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Human Leukocyte Antigen-G Level, Hematological and Biochemical Profile among Pregnant women with *Plasmodium falciparum* (*Pf*) infection: A case control study at the Bibiani Anhwiaso Bekwai Municipality, Ghana

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BY

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This 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 fulfillment of the requirements for the award of Master of Philosophy degree in Infection and Immunity

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis was dully conducted by me and that this research has never been submitted in part or whole in this university or any learning institution for another degree.

Student's Signature: ..... Date: .....

Name: Richmond Kwakye

Supervisors' Declaration

We equally declare that this thesis was supportively supervised by us under the supervision guidelines of the university of Cape coast, UCC. Ghana

Principal Supervisor's Signature: ..... Date: .....

Name: Rev. Dr. Benjamin Amoani

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Signature: ..... Date: .....

Prof. David Courtin

Signature: ..... Date: .....

Dr. Faustina Pappoe

ABSTRACT

Immune-suppressive ability of HLA-G and its binding to foetal maternal interface predispose pregnant women to *Plasmodium falciparum* (*Pf*) infection and pregnancy loss respectively. However, alterations in hematological parameters accelerate the severity of malaria infection. In addition, the effects of malaria infection on hepatocytes endanger the life of both the mother and the foetus to liver injury. The study assessed HLA-G levels, hematological and liver biochemical profile among *Pf* infected and uninfected pregnant women in the Bibiani Anhwiaso Bekwai Municipality, Ghana. The study was a case control one with 92 pregnant women consisting of 46 *Pf* infected (cases) and 46 *Pf* uninfected (as control) from the municipality. Convenience sampling and structured questionnaire were employed to collect socio-demographic, antimalarial intermittent preventive treatment (IPT-SP), insecticide treated mosquito net (ITN's) and miscarriage data. Using a first response *Pf* HRP2 RDT test kit, whole blood samples were tested for the presence of *Pf* parasites. Hematological and liver biochemical profile were done using automated hematology and chemistry analyzer respectively. Serological tests: Hepatitis B, Hepatitis C and Human Immunodeficiency Virus were done using the appropriate test kits/ strips following the manufacturer's protocol. Plasma HLA-G levels were assayed using sandwich ELISA (Maxisop Elisa plate) and corresponding concentrations were obtained using ADAMSEL Software. Clinical characteristics: IPT-SP ($p=0.007$) and Temperature ($p=0.045$) were significantly associated among the cases and the control group. However, there were no significant association between trimester, ITN's usage, age and educational status ($p>0.05$). HLA-G levels insignificantly increased among the cases as compared to the control group ($p>0.05$) and was significantly associated with miscarriage ($p=0.001$) with no association between the hematological parameters. Also, there were no statistical association between liver biochemistry and miscarriage ($p>0.05$). *Pf* infection was positively associated with ALT ($p=0.011$), ALP ($p=0.017$, $p=0.02$) with or without adjustment respectively and a significant association with percent lymphocytes ($p=0.04$) and percent granulocytes ($p=0.03$). *Pf* infection has effect on liver biomarkers (ALT and ALP) with decrease percent lymphocyte and an increase percent granulocyte among pregnant women with malaria infection. Again, higher plasma HLA-G was associated with previous miscarriage status among the pregnant women. However, the study shows increase levels of HLA-G among *Pf* infected pregnant women.

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I express my gratitude to the laboratory staff of Green Shield hospital for their contributions towards this work.

DEDICATION

I dedicate this thesis to my supportive parents: Mr James and Mrs Augustina Kwakye and my dear sisters, Blessing Mensah and Lydia Kwakye

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LIST OF ABBREVIATIONS

Ag	Antigen
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BABM	Bibiani Anhwiaso Bekwai Municipality
ELISA	Enzyme Linked Immunoassay
HBV	Hepatitis B Virus
HCT	Hematocrit
HCV	Hepatitis C Virus
HGB	Hemoglobin
HIV	Human Immunodeficiency Virus
HLA-G	Human Leukocytes Antigen
HRP2	Histidine -rich Protein 2
IPT	Intermittent Preventive Treatment
ITNs	Insecticide Treated Nets
K_3EDTA	Tripotassium Ethylenediaminetetraacetic Acid
LBW	Low Birth Weight
LFT	Liver Function Test
mRDT	Malaria Rapid Diagnostic Test
OD	Optical Density
PAM	Pregnancy Associated Malaria
PBS	Phosphate Buffered Saline
Pf	*Plasmodium falciparum*
PLT	Platelets

PRBC	Parasitized Red Blood Cells
RBC	Red Blood Cells
RDT	Rapid Diagnostic Test
sHLA-G	Soluble Human Leukocytes Antigen
SP	Sulfadoxine-Pyrimethamine
SST	Serum Separating Tubes
TB	Total Bilirubin
WBC	White Blood Cells

CHAPTER ONE

INTRODUCTION

This chapter elaborated on the background of study, problem statement, hypothesis, aim and objectives, significance of the study, limitations, delimitation and organization of the study.

Background of the study

Malaria, a parasitic infection is still a global burden and continues to cause wide range of morbidities and mortality in malaria endemic areas (Amoani et al., 2019a; Garcia et al., 2013). Despite preventive measures taken to reduce the impact of the infection in Sub-Saharan Africa, such as the use of insecticide-treated mosquito nets (ITNs), malaria nevertheless caused an estimated 229,000 cases and 409,000 deaths globally in 2019 (WHO, 2020; WHO, 2015). Transmission and severity of the diseases is associated with the parasite species, the human host (pregnant women, children and infants), host immune response, the environment and human leucocyte antigen, maternal risk factors such as gestational age, parity, gravidity (WHO, 2015; Sadissou et al., 2014,). More than 300 million new cases of malaria are reported each year, with 90% occurring in Africa (Maduka et al., 2008). In Ghana, the commonest species of malarial infection is *Plasmodium falciparum* and it infects both children and adults. Malaria is responsible for 3.4% of maternal deaths, 13.7% of hospitalizations, and 17.6% of OPD visits in Ghana (Anabire et al., 2019). Some clinical symptoms of malaria infection are chills, fever, waist pains, body pains, cough, and dizziness but in pregnancy some cases of malaria infection may be asymptomatic especially in the early gestational stage of the pregnancy. Pregnancy-related asymptomatic

malaria evades most fever-based surveillance systems but can nonetheless result in morbidities such as anemia and low birth weight (Afutu et al., 2020).

Pregnant women are more susceptible to *P. falciparum* infection due to their weak immune system, immunological changes induced by the pregnancy and some hormonal factors (Digban et al., 2017; Ndamukong-nyanga et al., 2020). *Plasmodium falciparum* infected erythrocytes bind to receptors like chondroitin sulphate A (CSA) and is sequestered in the placenta but rarely bind to receptors in non- pregnant women thus CD36 and intracellular adhesion molecule (ICAM-1) (Takem & D'Alessandro, 2013).

Moreover, Variant Surface Antigen (VAR2CSA), a specific variant of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is produced on infected erythrocytes and makes it easier for them to bind to Chondroitin Sulfate A (CSA), which improves the sequestration of the infected erythrocytes in the placenta (McLean et al., 2021). These are distinct from those that are exhibited in women who are not pregnant and in transmission that the immune system does not recognize (McLean et al., 2021). Malaria infection in pregnancy could result in death, low birth weight, spontaneous abortion, maternal anemia, fetal anemia, and cognitive impairments especially in infants as a result of fetal anemia during brain development process (Digban et al., 2017; Ndamukong-nyanga et al., 2020). One of the most alarming hematological alterations in pregnancy is anemia as defined by World health organization (WHO) as hemoglobin concentration being <11.0g/dl (WHO, 2015). Malaria caused by *Plasmodium falciparum* can induce hematological changes such as anemia, leucopenia, and lymphocytosis (Singh et al., 2015). These hematological changes may cause reduction in host immune

response especially in pregnancy making them more susceptible to viral and other parasitic infections (Sakzabre et al., 2020). Ndamukong-nyanga et al., (2020) reported significance association in white blood cells (WBC), hemoglobin (HGB), hematocrit and platelets concentrations between pregnant women with malaria and those without malarial infection. Hematological and biochemical changes coupled with high human leukocyte antigen- G (HLA-G) may endanger the life of the pregnant women and their newly born babies since low birth weight, as well as brain impairment can occur (Digban et al., 2017; Sakzabre et al., 2020).

During malaria infection, the normalcy of the body changes leading to immunological, biochemical and hematological alterations which accelerate the severity of the infection (Singh et al., 2015). Alterations in inflammatory cytokines (Ndamukong-nyanga et al., 2020) and hormonal changes trigger the release of human leukocytes antigen (HLA-G) which turns to suppress the immune strength of the individual increasing their risk and susceptibility to malaria especially in pregnant women (Sadissou et al., 2014; Singh et al., 2015). Human leukocytes antigen –G (HLA-G) is a non-classical HLA class I genes of Major Histocompatibility Complex (MHC) located on chromosome 6 in human and encodes proteins responsible for regulating immune response (Barbaro et al., 2023). Although HLA-G has identical structure as Class I Classical molecule, its main function is not antigen presentation but functions in immune response regulation (Sadissou et al., 2014). It can create tolerogenic environment due to its immunosuppressive properties which allow parasitic infections such as malaria to evade host immune response (Sadissou et al., 2014). HLA- G has been known to be a predisposing factor for maternal parasitaemia, foetal malaria and also inhibits

immune response by producing cells which can result in pregnancy loss or recurrent pregnancy loss by binding to foetal maternal interface (Najafi et al., 2021). Moreover, Pregnancy complications like pre-eclampsia and recurrent miscarriages are associated with HLA-G polymorphisms and altered HLA-G expression (Mosaferi et al., 2013).

It has been established that mothers with placenta malaria have high sHLA-G which increase infants malaria risk during the first two years of life (d'Almeida et al., 2019; D'Almeida et al., 2017). There are several isoforms of HLA-G but the soluble forms expressed in the plasma are HLA-G1and G5. During pregnancy, high HLA-G are expressed which increase maternal malaria susceptibility. Additionally, it has been discovered that HLA-G expression is high in persistent viral infections such as Hepatitis B, HIV and in cancers and could be a mechanism which pathogens evade immune response (Sadissou et al., 2014). A healthy pregnancy relies on the involvement of maternal immune cells and fetal trophoblasts obtained from the placenta (Laird et al., 2003). Meanwhile, Over 30% of conceptions experience pregnancy loss (Page & Silver, 2016) which is defined as the loss of three or more successive pregnancies in the first trimester of pregnancy (Laird et al., 2003). The cause of pregnancy loss is multifactorial since it is associated with factors like abnormal embryonic karyotype and maternal driven causes which alter placenta development (Laird et al., 2003; Page & Silver, 2016). HLA-G, due to its immunosuppressive ability play role in pregnancy loss as it binds to fetal maternal interface and mutations in HLA-G gene can alter the progress of pregnancy (Najafi et al., 2021). In addition, HLA-G polymorphism of

rs1736933 and rs2735022 also a contribute to recurrent pregnancy loss (Najafi et al., 2021).

Several studies elsewhere and in Ghana have shown that pregnant women with malaria have differential hematological and biochemical parameters such as white blood cells (WBC),hemoglobin (HGB), Urea, Creatinine and C - reactive protein and this is due to difference in parasitaemia and the significant changes in these parameters are due to the disruption of hematopoietic physiology (Al-Salahy et al., 2016a; Digban et al., 2017; Ndamukong-nyanga et al., 2020; Tobón-castaño et al., 2015; White, 2018). Alterations of liver markers have been solely attributed to viral hepatitis, alcoholism, drugs limiting the association of these markers to malaria infection, but, in adults with acute *Plasmodium falciparum* infection, Alkaline Phosphatase (ALP) has been proven to be an important biomarker for assessing the functionality of the hepatic drainage system in acute *Pf* infection (Anabire, 2017). Also biochemical parameters such as Alkaline Phosphatase (ALP), Alanine aminotransferase(ALT) and Total Bilirubin (TB) amongst pregnant women at the Northern part of Ghana with malaria infection has been reported to be higher than pregnant women without malaria infection (Anabire, 2017).

Hematological and biochemical profile such as WBC, anemia, urea, creatinine, have received a lot of attention in malaria infections and in pregnancy associated malaria (Digban et al., 2017; Rouamba et al., 2021) . Thus, this study aims to evaluate the comprehensive association of HLA-G level, liver-biochemical and hematological profiles among pregnant women with or without *Pf* infection in Ghana. This will guide clinicians to provide appropriate

management to pregnant women to reduce maternal death, early diagnose and treatment of malaria- related liver diseases, as well as initiating the need for liver function test (LFT) during pregnancy in Ghana.

Problem statement

Pregnancy reduces the immune strength of pregnant women making them more susceptible to infections such as malaria (Ndamukong-nyanga et al., 2020). Moreover, pregnancy associated malaria is still a global burden despite the preventive measures such as sleeping under treated mosquito net, intermittent preventive treatment (Dosoo et al., 2021). However, it is estimated that malaria infections in pregnancy resulted in 82,200 children with low birth weight and almost half of the children are in sub region of West Africa (WHO, 2020). Recent studies suggest that an early pregnancy *Plasmodium falciparum* infection could affect the development of the placenta and children born to infected mothers are at high risk of malaria infection since they acquire T lymphocytes that are specific for the maternal malaria antigen (Simon et al., 2021). This means that pregnant women with high level of HLA-G are at greater risk of maternal malaria due to the immunosuppressive ability of HLA-G and this will predispose their infants to malaria infection and low birth weight. Sadissou et al., (2014) established high relationship of HLA-G to an increased risk of infant malaria and low birth weight in pregnant women with high level of plasma HLA-G in Benin. Although, several studies have documented some complications such as foetal death, low birth weight, hematological alterations such as anemia of malaria infection in pregnancy, HLA-G, a predisposing factor for maternal malaria, infants' susceptibility, low birth weight and pregnancy loss is not well studied among

pregnant women in Ghana. However, role of HLA-G in pregnancy loss or miscarriage is under- researched despite some authors (Fan et al., 2014; Ober et al., 2003) elsewhere have reported the relationship of HLA-G-14bp polymorphism and -725 c/G of HLA-G in miscarriage. Therefore, this study seeks to investigate the comprehensive association of HLA-G level and pregnancy loss or miscarriage, hematological and biochemical profile of malaria infection (*Pf*) in pregnancy.

Hypothesis

Plasma HLA-G level, hematological and liver biochemical parameters (ALT, AST, ALP, TB) of *Pf* infected pregnant women will be higher than pregnant women without *Pf* infection.

Aim and Objectives of study

Aim

This study aimed at assessing the levels of Human Leukocytes Antigen- G (HLA-G), hematological and liver biochemical parameters among pregnant women with or without *P. falciparum* infection.

Objectives

i. To evaluate the hematological parameters and its anomalies among pregnant women with or without *Pf* infection.

ii. To assess the level of liver biochemical parameters: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Total Bilirubin (TB) among pregnant women with or without *Pf* infection.

iii. To determine plasma HLA-G level among pregnant women with or without *Pf* infection.

iv. To determine the association between HLA- G and liver biochemical profile among the pregnant women with or with *Pf* infection

v. To determine the association of HLA-G, hematological and liver biochemical profile among the pregnant women with or without malaria

vi. To determine the association of HLA-G and liver biochemical profile with miscarriage status among the pregnant women.

Significance of the study

The immunosuppressive ability of HLA-G allows *Plasmodium falciparum* to evade host immune response which predisposes pregnant women to maternal and foetal malaria, its role in recurrent pregnancy loss endangers the life of both the mother and the foetus (Sadissou et al., 2014). This makes the study of HLA-G very crucial in the Ghanaian populace especially among pregnant women. In Ghana, maternal death, spontaneous abortion continues to be very alarming. According to a recent assessment, Ghana has maternal mortality ratio (MMR) of 310, making it one of the world's high MMR countries (Ameyaw et al., 2021). HLA-G, coupled with malaria infection in pregnancy endangers the life of the pregnant women hence the need for this study since it would help clinicians to pay critical attention to women with high HLA-G level during pregnancy so an appropriate supportive and immunotherapeutic care can be given. In addition, this study would create awareness about the role of malaria infection in pregnancy-related-liver disease so liver function test (LFT) could be considered as an antenatal care (ANC) laboratory investigation especially for malaria infected

pregnant women since early detection of liver disease would help reduce maternal death in Ghana. However, this study would make scientific awareness about malaria infection and miscarriage: the relevance of HLA-G since pregnancy loss or miscarriage is a public concern to the Ghanaian society as Ghana recorded 10.8% prevalence of miscarriage as at 2017 (Ahinkorah et al., 2021). Findings from this study would again create awareness about the effects of the co-existence of high HLA –G level and malaria on the mother and the foetus.

Study limitation

One of the most targeted groups for malaria prevention programs in Ghana is pregnant women due to the maternal and foetal effects of the infection which has informed the choice of several preventives measure such as provision of insecticide treated mosquito net (ITN's), intermittent preventive treatment (IPT's). These measures have reduced the rate and spread of malaria infection in the communities which made getting positive malaria cases amongst the pregnant women very challenging and this really affected the sample size for the study. Moreover, some pregnant women didn't consent to the study amidst some untold cultural reasons with regards to blood sampling. Also, microscopic identification of *P. falciparum* was not done in this study.

Delimitation

The study site was expanded where pregnant women attending antenatal visit at three major different health facilities in the municipality were recruited. This was done to achieve the sample size so reliable conclusion can made from the study.

Organization of the study

This study has been grouped into five (5) chapters. Chapter one (1) described the background of the entire study. Chapter two (2) consist of literature review which reversed different information on malaria in pregnancy, the biology and epidemiology of malaria parasites, susceptibility and diagnosis of *Pf* infection, parasitaemia and hematological anomalies, the liver and malaria infection, HLA-G, its receptors with malaria and miscarriage. Chapter three (3) talks about the research methodology of which the study design, study site, sample collection and analysis were reported. Chapter four (4) consists of the results obtained and discussion for the study. Lastly, chapter five (5) talks about the summary, conclusion and recommendations.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter of the study reviewed previous articles on the biology and clinical manifestation of malaria parasites, malaria epidemiology and susceptibility, and diagnosis of malaria infection. This chapter further discussed the effects of parasitaemia on hematological parameters, hematological anomalies, histology of the liver and malaria infection, and pregnancy and liver diseases with its associated liver biomarkers. Again, this chapter discussed HLA-G and malaria infection and its association with miscarriage.

Biology and clinical manifestation of Malaria Parasites

There are six (6) species of malaria parasites namely: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale wallickeri, Plasmodium ovale curtisi, Plasmodium malariae, and Plasmodium knowlesi* (Milner, 2018) out of which *P.falciparum* is lethal and causes adverse clinical pathology in humans especially in pregnant women, the aged and children under 5yrs in Sub-Saharan Africa (Sinden, 2016). Malaria infection may be asymptomatic, thus infected individuals showing no signs and symptoms of the infection or symptomatic: showing clinical symptoms such as chills, fever, vomiting, waist pains, cough which is caused by the asexual intraerythrocytic development cycles(IDC) (Sinden, 2016; Thomson-luque et al., 2021). The parasites ring stage during the asexual stage develops into schizonts which upon replication releases a lot of merozoites which invades the red blood cells (RBC) of the individual (Sinden, 2016; Thomson-luque et al., 2021).

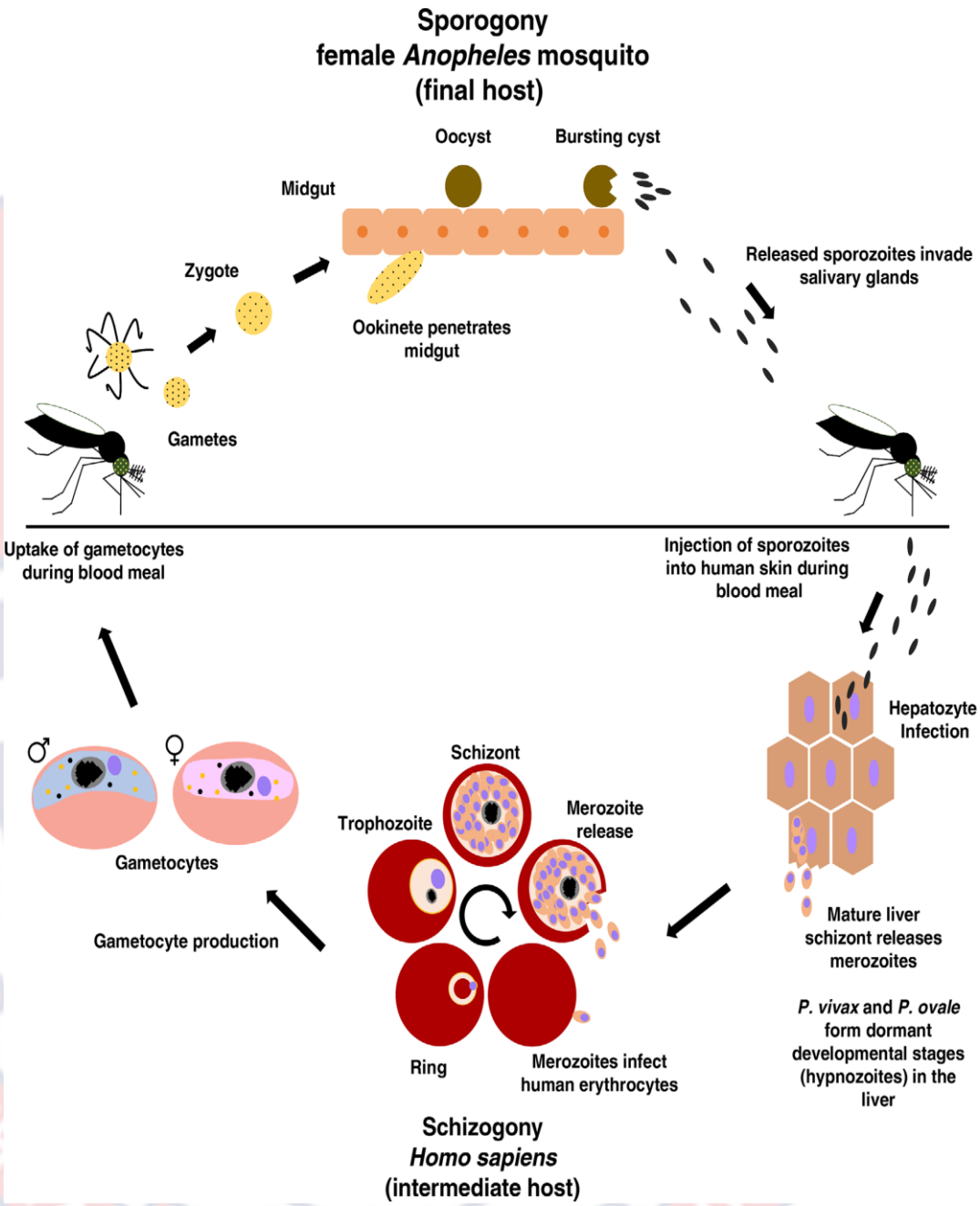

Figure 2. 1: Biological cycle of *Plasmodium* parasites (Gitta & Kilian, 2020)

Epidemiology of Malaria infection

Irrespective of several malaria preventive measures like intermittent preventive treatment with Sulfadoxine-Pyrimethamine (IPT-SP), insecticide treated Nets (ITN's), malaria particularly *Pf* still remains a significant health concern in Africa (Anto et al., 2019; Nyavor et al., 2017). According to Thomson-luque et al., 2021, annually over 200 million cases are reported worldwide of which approximately 2 million leads to severe diseases such as cerebral malaria, coma and even death. In 2019, over 400,000 death mostly amongst African children less than 5 years was reported (Thomson-luque et al., 2021).

About 90% of the 300–500 million cases and 2.7 million fatalities from malaria are thought to occur in Sub-Saharan Africa (Guprta Indrani, 2013). Its very alarming that malaria is considered second to HIV/AIDS representing 10.6% in Africa (Guprta Indrani, 2013). Predominantly *P. falciparum* causes 34% of all cases of malaria infection in Ghana seen at the outpatient's department (OPD), where 19% ends in admissions and 2% of total deaths with pregnant women constituting 3.9% of total suspected cases of malaria infection with 3.4% maternal death (Ampofo et al., 2022; Anabire et al., 2019).

Malaria infection in Ghana is highly dependent on epidemiological setting. Having three -main agro- ecological zones thus, the coastal zones, the forest zone and savanna accounts for different malaria cases in each region. Despite ecological changes, *Pf* represent 80-90% of the malaria cases. Taking Ghana's Northern and Ahafo region as an example, prevalence of malaria parasitaemia is 47% and 22.8% respectively (Asante et al., 2011; Clerk et al., 2009). Also the

prevalence of malaria has been reported to be 39.1% with 23% among females in Bibiani Anhwiaso Bekwai Municipality (Boadu et al., 2020).

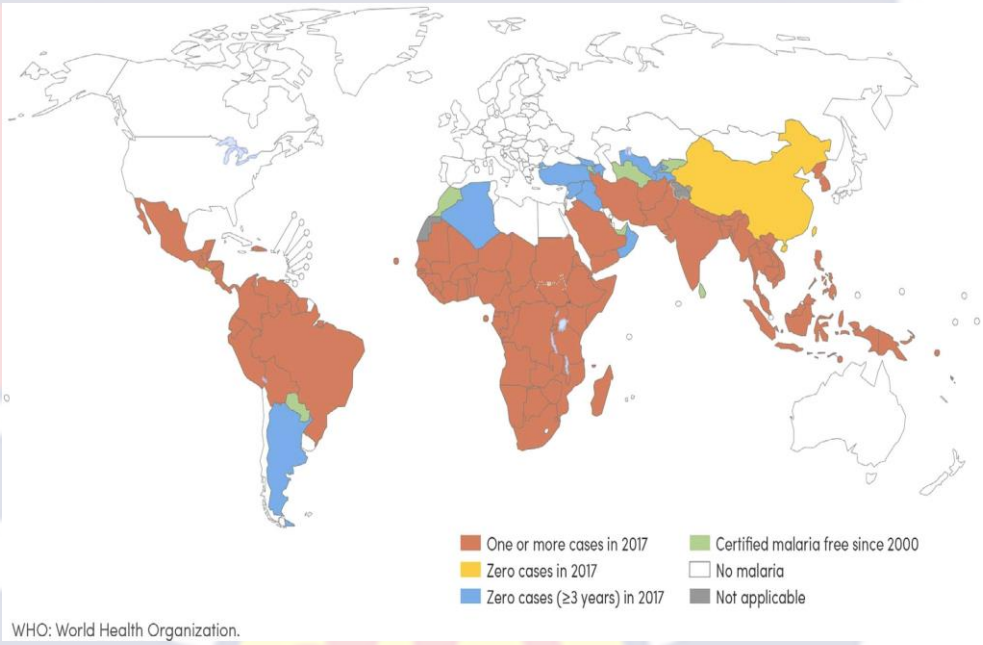

Figure 2. 2 : Global epidemiology of malaria infection. Obtained from (Gitta & Kilian, 2020)

Susceptibility of Malaria Infection in Pregnancy

Every year, about 25-30 million women in Africa's locations with a high prevalence of malaria become pregnant and women in their gestations get more attracted to mosquitoes than non-pregnant women (Rogerson, Mwapasa, et al., 2007). Pregnant women's susceptibility to malaria infection is probably due to pregnancy-related immunological and hormonal changes as well as infected red blood cells (RBCs') ability to sequester themselves in the placenta (Rogerson,

Hviid, et al., 2007). Moreover, studies have shown that antibodies that bind to the surface of infected RBCs in the placenta are mostly absent during the first pregnancy which increase their susceptibility (Rogerson, Mwapasa, et al., 2007)

Pregnant women in their first pregnancy are usually infected by malaria parasites and infection increases between 13 and 16wks and reduces according to term (Rogerson, Mwapasa, et al., 2007). Moreover, immune pregnant women are less susceptible to malaria infection as compared to non-immune pregnant women. These factors coupled with high HLA-G level increases the risk of malaria infection and may lead to foetal susceptibility, foetal anemia and even cerebral malaria. Immune balance in the placenta is decreased by placenta malaria (PM) which results in increase in synthesis of inflammatory cytokines like tumor necrosis factor-alpha (TNF-alpha), Interleukin-2 (IL-2), and interferon. Interestingly, high level of TNF-α has been associated with LBW and anemia. In Sub-Saharan Africa (SSA) alone, malaria infection affected approximately 11.6 million pregnancies in 2019 leading to an estimated 822, 000 LBW newborns. Nearly half (49%) of these infants were born in West Africa (Ampofo et al., 2022). Moreover, in west Africa, the prevalence of malaria infection in pregnancy is about 39.8% which resulted in 819,000 children with low birth weight (WHO, 2021).

Diagnosis of Malaria Infection

Detection of malaria parasites can be very challenging since the parasite density of a sick person can be as low as one (1) parasites/μl to tens of thousands (Gitta & Kilian, 2020). Moreover, naturally acquired immunity play role in asymptomatic malaria individuals making malaria diagnosis very crucial both

clinically and laboratory -wise (Gitta & Kilian, 2020). Clinical diagnosis of malaria infection may be achieved by clinicians due to clinical manifestation presented by the patient in the absence of laboratory service. This might lead to wrong diagnosis of malaria since some tropical diseases present symptoms equivalent to malaria infection (Gitta & Kilian, 2020). Clinical diagnosis, sometimes practiced by the patient due to signs and symptoms of malaria literally known by most Ghanaian population are error prone practices since it can lead to over diagnosis and overtreatment thereby contributing to antimalarial resistance (Gitta & Kilian, 2020).

On the other hand, some laboratory techniques used in the diagnosis of malaria are the use of Rapid Diagnostic Test (RDT's), Microscopic examination of both thick and thin film ,and PCR but in Ghana, the most widely used techniques are the RDT and microscopy using Giemsa stain (Gitta & Kilian, 2020; WHO, 2016,2021).

Microscopy of Thick and Thin Film

According to Gitta & Kilian, 2020 automated slide reading of malaria parasites have been developed and tested in some developed countries to enhance effective examination of malaria parasites. Nevertheless, the gold standard method for detecting parasitemia in patients' erythrocytes is still microscopic examination of thick and thin film with giemsa stain (Gitta & Kilian, 2020; WHO, 2016,2021). This approch of parasites detection helps to quantify and indentify parasites species in the Ghanaian population. In addition microscopy examination helps to monitor parasites treatment and prevent over treamtment of the malaria

parasites. It has been known in the sub-saharn Africa that the current available test with detection threshold of 200p/ul do not miss malaria parasite (WHO, 2021)

Rapid Diagnostic Test (RDTs) for Malaria Infection

Rapid diagnostic test (RDT) is a diagnostic tool used in Ghana at first-line facilities for healthcare lacking microscpic services in the diagnosis of malaria parasites. RDTs helps to dectect malaria antigen. For instance, *Pf* RDT kits detect HRP2 of *P.falciparum* immunochromatographically (Oyeyemi et al., 2015). In 2020, Ghana used the rule of test before treat to encourage the adoption of RDT for malaria diagnosis since RDT kits were made available at the majority of primary healthcare facilities with little resources (Boadu et al., 2016). In 2019, a minimum specfication and detection for RDTs was set by WHO as RDT which is able to detect 200 parasites per microliters(p/μl) (WHO, 2021) and this has been achived by RDTs kits used in Ghana for effective and reliable diagnosis of malaria infection. The first response malaria Ag.*P.falciparum* RDT is the most frequently used RDT in Ghana (HRP2)(The Global Fund, 2015).

Effects of Malaria infection on Hematological Parameters

During malaria infection, the entry of merozoites into the erythrocytes mostly increases the release of the pro-inflammatory cytokines IL-1 and IL-10, the activation of the coagulation cascade (caused by the consumption of platelets and endothelial damage), and a parasitized red blood cells (PRBCs) are sequestered. These with other mechanisms results in morphological and numerical changes of the various blood cells such as white blood cells (WBC), hemoglobin (HGB), and platelets (PLT) (Ndamukong-nyanga et al., 2020). Changes in these hematological parameters may reduce the immune strength of the pregnant

woman making them more susceptible to malaria infection. In addition, changes in numbers of RBC, WBC, and PLT are some common hematological alterations in malaria infection and play major role in malaria pathology (Ndamukong-nyanga et al., 2020).

Moreover, there is significant difference in WBC, RBC, HCT, PLTs, and HGB values between pregnant and non-pregnant women. Again, HGB level of malaria infected pregnant women has been significantly lower than non-infected pregnant women. Malaria anemia is multifactorial and usually due to bone marrow dysfunction and destruction of infected and uninfected RBCs. Moreover, it is also superimposed on micronutrients deficiency like iron and folic acid, infection such as HIV, hookworms. In addition, pigmented monocytes accumulated in the placenta also play a role in maternal anemia (Ndamukong-nyanga et al., 2020; Rogerson, Hviid, et al., 2007). A case control study conducted in the urban capital of Ghana: Accra by Acheampong et al., 2021 reported significant association of lymphocytes, HGB, HCT, PLT from peripheral blood of hyper-parasitaemia patients. Even though, some studies in Ghana and elsewhere have reported significant association of hematological parameters and parasitaemia, (Amoani et al., 2019b) reported insignificant association of WBCs, and PLT to parasitaemia in the Kintampo North municipality, Ghana.. In addition, (Sakzabre et al., 2020) reported lymphopenia (56.78%), anemia (55.51%), thrombocytopenia (47.46%), and leukocytosis(17.3%) as the common hematological anomalies in the Volta part of Ghana. This means that hematological alterations in malaria infection are highly affected by geographical settings. Below are some anomalies of hematological profiling in *Pf* infection.

Hematological Anomalies

Red cells anomaly

Anemia

Red blood cell (RBC) loss due to bleeding and hemolysis of parasitized RBC (PRBC) resulting in anemia (Kotepui et al., 2015) is one of the major complications of malaria infection in children, adults and in pregnant women in malaria endemic setting (Akinosoglou et al., 2012). Anemia can be severe, thus < 5g/dl, or <7g/dl, or <11.0g/dl in children, adults and pregnant women respectively (Akinosoglou et al., 2012). Anemia is a contributory factor to morbidity and mortality rate with 5.6-16% in children and 6% in pregnant women respectively particularly in primigravidae. In endemic regions of sub-Saharan Africa, Pf infection is the main cause of maternal and fetal anemia (Akinosoglou et al., 2012).

Bleeding Anomaly

Thrombocytopenia

Thrombocytopenia is been known to occur in about 50-80% of malaria cases as a result of endothelia damages (Akinosoglou et al., 2012). Thrombocytopenia caused by *Pf* and *P.malarie* (*Pm)* infection was found to be significant in the Ghanaian population (Sakzabre et al., 2020). Although, low PLT has been attributed to endothelia damage, some factors like immune mediated destruction of circulation PLT also contribute to malaria thrombocytopenia.

White Blood Cells (WBC) anomalies

White blood cells, or WBCs, are blood cells that are synthesized in the bone marrow with a life span of 14-21 days and are grouped into agranulocytes (lymphocytes and monocytes) and granulocytes (eosinophil, neutrophils and basophiles) (Mutua et al., 2018). WBCs are normally determined in pregnancy to diagnose infections and inflammations since maternal death is usually caused by wide range of infections (Dockree et al., 2021). During pregnancy the normalcy of the body changes as results of physiological stress leading to leukocytosis. Also elevation in leucocytes count during pregnancy is as a results of stimulatory signals as the pregnancy progresses from the first trimester throughout the pregnancy (Mutua et al., 2018). On the other hand, leucopenia is mostly occur in pregnancy associated malaria (McKenzie et al., 2005).

Lymphocytes count in malaria infection

Lymphocytes, a type of white blood cells have been reported recently in medical research to be involved in malaria infection and accounts 63% in *Pf* infection in malaria endemic countries(Van Wolfswinkel et al., 2013). According to Kotepui et al., 2015, there is significant association of lymphocytes and monocytes in malaria parasitaemia. Also a study conducted by Bun et al., 2022 elsewhere indicated statistical significance between parasites counts, lymphocytes and absolute lymphocytes.

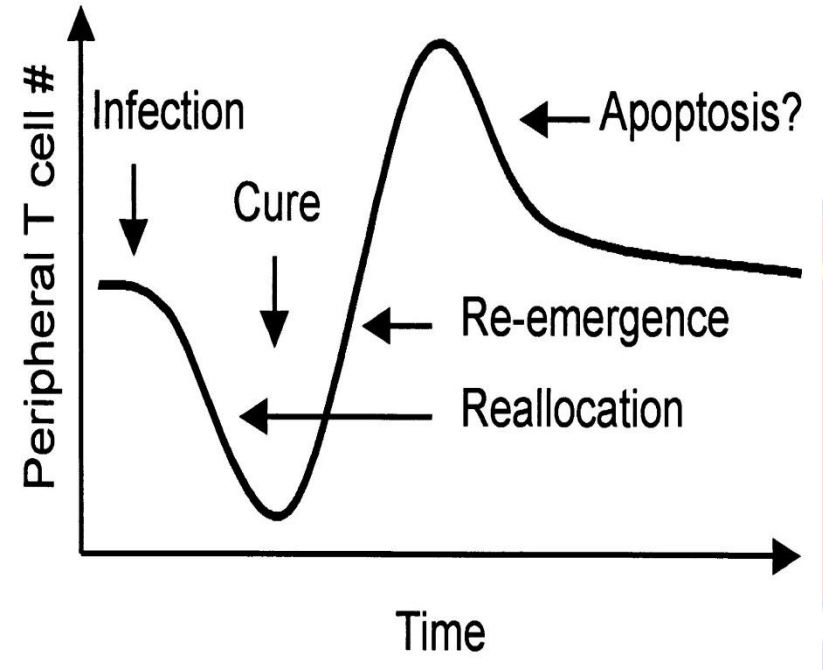

Figure 2. 3: Schematic diagram of perturbations in numbers of peripheral T cells following *Plasmodium* infection (Hviid, 2000).

Histology of the Liver in Malaria infection (*Pf*)

The liver is an anatomical organ which functions in several ways to sustain the life of humans. Some crucial functions of the liver are: excretion, secretions, and producing immunological factors to ward against infections and eliminating microorganisms from the bloodstream. Despite the liver ability to resist infections by making immune cells, some parasites such as malaria parasites evade the immune strength of the individual. Clear evidence of liver involvement in *Pf* infection is associated with total bilirubin levels (Viriyavejakul et al., 2014).

Hyperbilirubinemia (TB >51.3 μmol/L) has been reported in patients who had died from severe *Pf* infection as compared to patients who died without malaria infection. This means that malaria infection can cause a lot of

hepatocellular damage to infected liver. Moreover, increased liver enzyme concentrations such as transaminases and alkaline phosphatase has been reported in severe *Pf* infection (Viriyavejakul et al., 2014)

During the hepatic stage of malaria infection, sporozoites released develop in to merozoites which are subsequently released into the bloodstream and move into the erythrocytic stage, when PRBCs are confined to tiny blood vessels. Then, macrophages engulf the destroyed hemozoin pigment in *Pf* infections, common histopathological results of the liver are, reactive Kupffer cells, hemozoin pigment retention and sequestration of minimal PRBC's. high PRBC's load, hepatomegaly, and liver enzymes elevation, livers of malaria patients who had jaundice had been reported to have hyperbilirubinemia (Viriyavejakul et al., 2014).

Malaria infection has severe complications such as anemia, splenomegaly, hepatomegaly, hemoglobinuria from lysed red cells leaked into the urine throughout pregnancy and in children (Digban et al., 2017). Children born to mothers with higher parasitaemia and relapse malaria may experience developmental impairment. Jaundice, a clinical manifestation of completed malaria is an indication of liver involvement in malaria infection. *Pf* , the leading cause of malaria in the tropics cause jaundice in 2.5-5.3% of cases in malaria endemic area (Viriyavejakul et al., 2014).

Pregnancy and Liver Disease

In a normal pregnancy, without any pathogenic infections such as parasites, viral, pregnant women could experience some liver conditions such as intrahepatic cholestasis of pregnancy (ICP), HELLP Syndrome (Hemolysis,

Elevated Liver Enzymes, Low Platelet) and Acute Fatty Liver of Pregnancy (AFLP). These conditions might cause fetal and maternal distress, liver damage and sometimes hepatic failure. Destruction of hepatocytes by parasitic pathogens such as *Pf* leading to elevated enzymes coupling with these conditions might endanger the life of the fetus and the mother (Guarino et al., 2020).

Liver Biomarkers

Liver biomarkers are parameters that are determined to assess the functionality of the liver organ. Liver function test (LFT) is the test done to determine the levels of these biomarkers. Some of these are: Total bilirubin (TB), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT). Increased levels of these biomarkers especially ALT, AST give an indication of hepatocellular damage or liver injury. Liver injury is multifactorial since a lot of factors such as alcohol, drugs toxicity, and viral infections: HBV, HCV contributes. There has been numerous research done about the role of viral infections in liver diseases with less information about the role of hepatocellular damage by *Pf* infection. Liver enzymes such as AST, ALT, have been found mostly in the liver cells, thus the hepatocytes and their activities in hepatic cells are approximately 300 times higher than in circulation (WHO, 2020; Anabire, 2017). This means that during hepatocellular injury, serum AST, ALT doubles significantly as its leaks into circulation (Anabire,2017; WHO, 2020). Elevations in these enzymes may indicate hepatocellular injury but elevation within 1.5 times the upper limits of the normal does not necessary indicates liver injury (Guarino et al., 2020).

Aspartate aminotransferase (AST)

AST is a liver enzyme that is predominately located in the mitochondria (80%) but in lower concentrations in the hepatocytes (20%). AST can be found in the heart, liver, muscles and red blood cells with a half-life of ~18hours. AST, a widely distributed liver enzyme in the body is normally released into circulation from the hepatocytes during a severe liver injury especially when there is mitochondria injury by agents like alcohol. AST has been reported to be less specific for liver diseases and injury but mostly elevated in heart diseases such as ischemic heart diseases, hemolysis and muscle injury(WHO, 2020)

Alanine aminotransferase (ALT)

ALT is a biomarker that is predominately found in the liver. It's located in the cytoplasm of the hepatocytes with a half-life of ~48hrs. ALT is notable for being released into circulation during minor injury of the hepatocytes. This makes ALT a more sensitive and specific biomarker for liver injury (WHO, 2020)

Alkaline phosphatase (ALP)

ALP is a liver enzymes that is located in the cholangiocytes and mostly released into circulation when there is biliary obstruction (WHO, 2020). According to Fernandez & Kidney, (2007), increased levels of ALP has been found among women who are pregnant and it is linked to high-fat diets. Moreover, elevated levels of ALP have been reported in pregnant women in their third trimester due to the formation of placenta (Klinikleri & Gynecol, 2007).

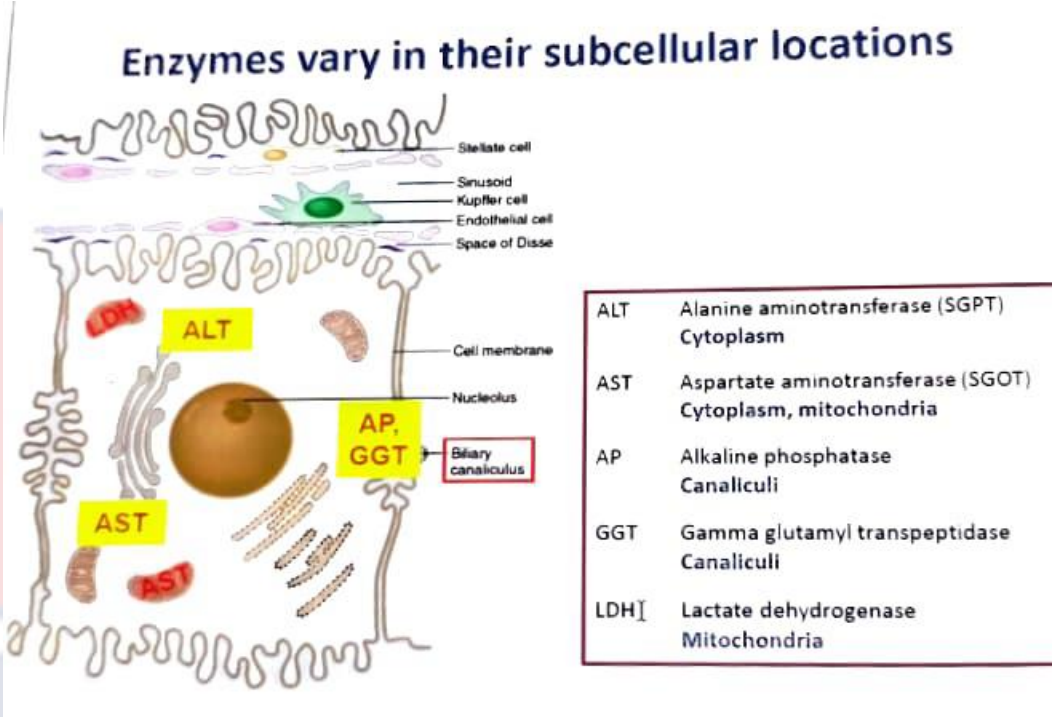

Figure 2. 4: Liver enzymes and their location. Source (WHO, 2020)

Serum Bilirubin

Serum bilirubin is an organic substance that is synthesized by reticuloendothelial system during normal or abnormal destruction of red cells (Anabire, 2017; WHO, 2020). Unconjugated (indirect) and conjugated (direct) bilirubin are the two types of serum bilirubin. As unconjugated bilirubin is released by red cells into the liver, the enzyme UDP gylcuronyltransferase convert it into conjugated in the liver and transported into the small intestine (Anabire, 2017; WHO, 2020). Bilirubin is excreted in the urine and gives the urine its distinctive yellow colour. Measurement of TB helps in the diagnosis of liver diseases, hemolytic anemia, and evaluation of jaundice. Clinical jaundice is

characterized by yellowish skin and elevated serum bilirubin level. Moreover, it's a primary clinical feature of liver impairment (WHO, 2020)

Some forms of jaundice are: obstructive jaundice, which is normally characterized by elevated TB level with conjugated bilirubin (direct) being the major cause. Hemolytic jaundice: this is where TB increase in the unconjugated (indirect). It has been noted that in viral hepatitis, both the conjugated and unconjugated increases and any diseases that affect bilirubin metabolism process thus: production of unconjugated bilirubin as old red cells break down, conversion of unconjugated into conjugated bilirubin in the liver and lastly the excretion of conjugated bilirubin into bile as pile pigment through the biliary tract may lead to jaundice. In addition some clinical conditions such as *Pf* infection causing hemolysis will lead to low hemoglobin or anemia which is also presented as yellowish skin (WHO, 2020).

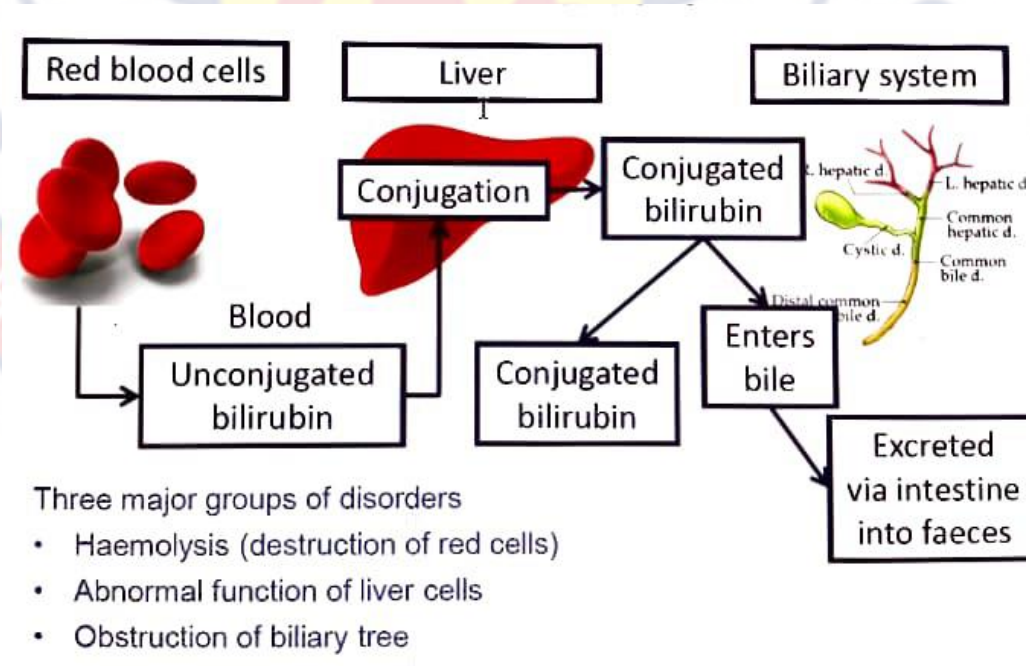

Figure 2. 5: Metabolism of bilirubin. Retrieved from (WHO, 2020)

HLA-G receptors and malaria infection

Human leukocytes antigen (HLA-G) is a tolerogenic molecule with immunosuppressive properties which differ from classical HLA Class I molecule by its lower genetic diversity, tissue expression and functions (Castelli et al., 2014). According to Alegre et al., 2014, there are about 50 alleles (IMGTHLA Database) and 16 proteins of HLA-G and seven isoforms are known. Four, (HLAG1,G2,G3, and G4) exist on the surface of the cells whereas HLA-G 5,G6, and G7 are soluble forms released from the cells but the isoforms present in the plasma of the human body are HLA- G1 and HLA-G5 (Sadissou et al., 2014).

HLA-G has multiple receptors which are expressed by the immune system. Some of these receptors are ILT2/CD85J, ILI14/CD85D binding to these receptors induces apoptosis of activated CD8+ T CELLS which accelerates its immune suppressive ability making pathogens to evade the immune strength of the individual predisposing them to parasitic infections such as malaria (Alegre et al., 2014).

Malaria infection is still of public health concern in malaria endemic areas due to its mode of transmission, morbidity and mortality rate amongst the populace especially children under 5 and pregnant women. It is established that the variability in clinical symptoms is partly due to host genetic polymorphism such as HLA-G class I, class II alleles which increase the risk of malaria susceptibility (Rizzo et al., 2009). Some studies have indicated the role HLA-G class I and class II alleles play in malaria susceptibility (d'Almeida et al., 2019; Sadissou et al., 2014). Even though causes of malaria infection are multifactorial, less has been documented about the role of HLA-G in malaria infection despite its

immunosuppressive modulatory properties (Sadissou et al., 2014). HLA-G is now known to cause variety of physio-pathological conditions such as tumor, inflammation and viral infections. Some viruses such is HIV, HCV induces changes in the level of HLA-G suppressing the function of the immunes cells (Rizzo et al., 2009).

According to d'Almeida et al., 2019, children born to mothers with high sHLA-G get malaria infection in the early stage of life due of its ability to suppress maternal immunity, HLA-G is important in fetal maternal immunological tolerance. Moreover, it has been established that the level of sHLA-G of a child is directly proportional to the level of sHLA-G of the mother at both the delivery and the entire pregnancy period. In addition, mothers with high HLA-G have a high probability of given birth to children with high HLAG level thereby increasing their susceptibility to malaria infection and higher chance of Low Birth Weight (LBW) (d'Almeida et al., 2019; D'Almeida et al., 2017).

Role of HLA-G in miscarriage

Miscarriage is one of the most common abnormalities that can happen during pregnancy which is very problematic to healthy women especially during their first pregnancy periods(Fan et al., 2014). Miscarriage can be recurrent thus three (3) or more consecutive first trimester or two (2) or more consecutive second trimester pregnancy loss before 20 weeks gestation (Bhalla et al., 2006). Recurrent miscarriage (RM) is likely to occur in 0.5-1% in couples desiring to have children (Fan et al., 2014). There are several factors such as chromosomal anomalies, uterine anatomic anomalies (Bhalla et al., 2006) that can cause RM. Couple not having these medical conditions tend to be overburdened about RM

but the human molecule, HLA-G tend to play role in RM due to its immune-suppressive ability at the maternal- fetal interface and placental angiogenesis (Fan et al., 2014).

HLA-G has seven isoforms of which HLA-G1,G5 are soluble forms found in the plasma of the human body with HLA-G1 occurring most in the placental tissues (Sadissou et al., 2014; Ober et al., 2003). However, during pregnancy, both G1 and G5 circulate and are responsible for wide range of functions at the maternal –fetal interface during the entire pregnancy (Ober et al., 2003).

According to two separate studies, HLA-G have been associated with RM depicting that low level of G1 protein may compromise the success of pregnancy.in addition, (Ober et al., 2003) reported that one polymorphism -725C/G is associated with fetal loss. Moreover, where both couples are having same -725G allele, there is a higher rate of RM than couples not carrying the same allele (Ober et al., 2003). Again, a metal analysis reported an association between HLAG 14bp insertion/deletion polymorphism with RM amongst patients who have had 3 or more miscarriages (Fan et al., 2014).

Chapter Summary

Several studies have discussed the pathogenicity of *P. falciparum*. This chapter of the study reviewed previous articles on the biology and clinical manifestation of malaria parasites, malaria epidemiology and susceptibility, and diagnosis of malaria infection. This chapter further discussed the effects of parasitaemia on hematological parameters, hematological anomalies, histology of the liver in malaria infection, pregnancy and liver diseases with its associated liver

biomarkers. Again, this chapter discussed HLA-G and malaria infection, and the role of HLA-G in pregnancy loss or miscarriage.

CHAPTER THREE

METHODOLOGY

Introduction

This chapter describes the study site, study design and selection criteria, sample size, participants' data collection, laboratory techniques, statistical analysis and ethnical clearance.

Study site

The study was conducted in the Bibiani Anhwiaso Bekwai Municipality (BABM). BABM is situated between latitude 6° N, 3° N and longitude 2° W, 3° W and bounded at the north of Atwima Mponua District in the Ashanti Region, South by the Wassa Amenfi Central in the Western Region, West by the Sefwi Wiawso Municipal Assembly in the Western North Region and East by the Upper Denkyira West and Amansie East districts in the Central and Ashanti regions, respectively (Boadu et al., 2020; Kumi & Daymond, 2015). In addition, the assembly is also found in the equatorial climatic zone with rainfall averages between 1200mm and 1500mm per annum with bimodal rainfall distribution thus, from March-August and September-October (Kumi & Daymond, 2015; GSS, 2014) .

The average birth rate in the assembly is 94.6 per 1000 women who are between the ages of 15 and 49. About four in ten (45.5%) of people who are at least 12 years old are married. The municipality has female (15-49) years population size of 30,844 (GSS, 2014). Pregnant women attending Antenatal Care

(ANC) at Green Shield Hospital (GSH), Anhwiaso Community Hospital (ACH) and Nana Amoako Diagnostic Centre (NADC) in the assembly were recruited for the study. GSH, ACH and NADC are core health facilities located within the BABM in the western north of Ghana legally licensed and operate under the ministry of health (MOH) to render quality healthcare service. Some core services that are provided by GSH are: out and in -patients' services, maternal care, eye clinic, general surgeries, orthopedics, urology, environmental and occupational health care, and medical diagnostics (X-ray, gynecology and obstetrics examinations, endoscopic and laboratory). GSH is located at Sefwi Bekwai opposite Alla junction. Also, ACH provides in- and out -patients' service, general surgeries, urology, maternal care and medical diagnostics (X-ray, gynecology and obstetrics examinations, laboratory). ACH is located at Sefwi Anhwiaso, adjacent Queen's secondary school. Moreover, some core health -care services that are provided by the NADC are: maternal health care or ANC, medical diagnostics (gynecology and obstetrics examinations), out- patient services to the general public in the BABM. NADC is located at Sefwi Bekwai adjacent Lord FM.

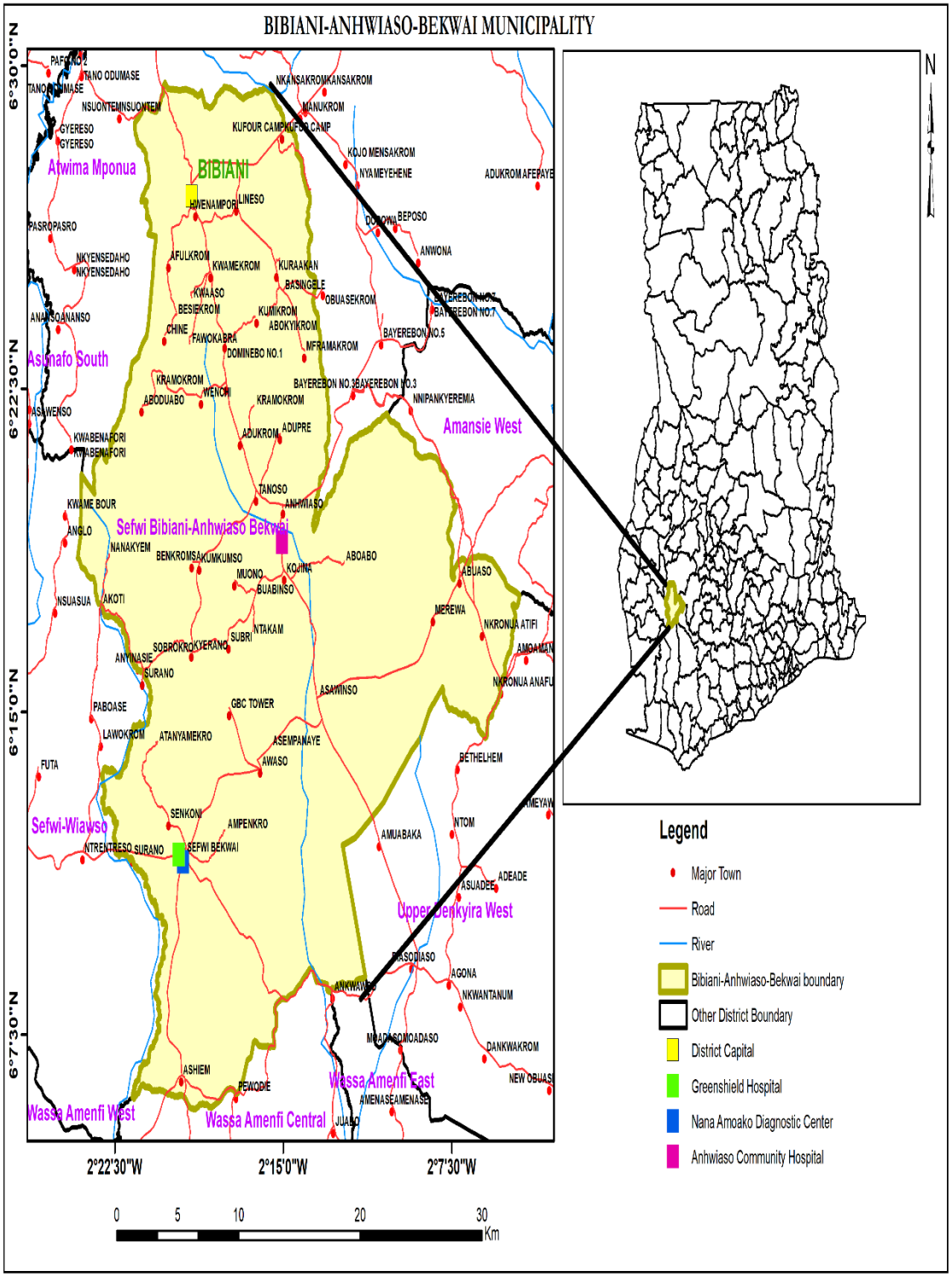

Figure 3. 1 Map of Bibiani Anhwiaso Bekwai showing the various study sites.

Retrieved from GIS/remote sensing unit, RMSC-forestry commission 2023.

Study design

A case control study of pregnant women with or without *Pf* infection was conducted in Bibiani Anhwiaso Bekwai municipality (BABM) to determine HLA-G, hematological and liver biochemical profile and their association among the two groups. The study population consisted of pregnant women attending antenatal care (ANC) at two (2) central hospitals, one (1) diagnostic center namely: GSH, ACH and NADC of the municipality respectively. The study consists of detailed flow chart **(figure 3.2).** Various study sites were visited on their ANC clinic days. GSH and NADC had their ANC clinic days on Wednesdays while ACH had Wednesdays and Fridays. Pregnant women, irrespective of their gestational period were educated about the study before their concerns were sought. Negative and positive results for HBV, HIV were prechecked from their ANC book. Pregnant women who tested positive for HBV and HIV from their ANC book were instantly excluded with no further engagement. On the other hand, consent was sought from pregnant women who tested negative from their previous results and first time ANC attendants. Structure questionnaire were administered and about 5mls venous blood samples were also taken from the study participants. Serological tests: HBV, HCV, and HIV were repeated in duplicates for all the study participants. The study was conducted at a four (4) month duration from March 2022 - June 2022. A total of 200 pregnant women consisting of 150, 35 and 15 from GSH, ACH and NADC respectively were recruited. About 20 pregnant women out of the 200 tested positive for serological tests. Thus, 15, 4 and 1 for HBV, HCV and HIV respectively. The remaining 180 were tested for *P. falciparum* using first response

Pf. Ag HRP2 test kit. About 46 tested positive for malaria (cases) whereas 134 tested negative, out of which 46 were selected as the control samples considering their trimester and IPT-SP status, preferably 3rd and 2nd or more doses respectively.

Sample Size

The study's estimated sample size was 272. This calculation was achieved at 95% and 23% confidence interval and prevalence respectively (Boadu et al., 2020).

Sample size estimation

$$n = \frac{Z^2\, pq}{d^2}$$

Where n is the sample size

Z is a constant of 1.96 representing 95% confidence interval

p is the prevalence = 23.0% (Boadu et al., 2020).

q is 1-p (1-0.23=0.77), d is the margin of error (5%). Therefore,

$$n \text{ (Minimum number of participants)} = \frac{1.96^2\ (0.23)(0.77)}{(0.05)^2} = 272$$

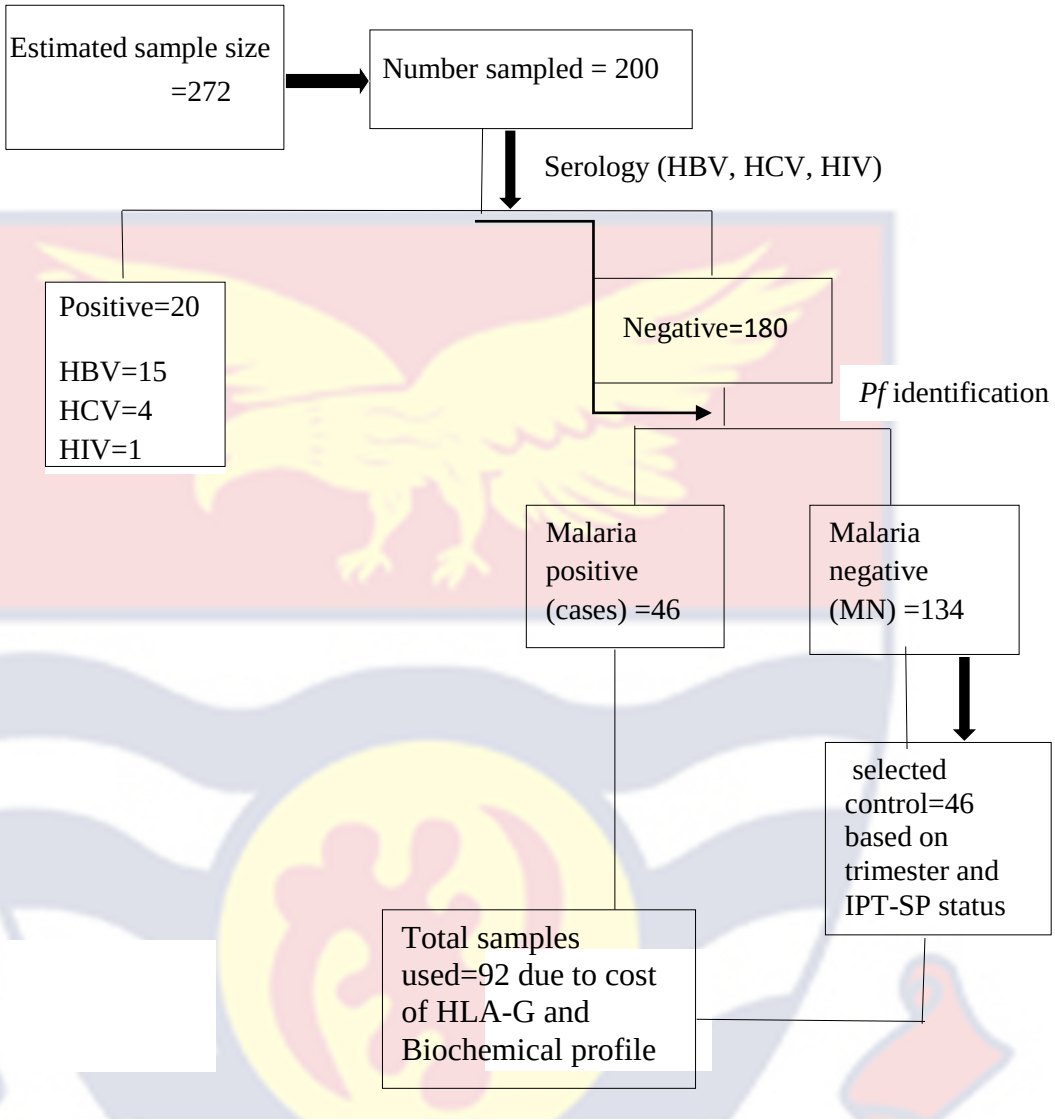

Figure 3. 2 Flow chart of the study participants

Selection criteria

Inclusion Criteria

Pregnant women with or without malaria infection (*Pf*) and had no medical condition of Hepatitis (B, C) or HIV who consented to participate in the study were recruited.

Exclusion criteria

Pregnant women with or without malaria infection (*Pf*) but had medical condition of HBV, HCV, and HIV were not included in the study.

Ethical consideration

Ethical approval was sought from UCC Institutional Review Board (UCCIRB/CHAS/2022/153). Internal approval was also obtained from the management of the various hospitals prior to data collection. Informed consent was obtained from the study participants after a candid explanation of the research protocol. All participants who could not read or write were guided by a witness and endorsed by thumb printing. Anonymity of participants was dully conserved.

Data Collection

Collection of Socio-Demographic Data

Socio-demographics such as age and level of education were collected from the study participants through structured questionnaire. The questionnaire had three (3) sections, thus: socio-demographic data, clinical history of pregnancy and IPT

Measurement of participants' axillary temperature

The body temperature of each participant was measured using an electronic thermometer. Temperature readings displayed were observed and recorded in degree Celsius (°C).

Blood Sample collection

About 5mls of venous blood were aseptically taken from each participant using sterile syringe. Three (3ml) was dispensed gently into ethylenediaminetetraacetic acid (EDTA) (K3EDTA:KOO291573, BDe glass) test

tube for the determination of hematological parameters, estimation of HLA -G level and screening for malaria infection. The remaining 2ml were also equally dispensed gently into serum separating tubes (SST) (SST: SG200404) and spun at 1500 rpm for 5mins to obtain serum for the determination of the liver biochemical parameters and serological tests. Pregnant women who tested positive for any of the serological tests were contacted to seek for treatment and management. The remaining whole blood in the EDTA test tubes was also spun at 1500rpm for 5mins to obtain plasma. Plasma for HLA-G were stored at -80°C in Cryo tubes and serum obtained as a result of centrifugation were also kept in Cryo tubes and stored at 2-8℃ until ready for immunological and biochemical analyses respectively.

Laboratory investigations

Determination of *Pf* infection

Pf infection was determined using a rapid diagnostics test kits (first response malaria *Ag P. falciparum*, HRP2) following the manufacturers manual. Briefly, 5µl of whole blood was slowly dispensed into the test well using a micro pipette (2-10µl; AU00204). Two (2) drops of the assay buffer was added to the assay buffer well. Results were then observed and recorded within 20 mins. Positive results appeared as two bands on the test kit while negative results appeared as only one band at the control line (C) region. Invalid tests were repeated for accuracy and reliability whereas valid tests were done in duplicates.

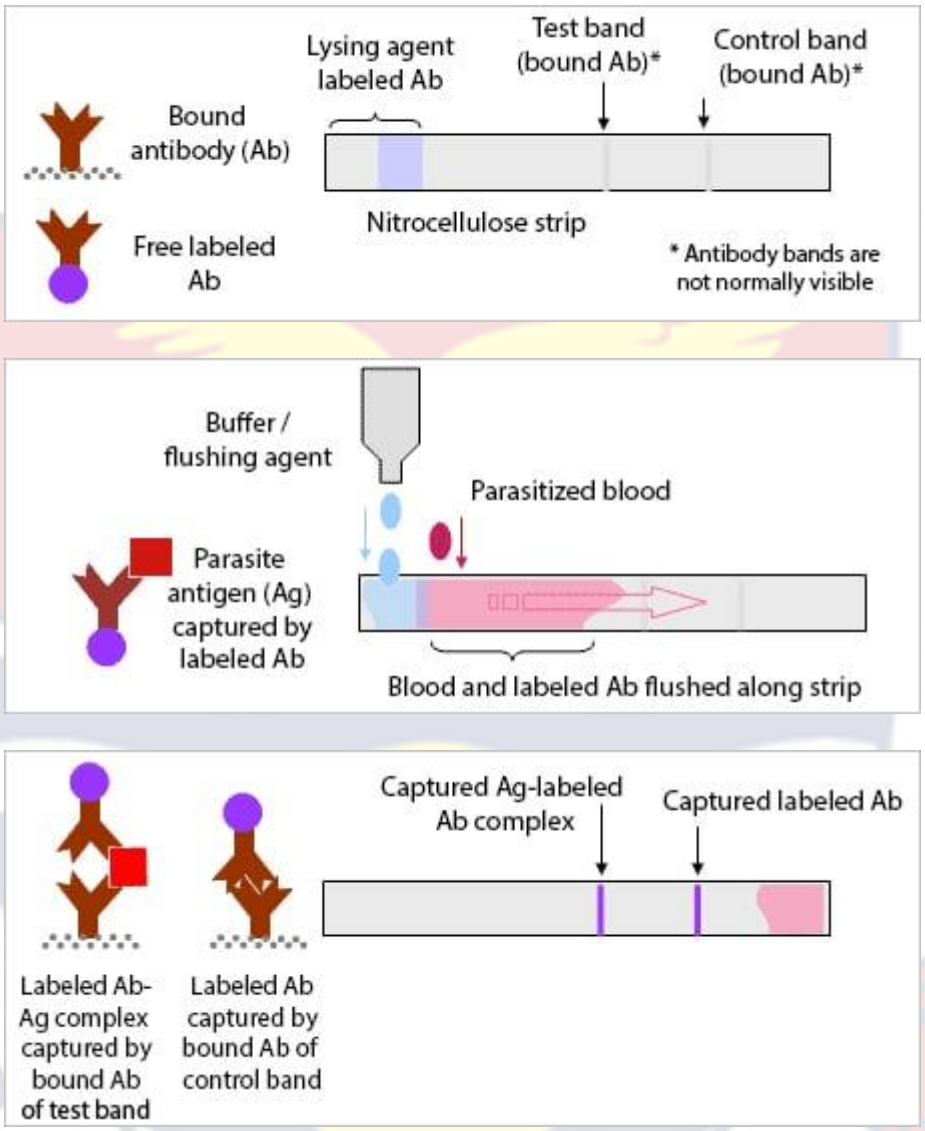

Figure 3. 3 Mode of action of malaria RDT procedure. Retrieved from (Wilson, 2012)

Determination of hematological parameters

Hematological parameters such as WBC, HGB, and PLT were estimated using an automated hematology analyzer (Mindray BC-2800). The machine operated on principles of impedance method for determining WBC, RBC, and PLT and the colorimetric method for determining HGB. Whole blood in K3EDTA

test tube was aspirated through the analyzer's probe, and within 2 minutes the differential results were displayed and printed (Fiseha et al., 2022).

Determination of liver biochemical parameters

All biochemical parameters were assayed using serum. The serum was obtained as a result of spinning clotted blood in SST tubes at 4000rpm for 5mins using 80-2B centrifuge. Serum that was not assayed within 24hrs were stored at 2-8℃ for a maximum of 3 days. Biochemical parameters that were assayed are Total Bilirubin (TB), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP). All the biochemical parameters were assayed using semi-automated chemistry analyzer (BC300 VS; 2.6.27.8-Smart-ver 2.0.4). Med source biochemical reagents were used. Below are the details of how each parameter was assayed.

Total Bilirubin (TB) (BIL-040721)

About 1000µl of total bilirubin reagent (R1) was pipetted into 2 test tubes for each sample and 50µl of R2 (T-nitrite Reagent) was added to the test tube (test tube B). The mixture was mixed thoroughly and incubated at room temperature (25℃) for 5 min. Then 100µl of the serum was added to the mixture and also incubated at 25℃ for another 5mins. The optical densities (OD) of the samples were measured against sample blank (test tubes without R2-T- nitrite reagent) at 546nm. Concentrations were recorded at a factor of 222.3 in µmol/L.

AST (ASAT-050621)

About 1000µl of AST working reagent was pipetted into the sample test tubes and 100µl of serum was added. The mixture was mixed well and incubated at (37℃) using thermostatic water bath for 1min. The change in OD was

measured photo -metrically at 340nm and concentrations were recorded at a factor 1746 of in IU/L.

ALT (ALT-091121)

About 1000µl of ALT working reagent was pipetted into test tubes (Lot: SG2800803). Then 100µl of serum was added. The mixture was incubated at 37℃ for 1min and the change in optical density was measured photometric ally at 340nm concentrations were recorded at a factor 1746 of in IU/L.

ALP (LOT: 16542)

About 20µl of serum was added to 1000µl of ALP working reagent. The mixture was mixed thoroughly and incubated at 37℃ for 5mins using digital thermostatic water bath (model: YSTE-SG043). The change in OD was measured at 405nm with its concentrations displayed at factor of 2764 and recorded in IU/L

HLA-G quantification

HLA-G concentration of the plasma samples were determined using Sandwich Enzyme-linked Immunoassay (ELISA) (Maxisorp Elisa Plate) by following the manufactures manual: Briefly, 100µl of capture antibody MEM-G/9 (lot:531294) was pipetted into 10ml of Phosphate Buffered Saline (PBS) solution in a 15ml falcon tube and vortexed for about 1min to obtain uniform solution. ELISA microliter plates (MAXISORP) were coated with 100ul of the solution containing PBS and MEM-G/9 and incubated over night at 4℃ without agitation. After the overnight incubation, each well of the plate was washed 4times with 250ul of a washing buffer (PBS tween20: 0.05%) with 1 min interval.

Additionally, 300µl of the diluent (a blocking agent) was transferred into each well of the ELISA plate and incubated for 1hour at room temperature with

shaking at 400RPM. Each well was again washed 4 times with the washing buffer. Further to this, 50µl of the prepared solution containing 100µl supernatant of the plasma samples and DAKO diluent respectively was transferred into the pre-washed ELISA plate and 50µl of the DAKO diluent was then added and incubated at room temperature for 2 hours with agitation at 400RPM. Standard was transferred in serial dilutions. Standards, positive and negative control, blanks and samples were transferred in duplicates. Each well was again washed 4times using 250µl of the washing buffer with 1 min interval after incubation. Also, 100µl of a secondary antibody solution containing 10ml of PBS buffer and 1µl of β_2 microglobulin was transferred into each well of the pre washed ELISA plate and incubated at room temperature for 1hour with shaking at 400RPM. Wells were again washed 4times with 250µl of washing buffer at 1 min interval. Then, 100µl of an enhancer containing 10ml of PBS and 50ul of DAKO envision System HRP (lot: 10151788: ref: k4003) was then added to each well and incubated for 1 hour with shaking at 400RPM. Each well was again washed 4 times with the buffer. Again, 150µl of the substrate Tetramethylbenzidine (TMB) was transferred into each well and incubated at ambient temperature in the Dark for 35mins without agitation. OD was then measured at 620nm and 100µl of HCL (IN) was transferred into each well to stop the enzymatic reaction. OD was again measured at 450nm. All ODs were measured using thermo-scientific Multiskan FC version 1.01.14 micro plate reader and converted into their corresponding concentration using the ADAMSEL FPLb040 software.

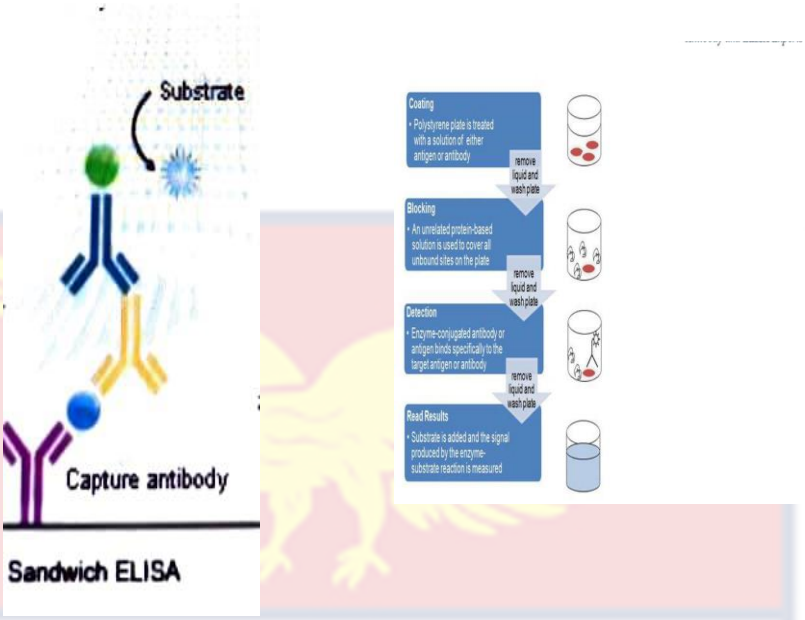

Figure 3. 4 Sandwich ELISA procedure for HLA-G retrieved from (Ave. et al., 2016)

Statistical analysis

Data obtained from the study was stored in Microsoft Excel 2010. IBM SPSS Statistics 21 and Graphpad Prisms 9.0 were used for the statistical analysis. T –test ([a]) reporting mean and standard deviations (SDs), chi-square ([b]) reporting frequencies and percentages were done for the socio-demographic and clinical characteristics of the study participants. Again, chi-square analysis was done for hematological anomalies amongst the study participants. Parametric and non-parametric analysis was done for hematological profile among pregnant women with or without malaria infection where comparison of the two groups was done using independent sample T- test and Mann-Whitney U test respectively. Multivariate regression model with or without adjustment was done to explore the association of HLA-G and biochemical levels among the pregnant women. Comparisons of the mean values of HLA-G and biochemical profile

among trimesters of the study participants were made using one-way analysis of variance (ANOVA). Linear regression of age and biochemical profile as well as association of HLA-G and biochemical levels with miscarriage status was done among the two groups using Mann-Whitney U test. Statistical significance was considered for all $p<0.05$.

Chapter summary

This is a case control study where study participants were recruited from the Western North Region of Ghana. A total of 200 participants were recruited using convenient sampling technique. About 20 pregnant women tested positive for serology which ascertained their exclusion. Auxiliary temperature of study participants was measured using electronic thermometer and recorded in degree Celsius. The remaining 180 participants were tested for *Pf* infection using first response *Pf. Ag*. HRP2 test kits. About 46 tested positive (cases) whereas 134 tested negative. About 46 participants, out of the 134 were selected as the control group considering their trimester and IPT-SP status preferably 3rd and 2nd doses respectively.

Ethical clearance was obtained from UCC institutional Review Board with clearance number (UCCIRB/CHAS/2022/153). Moreover, approval was sought from the management of the various sampling facilities.

Hematological and biochemical profiles were determined using automated hematology and chemistry analyzer respectively. On the other hand, HLA-G levels were obtained using sandwich Elisa.

Data obtained from the study was stored in Microsoft excel 2010. IBM SPSS statistics 21 and graph pad prism 9.0 were used for the statistical analysis.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

This chapter consist of two sections, thus results and discussions. The results include presentation of data obtained in tables and figures whiles discussion relates the findings to previous knowledge available.

Results

The study aimed at determining HLA-G levels, hematological and biochemical profile among pregnant women with or without malaria (*Pf*) infection. Socio-demographics and clinical characteristics of study participants were considered. Blood samples were collected for the laboratory examinations. HLA-G, biochemical profiles such as TB, AST, ALT, ALP and hematological parameters such as white blood cells, platelets, lymphocytes and granulocytes were determined. Appropriate statistical analysis was done and $p< 0.05$; or < 0.001 was considered significant.

Socio-demographic and clinical characteristics of the study participants

The mean ages obtained for the cases and control are 26.13 ± 5.54, 27.09 ± 6.47 respectively (Table 4.1). It was observed that 15(34.9%) and 31(63.3%) with or without IPT-SP respectively tested positive for *Pf*. Again, 28(65.1%) and 18(36.7%) of the participants with or without IPT tested negative for *Pf*. The use of IPT was statistically significant among the two groups (p=0.007). The mean temperature for the two groups, thus malaria cases and control were (36.56±0.74) and (36.41±0.47) respectively. Temperature amongst the two groups differ significantly (p=0.045). However, 45.2% and 54.8% among the cases and the

control group respectively had experienced miscarriage before with no significant association **(**Table4.2). In addition, the two groups were statistically insignificant for age, trimester, educational level and ITNs (p>0.05).

Table 4. 1: Socio-demographic characteristics of the study participants

Variable	**Cases**	**Control**	***p-value***
Age(years) Mean±SD	26.13 ± 5.54	27.09 ± 6.47	0.518[a]
Educational level			0.710[b]
None	9 (56.2)	7 (43.8)	
Primary	7 (50.0)	7 50.0)	
Junior high	15 (41.7)	21 (58.3)	
Secondary	11 (61.1)	7 (38.9)	
Tertiary	4 (50.0)	4 (50.0)	

[a] P-values were obtained by T-test, [b] p-values were obtained by Chi-square.

Table 4. 2: Clinical characteristics of the study participants

Variable	Cases	Control	*p-value*
Trimester			0.105[b]
1st	8 (57.1)	6 (42.9)	
2nd	19 (63.3)	11 (36.7)	
3rd	19 (39.6)	29 (60.4)	
IPT-SP status			**0.007**[b]
Yes	15 (34.9)	28 (65.1)	
No	31 (63.3)	18 (36.7)	
IPT-SP frequency			0.369[b]
Once	4 (50.0)	4 (50.0)	
Twice or more	11 (32.4)	23 (67.6)	
Temperature (℃) Mean±SD	36.56 ± 0.74	36.41 ± 0.47	**0.045**[a]
ITN'S usage			0.666[b]
Yes	30 (51.7)	28 (48.3)	
No	16 (47.1)	18 (52.9)	
Miscarriage status			0.508[b]
Yes	14 (45.2)	17 (54.8)	
No	32 (52.9)	29 (47.1)	

[a] P-values were obtained by T-test, [b] p-values were obtained by Chi-square.

IPT: Intermittent Preventive Treatment, ITN: Insecticide Treated Net

Hematological profile among pregnant women with or without malaria infection

Comparison of the infectious status of the study participants and hematological profile was done using Independent Sample T-test reporting Mean±SD and Mann Whitney U test reporting Median (IQR). It was observed that percent lymphocytes and percent granulocytes of white blood cell indices were statistically significant for the two groups (p=0.04, p=0.03) respectively (Table 4.3)

Table 4. 3: Hematological profile among pregnant women with or without malaria infection

Variables	Cases	Control	*p-value*	Reference ranges
Red blood cell indices				
RBC [b] x10^12/L	3.82(0.64)	4.00(0.62)	0.09	3.5 - 5.00
MCV[a] fL	78.91±8.02	77.86±7.44	0.52	80.0 -100.0
HCT[a] %	30.08±3.99	30.96±3.65	0.28	37.0 - 47.0
MCH[a] Pg	27.71±3.59	27.80±3.24	0.91	27.0 - 34.0
MCHC[a] g/dl	35.13±1.83	35.70±2.19	0.17	32.0 - 36.0
RDW-CV [b] %	14.20(1.25)	13.95(2.05)	0.43	11.0 - 16.0
RDW-SD [b] fL	39.00(6.05)	36.80(5.98)	0.12	35.0 - 56.0
HGB[a] g/dl	10.57±1.59	11.03±1.40	0.14	11.0 - 15.0
White blood cell indices				
WBC[a] x10^9/L	6.62±2.76	6.86±2.08	0.64	4.0 - 10.0
LYMPH (%) [b]	**28.20(11.75)**	**31.65(14.55)**	**0.04**	20.0 - 40.0
MID (%) [b]	6.70(3.98)	7.70(3.10)	0.17	3.0 - 14.0

GRAN (%) [b]	**65.00(13.80)**	**61.50(14.28)**	**0.03**	50.0 - 70.0
LYPM#[a] x10^9/L	1.78±0.72	2.16±0.68	0.11	0.8.0- 4.0
GRAN#[a] x10^9/L	4.37±2.35	4.11±1.65	0.55	2.0 - 7.0
MID#[b] x10^9/L	0.45(0.30)	0.50(0.40)	0.21	0.1 - 1.2
Platelet count				
PLT[a] x10^9/L	171.80±59.61	176.85±56.80	0.68	100 - 300

[a] Comparison of the two groups was done using Independent Sample T- Test
[b] Comparison of the two groups was done using the Mann-Whitney U Test. WBC-white blood cell count, Lymph#-Absolute lymphocyte count, MID#-Absolute mixed cell count, Gran# -Absolute granulocyte count, Lymph%-lymphocyte percentage, MID%-mixed cell percentage, Gran%-granulocyte percentage, HGB-hemoglobin, RBC- red blood cell count, HCT- hematocrit, MCV- mean cell volume, MCH- mean cell hemoglobin, MCHC- mean cell hemoglobin concentration, RDW-CV- red cell distribution width coefficient of variation, RDW-SD- red cell distribution width standard deviation, PLT-platelet count.

Hematological anomalies among pregnant women with or without malaria

Table 4.4 presents hematological anomalies among the study participants. Some anomalies that were investigated according to (Al-Mawali et al., 2018) and Green shield hospital laboratory reference ranges (GSHLR) are anemia, leukocytosis/leukopenia,thrombocytosis/thrombopenia,lymphocytosis/lymphopenia,andgranulocytosis/granulocytopenia. Lower and upper limits of the reference ranges were considered in determining these anomalies. There was no statistical difference among the hematological anomalies of the two groups (p>0.05).

Table 4. 4: Hematological anomalies among pregnant women with or without malaria

Variable	Cases (N = 46)	Control (N = 46)	*p-value*
Hemoglobin			0.210
Normal	28 (57.1)	21 (42.9)	
Anemia	18 (41.9)	25 (58.1)	
Leukocytes			0.338
Normal	36 (46.8)	41 (53.2)	
Leukocytosis	3 (60.0)	2 (40.0)	
Leukopenia	7 (70.0)	3 (30.0)	
Thrombocytes			0.473
Normal	41 (50.0)	41 (50.0)	
Thrombocytosis	0 (0.0)	1 (100.0)	
Thrombopenia	5 (55.6)	4 (44.4)	
Lymphocytes			0.235
Normal	30 (48.4)	32 (51.6)	
Lymphocytosis	8 (42.1)	11 57.9)	
Lymphopenia	8 (72.7)	3 (27.3)	
Granulocytes			0.189
Normal	26 (45.6)	31 (54.4)	
Granulocytosis	13 (68.4)	6 (31.6)	
Granulocytopenia	7 (43.8)	9 (56.3)	

p-values were obtained using chi-square.

Reference ranges: Haemoglobin: Normal ≥11.0g/dl, Anaemia <11.0g/dl, Leucocytes: Normal 4.0-10.0x10^9/L, Leucocytosis >10.0x10^9/L, leukopenia<4.0 x10^9/L Platelet: Normal 100.0-300.0 x10^9/L thrombocytosis >300.0 x10^9/L, Thrombopenia <100.0 x10^9/L, Lymphocytes: Normal 20-40% lymphocytosis >40%, lymphopenia <40%. Granulocytes: Normal 50-70%, Granulocytosis >70% granulocytopenia <50%.(Al-Mawali et al., 2018) and as defined by GSHL.

Mean HLA-G and biochemical profile among the study participants

Comparison of mean distribution of HLA-G and biochemical profile among the cases and controls of the study participants was done using independent sample T- test reporting mean±SD. Higher and lower mean HLA-G concentrations was observed between the cases and the controls respectively with no statistical significance ($p>0.05$). Also, insignificantly higher and lower concentrations of TB among the cases and controls respectively was observed among the two groups ($p>0.05$). However, lower and higher concentration of ALT among cases and control respectively with statistical significance was also observed ($p<0.05$). Lower concentrations of AST and ALP were observed among the two groups with no statistical significance ($p>0.05$) (Table 4.5).

Table 4. 5: Mean HLA-G and biochemical profile among the study participants

Variables	**Cases**	**Control**	***p-value***
HLAG (ng/ml)	54.48±32.42	50.43±40.72	0.67
TB (µmol/L)	14.33±10.91	10.53±7.50	0.06
AST (IU/L)	27.40±28.82	40.00±33.00	0.48
ALT (IU/L)	**11.80±7.29**	**17.32±10.94**	**0.01**
ALP (IU/L)	206.03±116.87	251.14±108.99	0.68

p-value was obtained using independent sample T-test. HLA-G- Human Leukocyte Antigen-G, TB-Total Bilirubin, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase.

Association of HLA-G and biochemical levels with *Pf* infection among the pregnant women with or without malaria infection

Multivariate regression analysis was done to assess the correlation of HLA-G and biochemical profile to malaria infection. Model 1 was the crude analysis for all the variables whereas model 2 was adjusted for age as a cofounder. The result was reported as Beta Coefficient (β) and *P*-values at 95% CI. It was observed in model 1 that both ALT (β =7.091, p=0.011) and ALP (β =13.3011, p=0.017) were up regulated with significance difference in pregnant women with malaria as compared to pregnant women without malaria. However, it was observed that, there was no significance difference (p>0.05) for HLA-G (β =0.321, p=0.3), TB (β =0.312,p=0.056), AST(β =2.455, p=0.059) even though they were up regulated in the pregnant women with malaria as compared to pregnant women without malaria. As the model was adjusted (model 2) for age as a cofounder, it was observed that ALP (β =17.524, p=0.02) was up regulated among pregnant women with malaria as compared to the negative control. There was significance difference for ALP (adjusted) among the study participants. HLA-G (β=1.234,p=0.861), TB (β =0.287,p=0.09), AST (β=1.739,p=0.28), ALT(β=5.248,p=0.056) were up regulated in pregnant women with malaria as compared to the negative control but with no significance difference (p>0.05) (Table 4.6).

Table 4. 6: **Association of HLA-G and biochemical levels with *Pf* infection among the pregnant women with or without malaria infection**

Variables	Crude [a] (Model 1)		Adjusted[b] (Model 2)	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
HLAG	0.405(0.05-3.068)	0.38	1.234(0.11-12.919)	0.861
TB	0.312(0.94-1.032)	0.056	0.287(0.67-1.234)	0.09
AST	2.455(0.967-6.232)	0.059	1.739(0.627-4.820)	0.28
ALT	**7.091(1.563-32.182)**	**0.011**	5.248(0.95-28.8)	0.056
ALP	**13.301(1.577-112.208)**	**0.017**	**17.524(1.534-200.20)**	**0.02**

Multivariate regression analysis with and without adjustment, estimated effect of covariate on HLA-G and Biochemical profile .CI: confidence interval. The model ([a]) crude analysis without adjustment and ([b]) adjusted for Age. HLA-G and biochemical were log10 transformed. The negative control (pregnant women without *Pf* infection) was set as reference for the model. HLA-G- Human Leukocyte Antigen-G, TB-Total Bilirubin, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase.

Association of HLA-G, hematological and biochemical profile of the study participants

Multivariate regression with estimated model coefficient (β) was done to assess the association between HLA-G, hematological and liver biochemical profile. It was observed that WBC inversely correlated with TB (β=-36.80*, $p<0.05$). MID# positively correlated with TB, AST, ALP (β=44.01*, β=140.08*, β=713.43, $p<0.05$) respectively whereas MID (%) inversely correlated with ALP and AST (β=-23.93, β=-7.45*, $p<0.05$) respectively. However, GRAN# positively correlated with TB (β=36.54*, $p<0.05$). Again, LYMP (%) had positive correlation with AST (β=1.77*, $p<0.05$). Moreover, PLT was negatively

associated with TB (β=-0.49*, p<0.05). However, HLA-G was not significantly correlated with the hematological parameters. All the variables were estimated at significance difference of p<0.05. Hematological variables: LYMPH#, HGB, RBC, RDW-CV, HCT, MCV, MCHC had no significance association among liver biochemical profile among the study participants, p>0.05 (Table 4.7).

Table 4. 7: Association of HLA-G, hematological and liver biochemical profile among the study participants

	HLA-G (ng/ml)	**TB (µmol/L)**	**AST (IU/L)**	**ALT (IU/L)**	**ALP (IU/L)**
Red blood cell indices					
RBC x10^12/L	-26.85	-7.65	-34.75	8.68	-198.57
MCV Fl	3.88	-1.22	4.12	-0.71	-8.23
HCT %	-0.09	0.09	-1.24	-2.73	-28.36
MCH Pg	-19.34	1.42	-16.9	1.80	-10.86
MCHC g/dl	10.88	-2.29	5.47	-3.22	-43.75
RDW-CV %	-5.21	-1.34	6.06	-0.27	-5.34
RDW-SD fL	1.40	0.35	-0.39	0.01	3.35
HGB g/dl	17.38	2.61	11.17	5.49	137.74
White blood cell indices					
WBC x10^9/L	7.25	**-36.80***	-28.89	27.59	-360.48
LYMPH (%)	0.28	0.03	**1.77***	-0.10	1.96
MID (%)	-0.12	-0.65	**-7.45***	0.05	**-23.93***
LYPM# x10^9/L	-15.00	32.19	10.55	-26.10	304.93
GRAN# x10^9/L	-10.01	**36.54***	28.14	-28.16	357.25
MID# x10^9/L	-4.58	**44.01***	**140.08***	-33.41	**713.43***
Platelet count					
PLT x10^9/L	0.03	**-0.49***	-0.03	-0.02	-0.23

Multivariate regression analysis with estimated model coefficients(**β**) *Significant at p<0.05.WBC-white blood cell count, Lymph#-Absolute lymphocyte count, MID#-Absolute mixed cell count, Gran# -Absolute granulocyte count, Lymph%-lymphocyte percentage, MID%-mixed cell percentage, HGB-hemoglobin, RBC- red blood cell count, HCT- hematocrit, MCV- mean cell volume, MCH- mean cell hemoglobin, MCHC- mean cell hemoglobin concentration, RDW-CV- red cell distribution width coefficient of variation, RDW-SD- red cell distribution width standard deviation, PLT-platelet count, HLA-G- Human leukocyte antigen-G, TB-Total Bilirubin, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase.

Effects of clinical characteristic (trimester) on HLA-G and biochemical profile among the study participants

Table 4.8 presents the mean values of HLA-G and each biochemical parameter among trimester of pregnancy of the study participants. Statistical significance between the means was determined by one-way analysis of variance (ANOVA). HLA-G was observed to decrease across trimester with no statistical significance (P>0.05). Among the biochemical profiles, only ALP increased across trimester of pregnancy with statistical significance (p<0.05) among 2nd and 3rd trimester (Table 4.8)

Table 4. 8: Mean HLA-G and biochemical profile among trimester of the study participants

Variables	1st trimester	2nd trimester	3rd trimester	*p-value*
HLA-G (ng/ml)	65.88±68.84 [a]	56.27±36.52 [a]	46.16±19.12 [a]	0.16
TB (µmol/L)	7.50±4.34 [a]	13.18±9.00 [a]	13.39±10.54 [a]	0.11
AST (IU/L)	36.07±45.62 [a]	31.86±24.42 [a]	34.16±31.12 [a]	0.91
ALT (IU/L)	17.12±13.19 [a]	14.14±9.48 [a]	14.07±8.62 [a]	0.56
ALP (IU/L)	**181.32±62.64[ab]**	**198.88±127.51[a]**	**260.94±109.76[b]**	**0.02**

p-value was obtained using one-way analysis of variance (ANOVA). Significant at p<0.05. Means with Superscripts of different letters are significant (posthoc test) HLA-G- Human Leukocyte Antigen-G, TB-Total Bilirubin, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase.

Effects of clinical characteristic (IPT-SP) on HLA-G and biochemical profile among the study participants

Comparison of the effect of intermittent preventive treatment (IPT-SP) on HLA-G and biochemical profile was done using independent sample T- test reporting mean±SD. It was observed that pregnant women who had taken IPT-SP had insignificantly lower levels of HLA-G as compared to pregnant women without IPT-SP ($p>0.05$). However, ALP was statistically significant among the two groups ($p<0.05$) Table 4.9

Table 4. 9: Mean HLA-G and biochemical profile with IPT status among the study participants

Variables	IPT-SP (Yes)	IPT-SP (No)	*p- value*
HLA-G (ng/ml)	45.03±**16.92**	58.97±**46.96**	0.06
TB (µmol/L)	12.43±**9.30**	12.42±**9.78**	0.9
AST (IU/L)	34.30±**32.68**	33.18±**30.67**	0.87
ALT (IU/L)	15.49±**9.09**	13.74±**10.14**	0.39
ALP (IU/L)	**257.14±125.98**	**203.53±98.29**	**0.03**

p-value was obtained using independent sample T-test. HLA-G- Human Leukocyte Antigen-G, TB-Total Bilirubin, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase, sulfadoxine-pyrimethamine intermittent preventative treatment (IPT-SP)

Association of HLA-G and Biochemical levels with Miscarriage status among the study participants

Among the study participants, 31 had experienced miscarriage before and 61 had not experienced any miscarriage before. Association of HLA-G and biochemical

levels with miscarriage status revealed that, the mean HLA-G for participants with miscarriage was higher than the mean HLA-G of participants without miscarriage**.** It was observed that there was significance difference in HLA-G level among pregnant women with or without miscarriage (p=0.001). However, there was no significance difference in the biochemical levels among the pregnant women with or without miscarriage (Table 4.9)

Table 4. 10: **Association of HLA-G and Biochemical levels with Miscarriage status among the study participants**

	Miscarriage status		
Variable	**Yes(N=31)**	**No(N=61)**	**P value**
HLA-G	**46.25(37.6)**	**39.33(21.29)**	**0.001**
TB	11.55(13.44)	8.64(9.94)	0.48
AST	19.69(19.83)	33.90(25.83)	0.34
ALT	10.67(9.68)	13.97(17.39)	0.54
ALP	185.04(152.77)	217.65(119.19)	0.92

Comparison of the two groups was done using the Mann-Whitney U Test. Significant at $p<0.05$ HLA-G- Human Leukocyte Antigen-G, TB-Total Bilirubin, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase.

Relationship between biochemical profile, HLA-G and age of pregnant women with or without malaria infection

A simple linear regression analysis was done to determine the correlation between liver biochemical levels and HLA-G with age. Although, HLA-G (R^2=0.004, p=0.57), AST (R^2=0.0031, p=0.09), ALT (R^2=0.014, p=0.26), ALP (R^2=0.000, p=0.94), TB (R^2=0.014, p=0.26) increased positively with age, their correlation was not statistically significant (p>0.05) (Figure 4.1).

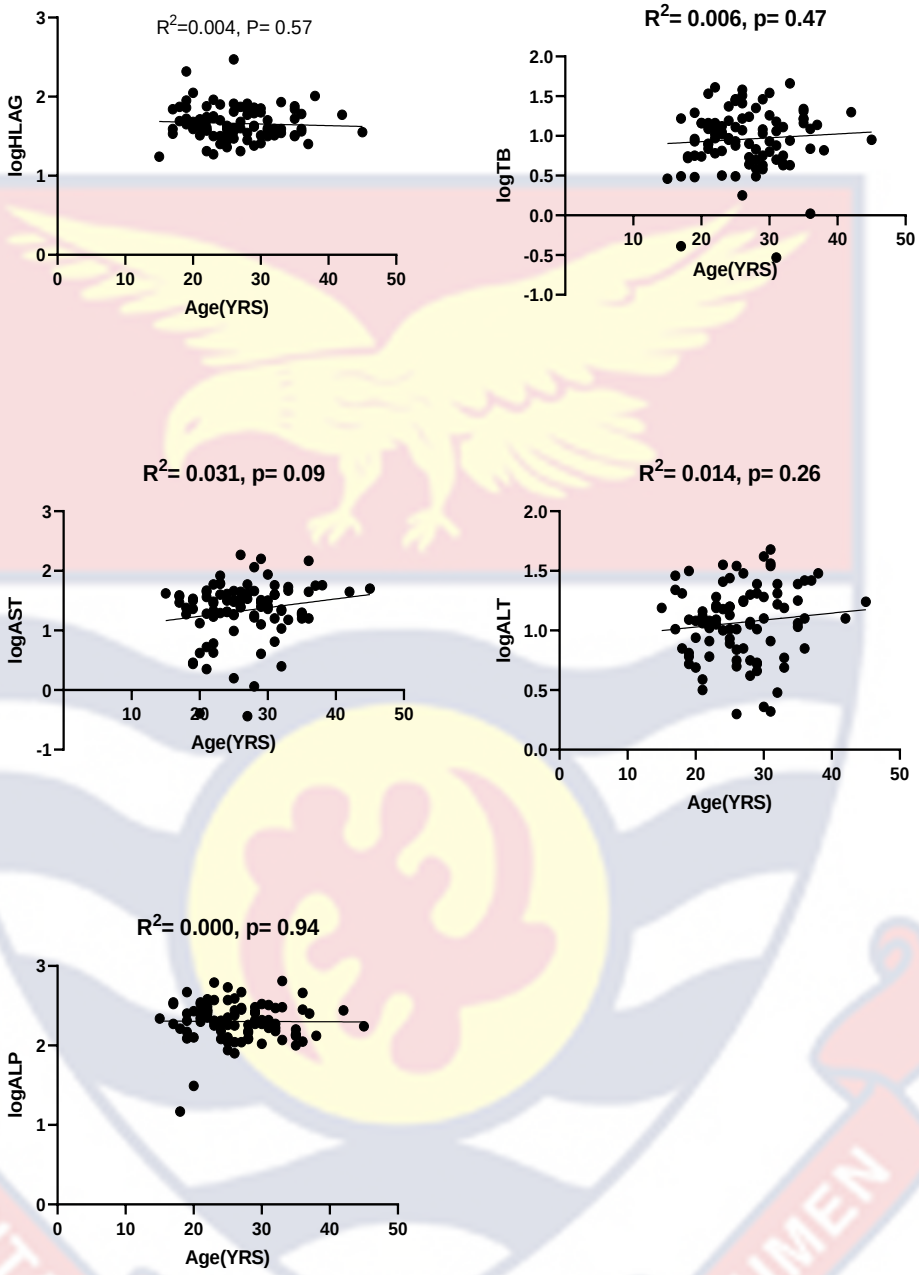

Figure 4. 1: Linear regression of Age, HLA-G and Biochemical profile of the study participants

Discussion

Pregnancy associated malaria (PAM) with its associated hematological alterations has been widely and globally studied due to its adverse effects such as low birth weight, spontaneous abortion and anemia on both the mother and the foetus (Digban et al., 2017; Ndamukong-nyanga et al., 2020). HLA-G is an immune-suppressor, and a predisposing factor for maternal parasitaemia, low birth weight, spontaneous abortion or miscarriage has received little attention in sub Saharan Africa especially Ghana (Sadissou et al., 2014; Najafi et al., 2021). The liver has been found to be essential in diseases pathogenesis. Previous studies have reported pregnancy associated liver conditions such as intra-hepatic cholestasis of pregnancy (ICP), Hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome (Guarino et al., 2020) and elevated levels of hepatocytes in parasitic infections such as malaria (Guarino et al., 2020; Anabire, 2017). This study hypothesized that plasma levels of HLA-G, hematological and biochemical parameters of *Pf* infected pregnant women will be significantly higher than pregnant women without *Pf* infection. Some previous studies done in Ghana revealed higher ALT,ALP, and TB (Anabire, 2017) and reported that pregnant women with malaria infection are more likely to have hematological abnormalities such thrombopenia and lymphocytosis than pregnant women without malaria infection (Akinosoglou et al., 2012; Sakzabre et al., 2020). Miscarriage in pregnancy is multifactorial but currently, little is known regarding the function of HLA-G in miscarriage especially in the Ghanaian populace. This study aimed at assessing the level of HLAG, biochemical profile and its

association with miscarriage among pregnant women with or without malaria infection.

Association of clinical characteristics among the pregnant women

The role of temperature in parasitic or vector disease such malaria has been established with some models revealing that mosquito maturation rate has a lower and maximum sensitivity at 24℃ and 30℃ respectively (Agusto et al., 2015). A study by (Oluleye & Akinbobola, 2010) recorded a significant association of temperature and malaria infection. This finding agrees with the present study which recorded a significant association of temperature and malaria infection. The mean temperature obtained agrees with (Agusto et al., 2015) who reported that the survival of malaria parasites is adversely affected by temperatures higher than 34°C leading to low parasitaemia since the lifespan of malaria parasites depends on temperature decreasing rapidly beyond 30℃, 32℃ (Agusto et al., 2015).

Using sulfadoxine-pyrimethamine intermittent preventative treatment (IPT-SP) in pregnancy to control malaria in pregnancy upon WHO revision of IPT policies was adopted in 2012 by Ghana (Dosoo et al., 2021) and achieving the global target of 80% for IPT-SP has been a challenge and this might be associated with low level of public education in Ghana (Ibrahim et al., 2017).

This study revealed 34.9% and 63.3% with or without IPT-SP tested positive for malaria respectively. Moreover, 50% and 32.4% who had one or more doses tested positive respectively. This is in contrast with the findings of (Dosoo et al., 2021) who recorded 16.1%, 16.8%, 10.4% and 9.7% of pregnant women who received ≤1, 2, 3, or ≥4 IPT-SP doses respectively. Even though these

findings differ, carry similar information thus, pregnant women who take ≤1 dose of SP are still at risk of malaria infection than those with ≥2 doses of SP. The use of IPT-SP among the two groups was statistically significant. Insecticide treated mosquito nets (ITN's) play role in malaria prevention (WHO, 2015) . However, 51.7% of ITN's users among the pregnant women had malaria infection. This could result from insecticides resistance by *P. falciparum* as recent report in Ghana indicated a surge of resistance to ITN's chemicals like pyrethroids (Baffour-Awuah et al., 2016). However, this study recorded insignificantly higher levels of AST and ALT among participants in their 1st trimester and this is similar to the findings of (Gok, 2023) who observed higher levels of AST and ALT with significance difference in the first trimester. Contrast in this study could be difference in sample size and geographical location. Also, Elevated mean ALP level of this study conforms with the findings of (Prakash & Pandeya, 2021) who reported slightly elevated mean ALP level in the 2nd trimester and highly increased level in the 3rd trimester with reduced ALT level in the 2nd and 3rd trimester among pregnant women.

Relationship between liver biochemical levels, HLA-G and age

Aging continues to be a risk factor in disease development including liver related diseases such as liver impairments or hepatocellular damage (Jin et al., 2020). However, age related liver injury and aging process of the liver remains unknown (Cieslak et al., 2016). Some authors have reported that decrease in hepatic blood flow, loss of liver volume and morphological changes are associated with old age especially at 65years as compared to those less than 40years (Cieslak et al., 2016; Jin et al., 2020).

This study recorded no correlation between age and the liver cells although a positive increase was observed. The study's findings are consistent and in contrast simultaneously with the findings of (Cieslak et al., 2016) which reported negative linear and no correction for group A and group B respectively in relation to age and liver function. Differences in these findings might be associated with age of participants involved in the studies since some liver parameters like bilirubin decrease with aminotransferase remaining normal as one gets older (Kim et al., 2016). Although HLA-G, was significantly associated with miscarriage, it had no correlation with age in this study despite its positive increase and this calls for further research with a larger sample size

Association of HLA-G and biochemical levels with *Pf* infection among pregnant women

HLA-G, a predisposing factor to maternal and infant parasitaemia, has been recently explored elsewhere which reported significance association of HLA-G and malaria infection among pregnant women (d'Almeida et al., 2019; D'Almeida et al., 2017). This study is the first to investigate the role of HLA-G in *Pf* infection and miscarriage in the Ghanaian population.

The present study reported no significance association of HLA-G and *Pf* infection among the pregnant women with or without malaria even though there was marginal increase of HLA-G levels among the cases. This finding is in contrast with earlier findings elsewhere which reported significance association of HLA-G and *Pf* infection (d'Almeida et al., 2019; D'Almeida et al., 2017). According to other studies, placental malaria may be associated with elevated

sHLA-G levels during pregnancy and inhibits the immune response by HLA-G expressions (D'Almeida et al., 2017).

This present study agrees to the findings of (Sadissou et al., 2014) which reported no significance effect of *Pf* infection on HLA-G levels. HLA-G levels could be influenced by a lot of factors such as viral infection, cellular stress, antimalarial preventive treatment during pregnancy as (Sadissou et al., 2014) reported that decreased levels of HLA-G was due to antimalarial preventive treatment given to pregnant women during their gestational period. This could be associated with the insignificance association obtained in this study since some of these factors were excluded in the previous studies (Sadissou et al., 2014).

Again, the exclusion of viral infection such as HBV, HCV, and HIV from this study could explain the variations in the findings with the others studies that established significant association between higher HLA-G level and viral infections (Sadissou et al., 2014). In addition, geographical location, sample size cannot be ignored in the contrast of these studies.

On the other hand, there was significant association of ALT without adjustment and ALP with or without adjustment in *Pf* infection whereas no significance association was observed for TB, AST with or without adjustment. This finding is similar to previous studies which reported significant levels of ALT, AST, TB with insignificant association of ALP among *Pf* infected pregnant women in the northern part of Ghana (Anabire, 2017) and (Uzuegbu & Emeka, 2011; Viriyavejakul et al., 2014) which reported significant association of transaminases (AST,ALT) and ALP in *Pf* infection. An increase levels of ALP in this study is not surprising and conforms with normal findings of ALP in

pregnancy since ALP is mostly associated with pregnancy due to the formation of placenta (Klinikleri & Gynecol, 2007; Sasamori et al., 2020). This indicates that rise in ALP does not indicate liver disorder in pregnancy. On the contrary, rise in ALT among *Pf* infected individuals indicates liver injury and should not be overlooked. This is because during normal pregnancy, ALT remains unchanged (Brady, 2020; Sasamori et al., 2020). The observed increase in ALT in this study could be associated with the release of hepatic cells as a result of injuries by the malaria parasites (Uzuegbu & Emeka, 2011) which agrees with previous suggestions that *Pf* infection is associated with liver injury (Sasamori et al., 2020). This is because ALT is a liver cell very sensitive to even minor injuries and mostly released into circulation, an indication of hepatic injury or disorder (WHO, 2020).

Association of HLA-G, hematological and biochemical profile among the study participants

Common symptoms of liver involvement in malaria infection include jaundice and increased aspartate and alanine transaminases (Al-Salahy et al., 2016b; Amor et al., 2017). However, another symptom of *P. falciparum* malaria is hyperbilirubinemia, which is partly caused by liver damage and is related to hemolysis of both infected and uninfected red blood cells. Malaria infection changes hematological and hepatic parameters, which aids in effective diagnosis and therapeutic intervention (Al-Salahy et al., 2016b; Amor et al., 2017).

In both in vitro and in vivo investigations, bilirubin, an end metabolic product of heme breakdown, has shown strong antioxidant and anti-inflammatory

characteristics and can restrict leukocyte or white blood cells migration (Amor et al., 2017; L. Zhang et al., 2019; X. Zhang et al., 2018).

In liver cells, bilirubin is conjugated with glucuronic acid with a strong anti-oxidant property against oxidants in the blood (Shaikh et al., 2020). This enhances the relevance of serum bilirubin investigation. This study recorded a significant inverse correlation between serum total bilirubin (TB) and white blood cell count (WBC). Findings from this study are consistent with earlier studies (Amor et al., 2017; Shaikh et al., 2020; L. Zhang et al., 2019; X. Zhang et al., 2018) which reported a significantly increase level of total bilirubin with decreasing levels of white blood cell counts (WBC). There is limited information about the mechanism of HLA-G and biochemical interaction with hematological parameters in malaria infections although alteration of hepatic parameters has been widely exploited in malaria infection by some authors (Al-Salahy et al., 2016a; Reuling et al., 2018).

Association of HLA-G and biochemical levels with miscarriage

Miscarriage is problematic especially during the first pregnancy periods (Fan et al., 2014). Some studies have explored factors such as chromosomal and uterine anomalies and immunological factors as the cause of miscarriage (Bhalla et al., 2006). Currently there is limited information about the role of HLA-G in miscarriage even though some studies elsewhere have reported the role of HLA-G in pregnancy loss (Barbaro et al., 2023; Moreau et al., 2008; Mosaferi et al., 2013). Interestingly, this study observed significant association of HLA-G with miscarriage. Findings from this study is in contrast to (Bhalla et al., 2006) which observed no significance association of HLA-G expression between women with

recurrent and normal pregnancies. On the other hand, this study is similar to the findings of (Fan et al., 2014) who reported significant association between HLA-G-14bp polymorphism and patients with three or more miscarriage. In addition, results of this study is again similar to (Ober et al., 2003) which observed a significant association of -725 c/G of HLA-G with fetal loss.

Moreover, a study conducted by (Najafi et al., 2021) also established a significant association of HLA-G genotypes (rs2735022 and rs1736933) with recurrent pregnancy loss (RPL). Also, some authors have discovered significant intraindividual differences of sHLA-G concentrations during pregnancy and have related these findings to prior miscarriages, according to (Barbaro et al., 2023). This is due to the fact that 8 of the 45 pregnant women studied had previous miscarriage. This result is consistent with the current study's observation as 31 pregnant women, out of 92 had previous miscarriage. Moreover, this study conforms with the findings of (Krop et al., 2022) who observed increased levels of HLA-G in pregnant women with previous miscarriage.

Factors responsible for miscarriage are multifactorial with limited information about the role of HLA-G. Nevertheless, some authors have suggested that the involvement of HLA-G in fetal immune tolerance and mutations in the HLA-G gene could affect the success of pregnancy (Najafi et al., 2021) and the immune suppressive ability at the maternal-fetal interface and placental angiogenesis could also affect the success of pregnancy(Fan et al., 2014; Mosaferi et al., 2013).

Surprising, no significant association was observed between the biochemical parameters (AST, ALT, ALP, and TB). This can be associated with

the non-tolerogenic effect of the biomarkers especially ALP which is mostly associated with pregnancy due to placental formation (Anabire, 2017; Sasamori et al., 2020).

Hematological profile and its anomalies amongst pregnant women with or without malaria infection

Several hematological alterations such as anemia, thrombopenia, leucopenia, and lymphopenia have been reported elsewhere and in Ghana (Sakzabre et al., 2020; Surve et al., 2017). Acheampong et al.,(2021) reported significantly lower levels of leucocytes, lymphocytes, MCH of infected individuals to uninfected and RBC count, HGB, HCT, PLT were significantly higher in malaria cases than the control group. Although findings from this study reported lower levels of WBC (6.62±2.76 vs. 6.86±2.08), HGB (10.57±1.59vs11.03±1.40), HCT (30.08±3.99 vs. 30.96±3.65), MCH (27.71±3.59vs 27.80±3.24), PLT (171.80±59.61 vs. 176.85±56.80) and RBC [3.82(0.64) vs. 4.00(0.62)] among *Pf* infected pregnant women (cases) to non-infected (control) respectively, there was no statistical difference between the groups. These findings are in contrast to the previous findings of Acheampong et al., (2021) who reported higher RBC,HGB, HCT levels with low WBC, MCH and lymphocytes among *Pf* infected individuals, differences in these results might be due to the study population, sites and some malaria control practices. However, This study agrees with the findings of (Sakzabre et al., 2020) which reported lower levels of platelet count among *Pf* infected adults in Ghana.

This study reported significantly lower and higher levels of percent lymphocytes and percent granulocytes among *Pf* infected pregnant women

compared to the control group. Lymphocytes account for 63% of *Pf* infection in endemic regions for malaria (Van Wolfswinkel et al., 2013). Mechanism of lymphopenia in malaria infection still remains debatable (Van Wolfswinkel et al., 2013) since lymphocytes are mostly known to be viral and bacterial fighters with limited role in parasitic infection (Mutua et al., 2018). Due to the re-emergence of lymphocytes in circulation, some authors have suggested the transient sequestration in malaria infection as the cause of lymphopenia. Others also suggested spontaneous apoptosis in vitro as a result of Fasligand (sFasL) interaction (Van Wolfswinkel et al., 2013). Malaria-related lymphopenia reveals disease-induced T cell redistribution to the site of inflammation (Hviid, 2000).

Again, the study observed 72.7% and 27.3% lymphopenia among *Pf* infected and non-infected pregnant women respectively. This findings agrees with previous study on lymphopenia by (Hviid, 2000) in acute *Pf* infection. A previous study elsewhere also recorded lymphopenia in about half of the study participants (42.9%) who tested positive for *Pf* infection (Bashawri et al., 2002).

Significant difference of percent granulocytes observed in this study takes in consideration the role of eosinophil, neutrophils in parasitic infection such as malaria. Involvement of neutrophils have been reported in several malaria related studies as (Mohammed, 2022) reported increased levels of neutrophils in *Pf* infected pregnant women. Moreover, a significantly higher levels of neutrophils was reported in high parasitaemia individuals to the uninfected (Kotepui et al., 2015).

Thrombopenia revealed among *Pf* infected individuals in this study is consistent with the findings by Kotepui et al.,(2015) who reported 73%

thrombopenia and increase level of parasitaemia leading to lower platelet count among *Pf* infected individuals. Thrombopenia, a second hematological anomaly in pregnancy is a complex disorder and occurs in 8% of all pregnancies as a result of laceration of blood vessels at the uterus which cause massive hemorrhage due to the expansion of uterine wall to accommodate the foetus. Moreover, hemodilution also contribute to Thrombopenia among pregnant women (Mutua et al., 2018).

Anemia, a hematological anomaly is still a challenge in pregnancy especially during the 1st and 2nd trimester (Mutua et al., 2018) and as reported by some previous studies elsewhere and in Ghana (Bashawri et al., 2002). The current study reported highest prevalence of anemia among the control group (58.1%). Pathogenesis of anemia is very complex and multifactorial since combination of hemolysis of parasitizes RBCS and removal of both PRBCS and non-PRBCS account of anemia (Bashawri et al., 2002). Moreover, during pregnancy, there is mostly more than 50% increase in blood volume leading to hemodilution as a result of plasma renin activity (Mutua et al., 2018). In addition, the higher prevalence of anemia among the control group might be as a result of iron and folic acid deficiency, nutritional challenges and even RBC loss by bleeding (Nyoni, 2020; Kotepui et al., 2015) and autoimmune hemolytic anemia (Sinha et al., 2021).

Chapter Summary

About 63.3% among the cases without IPT-SP tested positive with 65.1% among the control group testing negative for *Pf* infection. Most of the study participants with *Pf* infection had secondary education (61.1%). However, most pregnant women (60.4%) in their 3rd trimester tested negative for malaria

infection. A lower percent lymphocytes and higher percent granulocytes were recorded among the cases as compared to the control group. Also, high HLA-G with lower ALT levels were obtained among the cases as compared to the control group. About 31 pregnant women among the study participants had experienced previous miscarriage. Also, this chapter discussed the association of clinical characteristics, relationship between liver biochemical levels, HLA-G and age.

Moreover, this chapter discussed the association of HLA-G and biochemical levels with *Pf* infection. This chapter further looked at the association of HLA-G and biochemical levels with miscarriage. Lastly, this chapter discussed hematological profile and its anomalies among pregnant women with or without *Pf* infection.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Introduction

This chapter presents the summary of key findings and makes conclusion in accordance with the findings. Lastly, this chapter presents convincing recommendations directed at policy making and practices based on the outcome of the study. These recommendations include suggestions for future research

Summary of the study

The study aimed at assessing HLA-G levels, hematological and biochemical profile among *Pf* infected pregnant women considering some clinical characteristics such as IPT-SP, temperature, gestational period of the pregnancy. A case control study was conducted among 92 participants, consisting of 46 *Pf* infected and 46 *Pf* uninfected pregnant women. Whole blood samples were used for determination of hematological parameters, determination of malaria parasites by RDT. Plasma and serum obtained after centrifugation was used for HLA-G quantification, estimation of biochemical levels and serology respectively. Findings of the study were presented in the context of existing empirical findings. All statistical association was considered significant at 5% significant level.

Summary of the findings

Association of clinical characteristics of the study participants

There was high prevalence (63.3%) of *Pf* infection among pregnant women without IPT-SP intervention, with significance association among *Pf* infected and *Pf* uninfected pregnant women (p=0.007). Moreover, there was significant difference in temperature between the two groups (p=0.045). The use

of IPT-SP has insignificantly reduced effect on the HLA-G levels (p>0.05) with significantly increased levels of ALP among the pregnant women (p=0.03). However, pregnant women had insignificantly increased levels of HLA-G in the first trimester with significantly increasing levels of ALP in the 2nd and 3rd trimester (p=0.02).

Association of HLA-G and biochemical levels with Pf infection among pregnant women with or without malaria infection

ALT (β =7.091, p=0.011) and ALP (β =13.3011, p=0.017) were up regulated with significance difference in pregnant women with malaria as compared to pregnant women without malaria. However, it was observed that, there was no significance difference (p>0.05) for HLA-G (β =0.321, p=0.3), TB (β =0.312, p=0.056), AST (β =2.455, p=0.059) even though they were up regulated in the pregnant women with malaria as compared to pregnant women without malaria. In the adjusted model, only ALP showed significance difference (p=0.02). However, HLA-G increased insignificantly among cases and the control group (p>0.05) whereas ALT significantly reduced among the cases and the control (p=0.01).

Association of HLA-G and biochemical levels with miscarriage status among the study participants.

There was significant association of HLA-G and miscarriage among the study participants (p=0.001) whereas no significance association of liver biochemical parameters (AST, ALT, ALP and TB) was obtained (p>0.05).

Association of hematological profile among pregnant women with or without malaria infection

There were lower levels of percent lymphocytes with increased percent granulocytes among *Pf* infected pregnant women as compared to *Pf* uninfected pregnant women with significant association [28.20(11.75) vs. 31.65(14.55), p=0.04], and [65.00(13.80) vs. 61.50 (14.28), p=0.03] respectively.

Relationship between liver biochemical levels, HLA-G and age

HLAG (R^2=0.004, p=0.57), AST (R^2=0.0031, p=0.09), ALT (R^2=0.014, p=0.26), ALP (R^2=0.000, p=0.94), TB (R^2=0.014, p=0.26) increased positively with age, though no statistically significant (p>0.05)

Conclusion

Pf infection has effect on liver biomarkers (ALT and ALP) with decrease percent lymphocyte and an increase percent granulocyte among pregnant women with malaria infection. Again, higher plasma HLA-G was associated with previous miscarriage status among the pregnant women. However, the study shows increase levels of HLA-G among *Pf* infected pregnant women.

Recommendations

Recommendation for policy markers

Ministry of health and the Ghana health services should incorporate into the ANC routine investigations; liver function test (LFT) to help assess and provide early diagnoses of liver injury during pregnancy especially among *Pf* infected pregnant women.

Recommendation for future research

1. Further case control studies are recommended to explore the relationship of HLA-G and *Pf* infection among the Ghanaian population.

2. Further studies are recommended to explore the association of liver biochemical levels with miscarriage
3. Further studies are recommended to investigate the relationship between HLA-G and biochemical levels with age with larger sample size
4. Further studies are recommended to explore the relationship between HLA-G and miscarriage among pregnant women with or without pregnancy loss in the absence of malaria infection

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APPENDICES

Appendix A: Preparation of coating buffer; Phosphate Buffered Saline (5%PBS)

Components	Amount/Volume	Lot No:	Check steps done (✔)
PBS	2tablets		
Volumetric flask	1		
Measuring cylinder	1		
Distilled water	1000ml		
Magnetic stirrer	1		

PROCEDURE:

1. Add two (2) tablets of PBS into a volumetric flask containing 100ml of distilled water
2. Place the flask on a mixer with a stirrer to obtain a uniform mixture

Label:

PBS + date +initials

Storage condition:

At room temperature (RT)

Appendix B: Preparation of coating solution

Components	Amount/ Volume	Lot No:	Check steps done (✔)
PBS	10ml		
MEM-G/9	100ul	531294	
15ml falcon tube	1		
Serological pipette	1		
Micro pipette	1		

PROCEDURE:

1. Transfer 10ml of PBS into 15ml falcon tube
2. Add 100ul of antibody MEM-G/9
3. Place the tube on an electronic mixer and vortex for about 30s to obtain a uniform solution

Label:

Coating buffer +date +initials

Storage condition:

At 2-4℃

Appendix C: Preparation of washing buffer (PBS tween20, 5%)

Components	Amount/ volume	lot no.:	Check steps done (✔)
PBS	1L		
Tween 20	500ul	A49740500	
Micro pipette	1		

PROCEDURE:

1. Transfer 500ul of PBS tween into 1L PBS solution
2. Place the flask on a mixer with a stirrer to obtain a uniform solution

Label:

Washing buffer +date+ initials

Storage condition: RT

Appendix E: Preparation of secondary antibody

Components	Amount/ volume	Lot No:	Check steps done (✔)
PBS	10ml		
β2microglobulin	1ul	A0072	
Micro pipette	1		
15ml falcon tube	1		

PROCEDURE:

1. Add 1ul of B2microglobulin into a 15ml falcon tube containing 10ml PBS solution
2. Vortex it on an electric mixer to obtain a uniform solution

Label

2^0 antibody +date + initials

Storage condition:

At 2-4°C

Appendix F: Preparation of enhancer

Components	Amount/ volume	Lot No:	Check steps done (✔)
PBS	10ml		
DAKO envision sytem HRP	50ul	10151788	
Micro pipette	1		
15ml falcon tube	1		

PROCEDURE:

1. Transfer 10ml of PBS into 15ml falcon tube and add 50ul of DAKO envision system HRP
2. Vortex it using an electric mixer to obtain uniform solution

Label:

Enhancer +date +initials

Storage condition:

At 2-4℃

Appendix G: Preparation of stop solution

Components	Amount/ Volume	Lot No:	Check steps done (✔)
HCL(IN)	20ml		
Distilled water	230ml		
serological pipette	1		
Volumetric flask	1		

PROCEDURE:

Transfer 20ml of HCL (IN) into a volumetric flask containing 230ml of distilled water

Label:

Stop solution+ date +initials

Storage condition:

At RT

Appendix H: preparation of positive control/standard (lot: B171)

PROCEDURE:

1well:100ul

6well: x

x=600ul+100ul (extra)

Hence x=700ul

Using the dilution factor: C1V1=C2V2,

V1= (200ngx700ul)/507,100

=276.1ul of the positive sample/standard

Volume of DAKO to use: 700-276.1=423.9ul

Appendix I: Preparation of negative control (lot: 118)

100:400

x: 300

x= (300x100)/400, x=75ul of the negative sample

Volume of DAKO to use: 300-75=225ul

Appendix J: Preparation of working reagent for TB

Components	Amount/ Volume	Lot No:	Check steps done (✔)
R1(B reagent)	1000ul	BIL-040721	
R2(T-nitrite reagent)	50ul	BIL-040721	
Micro pipette	1		
Test tubes	2		

Procedure:

1. Transfer 1000ul of R1 into two (2) test tubes labeled test and blank and add 50ul of R2 into the sample test tube
2. Vortex gently to obtain a uniform solution

Label:

TB + date +initials

Storage condition:

2-8°C

Appendix K: Preparation of AST working reagent

Components	Amount/ Volume	Lot No:	Check steps done (✔)
R1	4000ul	ASAT-050621	
R2	1000ul	ASAT-050621	
Micro pipette	1		
Test tube	1		

Procedure:

1. Transfer 4000ul of R2 into a sterile container and add 1000ul of R1
2. Vortex gently to obtain a uniform solution

Label:

AST +date + initials

Storage:

2-8°C

Appendix L: Preparation of ALT working reagent

Components	Amount/ Volume	Lot No:	Check steps done (✔)
R1	4000ul	ALAT-091121	
R2	1000ul	ALAT-091121	
micro pipette	1		
Test tube	1		

Procedure:

1. Transfer 4000ul of R2 into a sterile container and add 1000ul of R1
2. Vortex gently to obtain a uniform solution

Label:

ALT + date + initials

Storage:

2-8°C

Appendix M: Preparation of ALP working reagent

Components	Amount/ Volume	Lot No:	Check steps done (✔)
R1	4000ul	ALP-16542	
R2	1000ul	ALP-16542	
micro pipette	1		
Test tube	1		

Procedure:

1. Transfer 4000ul of R2 into a sterile container and add 1000ul of R1
2. Vortex gently to obtain a uniform solution

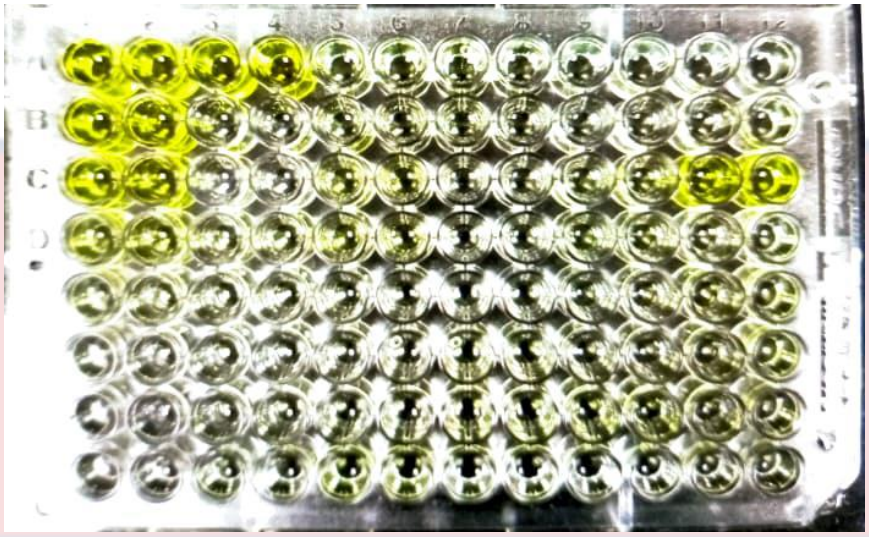
Label:

ALP + date + initials

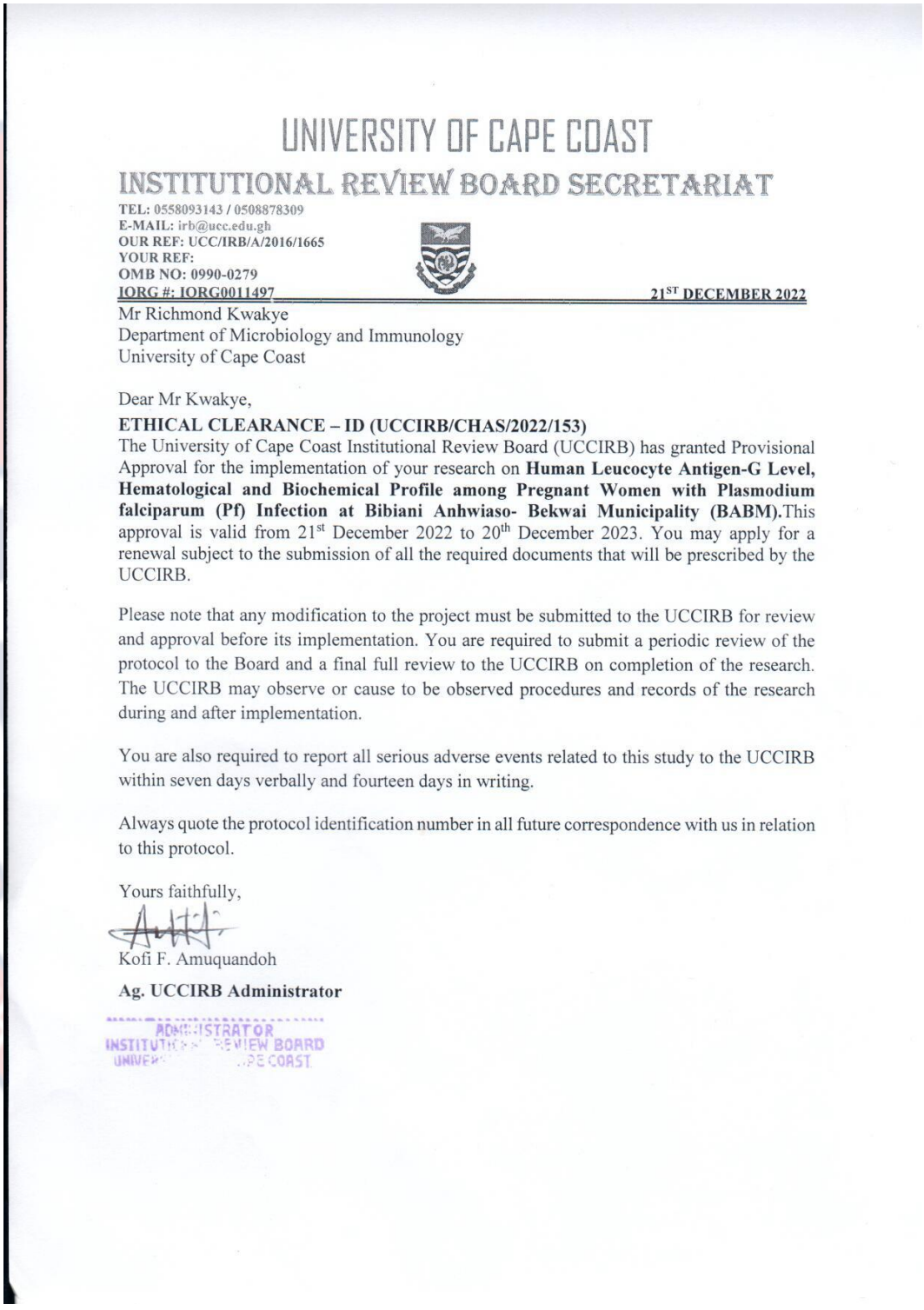
Storage:

2-8°C

Appendix N: Sample of HLA-G ELISA Reaction



APPENDIX O: Ethical clearance



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21ST DECEMBER 2022

Mr Richmond Kwakye
Department of Microbiology and Immunology
University of Cape Coast

Dear Mr Kwakye,

ETHICAL CLEARANCE – ID (UCCIRB/CHAS/2022/153)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted Provisional Approval for the implementation of your research on **Human Leucocyte Antigen-G Level, Hematological and Biochemical Profile among Pregnant Women with Plasmodium falciparum (Pf) Infection at Bibiani Anhwiaso- Bekwai Municipality (BABM).**This approval is valid from 21st December 2022 to 20th December 2023. You may apply for a renewal subject to the submission of all the required documents that will be prescribed by the UCCIRB.

Please note that any modification to the project must be submitted to the UCCIRB for review and approval before its implementation. You are required to submit a periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

Kofi F. Amuquandoh

Ag. UCCIRB Administrator

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