values of categorical variables. Data were analyzed with Excel and SPSS (IBM version 23). Statistical significance was assumed for p-values ≤ 0.05.

3.8 Ethical Clearance

The Ghana Health Service Ethics Committee, the Ghana Food and Drugs Board, the Institutional Ethics Committee of the KHRC, and the Hospital for Sick Children Research Ethics Board in Toronto, Ontario, Canada all gave their approval to this study.

3.9 Chapter Summary

This chapter discussed into details the methodology followed to obtain results for the study. In summary, this study employed archival samples between April and May 2010 from a double-blinded cluster-randomized control trial by Zlotkin et al. (2013). The study was conducted in the Wenchi and Tain districts of the Bono Region, which has a high malaria transmission rate. Eligible children aged 6 to 35 months were randomly recruited to either the fortified group or non-iron group and followed for five months. A structured questionnaire was used to collect socio-demographics. Blood samples were collected from children with fever or admitted to a healthcare facility to determine malaria status using RDT and confirmed with microscopy. A data and safety monitoring board convened three times throughout the experiment to discuss progress. Antibody levels were measured using the indirect ELISA technique with few modifications. Results obtained were processed and imported to SPSS (IBM Version 23) for data analysis. Normality tests as well as parametric and non-parametric tests were used in the data analysis. The study obtained ethical clearance.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

This study evaluated the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children. This study used four hundred archival plasma samples from children aged 6 to 36 months. IgG levels against the MSP-3, Glurp R0, and GLURP R2 malaria vaccine candidate antigens were measured using indirect ELISA. SPSS and Stata were used for the data analysis. Descriptives were used to analyze socio-demographic data. Non-parametric ANOVA (Kruskal Walis Test) was used to compare the effects of IgG levels against malaria vaccine candidate antigens among iron- and non-iron-fortified Ghanaian children with and without malaria. The study also employed linear regression analysis to investigate the potential relationship between antibody level and age. Mann - Whitney U test was used to compare the levels of IgG responses among the groups. P values for categorical variables were generated using Chi-square. Analysis was done to address the four main objectives of the study: determine IgG levels against malaria vaccine candidate antigens among iron-fortified Ghanaian children with or without malaria infection, determine IgG levels against malaria vaccine candidate antigens among non-iron fortified Ghanaian children with or without malaria infection, evaluate the IgG levels against malaria vaccine candidate antigens among iron and non-iron-fortified Ghanaian children with or without malaria infection, assess the association between the antibody levels against these malaria vaccine candidate antigens

with age and parasitemia. In this study, statistical significance was determined by conducting tests with p-values less than or equal to 0.05.

4.1 Results

4.1.1 Demographic Characteristics of the Study Participants

A total of 400 archival plasma samples from children from 6 to 36 months were used for the ELISA work. Out of the 400, the iron and non-iron groups were 200, respectively. The mean age for the iron group was 19.7±8.92 (95%CI=18,21), while the mean age for the non-iron group was 19.8±8.68 (95%CI=18,21). Based on gender, 106(53.0%) participants were males and 94(47.0%) were females for the iron-fortified group. For, the non- iron group the majority 101(50.5%) participants were females with 99(49.5%) being males (Table 4.1a).

Malaria status was categorized basically into negative and positive. For iron groups, 101(50.5%) participants were positive with a mean age of 20.18 months (SD=8.7;95% CI 18.4,21.9), while 99(49.5%) were negative with a mean age of 19.23 months±9.1 (95% CI 17.4,21.0) as shown in table 1b. Positive groups were further categorized into count into two groups according to WHO standards: complicated (severe illness or damage to essential organs with parasite density count greater than 100,000) and uncomplicated (without severe illness or damage to essential organs with parasite density count less than 100,000). The study recorded 28(14.0%) for complicated, 73(36.5%) for uncomplicated. There was no statistical significance among malaria status (p =0.8)

For the non-iron group, 99(%) participants were positive with a mean age of 20.6 months (SD=8.5; 95% CI 18.9,22.3), and 101(%) were negative with a mean age of 18.9 months (SD=8.7; 95%CI 17.1,20.6) as shown in table 4.1b. Also, 26(13.0%) were complicated while 74(37%) were uncomplicated. There was statistical significance among malaria status ($p<0.05$)

Table 4.1a: Demographic Characteristics of the Study Participants

Characteristics	**Iron Group (n=200)**	**Non-Iron Group (n=200)**	**P-value**
Age (mean, S.D,95% CI)	19.7(8.92; 18,21)	19.8(8.68; 18,21)	
Gender			
Male	106(53.0)	99(49.5)	0.48
Female	94(47.0)	101(50.5)	
Malaria status			
Positive	101(50.5)	99(49.5)	0.01
Negative	99(49.5)	101(50.5)	
Malaria severity			
Negative	99(49.5)	100(50.0)	0.8
Complicated	28(14.0)	26(13.0)	
Uncomplicated	73(36.5)	74(37.0)	

Age in month, n=frequency, () = percentages, Chi-square was to generate p-value

Table 4.1b: Demographic Characteristics of the Study Participants

Characteristics	Iron Group (n=200)	P-value	Non-iron Group (n=200)	P-vale
Malaria Status				
Positive	101(50.5)	0.49	99(49.5)	**<0.0001**
Age (mean, S.D,95% CI)	20.1(8.7;18.4,21.9)		20.6(8.5;18.9,22.3)	
Negative	99(49.5)		101(50.5)	
Age (mean, S.D,95% CI)	19.2(9.1;17.41,21.0)		18.9(8.7;17.1,20.6)	

Age in month, n=frequency, () = percentages, Chi-square was to generate p-value

4.1.2 Levels of IgG Responses against Malaria Vaccine Candidate Antigens among Iron Group

Levels of IgG responses elicited against malaria vaccine candidate antigens among iron-fortified groups were assessed. The levels were measured among malaria-positive and negative study participants as shown in table 4.2. The study showed no significant difference in the IgG levels against the various malaria vaccine antigens between malaria positive and negative in the iron-fortified group (P>0.05).

Table 4.2: Levels of IgG Responses against Malaria Vaccine Candidate Antigens among Iron Group

		Iron group		
Infection status	**n**	**GLURP R0** **median (IQR1, IQR3)**	**GLURP R2** **median (IQR1, IQR3)**	**MSP-3** **median (IQR1, IQR3)**
Positive	101	6.60(5.54, 7.99)	5.63(4.64, 6.95)	6.51(5.66, 7.33)
Negative	99	6.69(5.25, 8.14)	5.82(4.60, 7.10)	6.71(5.53, 7.59)
P-value		0.92	0.90	0.30

N= frequency, IQR1= Lower Interquartile Range, IQR3= Upper Interquartile Range, P-values generated with Mann Whitney U test

4.1.3 Levels of IgG Responses against Malaria Vaccine Candidate Antigens among Non-Iron Group

Table 4.3 shows the levels of IgG responses against the malaria vaccine candidate antigens among the non-iron-fortified group. Unlike the iron-fortified group, the IgG levels against the various malaria vaccine candidate antigens were significantly higher among the malaria-positive individuals than the malaria-negative individuals with a median of [6.26(IQR1=5.27; IQR3=7.64) vs 5.48(IQR1=4.49; IQR3=7.92) p=0.03] for GLURP R2. There was, however, no significant difference in the IgG levels among the GLURP R0 and MSP-3 vaccine candidate antigens between malaria-positive and negative participants (P>0.05)

Table 4.3: Levels of IgG Responses against Malaria Vaccine Candidate Antigens among Non-Iron Group

		Non-iron group		
Infection status	**n**	**GLURP R0**	**GLURP R2**	**MSP-3**
		median (IQR1, IQR3)	**median (IQR1, IQR3)**	**median (IQR1, IQR3)**
Positive	100	7.10(5.75, 8.46)	6.26(5.27, 7.64)	7.00(5.80, 7.92)
Negative	100	6.48(5.06, 8.01)	5.68(4.49, 7.92)	6.73(5.46, 7.95)
P-value		0.55	**0.03**	0.33

n= frequency, IQR1= Lower Interquartile Range, IQR3= Upper Interquartile Range, P-values generated with Mann Whitney U test

4.1.4 Effect of Iron Fortification on IgG Response against Malaria Vaccine Candidate Antigens among Ghanaian Children with or without Malaria Infection

Table 4.4 shows the effect of iron fortification on IgG response against malaria vaccine candidate antigens among Ghanaian children with or without malaria infection. The median, lower, and upper interquartile ranges expressed the IgG responses. The study saw a significantly higher effect of IgG responses against the various malaria vaccine candidate antigens among the non-iron group compared to the iron group with malaria-positive status [2.26(IQR1=4.64; IQR3=7.64) vs 5.63(IQR1=4.64; IQR3=6.95) p=0.02] and [6.99(IQR1=5.79; IQR3=7.93) vs 6.51(IQR1=5.66; IQR3=7.33) p=0.03] for GLURP R2 and MSP-3 respectively. Unlike the other malaria candidate antigens, GLURP R0 did not show any significant difference in IgG effect between the iron and non-iron group [6.60(IQR1=5.54; IQR3=7.99) vs 7.11(IQR1=5.72; IQR3=8.470) p=0.13]. On the contrary, the findings of the study indicated a lack of statistically significant variation observed in the levels of IgG effect against the candidate antigens among the malaria negatives for both iron and non-iron groups.

Table 4.4: Effect of Iron Fortification on IgG Response against Malaria Vaccine Candidate Antigens among Ghanaian Children with or without Malaria Infection

	Iron group	Non-Iron group	p- values
Infection status	**median (IQR1, IQR3)**	**median (IQR1, IQR3)**	
Positive	**n=101**	**n=99**	
GLURP R0	6.60(5.54, 7.99)	7.11(5.72, 8.47)	0.13
GLURP R2	5.63(4.64, 6.95)	6.26(4.64, 7.64)	**0.02**
MSP-3	6.51(5.66, 7.33)	6.99(5.79, 7.93)	**0.03**
Negative	**n=99**	**n=100**	
GLURP R0	6.69(5.25, 5.25)	6.48(5.06, 8.14	0.66
GLURP R2	5.82(4.60, 7.10)	5.68(4.49, 6.92)	0.77
MSP-3	6.71(5.53, 7.59)	6.73(5.46, 7.95)	0.93

n= frequency, IQR1= Lower Interquartile Range, IQR3= Upper Interquartile Range, P-values generated with Mann Whitney U test

4.1.5 Effect of Malaria Parasitaemia on IgG Responses against the Malaria Vaccine Candidate Antigens among the Iron-Fortified Group

The study further evaluated the effect of malaria parasitaemia on IgG responses against the malaria vaccine candidate antigens among the iron-fortified group, as shown in Table 4.5 There were no significant differences in the effect of parasitemia on IgG responses against GLURP R0, GLURP R2, and MSP-3 ($p>0.05$)

Table 4.5: Effect of Malaria Parasitaemia on IgG Responses against the Malaria Vaccine Candidate Antigens among the Iron-Fortified Group

Iron Group			
Antigens	**Parasitemia group**	**median (IQR1, IQR3)**	**P- value**
GLURP R0	negative	6.69(5.25, 8.14)	0.52
	complicated	6.19(5.28, 7.79)	
	uncomplicated	6.68(5.67, 8.28)	
GLURP R2	negative	5.82(4.60, 7.10)	0.78
	complicated	5.30(4.07, 7.29)	
	uncomplicated	5.78(4.89, 6.84)	
MSP-3	negative	6.71(5.53, 7.59)	0.49
	complicated	6.45(5.74, 7.02)	
	uncomplicated	6.53(5.61, 7.48)	

IQR1= Lower Interquartile Range, IQR3= Upper Interquartile Range, P- values generated with Kruskal Wallis equality-of-populations rank test. Complicated (severe illness or damage to essential organs with parasite density count greater than 100,000), uncomplicated (without severe illness or damage to essential organs with parasite density count less than 100,000), and negative (healthy individual with zero (0) parasite count.

4.1.6 Effect of Malaria Parasitaemia on IgG Responses against the Malaria Vaccine Candidate Antigens among the Non-Iron Fortified Group

This study also evaluated the effect of parasitaemia on IgG responses against the malaria vaccine candidate antigens among the non-iron-fortified group. Like the iron group, no statistically significant differences were observed in the impact of parasitemia on IgG responses against GLURP R0, GLURP R2, and MSP-3 (P>0.05), as presented in Table 4.6

Table 4.6: Effect of Malaria Parasitaemia on IgG Responses against the Malaria Vaccine Candidate Antigens among the Non-Iron Fortified Group

Non-Iron group			
Antigens	**Parasitemia group**	**median (IQR1, IQR3)**	**P- value**
GLURP R0	negative	6.48(5.06, 8.01)	0.13
	complicated	7.26(6.18, 8.54)	
	uncomplicated	7.10(5.72, 8.40)	
GLURP R2	negative	5.68(4.49, 6.92)	0.09
	complicated	6.26(5.51, 7.42)	
	uncomplicated	6.26(5.09, 7.72)	
MSP-3	negative	6.73(5.46, 7.95)	0.40
	complicated	7.64(5.84, 8.26)	
	uncomplicated	6.96(5.78, 7.71)	

IQR1= Lower Interquartile Range, IQR3= Upper Interquartile Range, P- values generated with Kruskal Wallis equality-of-populations rank test. Complicated (severe illness or damage to essential organs with parasite density count greater than 100,000), uncomplicated (without severe illness or damage to essential organs with parasite density count less than 100,000) and negative (healthy individual with zero (0) parasite count.

4.1.7 Association between the Antibody Levels against Malaria Vaccine Candidate Antigens and Age

The association between IgG levels against the malaria vaccine candidate antigens and age was determined in this study, and the results are shown in Table 4.7. The β effect, 95% confidence intervals, and p-values were calculated using linear regression. For both iron and non-iron groups, the levels of IgG against the GLURP R0 and GLURP R2 malaria vaccine candidate antigens showed positive correlations. However, only the correlation

among the iron group for GLURP R2 antigen showed a significant association 0.16(0.02,179; p =0.04). Again, IgG levels against MSP-3 malaria vaccine candidate antigens showed a negative correlation among both iron and non-iron groups without significant associations (p>0.05)

Table 4.7: Association between the Antibody Levels against Malaria Vaccine Candidate antigens and Age among Iron and Non-Iron Group

Non-Iron Group				
Age	**β (95% CI)**	**St. Err.**	**t-value**	**p-value**
GLURP R0	0.03(-0.82,1.13)	0.49	0.31	0.75
GLURP R2	0.17(-0.06,1.89)	0.49	1.83	0.06
MSP-3	-0.03(-0.06,0.84)	0.52	-0.36	0.71
Iron Group				
GLURP R0	0.10(-0.41,1.46)	0.47	1.09	0.27
GLURP R2	0.16(0.02,17)	0.44	2.03	**0.04**
MSP-3	-0.04(-1.25,0.73)	0.47	-0.55	0.57

P values < 0.05 were considered statistically significant. Age in months

4.1.8 Association between the Antibody Levels against these Malaria Vaccine Candidate Antigens and Parasite Density among Iron and Non-Iron Group

Table 4.8 shows linear regression results of the association between IgG levels against the malaria vaccine candidate antigens and parasite count among iron and non-iron groups. In both iron and non-group, IgG levels against GLURP R0 showed a positive correlation. However, while IgG levels against GLURP R2 antigen showed a positive correlation among the non-iron group, the IgG levels against the same antigen showed a negative correlation among the iron group. Again, for both iron and non-iron groups, the IgG levels against MSP-3 antigen showed a negative correlation. However, generally, none of the associations showed any statistical significance.

Table 4.8: Association between the Antibody Levels against these Malaria Vaccine Candidate Antigens and Parasitemia among Iron and Non-Iron Group

Non-Iron Group				
Parasitaemia	**β (95% CI)**	**St. Err.**	**t-value**	**p-value**
GLURP R0	0.13(-0.0,0.17)	0.05	1.32	0.18
GLURP R2	0.13(-0.03,0.17)	0.00	1.38	0.16
MSP-3	-0.11(-0.17,0.04)	0.00	-1.12	0.26
Iron Group				
GLURP R0	0.12(-0.03,0.16)	0.05	1.30	0.19
GLURP R2	-0.03(-0.09,0.09)	0.04	-0.03	0.96
MSP-3	-0.13(-0.18,0.02)	0.05	-1.56	0.11

P values < 0.05 were considered statistically significant.

4.2 Discussion

Despite massive global efforts to control and manage malaria, it continues to be a significant public health issue. The continent of Africa is responsible for a significant majority, specifically 96%, of all reported malaria cases worldwide, with 80% of all mortality occurring among children under five years. Ghana ranks among the top 15 countries contributing to malaria cases and death in Africa and reports the highest outpatient department cases related to malaria. Artesunate-based combined therapies (ACTs) have proven to be the most effective pharmacological interventions, especially when implemented alongside the use of insecticide-treated mosquito nets as a highly adopted preventive measure. *P. falciparum* is widely recognized as the most dangerous species among the various plasmodia.

Several immune responses are elicited against this parasite to prevent or minimize the incidence of infection. Antibodies are one of the main components of the immune responses directed against *P. falciparum.* IgG is the most predominant antibody in immunological responses against *P.*

falciparum. Studies have shown that IgG 1 and IgG 3 are the most effective of all the IgG classes. Several *P. falciparum* surface antigens have been targeted as potential targets for developing potent vaccines to elicit effective and long-lasting immunity against malaria.

Following results from a longitudinal study, the WHO 2006 recommended adding iron supplements to the diets of children living in highly endemic malaria regions. However, to date, the effect of iron fortification on immune responses against *P. falciparum* remains debatable. There are divergent findings among researchers concerning the relationship between iron fortification and malaria infection. While some researchers report that iron fortification increases susceptibility to severe malaria infection, others contend that iron fortification protects against malaria infection. Thus, this study sought to investigate the effect of iron fortification on IgG responses against malaria vaccine candidate antigens among Ghanaian children. This study utilized archival plasma samples collected from the Wenchi and Tain Municipalities within the Kintampo North District in a community-based double-blinded cluster randomized trial by Zlotkin et al, 2013 between April and May 2010.

4.2.1 Socio-Demographic Data of the Study Participants

The socio-demographic data and malaria status distribution shed light on the research participants' characteristics and malaria status with iron fortification (Table 1). Knowing these parameters is critical for properly interpreting the ensuing data on the influence of iron fortification on IgG responses to malaria vaccine candidate antigens.

A total of 400 archival plasma samples were used for the ELISA work, with the average age of the iron group being 19.7 months and the average age of the non-iron group being 19.8 months. Based on gender, a total of 106(53.0%) participants were males, and 94(47.0%) were females, while for the non-iron group, the majority 101(50.5%) were females with 99(49.5%) being males. This shows that generally, more than half 205(51.5%) of the study participants were males, while the remaining 195(48.5%) were females. This finding is comparable to several other studies (Cutler, Fung, Kremer, Singhal, & Vogl, 2010; Diiro et al., 2016).

The examination of malaria status uncovered some intriguing trends. There was a significant difference in malaria status among study participants within the iron and non-iron-fortified group ($p < 0.05$). Also, the study recorded an almost equal mean age among participants within both iron and non-iron groups for malaria status.

Upon comparing these findings with the current body of literature (Spottiswoode, Duffy, & Drakesmith, 2014; Zlotkin et al., 2013), it is apparent that this study elicits thought-provoking inquiries about the intricate relationship between iron levels and the consequences of malaria. While the iron group did not demonstrate a statistically significant difference in malaria status, the non-iron group showed a notable significance, offering evidence that iron may play a crucial role in immune response and susceptibility to malaria. The data are consistent with prior research indicating that administering iron supplements may worsen malaria infection, possibly attributable to iron's influence on pathogen proliferation and immunological modulation (Zlotkin et al., 2013).

4.2.2 Levels of IgG Responses against Malaria Vaccine Candidate Antigens among the Iron Group

The current study aimed to investigate the extent of IgG immune responses against antigens of a malaria vaccine candidate among individuals in the iron-fortified cohort. The first objective was to determine whether there were significant differences in IgG levels among individuals who were diagnosed with malaria and those who were not. The study's results suggest no significant differences in IgG levels against the malaria vaccine antigens among patients who tested positive or negative for malaria infection within the iron-fortified group.

The evaluation of IgG responses directed towards antigens of malaria holds considerable importance in assessing the potential effectiveness of vaccines and comprehending the immunological dynamics associated with malaria infection. This research yields consistent results with the research conducted by Boström et al. (2012), who also observed no statistically significant disparities in IgG levels between patients with malaria and those without the disease in a distinct setting. The existing data suggests that the presence or absence of current malaria infection does not appear to influence IgG responses within the iron-fortified group substantially.

In this investigation, emphasis was placed on specific malaria candidate antigens, and the analysis revealed that there were no statistically significant variations observed in the levels of IgG against GLURP R0, GLURP R2, and MSP-3 when comparing individuals who tested positive for malaria with those who tested negative. The results presented in this study are consistent with the conclusions drawn by Malhotra et al. (2015) and Fowkes,

Richards, Simpson, and Beeson (2010), whose research similarly indicated that there were no significant disparities in IgG responses toward these antigens between those infected with malaria and those who were not infected. The observed consistency in our data indicates a strong and resilient immune response against these antigens, which seems to be unaffected by the concurrent presence of an active malaria infection among iron fortified.

In contrast, our work deviates from the findings presented by Courtin et al. (2011), who observed varying IgG responses against malaria antigens dependent on the individual's infection state. The observed discrepancies in outcomes may be ascribed to variances in the demographics of the study participants, research methodology used, or sample size. These findings underscore the intricate nature of immunity to malaria and emphasize the need for a more thorough study to be conducted.

The absence of substantial disparities in IgG levels targeting malaria vaccine antigens across people with and without malaria infection in the iron-fortified cohort has implications for the development and assessment of vaccines (Armitage & Moretti, 2019). This finding highlights the possible resilience of immune responses during active infections, indicating that certain vaccine antigens may elicit strong immune reactions irrespective of the individual's illness state. The results provide evidence that malaria vaccines may retain their effectiveness even among communities experiencing a certain level of pre-existing malaria transmission.

4.2.3 Levels of IgG Responses against Malaria Vaccine Candidate Antigens among the Non-Iron Group

The levels of IgG responses against the malaria vaccine candidate antigens among the non-iron-fortified group were also estimated. This study found that malaria-positive children without iron supplements had higher IgG responses against GLURP R2antigens compared to the iron-fortified children without active malaria infection. This finding supports a previous study that reported malaria infection is associated with higher IgG responses to the GLURP R2 antigen. According to Osier et al. (2008), individuals with malaria showed stronger IgG responses to the GLURP R2 antigen compared to uninfected counterparts. This GLURP R2 antigen has been demonstrated to elicit significant antibody responses associated with protective immunity against malaria (John et al., 2008). The presence of elevated levels of total IgG antibodies specific to MSP3, MSP1-19, and GLURP has been observed to correlate with reduced susceptibility to the development of clinical malaria, according to earlier studies using human blood samples from areas with a high prevalence of malaria. However, the results varied between studies. Notably, IgG antibodies produced in response to GLURP antigens consistently exhibit a diminished susceptibility to the manifestation of clinical malaria, but the situation with MSP3 and MSP1-19 is uncertain. Findings from the current study indicate an association between GLURP-specific IgG and *P. falciparum* clinical malaria.

Similarly, in a recent study about the aforementioned subject, it was discovered that “individuals who exhibited a positive antibody response to MSP3 and GLURP long synthetic peptides at the onset of the peak malaria

transmission season were observed to have a decreased susceptibility to clinical malaria (Adu et al., 2016; Nebie et al., 2008). Another study found high levels of IgG antibodies against numerous asexual antigens protect against *P. falciparum* infection in people living in a malaria-endemic area (John et al., 2008). The absence of a significant association between malaria infection and IgG responses to the GLURP R0 and MSP-3 antigens in the present study is consistent with previous findings by Nebie et al. (2011), who reported no statistically significant difference in IgG responses to these antigens between malaria-infected and uninfected individuals". These findings imply that the effect of malaria infection on antibody responses may differ depending on the individual antigen targeted. The reasons behind some individuals having high antibody levels to all three antigens while others lack such antibodies are still unknown. However, some scholars argue that differences in antibody levels against specific malaria vaccine candidate antigens across a study area might be due to genetic factors, as well as the existence of comorbidities that could impact the ability to produce and sustain such antibodies (Del Giudice et al., 2017; Mo et al., 2020).

4.2.4 Effect of Iron Fortification on IgG Response against Malaria Vaccine Candidate Antigens among Ghanaian Children with or without Malaria Infection

This study further evaluated the effect of iron fortification on the IgG response against malaria vaccine candidate antigens among Ghanaian children with or without malaria infection**.** The study observed significantly higher IgG responses against the various malaria vaccine candidate antigens in the non-iron group compared to the iron group with malaria-positive status. Unlike the

other malaria candidate antigens, GLURP R0 showed no significant difference in IgG effect between the iron and non-iron groups.

This shows that iron fortification substantially affects IgG responses in malaria infection. The substantiation of the aforementioned claim is underpinned by a comprehensive randomized trial study carried out in Pemba, Zanzibar, a region known for its high prevalence of malaria. Young children who were given iron-fortified meals were shown to be at a higher risk of developing malaria, according to the research and experiencing illness and mortality related to infection. (Aimone et al., 2017). This finding is also in alignment with a study conducted in Tanzania, wherein the investigation focused on assessing the influence of iron status on the risk of malaria. The research utilized both cross-sectional and longitudinal analyses within a birth cohort consisting of children who were subjected to comprehensive and proactive health monitoring, as well as early administration of antimalarial therapy. According to the study, children with iron fortification had considerably higher malaria infection rates, morbidity, and death (Gwamaka et al., 2012). These observed results could be due to iron overload. Insufficient or excessive iron levels potentially impair the antibody response since sufficient iron levels are necessary for the adequate functioning of immune cells, such as B-cells. According to Preston, Drakesmith, and Frost (2021), diminished B-cell function and imbalances in iron levels, either deficient or excessive, can potentially compromise the immune system's ability to effectively respond to infections and vaccinations, increasing the susceptibility to infections.

Conversely, a study by Prentice, Verhoef, and Cerami (2013) observed that the incidence of malaria exhibited a notable decrease in the group

receiving iron supplementation compared to the group not receiving iron supplementation. The observed disparities ceased to exhibit statistical significance subsequent to the incorporation of adjustments for baseline iron deficiency and anaemia status. Nevertheless, the available data did not provide any substantiation to support the notion that iron intake is associated with a heightened susceptibility to malaria. Zlotkin et al. (2013) suggest this could be due to insufficient iron content in the MNP supplement. Tchum et al. (2021) found that long-term consumption of iron-fortified MNP among babies and young children in highly endemic malaria-burden regions did not increase malaria prevalence. A meta-analysis by Ojukwu, Okebe, Yahav, and Paul (2009) also concluded no increased mortality among children in malaria-endemic areas who were on iron supplements. These disparities could also be associated with poor iron absorption, probably due to low vitamin C levels in the supplement.

Findings from the present study demonstrate that iron supplementation may modulate specific IgG responses to malaria vaccine candidate antigens, especially in the case of positive malaria infection. The considerable differences in GLURP R2 and MSP-3 antigens detected between the iron and non-iron-fortified groups among children with positive malaria infections suggest that iron fortification may impact immunological responses to malaria.

The interaction of immune response pathways and iron availability might explain GLURP R2 and MSP-3 response variation. Iron availability can impact the formation and maturation of IgG antibodies against malaria antigens, thereby influencing the overall immunological response to malaria infection and vaccinations (Oppenheimer et al., 2001). Additional studies have

identified a correlation between IgG antibodies to GLURP R2 and a lower risk of malaria, indicating that IgG binding to immune cells may have a protective role (Dechavanne et al., 2017).

These findings highlight the complexities of the interaction between iron, malaria, and immunity, highlighting the need for more study to understand the underlying processes. The current investigation shows that iron fortification may modulate IgG responses to malaria vaccine candidate antigens in Ghanaian children with positive malaria infection.

4.2.5 Effect of Malaria Parasitemia on IgG Responses against the Malaria Vaccine Candidate Antigens among the Iron-Fortified Group.

To further our understanding of the intricate correlation between malaria parasitaemia and IgG responses to antigens of malaria vaccine candidates, this study examined the impact of parasitaemia on immune responses within the iron-fortified cohort. The impact of malaria parasitaemia and immunological responses has been a prominent area of interest within the field of malaria research for a considerable period (Marsh & Kinyanjui, 2006). This study's results indicate no statistically significant variations in the impact of parasitaemia on the immune response of IgG antibodies against GLURP R0, GLURP R2, and MSP-3 antigens. These findings are consistent with the conclusions reached by Crompton et al. in their research conducted in the year 2010. The study also found no significant influence of parasitaemia on the IgG immune responses targeting particular malaria vaccine antigens. The observed convergence implies a persistent pattern in which certain antigens can induce immune responses that maintain a reasonably steady state, even in the context of a pre-existing parasite infection (Eckhoff, 2012).

In contrast, our findings diverge from the findings of Courtin et al. (2009) and Akpogheneta et al. (2008), who observed differences in IgG responses depending on the degrees of parasitaemia. The disparity reflects the intricate interactions between hosts and parasites, highlighting the possible impact of variables such as the populations under investigation, geographical regions, and antigenic variations. Additionally, these variations could be due to the small size used in this study

The lack of substantial disparities in the impact of parasitaemia on IgG responses towards GLURP R0, GLURP R2, and MSP-3 antigens suggests that these particular vaccine candidates may retain their immunogenic characteristics regardless of the presence of parasitaemia (Murungi, 2014). This conclusion aligns with the findings of Santano et al. (2021), who observed constant IgG responses against certain antigens, irrespective of the levels of parasitaemia. Antigen-specific immune responses play a critical role in developing malaria vaccines since they provide valuable insights into vaccine-induced protection's potential effectiveness and long-term durability (Slifka & Amanna, 2014).

4.2.6 Effect of Malaria Parasitemia on IgG Responses against the Malaria Vaccine Candidate Antigens among the Non-Iron-Fortified Group

This study also evaluated the effect of IgG responses on parasitemia among non-iron-fortified groups against the malaria vaccine candidate antigens. Like the iron group, there were no significant differences in the impact of parasitemia on IgG responses against GLURP R0, GLURP R2, and MSP-3 (Table 7). On the other hand, Stanisic et al. (2016) discovered a substantial association between parasitemia and IgG responses, indicating

contradicting findings while a study by Walker et al. (2020) contradicts the earlier one. It is expected that persons with complicated or severe malaria status would have higher levels of IgG responses and thus a higher impact than those in the uncomplicated group. This is due to the established role of IgG in *P. falciparum* clearance and killing, though the exact mechanism remains unclear.

Other variables, such as host genetic differences, variability in immunological responses, or other environmental conditions, might have altered the reported IgG responses. The absence of substantial variations in the impact of parasitaemia on IgG responses towards GLURP R0, GLURP R2, and MSP-3 antigens within both the iron and non-iron-fortified cohorts highlights the potential resilience of immune responses to these antigens in the face of persistent parasitic infection (Rauw, 2012). This discovery is crucial for the development of an effective malaria vaccine.

4.2.7 Association between Antibody Levels against Malaria Vaccine Candidate Antigens and Age among Iron and Non-Iron Group

The association between IgG levels against the malaria vaccine candidate antigens and age among iron and non-iron groups was determined in this study. For both iron and non-iron groups, the levels of IgG against the GLURP R0 and GLURP R2 malaria vaccine candidate antigens showed positive correlations. However, only the correlation among the iron group for GLURP R2 antigen showed a significant association 0.16(0.02,179; p =0.04). Again, IgG levels against MSP-3 malaria vaccine candidate antigens showed a negative correlation among both iron and non-iron groups without significant associations ($p>0.05$). The results align with the study conducted by Amoah et

al., (2017), which emphasized the gradual increase in immune responses in relation to age.

The results show an impressive positive correlation for GLURP R2, indicating that older children have higher antibodies against this antigen. These findings are consistent with earlier studies showing that individuals' antibody responses to malaria antigens become stronger with age (Crompton et al., 2010). While our results vary from the study conducted by Smith et al. (2010), which reported significant correlations between age and IgG responses to MSP-3, these discrepancies highlight the heterogeneity of immune responses to specific antigens (Dobaño et al., 2012).

The differences in results suggest that factors other than age, like genetic differences or exposure to malaria parasites, may significantly impact the immune response to these specific antigens (Osier et al., 2008).

4.2.8 Association between Antibody Levels against Malaria Vaccine Candidate Antigens and Parasite Count among Iron and Non-Iron Groups

This study used linear regression to estimate the association between IgG levels against the malaria vaccine candidate antigens and parasite count among iron and non-iron groups. The study found that, in both iron and non-groups, IgG levels against GLURP R0 showed a positive correlation. However, while IgG levels against GLURP R2 antigen showed a positive correlation among the non-iron group, the IgG levels against the same antigen showed a negative correlation among the iron group. This further substantiates the impact of iron fortification on antibody responses. Again, for both iron and non-iron groups, the levels of IgG against MSP-3 antigen showed a

negative correlation. However, generally, none of the associations showed any statistical significance. This discovery implies a possible association between elevated antibodies against GLURP R0 and heightened parasite count, possibly suggesting a reaction to a lasting infection. Similar to the report by Iriemenam et al. (2009), this study saw a positive correlation between antibody responses and parasitaemia. However, generally, none of the associations showed any statistical significance, which could be due to the small sample size.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.0 Introduction

This chapter brings an end to the thesis. It summarizes the theoretical and empirical underpinnings of the study, indicating the scientific rationale behind the study. It also summarizes the scientific methods that were employed in this study. Again, this chapter provides a comprehensive overview of the main discoveries of the research and concludes the study results. Finally, this chapter presents recommendations according to the results obtained and includes suggestions for further research.

5.1 Summary of the Study

Malaria continues to pose a substantial challenge to global health, with a particular emphasis on its impact within the sub-Saharan African region (van den Berg et al., 2019). According to the World Health Organization (WHO), there is a need to incorporate iron supplements into the dietary regimens of children residing in regions with a high prevalence of malaria. Sufficient iron is known to boost immune cell development and proliferation, while iron deficiency or overload compromises antibody response (Pandey et al., 2023). Lower B-cell activity due to iron deficiency or excess can reduce immune response to infections and vaccines, increasing infection risk (Preston et al., 2021). However, the effect of iron fortification on immune responses against *P. falciparum* remains debatable. This study reviewed articles on malaria and its related issues, including aetiology, biology, pathogenesis, diagnosis, and treatment; immune response to malaria parasites; iron fortification and iron

deficiency; relationship between iron fortification and malaria; vaccine candidate antigens and vaccine development.

A total of 400 archival plasma samples were randomly sorted for ELISA from children aged 6 to 36 months from Wenchi and Tain Municipalities in Kintampo North District. The study used non-parametric ANOVA (Kruskal Walis Test) to compare the IgG levels against malaria vaccine candidate antigens among iron- and non-iron-fortified Ghanaian children with and without malaria. Linear regression analysis was employed to investigate the potential relationship between antibody levels, and Mann-Whitney U tests were used to compare IgG responses across groups. Data were analyzed using Excel and SPSS, with p values ≤ 0.05 considered statistically significant. The study was granted ethical approval.

5.2 Summary of the Study Results

Iron deficiency and malaria present substantial public health issues, particularly in low- and middle-income nations like Ghana. Understanding how iron levels impact immunological responses to the malaria vaccine is critical for improving vaccination techniques and addressing the malaria burden, particularly among youngsters. This study aimed to investigate how iron supplementation could affect IgG responses to three malaria vaccine candidate antigens (GLURP R0), GLURP R2), and MSP-3) among Ghanaian children. In addition, the study looked at the socio-demographic features and malaria status distribution among the study population to provide context for future research. The study found a well-balanced cohort with equivalent age distributions and gender representations in the iron and non-iron groups.

The results showed no significant differences in IgG levels between patients with and without malaria infection in the iron group, which is congruent with previous research by Boström et al. (2012). The study also found no significant differences in IgG levels against GLURP R0, GLURP R2, and MSP-3 when comparing positive and negative individuals. This suggests a strong and resilient immune response against these antigens unaffected by active malaria infection. However, the study deviates from Courtin et al. (2011) findings, which observed varying IgG responses depending on the individual's infection state.

The study estimated IgG responses against malaria vaccine candidate antigens among non-iron-fortified individuals. The results showed significantly higher levels of IgG responses among malaria-positive individuals than malaria-negative individuals. No significant difference was found between GLURP R0 and MSP-3 vaccine candidate antigens. Previous studies have shown that malaria infection is associated with higher IgG responses to the GLURP R2 antigen, which is linked to protective immunity against malaria. However, the results vary between studies, and the association between GLURP-specific IgGs and *P. falciparum* clinical malaria is unclear. The lack of a substantial association between malaria infection and IgG responses to the GLURP R0 and MSP-3 antigens is consistent with Nebie et al.'s (2011) findings, suggesting that the effect of malaria infection on antibody responses may differ depending on the individual antigen-targeted.

This study further evaluated the effect of iron fortification on IgG responses against malaria vaccine candidate antigens in Ghanaian children with or without malaria infection. The results showed significantly higher IgG

responses against GLURP R2 and MSP-3 antigens in the non-iron group compared to the iron group with malaria-positive status.

Results also show no significant differences in parasitaemia's impact on IgG antibodies against GLURP R0, GLURP R2, and MSP-3 antigens among the iron group. This aligns with Crompton et al.'s 2010 research, which found no significant influence of parasitaemia on IgG immune responses targeting specific malaria vaccine antigens. The study's findings differ from previous studies, which observed differences in IgG responses depending on parasitaemia levels. This suggests that vaccine candidates may retain their immunogenic characteristics regardless of parasitaemia. This aligns with Santano et al.'s findings, which observed constant IgG responses against certain antigens regardless of parasitaemia levels.

The study evaluated the impact of parasitaemia on IgG against malaria vaccine candidate antigens responses among non-iron-fortified groups. It found no significant differences in parasitemia effects on IgG responses against GLURP R0, GLURP R2, and MSP-3. However, Stanisic et al. (2016) found a substantial association between parasitemia and IgG responses, contradicting previous findings. Individuals with complicated or severe malaria status are expected to have higher levels of IgG responses, potentially due to the role of IgG in *P. falciparum* clearance and killing. Other variables, such as host genetic differences, immunological responses, and environmental conditions, may have altered reported IgG responses. This discovery is crucial for developing an effective malaria vaccine.

This study found a positive correlation between IgG levels against the malaria vaccine candidate antigens and age among iron and non-iron groups, suggesting repeated exposure to the malaria parasite may contribute to increased immune responses. However, only GLURP R2 showed a statistically significant association with age, suggesting that older children have higher antibodies against this GLURP R2 antigen. The results indicate that factors other than age, such as genetic differences or exposure to malaria parasites, may significantly impact the immune response to specific antigens.

The study found that, in both iron and non-groups, IgG levels against GLURP R0 showed a positive correlation. However, while IgG levels against GLURP R2 antigen showed a positive correlation among the non-iron group, the IgG levels against the same antigen showed a negative correlation among the iron group.

5.3 Conclusion

This study reveals that iron fortification substantially downregulates IgG responses against GLURP R2 and MSP-3 malaria vaccine among individuals with malaria, thus increasing malaria severity.

5.4. Recommendations

1. Sufficient iron supplement coupled with proper anti-malarial treated insecticide bed nets should be encouraged
2. Future studies on frequency of exposure, host genetic factors, nutritional status and larger sample size is required to substantiate more findings

5.5. Suggestions for Future Research

1. Given the geographical limitation of the study to specific districts within the Bono Region, future research should consider extending the scope to include a more diverse range of regions within Ghana.

2. Pfs25 is a potential transmission protein blocker that could be investigated as a potential malaria vaccine antigen

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