CLINICAL UTILIZATION OF CHONDROITIN SULPHATE-A TO DETERMINE THE SUSCEPTIBILITY OF PREGNANT WOMEN TO MALARIA

BY

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DECLARATION

This thesis is a presentation of my original research work. Wherever contributions from other persons are involved, every effort was made to indicate that clearly, with due reference to literature and acknowledgement of collaborative research and discussions.

The work was done under the guidance of Dr Alex Yaw Debrah. This work has not been submitted for any other degree.

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ABSTRACT

People living in areas with stable transmission of *Plasmodium falciparum* parasites acquire protective immunity to malaria following multiple disease episodes. Immunity acquired this way is mediated by IgG with specificity for parasiteencoded, clonally variant surface antigens (VSA) on the surface of infected erythrocytes (IEs). However, women in endemic areas become susceptible to *P. falciparum* infection during pregnancy, particularly for the first time, regardless of acquired protective immunity. Aggregation of erythrocytes infected by *P. falciparum* in the intervillous spaces of the placenta using chondroitin sulphate A (CSA) as the dominant placental adhesion receptor has been identified. Chondroitin sulfate A is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars. It is usually found attached to proteins as part of a proteoglycan. This study sought to investigate the role of chondroitin sulphate A in malaria susceptibility in pregnant women.

A total of 160 clients attending two selected health facilities, namely Kaneshie Polyclinic and Our Lady of Grace Hospital, were recruited. They were stratified into three groups: Pregnant women with malaria (n=80), non-pregnant women with malaria (n=40) and pregnant women without malaria (n=40). Full blood count (FBC), malaria RDT, thick film for malaria parasites and ELISA for chondroitin sulphate-A concentration were conducted for each volunteer. The mean haemoglobin count in pregnant women with malaria was 9.6 ± 1.4 g/dl and that of non-pregnant women with malaria was 10.3 ± 1.6 g/dL and the pregnant women without malaria was 11.5 ± 1.1 g/dL. The median CSA concentration in nonpregnant women with malaria was 2.24 ng/mL; that of pregnant women with malaria was 50.76 ng/mL, and pregnant women without malaria was 44.94 ng/mL. There were significant differences between non-pregnant women with malaria and pregnant women with malaria (P=0.0001) and also between nonpregnant with malaria and pregnant women with no malaria (P=0.0001) but no difference between pregnant women with malaria and pregnant women without malaria (P=0.084). Haemoglobin and haematocrit concentrations were significantly associated with malaria infection. The parasite count in pregnant women with malaria compared with that in non-pregnant women with malaria was significantly lower (P=0.0001). Malaria infection was associated with reduction in haemoglobin concentration, which worsened when pregnancy was present. In conclusion, CSA was elicited in pregnant women but had inverse correlation with peripheral malaria parasitaemia.

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LIST OF ABBREVIATIONS

CD – cluster of differentiation

CIDR – cysteine rich inter-domain region

CSA – chondroitin sulphate

DBL – Duffy- binding-like

EBL – erythrocyte binding ligands

GAG – glycosaminoglycan

GAG – glycosaminoglycan

GalNAc – N-acetylgalactosamine

HA – hyaluronic acid

HRP – Horseradish Peroxidase HRP

2 – histidine-rich protein 2

ICAM-1 – intracellular adhesion molelcule-1

IE – infected erythrocyte

IgG – immunoglobulin

IPT – intermittent preventive

treatment iRBC – infected red blood

cell ITN – insecticide treated net

IUGR – intrauterine growth retardation (IUGR)

LBW – low birth weight

MG – multigravid

NTS – N-terminal segment PAM – pregnancy associated malaria

pfEMP1 – plasmodium falciparum erythrocyte membrane protein

1

PG – primigravid

PIEs – parasite-infected erythrocytes

pRBC – parasitized red blood cell

PTD – preterm delivery

PV – parasitophorous vacuole

RBC – red blood cell

TNF – tumour necrotic factor

UNICEF – United Nations Children's Fund

VSA – variant surface antigen

WHO – World Health Organization

Chapter 1 INTRODUCTION

1.1 BACKGROUND

Malaria is a major cause of death, and according to the World Malaria Report in 2011, there were about 216 million cases of malaria and an estimated 655 000 deaths in 2010. Malaria has been recognized as a human disease for thousands of years and remains one of the most common diseases affecting humans worldwide. Its impact falls almost entirely on developing countries, with the heaviest toll on Africa (Hay *et al.,* 2004). Over half of the world's population is thought to be exposed to the risk of contracting malaria (Hay *et al.*, 2004). Apart from its direct health cost, it carries a significant economic burden in countries where the disease is endemic (WHO, 2013).

Malaria slows economic growth in Africa, fuelling the vicious cycle which perpetuates poverty (UNICEF, 2012). Malaria demands up to 5% of the gross domestic product in sub-Saharan Africa (WHO, 2013). In Africa, it accounts for about 40% of public health expenditure and 25% of the household income (African Medical and Research Foundation, 2012). Malaria deters investment and tourism and reduces labour-intensive cash-crops (African Medical and Research Foundation, 2012).

During the 1960s and 1970s, there was optimism that malaria could be eradicated. However the 1980s and 1990s saw serious setbacks, such as the development of resistance to commonly used drugs and insecticides, as well as the breakdown of control programmes and local primary health services, often in the context of regional political and economic collapse. Child deaths due to malaria doubled in sub-Saharan Africa in the 1990s and malaria re-emerged in Central Asia, Eastern Europe and previously cleared areas of Southeast Asia (Murray *et al.*, 2014). Malaria occurs almost exclusively in the tropics and subtropics and approximately 40% of the world's population, mostly those living in the world's poorest countries, are at risk of contracting malaria (WHO, 2013).

The groups most at risk of developing severe disease are the poor. About 60% of deaths from malaria worldwide occur in the poorest 20% of the population, due to lack of access to effective treatment (Suh *et al.*, 2004). Young children and infants, pregnant women (especially primigravidae), elderly people and non-immune people (eg, travellers, foreign workers) are all at risk (Health Protection Agency, 2011). Clinical immunity to malaria is acquired during childhood in areas of intense *falciparum* transmission, and adults are generally protected against malaria (Christophers, 1924; Riley *et al.*, 1994). Pregnant women, in whom malaria is both more prevalent and more severe than in non-pregnant women, constitute an important exception to this rule (Brabin, 1983).

Malaria is caused by a protozoan parasite of the *Plasmodium* species belonging to the phylum apicomplexa. There are four different *Plasmodium* species namely *P. falciparum, P. ovale, P. vivax and P. malariae,* which can infect humans (Medical Microbiology 4th edition). *P. falciparum* is the most harmful and the most widespred d (Health Protection Agency, 2007). Malaria is a mosquito-borne infectious disease of humans and other animals (WHO, 2013). Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases it can cause yellow skin, seizures, coma or death (Caraballo and King, 2014). The disease is transmitted by the biting of infected anopheles mosquitoes, and the symptoms usually begin 10 to 15 days after being bitten. In those who have not been appropriately treated, the disease may recur months later (WHO, 2013). In those who have recently survived an infection, re-infection typically causes milder symptoms. This partial resistance disappears over months to years if there is no ongoing exposure to malaria (Caraballo and King, 2014) .

About 50 million women living in malaria endemic areas become pregnant each year and are at risk of the adverse health impact of malaria (Menendez, 2006) . Approximately half of them live in sub-Saharan Africa and most of them in areas of intense *falciparum* transmission where there is substantial maternal and especially fetal and infant morbidity, causing 75,000 – 200,000 infant deaths every year due to combination of preterm deliveries and fetal growth restrictions

(Steketee *et al.*, 2001). Effect on miscarriage is unknown, but adequate control alone could prevent 3-8% of infant deaths (Steketee *et a*l., 2001). Indeed, malaria also causes several other prenatal and maternal complications including abortion, stillbirth, low birth weight and even death (Brabin, 1983; McGregor *et al.*, 1983)

Women in their first and second pregnancies are at particular risk of infection with *Plasmodium falciparum.* Selective accumulation of parasites in the placental space results in maternal anaemia (Shulman *et al.*, 1996) and infant low birth weight (LBW) (Brabin, 1983; McGregor *et al.*, 1983; Steketee *et al.*, 1996; Nosten *et al.*, 1999) through preterm delivery (PTD) (Steketee *et al.*, 1996) and intrauterine growth restriction (IUGR) (Brabin, 1983; McGregor *et al.*, 1983; Steketee *et al.*, 1996). Regardless of the extent of previous exposure to *P. falciparum* during pregnancy, all pregnant women are at increased risk of malaria and appear to be more attractive to mosquitoes because of physiological and behavioural changes that occur during pregnancy are known to be responsible for increased attractiveness to the mosquitoe (Ansell *et al.*, 2002; Abrams *et al.*, 2003). Marked differences in symptoms are apparent between varying levels of transmission.

Pregnancy-associated malaria (PAM) infection is one example of a severe malaria syndrome, mediated by the surface expression of variant surface antigens (VSAs) of *P. falciparum*-parasitized red blood cells (pRBC) that allow adherence to vascular endothelium (Kane and Taylor-Robinson, 2011). In non-pregnant individuals, VSAs adhere to the ubiquitous endothelial surface proteins intercellular adhesion molecule-1 (ICAM-1) and CD36, and to other pRBC, or form rosettes around noninfected RBC (Kane and Taylor-Robinson, 2011). Under high transmission settings with favourable breeding sites for the vector Anopheles mosquito, adults acquire natural immunity to VSAs, rendering them asymptomatic (Taylor-Robinson and Smith, 1999; Doolan *et al.*, 2009). Red blood cells (RBC) infected by the late developmental stages of *P. falciparum* parasites are not found in the peripheral circulation, as they adhere to receptors on the endothelial lining. This adhesion, called sequestration, is mediated through parasite-encoded, clonally variant surface antigens (VSA) inserted into the membrane of the infected RBC

(iRBC) and is thought to be an immune evasion strategy, possibly evolved to avoid splenic clearance (David *et al.*, 1983; Barnwell *et al.*, 1989; Berendt *et al.*, 1990).

Pregnancy-associated malaria is characterized by marked accumulation of parasites in the intervillous space of the placenta, and it is the cause of maternal anaemia as well as low birth weight, prematurity and increased infant mortality (McGregor *et al.*, 1983). In endemic areas, pregnancy associated malaria is concentrated in primigravid women, which indicates that protective immunity to PAM is acquired as a function of parity. In contrast, women who are immune to these parasites also display adverse consequences of infection when they become pregnant (Nathwani *et al.*, 1992) and this affirms the previous belief that pregnancy represented an immunocompromised state (Currie, 1968).

Apart from this immunocompromised state of pregnancy, there is the aggregation of erythrocytes infected by *falciparum* in the intervillous spaces of the placenta *(S*rivastava *et al.,* 2010*)*. These infected erythrocytes in the placenta adhere to glycosaminoglycan receptors not exploited by other infected erythrocyte.

The presence of symptoms results in prompt diagnosis and management which reduces the incidence of unfavourable pregnancy outcomes (Nosten *et al.*, 2007). In contrast, women living in areas endemic for malaria and hence possessing preexisting immunity, tend to be asymptomatic in pregnancy but harbour high, undetected parasite levels in the placenta (Doolan *et al.*, 2009; Recker *et al.*, 2009). PAM affects these women in a gravidity-dependent manner: primigravid (PG) women are more susceptible than multigravid (MG) women (Brabin and Rogerson, 2001). After correction for age-related susceptibility, this trend has been reported consistently and is more pronounced with increasing transmission (Baird, 1995; Megnekou *et al.*, 2009). PAM is managed during pregnancy with intermittent preventive strategies using chemotherapeutic medications or insecticide-treated nets. The World Health Organization recommends that insecticide treated nets and intermittent preventive treatment (IPTp) should be used during pregnancy (Nosten *et al.*, 2007). IPTp consists of two doses of sulfadoxine and pyrimethamine in the second and third trimesters (Rogerson *et al.*, 2003b). While this treatment

remains effective in West Africa, and more so in three doses than two (Diakite *et al.*, 2011) there is a need for novel interventions. Current efforts to control the incidence of malaria infection are being hampered by rapidly increasing numbers of insecticide-resistant mosquitoes and treatment-resistant parasites (Sevene *et al.*, 2010). Hence, production of a vaccine to protect women in high risk areas is an urgent public health priority.

Women in their first and second pregnancies are more susceptible as anti adhesion antibodies against Chondroitin Sulphate-A (CSA) binding parasites develop after successive pregnancies (Duffy and Fried, 1999). The presence of parasites in the peripheral blood without symptoms is common in hyper-endemic areas, and is associated with chronic anaemia and placental sequestration (Nwagha et al., 2009). By this, the parasite interferes with transmission of vital substances through the fetal placenta, often resulting in stillbirth, spontaneous abortion, or dangerously low birth weight (Duffy, 2001).

Chondroitin sulphate-A is a sulphated glycosaminoglycan (GAG) present on the syncytiotrophoblast in the intervillous space of the placenta, located as a side chain on the tissue anticoagulant thrombomodulin (Salem *et al.*, 1984). It appears during the 16th week of pregnancy with the completion of the placenta. A plethora of functions for proteoglycans have been identified, including regulation of cell proliferation, differentiation and adhesion, as reviewed by some authers (Schaefer and Schaefer, 2010). Binding to placental CSA may alter gene transcription, signal transduction, or activate intracellular signalling mechanisms that lead to increased expression of inflammatory mediators (Lucchi *et al.*, 2006; Lucchi *et al.*, 2008; Schaefer and Schaefer, 2010). CSA is expressed by nearly all cells but it is unclear why parasites only bind to CSA expressed by the placenta, although this may be explained by the specific patterns of sulphation of placental CSA and the structure it forms on the trophoblast membrane (Dahlback *et al.*, 2010). Previously, other placental molecules, such as hyaluronic acid (HA) (Matejevic *et al.*, 2001), have been implicated in placental binding of pRBC (Beeson *et al.*, 2000) and some parasite lines have been shown to have affinity for three receptors; CSA, HA and CD36.

However, the strength of binding, as determined by a *P. falciparum* cytoadherence assay performed under static conditions, does not match that of CSA-parasite adherence alone (Hommel *et al.*, 2010).

Antibodies that inhibit *P falciparum* adhesion to CSA are associated with a reduced risk of placental malaria (Duffy and Fried, 2003). Hence, susceptibility to *Plasmodium* parasitaemia has been linked to the level of antibodies to placental sequestrated parasites (Elliott et al., 2005).

The proteoglycan chondroitin sulphate A can mediate parasite adhesion *in vitro* (Robert *et al.*, 1995; Rogerson *et al.*, 1995) and, although CSA-adhering parasites are rarely found in non-pregnant hosts, placental parasites preferentially or perhaps even exclusively bind to CSA (Fried and Duffy, 1996; Achur *et al.*, 2000). It has thus been proposed that the placenta constitutes a niche for antigenically distinct VSA that have evolved to mediate sequestration exclusively at this site (Molecular Microbiology, 2003).

Parasites causing PAM must thus be able to escape the immunological effector mechanisms that control parasite multiplication in immune hosts. This seems to be explained by the observation that VSA expressed by parasites selected for strong adhesion to CSA *in vitro* are not recognized by plasma IgG from clinically immune adult males, although those of the parental unselected lines are well recognized (Ricke *et al.*, 2000; Staalsoe *et al.*, 2001).

There is thus a gender-specific recognition by antibodies of CSA-selected and placental parasites. Another significant characteristic shared by VSA expressed by CSA-selected and placental parasites is that the levels of CSA adhesion-blocking plasma IgG in malaria-exposed women depend on parity (parity-dependent IgG recognition) (Fried *et al.*, 1998a; Ricke *et al.*, 2000; Staalsoe *et al.*, 2001). This adhesion-blocking capacity is independent of the geographical origin of plasma as well as parasites (Fried *et al.*, 1998a). Together, these data suggest that the VSA responsible for placental adhesion to CSA (VSA_{PAM}) are functionally and

antigenically distinct from other molecules present at the iRBC surface, and that they share relatively conserved antigenic determinants (Ricke *et al*., 2000).

Plasmodium falciparum erythrocyte membrane protein1 (PfEMP1) can mediate iRBC adhesion to a range of host receptors and are encoded by the *var* gene family containing 50–60 members per haploid parasite genome (Baruch *et al.*, 1995; Su *et al.*, 1995; Gardner *et al.*, 2002). Different PfEMP1 molecules have different receptor specificities, and clonal switching between *var* genes that leads to expression of the various PfEMP1 molecules in a mutually exclusive manner (Chen *et al.*, 1998; Scherf *et al.*, 1998) allows the parasite to modify its adhesion properties (Smith *et al.*, 1995). To date, several *var* genes encoding PfEMP1 domains with affinity for CSA have been described (Buffet *et al.*, 1999; Reeder *et al.*, 1999; Degen *et al.*, 2000; Khattab *et al.*, 2001).

Although some progress has been accomplished in recent years, resulting in the identification of intermittent preventive treatment (IPT) and insecticide treated nets (ITNs) as key strategies to control malaria in pregnancy and the use of antimalarials such as sulphadoxine-pyrimethamine, much work needs to be done to control malaria effectively in this high at risk group. There are still many gaps in knowledge that need to be addressed regarding the biological mechanisms that explain the increased susceptibility during pregnancy. Determining the susceptibility of pregnant women to malaria by measuring their CSA levels will provide more support to the development of anti-adhesion vaccines to prevent poor fetal outcomes due to pregnancy malaria.

1.2 PROBLEM STATEMENT

Malaria severely affects pregnant women and children. Despite immunity through lifelong exposure to malaria, pregnant women become susceptible to the disease causing anaemia, abortions and low birth weight (Steketee *et al.*, 2001). They experience massive accumulation of infected erythrocytes (IEs) in the placenta. Adhesion of IEs to host endothelial receptors is mediated by members of a large diverse protein family called *P. falciparum* erythrocyte membrane protein 1

(PfEMP1). Pregnancy malaria is generally associated with the emergence of a distinct subset of parasites expressing a unique PfEMP1 that binds to the hostreceptor chondroitin sulfate A (CSA) (Barfod *et al.*, 2010). The absence or rare occurrence of CSA-binding parasites in malaria patients (children, men and nonpregnant women) suggests that these parasites become virulent only during pregnancy (Duffy and Fried, 2003).

The exact mechanism for the increase in susceptibility and frequency of complications during pregnancy, as well as the preferential sequestration of *falciparum* in the placenta, remains partially unclear. Cytoadherence of a subpopulation of *P. falciparum* to chondroitin sulphate A in the placenta (Fried and Duffy, 1996) and increased attractiveness of pregnant women to malaria-carrying mosquitoes (Lindsay *et al.*, 2000) are a few mechanisms that have been postulated.

1.3 JUSTIFICATION

Malaria in pregnancy results in such negative outcomes as maternal anaemia and reduction in birth weight, which in turn leads to increase in maternal and infant mortality. Adverse outcomes of pregnancy-associated malaria (PAM) include low birth weight neonates, foetal loss, increased perinatal and maternal mortality, maternal anaemia and the risk of hypertension in first-time pregnant mothers. Although some other infectious diseases are also worse in pregnancy, malaria seems to be a special case, affecting an estimated 24 million pregnant women in sub-Saharan Africa annually. This work investigates the basis for the susceptibility to pregnancy-associated malaria and of the mechanisms by which the disease could lead to morbidity and mortality, and discusses how these insights can help us to develop more effective strategies to prevent and treat the disease.

1.4 AIM

The aim of this study is to investigate if chondroitin sulphate A contributes to increased susceptibility to malaria in pregnant women

1.5 SPECIFIC OBJECTIVES

The specific objective objectives of this study are:

1. To assess paracetaemia load and the level of chondroitin sulphate A in pregnant women.
2. To determine the impact of chondroitin sulphate A in protecting against malaria in pregnant women.

Chapter 2 LITERATURE REVIEW

Malaria is caused by a protozoan parasite of the *Plasmodium* species belonging to the phylum apicomplexa. There are four different *Plasmodium* species namely *P. falciparum, P. ovale, P. vivax and P. malariae,* which can infect humans (Daneshvar *et al.*, 2009)

2.1 LIFE CYCLE OF PLASMODIUM FALCIPARUM

A human being is infected when an infected female Anopheles mosquito takes a blood meal, and sporozoite-stage parasites of *P. falciparum* are injected into the human skin (Rowe *et al.*, 2009). The sporozoites migrate into the bloodstream and then invade liver cells. Parasite stages in the liver are clinically silent. The parasite grows and divides within the hepatocytes in 8–10 days. This asexual replication is known as exoerythrocytic schizogony and culminates in the production of merozoites which are released into the bloodstream, where they rapidly invade erythrocytes (Rowe *et al.*, 2009). The early trophozoite is often referred to as 'ring form' because of its morphology (Lee *et al.*, 2007). Trophozoite enlargement is accompanied by an active metabolism including the ingestion of host cytoplasm and the proteolysis of hemoglobin into amino acids. The haemoglobin in the red blood cell provides the main source of protein necessary for the parasites development (Larzarus *et al.,* 2008). During that process the iron in the haem rapidly oxidizes and haem monomers are converted into an inert crystalline and paramagnetic material named haemozoin or malaria pigment which accumulates in infected erythrocytes (Hempelmann and Marques, 1994; Egan, 2008). The rate of haemozoin formation correlates with parasite metabolic activity and peaks at the late trophozoite stage of development. The end of the trophic period is manifested by multiple rounds of nuclear division without cytokinesis resulting in a schizont (Miller *et al.,* 2002)

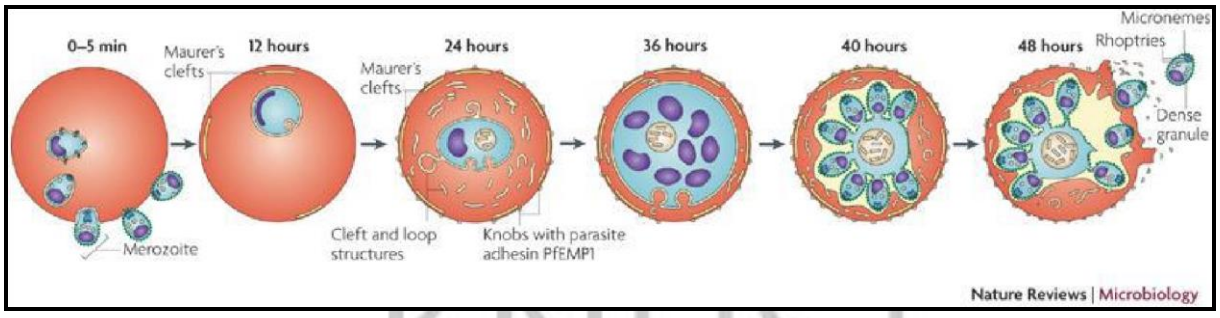

Figure 2-1: The different stages of *Plasmodium falciparum* development (source: Nature Reviews of Microbiology, 2007)

P. falciparum merozoites attach to and invade mature human red blood cells (RBCs) and the parasite develops in a parasitophorous vacuole (PV) through the ring (0–24 hours), trophozoite (24–36 hours) and schizont stages (40–48 hours). In maturestage parasites (>24 h), membrane-bound structures appear in the RBC cytoplasm and knobby deformations are formed at the RBC membrane. After approximately 48 hours the infected RBC ruptures,releasing 16–32 daughter merozoites. Degradation of haemoglobin results in the deposition of crystals of haemozoin in a digestive vacuole (Maier *et al.*, 2009).

The asexual intraerythrocytic cycle lasts for approximately 48 hours, and is completed by the formation and release of new merozoites that will re-invade uninfected erythrocytes (Expert Reviews in Molecular Medicine, 2009). It is during this asexual bloodstream cycle that the clinical symptoms of malaria (fever, chills, impaired consciousness, etc.) occur (Center for Disease control, 2010). During the asexual cycle, some of the parasite cells develop into male and female sexual stages called gametocytes that are taken up by feeding female mosquitoes. The gametocytes then undergo further development in the mosquito, resulting in the presence of sporozoites in the mosquito salivary glands, ready to infect another human host (Rowe *et al.*, 2009).

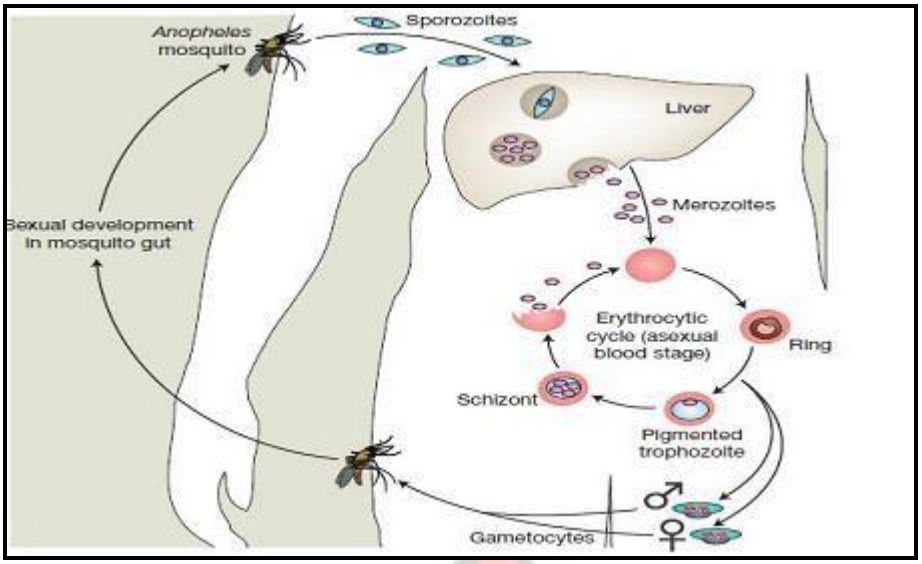

Figure 2-2 Life cycle of *P. falciparum* (*Source: expert reviews in Molecular Medicine, 2009).*

2.2 PATHOGENESIS OF PLASMODIUM FALCIPARUM

Adhesion of erythrocytes infected with *Plasmodium falciparum* to human cells has a key role in the pathogenesis of life-threatening malaria. Parasite adhesion interactions include binding to endothelial cells (cytoadhesion), rosetting with uninfected erythrocytes and platelet-mediated clumping of infected erythrocytes (Rowe *et al.*, 2009).

The presence of the parasite and the resulting host inflammatory responses leads to high fevers and associated 'flu'-like symptoms (Marsh *et al.*,1995).The incubation period before the appearance of symptoms varies from 12 days to over a year. In some cases of infections a life-threatening illness develops, characterized by various clinical features, including impaired consciousness, coma, breathing difficulties, severe anaemia and multi-organ failure (Marsh *et al.*, 1995; Dondorp *et al.*, 2008). These clinical manifestations of severe malaria are thought to occur because of a combination of a high parasite burden and the sequestration of mature *P. falciparum*-infected erythrocytes (IEs) in microvascular beds throughout the body (Miller *et al.*, 2002). The sequestered mass of IEs leads to microvascular obstruction (Dondorp *et al.*, 2004; Dondorp *et al.*, 2008) metabolic disturbances, such as acidosis (Planche and Krishna, 2006) and release of damaging

inflammatory mediators (van der Heyde *et al.*, 2006; Schofield, 2007) which can combine to cause severe disease and death of the human host. Sequestration is thought to benefit the parasite by allowing it to avoid the host's normal splenic clearance mechanisms that remove aged or damaged erythrocytes (Mebius and Kraal, 2005).

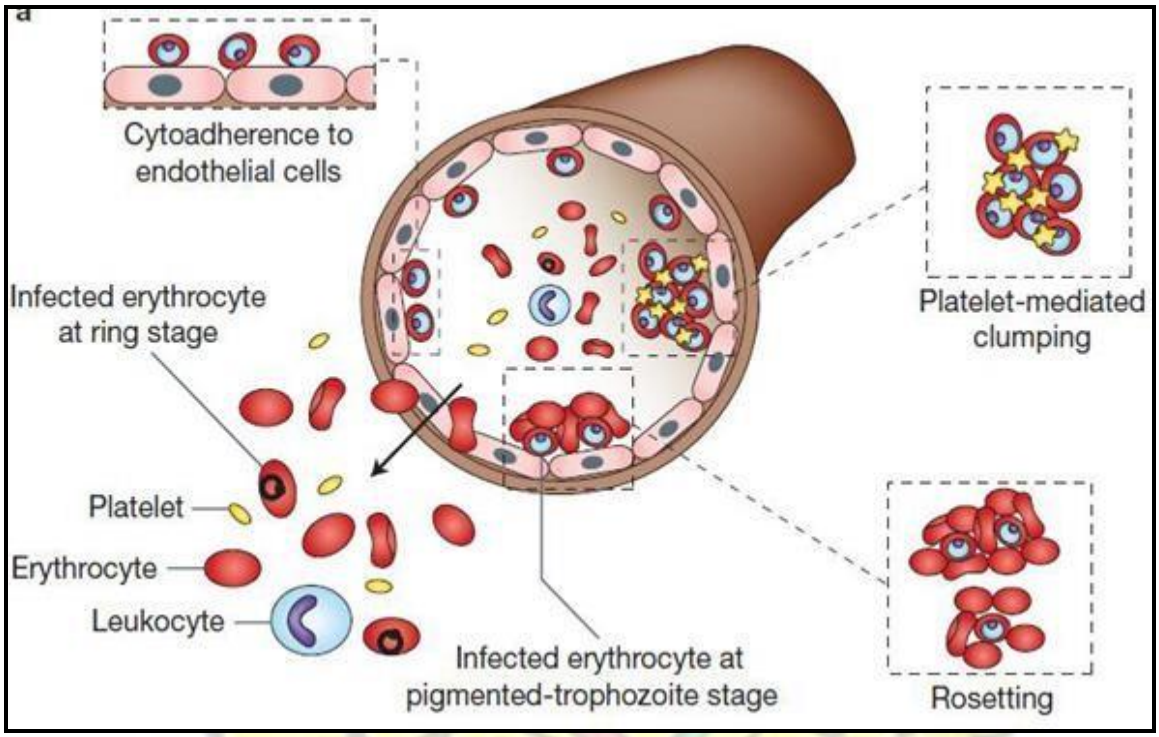

Figure 2-3: Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells, including cytoadherence to endothelial cells, platelet-mediated clumping and resetting; *Source:* (Rowe *et al.*, 2009)

2.3 MALARIA IN PREGNANCY

In malaria-endemic areas, the frequency and severity of malarial infections are greater in pregnant women than in their non-pregnant counterparts (Monif and Baker, 2004).

In sub-Saharan Africa, where the largest burden of malaria occurs, about 25 million pregnant women are at risk of malaria infection each year, and have 2-10-fold higher mortality than non-pregnant women (Steketee *et al.*, 2001; Doolan *et al.*, 2009). Both *P. falciparum* and *P. vivax* infections can cause adverse pregnancy outcomes (Nosten *et al.*, 1999; Steketee *et al.*, 2001; Desai *et al.*, 2007).

Infection rates have been consistently demonstrated to be highest in women in their first and second pregnancies, with lower rates in later pregnancies (Nosten *et al.*, 1999; Steketee *et al.*, 2001). The prevalence of infection and the parasite density in peripheral blood both peak in the first half of the gestation period and then decrease progressively until delivery. An increased risk of recurrent and new *P falciparum* malaria occurs in the first 2–3 months (Diagne *et al.*, 2000; Nahlen, 2000; Ramharter *et al.*, 2005), then there is a rapid clearance of peripheral parasitaemia within 2 days of delivery (Nguyen-Dinh *et al.*, 2008). The risk of maternal infection has been found to be highest during the second trimester (Schantz-Dunn and Nour, 2009). Apart from primigravidas, adolescents (Leenstra *et al.*, 2003; Rogerson *et al.*, 2003a) and those co-infected with HIV also suffer a high risk of infection and morbidity (Brentlinger *et al.*, 2006).

Pregnancy- associated malaria is not caused by the presence of more parasite types in pregnancy, but instead is caused by an increase in density of parasitaemia. Hence, women who remain able to control parasite density might not experience adverse consequences of pregnancy-associated malaria (Mockenhaupt *et al.*, 2000; Mankhambo *et al.*, 2002; Walker-Abbey *et al.*, 2005). Because of high rates of parasitaemia in pregnancy, particularly in many settings in sub-Saharan Africa, the World Health Organization has recommended presumptive malarial treatment followed by additional prevention measures during pregnancy (WHO, 1986).

2.4 FACTORS ASSOCIATED WITH THE SUSCEPTIBILITY OF PREGNANT WOMEN TO MALARIA

Pregnancy causes a number of physiological changes that affect the way the *Plasmodium* parasite invades its host. Several hypotheses have attempted to explain the increased susceptibility of pregnant women to malaria and the high frequency of placental infection. It has been proposed that pregnancy malaria is facilitated by hormone-dependent, depression of the immune system or non-specific effector mechanisms (The immunology and pathogenesis of malaria ,2005).

However, it has been shown that despite this susceptibility, the maternal immune system continues to respond positively to the parasite resulting in better fetus outcomes (Duffy and Fried, 2003). Furthermore, the fact that women who have had multiple pregnancies (multigravids) are at a lesser risk than those in their first pregnancy (primigravids) (Steketee *et al.*, 2001) suggests that immune build-up is achieved after several pregnancies and infections.

2.4.1 Increased Cortisol Concentrations

Pregnancy has been viewed as a period of generalized immunosuppression which is mainly sustained by increased blood levels of cortisol (Elenkov, 2004). It has been shown that cortisol levels are higher in pregnant women with malarial infection than in those without (Adam *et al.*, 2007), suggesting that a sustained increase in cortisol levels underlies the increased susceptibility of pregnant women, particularly primigravidae women, to malaria. This hypothesis has been corroborated by the discovery of a significant association between cortisol concentration and *P. falciparum* infection, on one hand, and a strong correlation between parasite load in *P. falciparum*-infected primigravidae women, on the other hand (Bouyou-Akotet *et al.*, 2005). While cortisol levels would explain an increased susceptibility to malaria, they do not explain why parasites have a preference for growing and multiplying in the placenta.

2.4.2 Decreased Cell-mediated Immunity

Studies of immune function have demonstrated a transient depression of cellmediated immunity in pregnant women, particularly in the second and third trimesters. This impairment of cell-mediated immunity allows fetal allograft retention and allows pregnancy to continue without rejection (Meeusen *et al.*, 2001). However, there is interference with resistance to various infectious diseases including malaria. Cell-mediated immune responses to malarial antigens are more markedly suppressed in first pregnancies than in subsequent ones (Riley *et al.*, 1989; Fievet *et al.*, 1995).

2.4.3 Inhibition of Type-1 Cytokine Responses

Th1-type responses are of parasitological importance. For example, substantial increases in tumour necrosis factor (TNF)-α (Fried *et al.*, 1998b; Rogerson *et al.*,

2003a), interferon-γ (Fried *et al.*, 1998a; Moore *et al.*, 1999), interleukin 1β (Moormann *et al.*, 1999), and interleukin-2 (Fried *et al.*, 1998b) have been found in placental blood or tissue in response to malaria parasite infection. These cytokines are known to aid in the elimination of parasites from the placenta by enhancing phagocytic activity of macrophages, generating reactive oxygen intermediates and L-arginine-derived nitric oxide, and stimulating the proliferation of T cells (TaylorRobinson and Smith, 1999). However, overproduction of Th-1 cytokines can compromise the pregnancy by causing maternal anaemia, spontaneous abortions, and premature deliveries (Kwak-Kim *et al.*, 2005). Hence pregnant women have an immune system which is biased towards type-2 humoral defense mechanisms and away from type-1 Cellular responses. Pregnant women would therefore be more susceptible to malaria because of the inhibition of type-1 cytokine responses (Wegmann *et al.*, 1993).

2.4.4 Naturally acquired immunity to malaria

In malaria endemic areas young children are very susceptible, and it has been estimated that a quarter of all infancy deaths are due to this infection (SchantzDunn and Nour, 2009). Immunity is gradually built after exposure to several *P. falciparum* infections; however, sterile immunity is most likely never achieved, meaning that a lower number of parasites are still able to infect the adult but without causing symptoms (Doolan *et al.*, 2009). From human studies it is observed that although protective immunity to malaria is achieved slowly and following regular exposure to *P. falciparum* parasites, immunity to death is acquired faster and may be significant after a single episode (Gupta *et al.*, 1999a).

When the parasite enters the human body, it is immediately exposed to the immune system that would potentially fight any foreign organism, but studies show that natural immune responses to the pre-erythrocytic stages are of lower importance to prevent disease (Owusu-Agyei *et al.*, 2001). In blood-stage immunity, given that infected erythrocytes (IE) lack antigen presenting cell surface proteins, the humoral response is the main actor, particularly IgG is considered to have a crucial role (Cohen *et al.*, 1961). Antibodies are effective through 3 different

mechanisms: blocking the IE invasion (Blackman *et al.*, 1990); inducing opsonization and phagocytosis (Bouharoun-Tayoun *et al.*, 1995) and binding to IE surface antigens contributing to parasite clearance (Bull *et al.*, 1998).

It is not totally established which of the three mechanisms is more important in the development of immunity. Nevertheless, evidence supporting the importance of variant surface antigens (VSA) comes from studies where children with *P. falciparum* infection lacked VSA-specific antibodies, while immune adults had antibodies targeting the surface of the children isolates; later, the same children recovered from disease and their sera effectively recognized the IE that had caused the infection (Bull *et al.*, 1998; Ofori *et al.*, 2002).

From these experiments, it is clear that clinical immunity is achieved with the acquisition of an antibody repertoire that targets different VSA's, being a likely explanation for the slow development of immunity (Marsh and Howard, 1986). VSAs mediate the adhesion of IE to specific receptors in different tissues allowing the parasites to escape from circulation, evade immunity and reach high parasitaemias (Chan *et al.*, 2014). Since acquisition of immunity to severe disease is acquired before the immunity to uncomplicated infection, (Gupta *et al.*, 1999b) and VSAs expressed by patients infected with severe disease (VSASM) differ in terms of IgG recognition from the ones expressed by patients infected with uncomplicated malaria (VSAUM) (Nielsen *et al.*, 2002; Ofori *et al.*, 2002), it is apparent that there is limited antigen diversity in parasites causing severe malaria. A vaccine based on VSASM antigens would protect against severe disease, still allowing parasite infection causing mild symptoms, which would re-boost the immune response and maintain protection.

Almost all late stage IE are adhesive with differences in specificity as well as affinity. Sequestration enables the parasite to avoid spleen-dependent killing mechanisms and it is an important virulence factor (David *et al.*, 1983). The cytoadhesion to human endothelial receptors is allowed by the different morphology and composition of the erythrocyte membrane due to *P. falciparum* infection.

Several host receptors have been implicated in IE cytoadherence and these include: Thrombospondin (TSP) (Roberts *et al.*, 1985); CD36 (Barnwell *et al.*, 1989); ICAM-1 (Berendt *et al.*, 1989); vascular cell adhesion molecule-1 (VCAM-1) (Ockenhouse *et al.*, 1992); platelet/endothelial cell adhesion molecule (PECAM-1) (Treutiger *et al.*, 1997) and Chondroitin sulphate A (CSA) (Rogerson *et al.*, 1995) but the significance of these receptors in disease pathogenesis remains unclear.

Surface molecules are highly immunogenic and in order to escape the immune system, these proteins evolved towards belonging to large, diverse and varying families. The VSA families described until now are: PfEMP1, RIFS, STEVOR and SURFIN, respectively belonging to the gene families: var, rif, stevor and surf (Bull *et al.*, 1998). *Plasmodium falciparum* erythrocyte membrane protein one (PfEMP1) is the only described VSA holding binding properties that explain the sequestration pathogenic feature of *P. falciparum* infection (Bull *et al.*, 1998). Antibodies against PfEMP1's have shown to interfere with parasite sequestration (Bull *et al.*, 1998). IE display a range of binding properties differing in their receptor specificity depending upon the expressed PfEMP1 (Smith *et al.*, 2001). PfEMP1 proteins are encoded by approximately 50-60 var genes that are present in the genome of each parasite (Kyes *et al.*, 2001).

The great antigenic diversity characteristic of this family is achieved through high levels of gene recombination (Taylor *et al.*, 2000). var genes are typically large (6-13 kb) and have two exons (Su *et al.*, 1995), the first encodes for the variable extracellular binding region and a transmembrane domain, and the second encodes for a more conserved intracellular part. The PfEMP1 extracellular binding region includes four different types of domains: the N-terminal segment (NTS), the Duffy-binding-like (DBL) domain, the cysteine rich inter-domain region (CIDR) and the C2 domain. DBL domains are classified into five different groups that share sequence types and amino acid features (Smith *et al.*, 2000). DBL and CIDR domains are described as being the regions of PfEMP1 responsible for the binding functionalities (Smith *et al.*, 2001).

Importantly, DBL domains are a common module responsible for adhesion found in two distinct families of parasite ligands, the erythrocyte binding ligands (EBL) essential for invasion of erythrocytes (Adams *et al.*, 2001) . Specific sequence signatures allow the classification of these adhesive domains in different classes. The CIDR has a three-helix bundle that exhibits less than 20% sequence identity with the three-helix bundles of Duffy-binding like (DBL) domains, but the two kinds of bundles are almost identical. In addition, the three-dimensional structures of four DBL domains were solved recently, and showed that even though there is very limited sequence similarity among these domains. their structure is overall highly similar (Tolia *et al.*, 2005; Singh *et al.*, 2006; Higgins, 2008).

Although sequence diversity is a very important characteristic of the var family, it is possible to organize the genes in groups based on sequence alignments, promoter regions and chromosome locations. As a result, three major groups are described (group A, B and C) and two intermediate groups B/A and B/C that represent transitions between the three main groups (Malaria Journal,2003). There are two other independent groups formed by genes exceptionally conserved across parasite isolates (var1 and var2) (Lavstsen *et al.*, 2003).

This genomic organization indicates that recombination events might happen mainly within each group, allowing the maintenance of group characteristics. var genes are expressed in a mutually exclusive manner, with only one PfEMP1 protein expressed at a time by each parasite (Scherf *et al.*, 1998). During the 48 h blood-stage asexual part of the parasite cycle, transcription of a var gene is initiated early. The levels of the var transcript peak at 12 h post-erythrocyte invasion and decline rapidly (Kyes *et al.*, 2000). However, PfEMP1 does not appear on the infected RBC surface until approximately 16 h post-invasion (Gardner *et al.*, 2002), meaning that PfEMP1 is synthesized early but follows a slow trafficking pathway to reach the IE surface (Kriek *et al.*, 2003).

2.5 CHONDROITIN SULPHATE

Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid(Baeurle *et al.*, 2009). It is usually found attached to proteins as part of a proteoglycan. A chondroitin chain can have over 100 individual sugars, each of which can be sulfated in variable positions and quantities. Chondroitin sulfate is an important structural component of cartilage and provides much of its resistance to compression (Baeurle *et al.*, 2009). Chondroitin sulfate A is carbon 4 of the Nacetylgalactosamine (GalNAc) sugar, i.e. chondroitin-4-sulfate (Barnhill *et al.*, 2006).

Chondroitin's functions depend largely on the properties of the overall proteoglycan of which it is a part. Chondroitin sulfate is a major component of extracellular matrix, and is important in maintaining the structural integrity of the tissue. Chondroitin sulfate is a major component of cartilage (Baeurle *et al.*, 2009). The tightly packed and highly charged sulfate groups of chondroitin sulfate generate electrostatic repulsion that provides much of the resistance of cartilage to compression (Baeurle *et al.*, 2009).

Chondroitin sulfate readily interacts with proteins in the extracellular matrix due to its negative charges. The levels of chondroitin sulfate proteoglycans are vastly increased after injury to the central nervous system where they act to prevent regeneration of damaged nerve endings (Forsyth *et al.*, 2006). In cortical development, chondroitin sulfate is expressed by the Sub Plate and acts as a stop signal for neurons migrating from the Ventricular Zone (Forsyth *et al.*, 2006). Neurons stopping here may then be programmed for further migration to specific layers in the cortical plate (Jordan *et al.*, 2003). Chondroitin sulfate (CS) was originally isolated well before the structure was characterized, leading to changes in terminology with time. Early researchers identified different fractions of the substance with letters. So far four CS are known namely Chondriotin sulphate A, Chondriotin Sulphate C, Chondriotin Sulphate D and Chondriotin Sulphate E as shown in the table below:

Table 2-1 Fractions of chondroitin sulfate (Lavene *et al*,1931)

Letter Identification	Site of sulfation	Systematic name
Chondroitin sulfate A	Carbon 4 of the N-acetylgalactosamine (GalNAc) sugar	chondroitin-4sulfate
Chondroitin sulfate C	Carbon 6 of the GalNAc sugar	chondroitin-6sulfate
Chondroitin sulfate D	carbon 2 of the glucuronic acid and 6 of the GalNAc sugar	chondroitin-2,6sulfate
Chondroitin sulfate E	carbons 4 and 6 of the GalNAc sugar	chondroitin-4,6sulfate

2.6 PLACENTAL MALARIA

Placental malaria is caused primarily by infection with P. falciparum, the most dangerous of the four species of malaria parasites (WHO, 2001). Although *P. vivax* can also be found in the placenta *P. falciparum* is sequestered there more frequently and at a higher frequency (McGready *et al.*, 2004). Placental malaria has long been recognized as a complication of malaria in pregnancy in areas of stable transmission, and is particularly frequent and more severe in primigravidae (Matteelli *et al.*, 1997). Women experiencing placental malaria may exhibit normal symptoms of malaria, but may also be asymptomatic or present with more mild symptoms, including a lack of the characteristic fever. This may prevent a woman from seeking treatment despite the danger to herself and her unborn child (Desai *et al.*, 2007).

Interestingly, the greatest degree of placental infestation is seen in women who have the highest level of immunity, leading to milder maternal symptoms and a disproportionate increase in fetal complications (Monif and Baker, 2004). It could be hypothesized, therefore, that although primigravidas may develop the clinical symptoms of malaria, women with higher immunity may not demonstrate

symptoms, will not receive treatment, and will build a higher placental parasite burden (Schantz-Dunn and Nour, 2009).

P. falciparum expresses proteins on the surface of parasite-infected erythrocytes (PIEs) helping them to bind to chondroitin sulphate A (CSA) in the placental intervillous space (Fried and Duffy,1996; Neilson *et al.*,2009). *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) variant VAR2CSA enables adhesion of P. falciparum-infected erythrocytes to chondroitin sulfate A (Barfod *et al.*, 2010). By this process, the parasite avoids being filtered through the spleen where it would be cleared from the bloodstream and killed (Desai *et al.*, 2007).

Chondroitin sulphate is present in the placenta as a glycosaminoglycan side-chain to the tissue anticoagulant thrombomodulin (Salem *et al.*, 1984) and as part of a secreted low-sulphated aggrecan in the intervillous space, postulated to function as a reversible immobilizer of hormones, cytokines, and other molecules (Muthusamy *et al.*, 2004). Although CSA is a common component of the extracellular matrix and is found widely in the body, it is proposed that the placenta is the only site where interaction between CSA and the parasitized erythrocytes can actually occur (Fried and Duffy, 1996).

2.6.1 Characteristics of Malaria-infected Placenta

The placenta is a complex, sophisticated organ with several important functions throughout gestation, with the primary purpose of providing sustenance for the developing fetus. The placenta is also a site for P. falciparum sequestration (Matteeli *et al.*,1997). Many hypotheses, based on a systemic or local failure of the immunological response to malaria, have been proposed to explain the preference of the parasites for replication in the placenta; some have been reviewed elsewhere (Matteelli *et al.*, 1997). At term, the placenta is usually a circular-shaped structure measuring approximately 20 cm in diameter and 2.5 cm in thickness. The fetal portion of the placenta consists of the chorionic plate and its villi and the peripheral trophoblastic shell that surrounds the intervillous space and covers the maternal tissue. Anchoring villi contact the decidua basalis. The decidua basalis (maternal

portion of the placenta) rests on the stratum basalis and is characterized by large, polyhedral pale blue stromal (decidua) cells (Walter *et al.*, 1982; Fried *et al.*, 1998a).

The placental sequestration of *P. falciparum* is characterized by the accumulation of parasitized erythrocytes in the intervillous space (Fried *et al.*, 1998a). Malaria pigment can also be found concentrated in the macrophages in the intervillous spaces, in the trophoblasts and fribrin deposits (Matteelli *et al.*, 1997). Other histopathological changes that have been reported are the proliferation of cytotrophoblastic cells (suggesting a non-specific response to trophoplast damage), thickening of the trophoblastic basement membrane, and the presence of large numbers of leukocytes in the intervillous spaces. There is also infiltration by inflammatory cells, and release of pro-inflammatory mediators, which cause pathologic alterations (Walter *et al.*, 1982; Ordi *et al.*, 1998).

Bulmer *et al.*, (1993) indicated in their study that, the malaria pigment concentrated in circulating cells was associated with active infection, pigment in fibrin and fibrinoid necrosis of chorionic villi were features of active chronic infections, while thickening of the trophoblast basement membrane was common in both active and past infections. Also, perivillous fibrin and cytotrophoblast prominence were not associated with malarial infection (Bulmer *et al.*, 1993).

The exact mechanisms underlying histological changes of malaria-infected placentas are still being investigated. However, since the parasites are limited to the intervillous spaces they are unlikely to determine placental pathology directly. Leucocytes in the intervillous space seem to be a hallmark of active placental infection, and might be associated with the thickening of the trophoblastic basement membrane (Matteelli *et al.*, 1997), through the production of nonchemotactic cytokines by inflammatory reactions in the intervillous space (Leopardi *et al.*, 1996). The thickening of the trophoblast basement membrane produces a mechanical blockage adversely affecting oxygen and nutrient transport across the placenta. Compounding the situation are nutrients used by the developing and replicating parasites, and poor oxygen and glucose transfer from the parasitized erythrocytes sequestered in the placentas (Steketee *et al.*, 1996). The

high frequency of adverse perinatal outcomes, including, but not restricted to, premature, hypotrophic neonates, and stillbirths in the malarial population, has been linked to the intervillous macrophages, which decrease the maternal blood output, and perivillous excess of fibrin, which reduces the materno-fetal exchanges (Philippe and Walter, 1985).

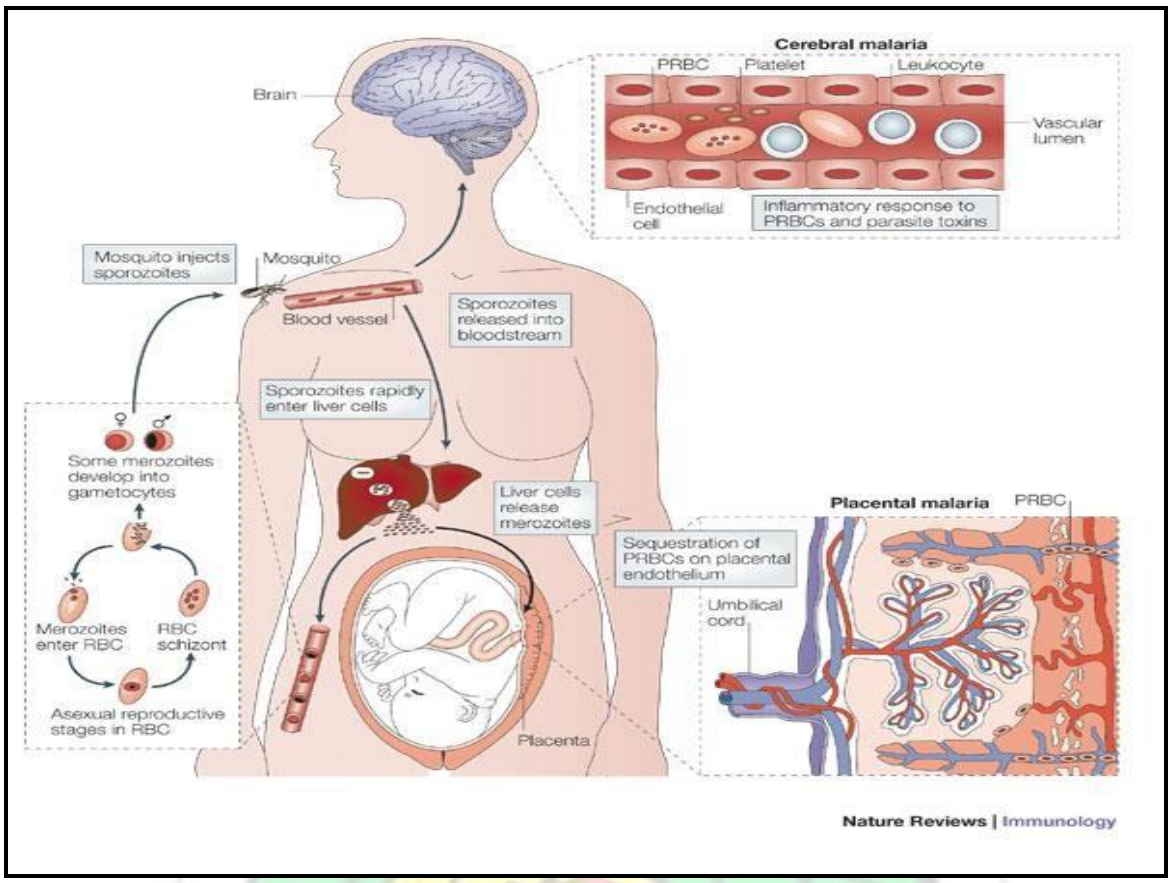

Figure 2-4: Mechanisms of malaria infection (*source: nature reviews/immunology; (Antelman et al., 2000)*)

2.7 CONSEQUENCES OF MALARIA IN PREGNANCY

2.7.1 Maternal Health and Malaria

Although pregnant women in malaria endemic areas have higher rates of parasitaemia and parasite density compared with non-pregnant women, infection is largely asymptomatic because some degree of pre-existing immunity is retained during pregnancy (Dorman and Shulman, 2000). However, even malaria-immune women (i.e., those who have evolved some level of immunity against severe infection as a result of long residence in areas of stable malaria transmission) are susceptible to placental malaria (Menendez *et al.*, 2000; Tako *et al.*, 2005). Because

so many parasites become sequestered within the placenta, peripheral blood smears often fail to detect evidence of infection (Dorman and Shulman,2000).

The resulting lack of appropriate or timely treatment may lead to adverse pregnancy outcome, including severe anemia, which is the main maternal consequence of malaria and can be deadly (Dorman and Shulman, 2000). Apart from anemia, malaria may contribute to maternal mortality by increasing the risk and severity of obstetric conditions such as pre-eclampsia/eclampsia and postpartum hemorrhage by as much as 50% (Brabin *et al.*,2005).

2.7.2 Effects on Birth Outcome

The effect of pregnancy malaria on the new born is very devastating. Low birth weight (defined as birth weight <2500g) is associated with a marked increase in infant mortality (Greenwood *et al.*, 1992; Luxemburger *et al.*, 2001). In areas of high malaria transmission in Africa, the risk of low birth weight approximately doubles if women have placental malaria with the greatest effect in primigravidae (Guyatt and Snow, 2004).

In sub-Saharan Africa, nearly 20% of low-birth weight deliveries are attributable to malaria in pregnancy and this is 35% of preventable low birth weight in women of all pregnancy disorders (Steketee *et al.*, 2001; Guyatt and Snow, 2004). Malaria induced low birth weight is estimated to be responsible for between 62, 000 and 363, 000 infant deaths every year in Africa, which translates to three to 17 deaths per 1000 live births (Murphy and Breman, 2001). Another estimate suggests that 11.4% of neonatal deaths and 5.7% of all infant deaths in malaria endemic areas of Africa may be caused by malaria in pregnancy-associated low birth weight, which translates to around 100 000 infant deaths (Guyatt and Snow, 2004). Not surprisingly, this effect is greatest in infants born to primigravidae at 17.6% of neonatal deaths and 9.8% of infant deaths (Guyatt and Snow, 2004).

The relative contribution of intrauterine growth retardation (IUGR) or preterm delivery in causing low birth weight varies by the level of malaria endemicity as well as other factors, such as access to prompt treatment and spread of HIV (Desai

et al., 2007). In areas of high malaria transmission where women are exposed to a greater frequency of antenatal infections and may have acquired immunity to prevent most febrile episodes that cause preterm delivery, IUGR is likely to be the predominant cause of malaria associated low birth weight (Brabin and Rogerson, 2001).

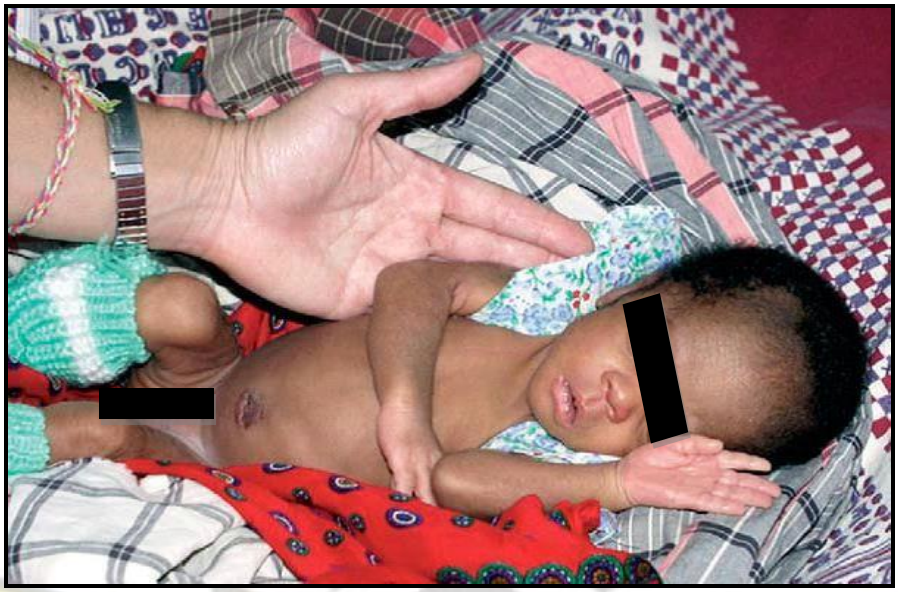

Figure 2-5: A low-birth weight baby born to a woman with an infected placenta
***Source:* (Brabin and Rogerson, 2001)**

Malaria in pregnancy in these settings may be responsible for up to 70% of IUGR, whereas its contribution to preterm delivery, although still substantial, is relatively lower at up to 36% (Steketee *et al.*, 2001)

Until recently, the link between placental malaria and stillbirths in endemic settings was unclear. However, a recent review of nine mainly hospital-based studies showed that placental malaria was associated with twice the risk for stillbirth; although the review did not take into account the effect of gravidity, it is likely that the effect is stronger in paucigravidae (van Geertruyden *et al.*, 2004). Furthermore, systematic reviews of randomized controlled trials have shown that successful prevention of malaria in pregnancy among paucigravidae with antimalarial prophylaxis, IPT, or insecticide-treated bednets, result in substantial reductions in perinatal mortality (27%) and spontaneous abortions and stillbirths (33%) (Garner and Gulmezoglu, 2006).

2.7.3 Effect on Infant Outcomes

The prevalence of foetal anaemia at birth is high in malaria-endemic areas, and the risk is associated with the presence of high-density parasitaemia in the mother at delivery (Brabin *et al.*, 2004). Few studies report the effect of malaria in the pregnant mother on anaemia or malaria in the infant(Epidermiology and Burden of malaria in pregnancy,2007). Some studies have now shown that the risk of allcause anaemia is estimated to be three times higher among infants born to mothers with placental parasitaemia, even after adjusting for environmental and ecological confounders (Cornet *et al.*, 1998; van Eijk *et al.*, 2002).

Recent evidence also indicates an association between placental malaria and diminished development of cellular and antibody responses to *P falciparum* epitopes in infants (Bonner *et al.*, 2005; Brustoski *et al.*, 2006). A birth cohort study from Tanzania reported a 41% increased risk of malaria infection in infants born to mothers with placental malaria (Mutabingwa *et al.*, 2005). This study shows that multigravidae are also at increased risk, implying that offspring of multigravid women with malaria may have greater clinical consequences than previously appreciated, even after adjusting for the effect of HIV on malaria in pregnancy, which is more pronounced in multigravidae (ter Kuile *et al.*, 2004).

These are important observations, because if placental malaria indeed affects infant morbidity in multigravidae, then the burden of malaria in pregnancy in Africa extends beyond that observed in paucigravidae, and the total burden may have been vastly underestimated. This is important to confirm in prospective studies. Placental malaria also reduces infant transplacental transfer of maternal antibodies and cellular immune responses in the infant to several other infectious diseases, including measles, Streptococcus pneumonia (de Moraes-Pinto *et al.*, 1998) and tetanus (Brair *et al.*, 1994).

Congenital malaria in the indigenous populations of malaria-endemic areas is generally reported as rare (Covell, 1950) and more frequent in offspring of nonimmune mothers with malaria (Quinn *et al.*, 1982). However, more recent reports from both malaria-endemic and non-endemic areas show higher

prevalences of congenital malaria ranging from 8% to 33%(Epidermiology of malaria in pregnancy,2007). The apparent increasing trend in the incidence of congenital malaria may be the result of increasing drug resistance, increasing virulence of the parasite, HIV, or increased reporting or detection of cases by use of PCR (Akindele *et al.*, 1993; Egwunyenga *et al.*, 1996).

2.7.4 Maternal Anaemia

Anemia is the most common consequence of P. falciparum malaria infection (Steketee *et al.*, 2001). In sub-Saharan Africa, it is estimated that between 200,000 and 500,000 pregnant women develop severe anemia as a result of malaria (Steketee *et al.*, 2001) and P. falciparum malaria in pregnancy is the primary cause of up to 10,000 maternal anemia-related deaths in sub-Saharan Africa annually (Anagnos *et al.*, 1986; Fleming, 1989a).

However, there have been conflicting reports from parts of sub-Saharan Africa on the relationship between placental malaria and maternal anemia. An earlier report from the Ubangi district of Zaire noted that malarious placentas had no consistent relationship to maternal anemia (Anagnos *et al.*, 1986). In other studies, maternal anemia and placental malaria were associated in all gravidity and age groups, with maternal anemia higher among women with placental malaria than those without placental malaria(Tako *et al.*,2005).

In most areas of malaria endemicity, many other causes of anemia have been identified, including both nutritional (iron, folate and protein deficiency) and nonnutritional (hookworm or HIV infection, hemoglobinopathy) factors (Fleming, 1989a; Antelman *et al.*, 2000). Since many of these causes of anemia occur concurrently in pregnancy and no unique hallmarks of malaria-driven anemia have been identified, it is difficult to evaluate the contribution made to anemia in pregnancy by placental malaria infection (Matteelli *et al.*, 1994).

Apart from its significant contribution to maternal mortality and both maternal and fetal morbidity, anemia in pregnancy is a risk factor for infant iron deficiency anemia (Colomer *et al.*, 1990) that, if left uncorrected, can be associated with

adverse behavioral and cognitive development. Severe anemia in pregnancy is an important direct and indirect cause of maternal death. During pregnancy, severe anemia may result in circulatory changes associated with an increased risk of heart failure and acute onset of anemia due to rapid cardiac decompensation and decreases in hemoglobin (Hb) concentration to < 80g/L (Achidi *et al.*, 2005).

Such changes can result in the failure of compensatory mechanisms, accumulation of lactic acid and breathlessness at rest (Achidi *et al.*, 2005). Furthermore, during labor, women with severe anemia are less able to endure even moderate blood loss and, as a consequence, are at a higher risk of requiring a blood transfusion during delivery (Fleming, 1989a). For the fetus, severe maternal anemia may result in intrauterine growth retardation, still birth, and low birth weight (Hoestermann *et al.*, 1996; Brabin and Piper, 1997; MacLeod and Rhode, 1998). The mechanism of malaria-driven anemia can be described in association with iron status in pregnancy. The iron status in pregnancy is affected by malaria parasites, which influence the anemia observed in pregnancy(Brabin and Piper,1996) . P. falciparum may affect iron status through reducing intestinal iron absorption, sequestrating iron within the malarial pigment haemeozoin, consuming iron for its own metabolism, promoting/stimulating the mobilization of iron to body stores, and releasing iron into the circulation during intravascular haemolysis (Fleming, 1989b; Fleming, 1989a)

2.8 CONGENITAL MALARIA

Malaria during pregnancy may result in fetal exposure to malaria if parasites are transmitted across the placenta and could result in congenital malaria. Transplacental transmission of *P. falciparum* has been well described, and the reported frequency of this event in babies born in malaria-exposed pregnancies has ranged from 0% to more than 25% (Riley *et al.*, 1989; Fievet *et al.*, 1995; Matteelli *et al.*, 1997; Steketee *et al.*, 2001). Thus, placental malaria is known to be a major determinant of congenital malaria.

Although previously thought to be a rarity in sub-Saharan Africa, a recent review has indicated that congenital malaria is more common than previously thought (Fried *et al.*, 1998a). While still debatable, congenital malaria may be defined as the presence of asexual stages of *P. falciparum* in cord blood smear at delivery or in peripheral blood smear of the infant in the first seven days of life, irrespective of clinical symptoms (Fried *et al.*, 1998a). Normally, symptoms occur 10 to 30 days postpartum; however, the disease may be seen in a day-old infant or appear after weeks to months (Meeusen *et al.*, 2001).

Interestingly, earlier reports from sub-Saharan Africa rarely identified clinical disease as a consequence of congenitally acquired malaria, and only a few reports before the 1970s documented detectable parasitaemia in infants younger than one month (Walter *et al.*, 1982; Riley *et al.*, 1989). These studies were interpreted as showing either that transplacental transmission of malaria occurs infrequently or that after transplacental transmission some elements of immunity acquired from the mother protects infants (Fievet *et al.*, 1995). It has been demonstrated that in hyperendemic areas, newborns more rarely become ill with malaria because of passive maternal antibody and high levels of fetal hemoglobin (Yamada *et al.*, 1989; Ordi *et al.*, 1998).

Postulated mechanisms for congenital transmission include maternal transfusion into the fetal circulation either at the time of delivery or during pregnancy, direct penetration of the chorionic villi, or penetration of premature separation of the placenta (Bulmer *et al.*, 1993). However, the effectiveness of the placenta to restrain malaria parasite passage to the fetus and the remarkable capacity of the fetus to resist infection has been demonstrated (Philippe and Walter, 1985). This resistance may reflect, among other things, the physical barrier of the placenta to infected red cells, the passive transfer of maternal antibodies, and the poor environment afforded by fetal erythrocytes for plasmodial replication due to their fetal hemoglobin composition and low free-oxygen tension (Philippe and Walter, 1985; Bulmer *et al.*, 1993).

It has been implied that transplacental transmission of malaria parasite and, consequently, congenital malaria seemed to occur rarely(Uneke CJ,2007). However, increasing reports from many parts of sub-Saharan Africa consistently have indicated high prevalence of umbilical cord parasitaemia ranging from 1.5% to 54.2%, and in some of these studies, there was a strong association between placental malaria and umbilical cord parasitaemia (Fried *et al*., 1998a).

Until recently, it was unclear whether the presence of *P. falciparum* malaria parasites in umbilical cord blood is an indication of antenatally acquired infection or contamination with infected maternal blood at delivery. In a study in Kenya, it was unequivocally shown that malaria parasites identified in cord blood are acquired antenatally by transplacental transmission of infected erythrocytes and primigravid and secundigravid women with placental malaria are at increased risk for congenital infection (Sartelet *et al*., 1997). The high rate of transplacental transmission of malaria appears to suggest the placental barrier is not very effective when infected with malaria parasites (Fievet *et al*., 1995).

2.8.1 Preterm delivery and intrauterine growth retardation

The relationship between placental malaria and preterm delivery (PTD)/intrauterine growth retardation (IUGR) has been evaluated in various studies. Two earlier studies among semi-immune women failed to show a difference in the proportion of PTD among infected and non-infected mothers (Uneke, 2008) but other reports across sub-Saharan Africa have shown that placental malaria was significantly associated with PTD and IUGR (Fleming, 1989a; Shulman, 1999; Guyatt and Snow, 2001; Etard *et al*., 2003). This is in contrast with reports from Yaoundé, Cameroon, Malawi, and The Gambia, where, after adjusting for potentially confounding variables, the association of placental malaria with PTD was not found to be significant (Dorman and Shulman, 2000; Brabin and Johnson, 2005).

Although the precise effect of malaria-parasitized placentas on PTD is uncertain, malaria-infected placentas frequently carry antibodies, cytokines, and macrophages, which are indicative of an active immune response, and this

immune response may stimulate early labor (Rogerson *et al.*, 2003b). The biological processes that mediate IUGR as a result of placental malaria remain uncertain, given that they can be studied only after the placenta has been delivered.

However, the IUGR effect appears to relate to nutrient transport to the fetus (Rogerson *et al.*, 2003b). A high density of parasites and chronic parasite infection in the placental blood and the associated cellular immune response may result in consumption of glucose and oxygen that would have gone to the fetus. Histopathological studies of infected placentas have found thickening of the cytotrophoblastic membranes, which may interfere with nutrient transport (Rogerson *et al.*, 2003b).

Although IUGR is more common than PTD with chronic placental infection, chronic infection of the placenta (with pigment and parasites) may be associated with low birth weight (LBW), through both prematurity and IUGR (Ismail *et al.*, 2000). Active placental infections were associated with a statistically significant lower risk of LBW as a result of IUGR and with a non-significant increase in the risk of LBW as a result of prematurity (Ismail *et al.*, 2000). This finding suggests that acute infections toward the end of pregnancy may play an important role in the induction of PTD, consistent with higher rates of abortions and preterm deliveries that have been observed during malaria transmission seasons (Anagnos *et al.*, 1986). Because premature infants are more likely to die than IUGR babies, the prevention of placental malaria particularly toward the end of gestation in malarious areas becomes absolutely imperative (Antelman *et al.*, 2000).

2.8.2 Effects on Neonatal Anthropometric Parameters

Neonatal anthropometric parameters such as neonatal length, head circumference, and placental weight have been related to placental malaria. In southeastern Tanzania, chronic ongoing malaria infection of the placenta was associated with significant reductions in mean head circumference, neonatal length, and body index (weight/length), whereas past infections were associated only with reduced mean length at birth (Ismail *et al.*, 2000).

In southeastern Nigeria, a slightly higher proportion of infected placenta was not significantly associated with lower neonatal length and lower head circumference; in the Ubangi district of Democratic Republic of Congo, malarious placentas had no consistent relationship with neonatal length or head circumference (Anagnos *et al.*, 1986). Reduction in newborn length and head circumference associated with chronic infections probably indicates a prolonged effect on fetal nutrition, which previously has been suggested (Matteelli *et al.*, 1994; Antelman *et al.*, 2000). Similarly, it has been suggested that the reduction in the body mass index may reflect the severity and duration of fetal malnutrition (Ismail *et al.*, 2000).

Two earlier studies evaluated the relationship between placental malaria and placental weight in Gabon. The mean weight of term placentas with malarial changes was significantly less than that of placentas without such changes (Colomer *et al.*, 1990). In southeastern Nigeria, placental malaria was significantly associated with lower placental weight. The reason for this reduction is not fully known, but it may be associated with changes in the placenta, including the presence of parasitized erythrocytes and malarial pigment particles in the intervillous space, chronic basal villitis, malarial pigment deposits in the trophoblasts, trophoblastic damage with focal necrosis, partial loss of microvilli, and thickening of the trophoblastic basement membrane (Achidi *et al.*, 2005).

As a result of these changes, a high density of parasites and chronic parasite infection in the placental blood and the associated cellular immune response may result in consumption of glucose and oxygen that would have gone to the fetus (Rogerson *et al.*, 2003b). Placental insufficiency also could be attributable to physical blockage by parasitized red blood cells and the massive monocyte infiltration of the intervillous spaces. Such infiltrate is likely to be a source of cytokines, including interferon-g, interleukin-2, interleukin-6, and tumor necrosis factor–a, which are considered detrimental to pregnancy because they are associated with growth retardation (Hoestermann *et al.*, 1996).

2.8.3 Fetal anemia

The prevalence of fetal anemia, defined as cord hemoglobin level < 12.5g/dl, is reportedly very high in sub-Saharan Africa. In two separate studies conducted in southern Malawi, fetal anemia prevalences of 23.4 percent (MacLeod and Rhode, 1998) and 23.3 percent (Brabin and Piper, 1997) were recorded, while in Maputo Mozambique, up to 93 percent of newborns were found to have fetal anemia (Brabin and Piper, 1997). Interestingly, a statistically significant link was established between fetal anemia and maternal malaria infection in all of these studies. The contributory role of placental malaria to fetal anemia has been evaluated in a number of studies with varying results.

In southern Malawi, a higher prevalence of fetal anemia occurred with increasing peripheral *P. falciparum* parasite density, and geometric mean placental parasite densities were higher in babies with fetal anemia than in those without it (MacLeod and Rhode, 1998). Other studies have found no statistically significant connection between evidence of malaria infection and fetal anemia (Bouyou-Akotet *et al.*, 2003).

This lack of consistency in the findings from various studies may be explained by the fact that malaria in pregnancy varies with transmission intensity, access to treatment, coverage and quality of antenatal services, and drug resistance, among other factors (Steketee *et al.*, 1996; Rogerson *et al.*, 2000). The etiology of fetal anemia is complex and multifactorial; placental malaria could play either a major or minor role, depending on the local epidemiological situation (Dicko *et al.*, 2003). It has been suggested that exposure of the fetus to malaria antigens due to damage of the placental barrier may make the newborn more susceptible to immunologically mediated haemolysis or to dyserythropoiesis.

Chapter 3 MATERIALS AND METHODS

3.1 STUDY DESIGN

This is a cross-sectional study involving data collection from pregnant women attending ante-natal clinic and non-pregnant women attending the out patient department.

3.2 STUDY SITES

The study was carried out at the Kaneshie Polyclinic in Accra and Our Lady of Grace Hospital in the Central region. The Kaneshie Polyclinic is a community health center situated at Kaneshie, a suburb of Accra. Patients attending the hospital are covered by the National Health Insurance Scheme (NHIS), which grants access to free basic medical care. An average of 148 pregnant women attend the polyclinic for antenatal care each month. This center was selected primarily because of its convenient location in the middle of the town making it easily accessible to pregnant women from all directions in the locality. With as many as 8 midwives and 5 medical officers, the polyclinic prioritizes women in pregnancy.

Our Lady of Grace Hospital is located at Bremen Asylum in the Asikuma-OdobenBrakwa district in the Central region. The hospital is a member of the Christian Health Association of Ghana. It is the biggest hospital in the district serving the people of Asikuma and neighboring towns. Unlike the Kaneshie Polyclinic, Our Lady of Grace hospital is a 24-hour facility that also offers a wide range of services including diagnostics. It has a capacity of 104 beds, with 6 medical officers and 8 midwives. An average of 160 pregnant women visit the hospital for antenatal care each month. The hospital, also NHIS accredited, was selected because it was the main health center in the district, providing health care to the surrounding communities.

3.3 STUDY SUBJECTS AND SAMPLE SIZE

3.3.1 Study Subjects

The study population comprised pregnant women attending the ante-natal clinic of the selected health facilities and non–pregnanat women attending the out patient departments of the health facilities served as controls.

3.3.1.1 Inclusion Criteria

The inclusion criteria included:

- Pregnant women who consented to the study
- Non-pregnant women who consented to the study

3.3.1.2 Exclusion Criteria

The exclusion criteria inclucluded:

- Women who would not consent to the study
- Pregnant women who had other complications like HIV and heart related diseases
- Children and males

3.3.2 Sample Size

Sample size was determined by the Cochran's method(Cochran,1934). A sample size of 384 was obtained using a prevalence rate of 50% since the study had not been carried out in Ghana before. A finite population correction was done based on the average number of pregnant women seen at the two facilities together within a month (i.e. 250) and this yielded a sample size of 151 which was approximated to 160 to adjust for drop-out or volunteers who might opt out of the study.

3.3.2.1 Sample size calculation (Cochran, 1934)

$$n_o = \frac{Z^2 \times p(1-p)}{e^2}$$

Where: n_o = sample size

Z^2 = Abscissa of the normal curve that cuts off an area (α) at the tails (**1.96**)

e = the acceptable sampling error at 95% confidence interval (**0.05**) p = the estimated proportion of the attribute present in the population (**50**%)

$$n_o = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2}$$

$$n_o = \frac{(3.8416) \times 0.25}{2.5 \times 10^{-3}}$$

$$n_o = \frac{0.9604}{2.5 \times 10^{-3}}$$

$$n_o = 384.16$$

3.3.2.2 Finite population correction for proportions

Final population correction was calculated to adjust the estimated sample size calculated above to fit the actual estimates of pregnant women visiting the facilities enrolled in the study. The final population correction for proportion formular is as follows:

$$n = \frac{n_o}{1 + \frac{(n_o - 1)}{N}}$$

Where: $\mathbf{n_o}$ = the initial sample size (384.16) **n**

= adjusted sample size

N = the population size (estimated to be 250; representing the average number of pregnant women visiting the respective facilities in a month)

$$n = \frac{384}{1 + \frac{(384-1)}{250}}$$

$$n = \frac{384}{1 + \frac{(383)}{250}}$$

$$n = \frac{384}{1 + 1.532}$$

$$n = \frac{382}{2.532}$$

$$n = 150.86 \cong 151$$

3.4 DATA COLLECTION AND ETHICAL CLEARANCE

Data collection took place from September 2013 to July 2014. Pregnant women attending antenatal clinic of the Kaneshie Polyclinic and Our Lady of Grace Hospital were approached to participate in the study. In all, a total of 160 study participants were recruited into the study. These were categorized into three groups comprising 80 pregnant women with malaria within the case group and 40 pregnant women each without malaria and non-pregnant women with malaria respectively within the control group. Verbal explanation of the aim of the study was given to potential participants and those who accepted to partake in the study were made to sign written consent forms. For participants who consented to partake, questionnaires were administered requesting demographic information on age and number of times participants had been pregnant regardless of whether these pregnancies were carried to term. Healthy non-pregnant women were recruited into the study following the same protocol to serve as parallel controls in addition to the case control design of the study. Clearance for sampling was sought from the the Directors and the Institutional Ethical Boards of the two health facilities that were involved in the research. For each of the study participants who agreed to partake in the study, the aims and objectives of the study were explained to them and a written or verbal consent taken prior to data collection and documented.

3.5 LABORATORY PROCEDURES

Capillary blood samples were first collected and used for the rapid diagnostic test (RDT) as a test preliminary to screen for both positive and negative cases. A thick blood film was prepared from the capillary blood samples of subjects who tested

positive for malaria RDT. This was used as a screening test before 6ml of venous blood samples were collected from each study subject: 2ml was dispensed into one ethylene diamine tetraacetic acid (EDTA)-containing tube (Vacutainer; BD Biosciences, San Jose, CA, USA), and 4 ml into a second EDTA tube. The tubes were disposable, non-pyrogenic, and non-endotoxin. Samples in the EDTA tubes were well mixed and the first 2ml tube was used for the full blood count. Also from the blood sample in the second EDTA tube, plasma was isolated by centrifugation for 15 minutes at 1000xg within 30 minutes of sample collection. The plasma was pipetted into 1000µL pyrogen-free plain tubes leaving the blood-cell sediment which was discarded. At least four aliquots of plasma were collected from each sample and were frozen until assayed for CSA concentrations. Samples were frozen and thawed only once prior to analysis.

The following laboratory tests were carried out:

- Rapid Diagnostic Test (RDT) for malaria
- Giemsa staining of thick blood films
- Full blood counts
- ELISA for chondroitin sulphate A levels

3.5.1 Rapid Diagnostic Test (RDT) for Malaria

The RDT was used as a screening test for both positive cases specific for *P. falciparum* and the negative cases which were used for controls before blood samples were taken into EDTA tubes.

This method was for rapid qualitative detection of malaria histidine-rich protein 2 (HRP2) in human blood as an aid in diagnosing *P. falciparum* malaria infection. The RDT kit comprised: test device, dropper (mini pipette) for transferring blood, assay buffer, sterile blood lancets, and alcohol swabs.

Figure 3-1: Malaria RDT kit

3.5.1.1 Procedure

A sterile blood lancet was used to prick the finger to obtain blood after cleaning the finger with the alcohol swab. By applying gentle pressure to the finger the first drop of blood was expressed and wiped away with a dry piece of cotton wool. Using the dropper 2 drops of blood from each subject was collected and added onto the sample well of one labelled RDT test device. Three (3) drops of the assay buffer was also added to the buffer well and results read after 20 minutes. The results were interpreted as follows: Red lines at the test 'T' and control 'C' regions indicated sample was positive for *P. falciparum* whilst one red line only at the control 'C' indicated sample was negative for *P. falciparum*.

3.5.2 Thick Blood Films for Malaria Parasites

Pre-cleaned frosted end slides were first labelled according to the subjects. Using a micropipette, 6 µl of blood was then collected from each subject directly from the finger onto a labeled glass slide to prepare a thick film of size 12 mm. The smears were then air-dried on a slide rack on a flat surface and protected from dust and flies. Smears were stained within 72 hours of preparation using Giemsa stain of 10% concentration and buffered pH 7.0 – 7.2, for 10 minutes. During the staining process, the slides were arranged on a staining rack with the smears facing upwards. The smears were then flooded with the prepared stain and timed

appropriately for a staining period of 15 minutes. The slides were washed gently and individually, after which they were air-dried and examined using the x100 oil immersion lens.

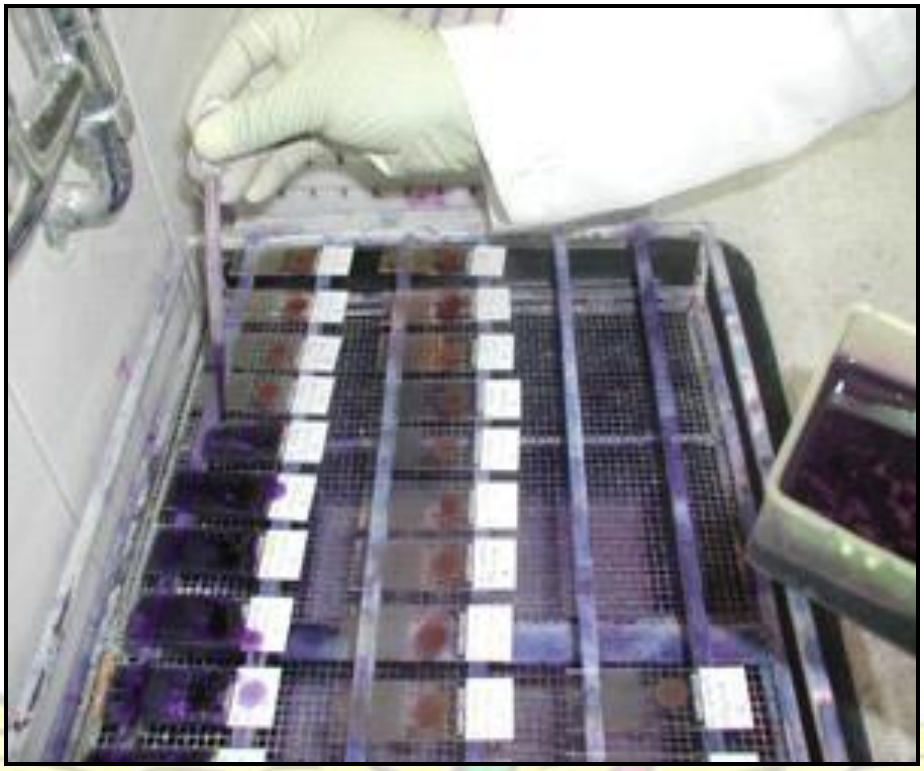

Figure 3-2: Preparation and staining of blood films

The parasite densities of the positive films were determined by counting the number of parasites against the white blood cells until at least 200 WBCs were counted. The parasites were quantified using the formula:

$$Parasite\ count = \frac{Number\ of\ malaria\ parasites\ counted}{WBCs\ counted} \times Total\ WBC\ Count$$

3.5.3 Full Blood Count

For full blood counts blood in the EDTA-containing tubes was processed within two hours of handling. Assessment of haemoglobin level, the quantification of blood platelets and white blood cell numbers, and differential counts were done using the auto haematology analyzer, Sysmex KX-21N (Sysmex Asia Pacific Pte Ltd and Sysmex Corporation of Japan, Kobe, Japan). The Sysmex KX-21N is an

automated hematology analyzer that provides primary white blood cell (WBC) count, all differential information and reticulate analysis. In this test, each whole blood sample was mixed thoroughly by gentle agitation and then fed to an aspirator on the Sysmex KX-21N machine after which results were produced and printed out within minutes.

Figure 3-3 Sysmex KX-21N

3.5.4 Assaying for Chondroitin Sulphate A using ELISA

The quantitative determination of Chondroitin Sulphate A (CSA) concentrations was done using a commercial Enzyme Linked Immunosorbent Assay (ELISA) kit, (Human CS ELISA, Elabscience Biotechnology Co. Ltd., Wuhan, Hubei Province, China). This ELISA kit applied to the *in vitro* quantitative determination of human CSA concentrations in serum, plasma and other biological fluids. It had a detection range of 0.31 – 20 ng/mL. The kits componenets were:

Table 3-1 ELISA Kit components

ITEM	Specifications
Micro ELISA Plate	8wells x 12 strips
Reference Standard	2 vials
Reference Standard & sample Diluent	1 Vial 120 mL
Concentrated Biotinylated Detection Ab	1 Vial 120 µL
Biotinylated Detection Ab Diluent	1 Vial 10 µL

Concentrated HRP Conjugate	1 Vial 120 µL
HRP Conjugate Diluent	1 Vial 10 mL
Concentrated Wash Buffer (25x)	1 Vial 30 mL
Substrate Reagent	1 Vial 10 mL
Stop Solution	1 Vail 10 Ml
Plate sealer	5 pieces
Manual	1 copy
Certificate of Analysis	1 copy

The kit employed the Sandwich- ELISA method. The micro ELISA plate had been pre-coated with an antibody specific to CSA. Standards and samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for CSA and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well successively and incubated, after which the substrate solution was added to each well. Only those wells that contained CSA, biotinylated detection antibody and Avidin- HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of a 2% sulphuric acid solution and the color turned yellow. The optical density (OD) was measured spectrophometrically at wavelength of 450 nm. From the results obtained, a standard curve was run using Microsoft Office Excel 2013 (Microsoft, Redmond, Washington, USA). The concentration of CSA in the samples were calculated by interpolation from the standard curve. The ELISA was done at Kumasi Center for Collaborative Research (KCCR) laboratory at the Kwame Nkrumah University of Science and Technology (KNUST).

3.6 STATISTICAL ANALYSIS

Raw data obtained from the study respondents were entered in Microsoft Excel® and cleaned for duplicate entries and outliers. Data were presented as mean ± SD using ANOVA, linear regression and logistic regression for univariate and multivariate analysis. Box and whisker plots were explored to present the central

tendencies of data were appropriate. Univariate logistic regression was performed with malaria as the dependent variable, using age, gravidity, parasite count, chondroitin sulphate concentration, WBC count, RBC count, haemoglobin concentration, haematocrit, MCV, MCH, MCHC and platelet count as independent variables. Age was categorized as ≤ 25 years versus >25 years; gravida was categorized as primigravidae, secungravidae, multigravidae 3 – 5 and grandmultigravidae >5; haemoglobin concentration was categorized as <11 g/dL and ≥11 g/dL; haematocrit was categorized as ≤33% and >33%; MCV was categorized as ≤70 fL and >70 fL; MCH was categorized as ≤23 pg/cell and >26 pg/cell and MCHC as ≤31 g/dL and >31 g/dL. Parasite count, chondroitin sulphate, WBC and RBC were analyzed on a continuous scale. All data were analyzed using GraphPad Prism version 5.01 (*www.graphpad.com*) and for all comparisons, a p-value <0.05 was considered significant.

Chapter 4 RESULTS

The study population comprised 160 clients out of which 80 were pregnant women with malaria, 40 were pregnanat women without malaria and 40 were non- pregnanat women with malaria.

4.1 AGE AND HAEMATOLOGICAL CHARACTERISTICS OF STUDY SUBJECTS

The age and haematological characteristics of the pregnant women with malaria group (80) as compared with the non-pregnant women with malaria group (40) is as shown in Table 4-1. The mean age of the pregnant women with malaria group was 24.2 ± 6.2 years compared to the non-pregnant women with malaria group which was 27.0 ± 9.5 years (p=0.057) and it was not significantly different. The haemoglobin concentration in the non-pregnant with malaria group was 10.3 ± 1.6 g/dL was significantly higher compared with that of the pregnant with malaria group was 9.6 ± 1.4 g/dL(p=0.008). Mean cell volume was 80.9 ± 6.6 vs 76.5 ± 4.5 fL (p<0.0001) and mean cell haemoglobin concentration was 31.7 ± 2.2 vs 30.8 ± 1.7 g/dL(p=0.016) as measured in the non-pregnant with malaria group were significantly higher when compared with the pregnant with malaria group.

Contrarily, the platelet count as estimated in the pregnant with malaria group was 146.3 ± 67.6 $x10^9$/L when compared with the non-pregnant with malaria group which was 114.7 ± 71.6 $x10^9$/L (p=0.020). This was significantly higher. A comparison of WBC, lymphocytes count, neutrophil count, RBC count, haematocrit and mean cell haemoglobin between the two groups as shown in Table 4-1 showed no statistically significant differences.

Table 4-1: Age and haematological characteristics of pregnant women with malaria and non-pregnant women with malaria groups

Parameter	Pregnant with malaria (n = 80)	Non-pregnant with malaria (n=40)	*p-value*
Age (years)	24.2 ± 6.2	27.0 ± 9.5	0.057
WBC (k/µL)	5.9 ± 2.2	7.0 ± 4.2	0.081
Lymphocytes (%)	28.2 ± 10.5	32.4 ± 17.5	0.097
Neutrophils (%)	63.4 ± 11.7	58.6 ± 19.1	0.090
Hb (g/dL)	9.6 ± 1.4	10.3 ± 1.6	0.008
RBC ($x10^{12}$/L)	3.7 ± 0.8	3.9 ± 0.5	0.054
HCT	31.6 ± 6.2	32.4 ± 4.7	0.474
MCV (fL)	76.5 ± 4.5	80.9 ± 6.6	< 0.0001
MCH (pg/cell)	26.8 ± 2.7	26.4 ± 2.6	0.466
MCHC (g/dL)	30.8 ± 1.7	31.7 ± 2.2	0.016
PLT ($x10^9$/L)	146.3 ± 67.6	114.7 ± 71.6	0.020

Data are presented as Mean ± SD. WBC-white blood cell; Hb-haemoglobin; RBC-red blood cell; HCT-haematocrit; MCV-mean cell volume; MCH-mean cell haemoglobin; MCHC-mean cell haemoglobin concentration; PLT-platelet; p-value = defines the level of significance when pregnant participants with malaria were compared with non-pregnant participants with malaria (unpaired t-test)

Table 4-2 summarizes the age and haematological characteristics of the pregnant women with malaria group (80) and the pregnant women without malaria group (40). There was no significant difference in age among the two groups. The total WBC count was 5.9 ± 2.2 k/µL in the pregnant with malaria group compared to the pregnant without malaria group which was 3.8 ± 0.5 k/µL ($p<0.0001$).This was significantly higher . The lymphocyte count in the pregnant with malaria group which was 34.6 ± 10.2 compared to that estimated for the pregnant with malaria group which was 28.2 ± 10.5% ($p=0.002$) was significantly higher.

The mean haemoglobin count was 9.6 ± 1.4 vs. 11.5 ± 1.1 g/dL (p< 0.0001), haematocrit was 31.6 ± 6.2 vs. 34.4 ± 3.3 (p = 0.007), MCV was 76.5 ± 4.5 vs. 8.4 ± 9.6 fL (p< 0.0001) and platelet count was 146.3 ± 67.6 vs. 216.3 ± 53.4 $x10^9$/L (p=0.02) as estimated in the pregnant with malaria group were significant reduced when compared to the mean values estimated for the pregnant without malaria group.

The neutrophil count, MCH and MCHC concentrations did not show any significant differences between the two groups.

Table 4-2: Age and haematological characteristics of pregnant women with malaria and pregnant women without malaria groups

Parameter	Pregnant with malaria (n = 80)	Pregnant without malaria (n = 40)	*p-value*
Age (years)	24.2 ± 6.2	25.0 ± 7.9	0.546
WBC (k/µL)	5.9 ± 2.2	3.8 ± 0.5	< 0.0001
Lymphocytes (%)	28.2 ± 10.5	34.6 ± 10.2	0.002
Neutrophils (%)	63.4 ± 11.7	63.4 ± 10.5	0.991
Hb (g/dL)	9.6 ± 1.4	11.5 ± 1.1	< 0.0001
RBC ($x10^{12}$/L)	3.7 ± 0.8	3.0 ± 0.7	< 0.0001
HCT	31.6 ± 6.2	34.4 ± 3.3	0.007
MCV (fL)	76.5 ± 4.5	88.4 ± 9.6	< 0.0001
MCH (pg/cell)	26.8 ± 2.7	26.9 ± 2.2	0.798
MCHC (g/dL)	30.8 ± 1.7	31.4 ± 1.9	0.080
PLT ($x10^9$/L)	146.3 ± 67.6	216.3 ± 53.4	< 0.0001

Table 4-3 summarizes the age and the haematological characteristics of the non-pregnant with malaria group and the pregnant without malaria group. A

comparison of age between the two groups showed no statistically significant difference. The total white blood cell count (WBC) of the non-pregnant with malaria group was 7.0 ± 4.2 k/µL compared with the pregnant without malaria group which was 3.8 ± 0.5 k/µL ($p<0.0001$) was significantly higher. The mean haemoglobin concentration was 10.3 ± 1.6 vs. 11.5 ± 1.1 ($p=0.002$), mean haematocrit concentration was 32.4 ± 4.7 vs. 34.4 ± 3.3 ($p=0.026$), mean MCV was 80.9 ± 6.6 vs. 88.4 ± 9.6 fL ($p=0.0001$) and the mean platelet count was 114.7 ± 71.6 vs. 216.3 ± 53.4 $x10^9$/L ($p<0.0001$) as estimated in the non-pregnant with malaria group was significantly lower when compared with the mean values estimated for the pregnant without malaria group. The mean RBC count estimated in the non-pregnant with malaria group was 3.9 ± 0.5 $x10^{12}$/L compared to the mean count in the pregnant without malaria group which was 3.0 ± 0.7 $x10^{12}$/L ($p<0.0001$) was significantly higher. The mean lymphocyte count, mean neutrophil count, MCH and MCHC values for the non-pregnant with malaria group showed no statistically significant difference from that estimated for the pregnant without malaria group.

Table 4-3: Age and haematological characteristics of non-pregnant women with malaria and pregnant women without malaria groups

Parameter	Non-pregnant with malaria (n = 40)	Pregnant without malaria (n = 40)	*p-value*
Age (years)	27.0 ± 9.5	25.0 ± 7.9	0.314
WBC (k/µL)	7.0 ± 4.2	3.8 ± 0.5	< 0.0001
Lymphocytes (%)	32.4 ± 17.5	34.6 ± 10.2	0.501
Neutrophils (%)	58.6 ± 19.1	63.4 ± 10.5	0.161
Hb (g/dL)	10.3 ± 1.6	11.5 ± 1.1	0.002
RBC ($x10^{12}$/L)	3.9 ± 0.5	3.0 ± 0.7	< 0.0001
HCT	32.4 ± 4.7	34.4 ± 3.3	0.026

MCV (fL)	80.9 ± 6.6	88.4 ± 9.6	0.0001
MCH (pg/cell)	26.4 ± 2.6	26.9 ± 2.2	0.351
MCHC (g/dL)	31.7 ± 2.2	31.4 ± 1.9	0.551
PLT (x10^9/L)	114.7 ± 71.6	216.3 ± 53.4	< 0.0001

Data are presented as Mean ± SD. WBC-white blood cell; Hb-haemoglobin; RBC-red blood cell; HCT-haematocrit; MCV-mean cell volume; MCH-mean cell haemoglobin; MCHC-mean cell haemoglobin concentration; PLT-platelet; p-value = defines the level of significance when nonpregnant participants with malaria were compared with pregnant participants without malaria (unpaired t-test).

4.2 GRAVIDITY OF STUDY SUBJECTS

The pregnancy outcomes of the study respondents regardless of whether such pregnancies were carried to term were assessed from antenatal records. The box and whisker plot showing the minimum (*lower whisker*), 25th percentile, median, 75th percentile and maximum (*upper whisker*) gravid status is shown in Figure 4-1. Women across the three categories had a median gravid status of 1. Pregnant women with malaria had a mean gravid status of 2 and this was significantly higher when compared with the non-pregnant with malaria mean of 1($p<0.0001$) and pregnant without malaria mean = 1; $p<0.0001$) groups respectively (*Tukey's post-test*).

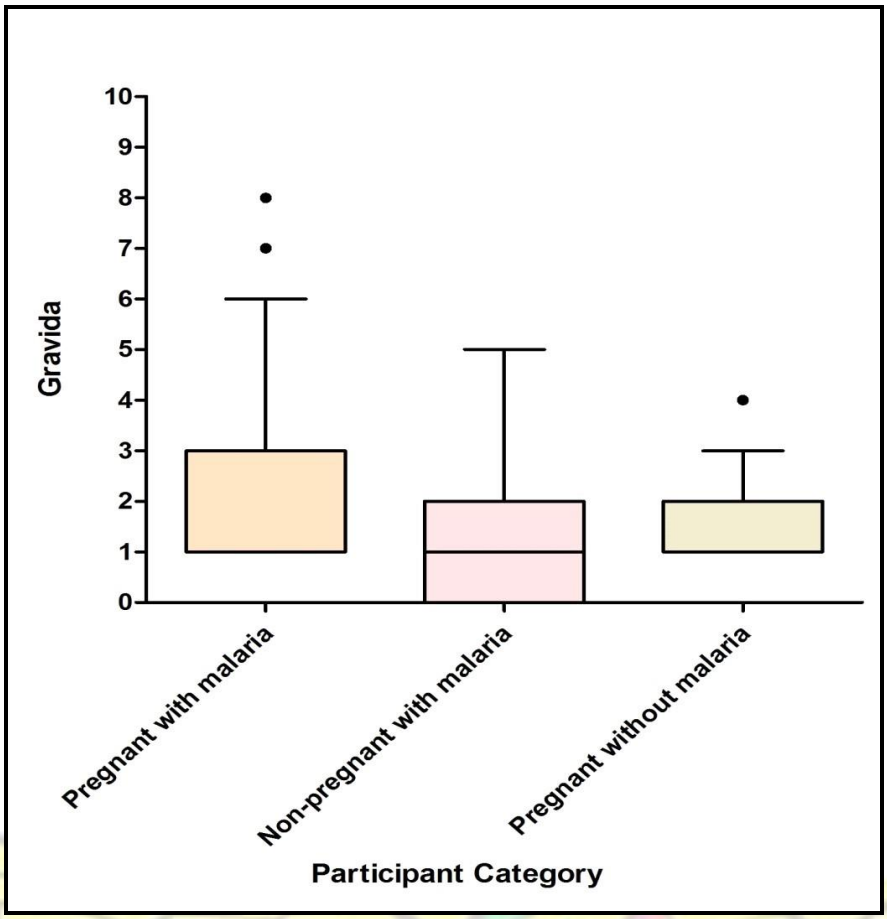

Figure 4-1: Box and Whisker plot of gravid status of the study participants stratified by category

4.3 CHONDROITIN SULPHATE A CONCENTRATION OF STUDY SUBJECTS

Chondroitin sulphate A (CSA) concentration among the study participants stratified by the presence or absence of pregnancy and/or malaria is as depicted in Figure 4-2. The median CSA concentration in pregnant women with malaria was 50.76 ng/mL and that of pregnant women without malaria was 44.94 ng/mL.There was no significant difference (p=0.084) The median CSA concentration in the non-pregnant women with malaria was 2.24 ng/mL when compared with the other two categories of respondents and this was significantly lower ($p<0.0001$).

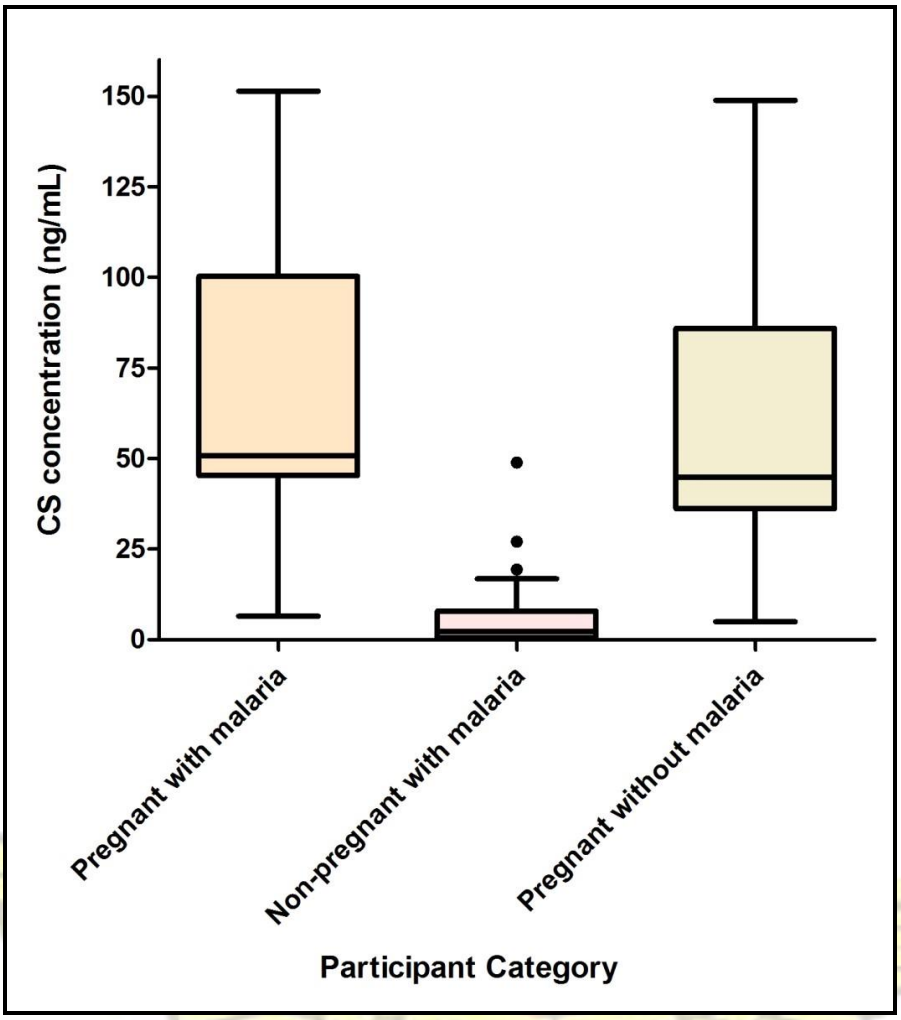

Figure 4-2: Box and Whisker plot of chondroitin sulphate - A concentration among the study participants stratified by category

4.4 MALARIA PARASITE BURDEN AMONG THE STUDY RESPONDENTS

The malaria parasite burden in study respondents expressed as number of parasites per microlitre of blood is as shown in the box and whisker plot in Figure 4-3. The median parasite count in pregnant women with malaria was 1532 parasites/µ which compared with that in non-pregnant women with malaria (4077 parasites/µL) was significantly lower ($p<0.0001$). The maximum parasite count, the 75th percentile, the 25th percentile and minimum parasite count in pregnant women with malaria were 18, 460 parasites/µL, 5655 parasites/µL, 624.5 parasites/µL and 66 parasites/µL respectively. Those in

non-pregnant women with malaria were 20, 916 parasites/µL, 12, 096 parasites/µL, 1, 176 parasites/µL and 129.0 parasites/µL respectively.

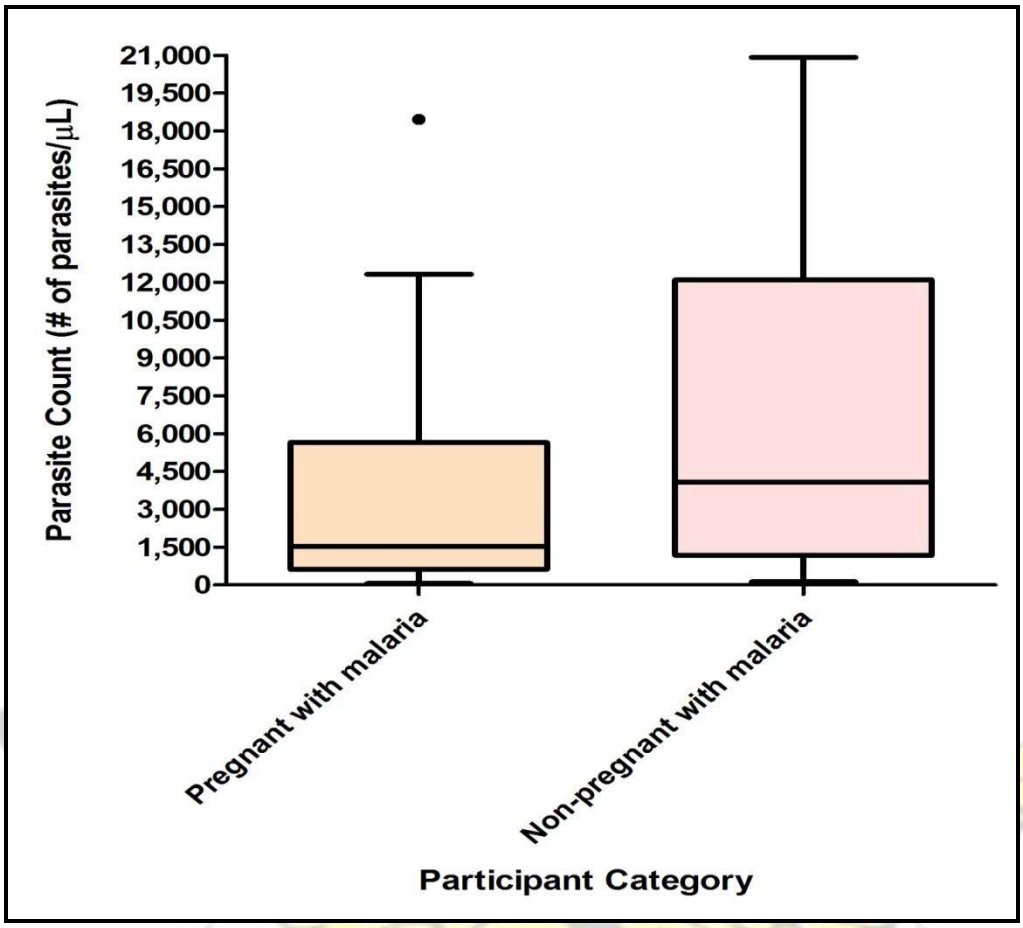

Figure 4-3: Box and Whisker plot of malaria parasite count in pregnant women with malaria and non-pregnant women with malaria.

4.5 RISK FACTORS ASSOCIATED WITH MALARIA

Univariate analyses of risk factors associated with malaria are as depicted in Table 4-4. From the parameters analyzed, the association between haemoglobin concentration less than 11 g/dL with an odd ratio of 6.5 (CI =2.98 -14.25) and haematocrit concentration less than 33% with an odd ratio of 4.6 (CI = 2.09 - 9.89) were both significant (p=0.0001). White blood cell count with an odd ratio of 2.3 (CI=1.62-3.13) and red blood cell count with an odd ratio of 5.5 (CI=2.77-11.06) assessed on a continuous scale were also significantly associated with malaria infection (p=0.0001).

Malaria infection among the study participants was adjusted for pregnancy in the multivariate analyses to assess the risk association of each parameter when pregnancy co-exists with malaria. The 6.5 risk for malaria infection associated with haemoglobin concentration (<11 g/dL) in the univariate analysis rose to 10.7 (CI = 4.40 - 26.04, p = 0.0001) when pregnancy was associated with malaria. Likewise, the 4.6 risk of malaria infection associated with haematocrit in the univariate analysis marginally rose to 5.7 (CI = 2.47 - 13.06, p = 0.0001) when the pregnancy co-existed with malaria. Mean cell haemoglobin concentration which was hitherto not significantly associated with malaria infection in the univariate analysis showed an odds of 2.3 (CI = 1.04 - 4.98, p = 0.041) when pregnancy is coexisting with malaria. There was an inverse relation was obserserved between platelet concentration with pregnancy coexisting with malaria with an odd ratio of 0.98 (CI = 0.98 - 0.99, p = 0.0001).

Table 4-4: Risk factor analysis

	Univariate			Multivariate (adj: pregnancy)		
Parameters	**OR**	**95%CI**	***p-value***	**OR**	**95%CI**	***p-value***
Age (>25 years)	1.5	0.70 - 3.24	0.300	1.3	0.59 – 3.00	0.497
HB (<11 g/dL)	6.5	2.98 - 14.25	0.0001	10.7	4.40 - 26.04	0.0001
CSA	1.0	0.98 - 1.00	0.123	1.0	1.00 - 1.02	0.118
WBC	2.3	1.62 - 3.13	0.0001	2.5	1.69 - 3.76	0.0001
RBC	5.5	2.77 -11.06	0.0001	4.2	2.04 - 8.66	0.0001
HCT (≤33 %)	4.6	2.09 - 9.89	0.0001	5.7	2.47 - 13.06	0.0001
MCV (≤70 fL)	4.0	0.50 - 31.78	0.194	5.0	0.61 - 41.06	0.133
MCH (≤26 pg/cell)	2.3	0.50 - 10.81	0.280	2.4	0.50 - 11.89	0.269
MCHC	1.7	0.83 - 3.65	0.144	2.3	1.04 - 4.98	0.041
PLT	1.0	0.98 - 0.99	0.0001	0.98	0.98 - 0.99	0.0001

Adj: pregnancy-adjusted for pregnancy; OR = odds ratio; CI = confidence interval

Chapter 5 DISCUSSION

5.1 DISCUSSION

Malaria in pregnancy still remains an important public health problem that has proven difficult to tackle despite numerous studies conducted over the last decades (De Beaudrap *et al.*, 2013). Pregnant women are more susceptible to malaria than their non-pregnant counterparts (Boel *et al.*, 2012) and therefore the extent of utilization of malaria preventive measures may impact on the burden of malaria in pregnancy (Tongo *et al.*, 2011). Maternal, placental and foetal malaria infection during pregnancy adversely affects development and survival of foetus through low birth weight, maternal anaemia and possibly abortion and stillbirth. These malaria-induced medical problems constitute major clinical, public health and research challenges (Murphy and Breman, 2001). Pregnancy causes significant changes in metabolism, fluid balance, organ function and blood circulation which are mainly driven by oestrogen and the presence of the foeto-placental unit (Elgari, 2013). Such dramatic changes influence a wide variety of haematological parameters and as such acknowledging these changes is essential when interpreting the result of haematological investigations to diagnose or monitor illness in pregnant women.

5.2 HAEMATOLOGICAL OUTCOMES

The World Health Organization (WHO) has suggested the presence of anaemia in pregnancies when haemoglobin (Hb) concentration is less than 11 g/dL (Sharma, 2003). This current study showed a significant decrease in Hb concentration in the pregnant women with malaria group, which was 9.6 ± 1.4 g/dL compared to that in the non-pregnant women with malaria which was 10.3 ± 1.6 g/dL ($p=0.008$) and pregnant women without malaria which was 11.5 ± 1.1 g/dL ($p<0.0001$) groups respectively. Furthermore, in analyzing risk factors associated with malaria in this study, a 6.5 times risk of Hb <11.0 g/dL ($p<0.00001$) was significantly associated with malaria and this risk increased to

10.7 when adjusted for pregnancy. The outcome of the Hb results brings to bare t`he devastating effects of cohabitation of malaria with pregnancy which induced anaemia in the pregnant women with malaria group as defined by the WHO.

On the contrary however, no significant changes were observed in the mean haematocrit (HCT) concentration in the pregnant women with malaria group and the non-pregnant women with malaria group (p=0.474). The HCT concentration in the pregnant women without malaria group was significantly higher when compared to the other two study groups (p=0.007 and p=0.026 respectively). Risk factor analysis from the study showed that malaria infection was strongly associated with a 4.6 times reduction in HCT (<33%) and this risk was observed to increase to 5.7 times when adjusted for pregnancy which further confirms the devastating effect of combination of pregnancy and malaria on HCT concentration. This significant finding is consistent with other reports by Bashiri *et al.* (2003), Ruchi *et al.* (2013) and Elgari (2013) which, in separate studies, reported that decrease in Hb and HCT concentration were common findings in pregnancy and attributed this to increased plasma volume combined with poor iron intake. Ogbodo *et al.* (2009) suggested that the observed anaemia could be due to increased red blood cell breakdown due to increasing parasite density. This finding cannot be excluded in the multiplicity of factors associated with the observed anaemia and decrease in HCT in this study.

From the findings of the study, it is most important to mention the significant observation of rise in red blood cell concentration in the pregnant women with malaria which was $3.7 \pm 0.8 \times 10^{12}/L$ and non-pregnant with malaria which was $3.9 \pm 0.5 \times 10^{12}/L$ groups respectively and these increases were significantly higher when compared with the pregnant without malaria group ($3.0 \pm 0.7 \times 10^{12}/L$; $p<0.0001$ and $p<0.0001$). This finding gives credence to the body's ability to replenish the circulating red cell capacity in the face of lyses as a result of parasite invasion. As expected, the mean MCV was significantly lowered in

the pregnant women with malaria and non-pregnant women with malaria groups compared to the pregnant women without malaria group. The reduced MCV therefore shows that most of the circulating red blood cells were immature thus confirming the argument of the body to restore oxygen carrying capacity in the face of malaria in pregnancy.

White blood cells(WBC) are responsible for the body's defense and WBC has been reported to be elevated during pregnancy (Pitkin and Witte, 1979). Total leukocyte count has been reported to rise in early pregnancy which remained elevated through out pregnancy. From this study, the WBC count in the pregnant women without malaria group was significantly lower compared with the pregnant women with malaria ($p<0.0001$) and non-pregnant women with malaria groups respectively. The finding of increases in WBC count in the malaria infected group from this study supports the argument of immunity building in the face of infection which is achieved by a state of selective immune tolerance (Elgari, 2013).

Matthews *et al.* (1990) reported that platelet count remains in the normal pregnant range in most women during uncomplicated pregnancies and Verdy *et al.* (1997) reported that mean platelet counts of pregnant women may be slightly lower than in healthy non-pregnant women. From this study, the mean platelet count in the pregnant without malaria group was significantly higher compared with those pregnant with malaria ($p<0.0001$) and non-pregnant women with malaria groups respectively(tables 4-2 and table 4-3). The observed slightly lower mean platelet counts in the pregnant with malaria and non-pregnant with malaria groups in this study obviously points to the role of malaria infection as a major cause of the observed decrease in mean platelet count agreeing with that of the Verdy *et al.* (1997) who reported slightly lower mean platelet counts in pregnant women compared to healthy non-pregnant women.

5.3 ROLE OF CHONDROITIN SULPHATE A

Cytoadherence of *Plasmodium falciparum*-infected erythrocytes (IEs) has been studied in vitro using cell lines such as human endothelial cells (Udeinya *et al.*, 1981) and C32 melanoma cells and a variety of glycoprotein receptors (Udeinya *et al.*, 1981). Thrombospondin (Roberts *et al.*, 1985), CD36 (Ockenhouse *et al.*, 1992), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 and E-selectin (Ockenhouse *et al.*, 1992) have all been implicated as receptors on host cells but the adherence patterns observed in other studies suggest that additional as yet unidentified receptors exist (Rogerson *et al.*, 1995). Sulphated glycoconjugates, including the glycosaminoglycan (GAG) heparin, interfere with attachment of *Plasmodium* sporozoites to the hepatocyte surface and may decrease sporozoite infectivity in mice (Berendt *et al.*, 1989).

It has been found that GAGs may be involved in cell-cell interactions in asexual stages of *Plasmodium falciparum* with IEs bound to cell-associated chondroitin sulphate (CS) and to immobilized chondroitin sulphate A (CSA) (Rogerson *et al.*, 1995). This binding is inhibited by hydrolysis of cell surface CS, or by free CSA. Adhesive PfEMP1 proteins are displayed on the surface of malariainfected red blood cells and these play a critical role in disease, tethering infected cells away from destruction by the spleen and causing many severe symptoms (Higgins, 2008). Susceptibility to *Plasmodium* parasitaemia has been linked to the level of antibodies to placental sequestrated parasites (Elliott *et al.*, 2005). Indeed these parasites preferentially adhere to chondroitin sulphate-A receptors (CSA) expressed by the syncytiotrophoblasts in the placenta (Fried *et al.*, 1998a). Antibody measurements were assessed among the study participants to indirectly estimate the concentrations of CSA and to further determine susceptibility to *Plasmodium* parasitaemia. It was most striking to observe that the CSA concentration in the non-pregnant women with malaria group was 2.24 ng/mL, compared to the pregnant women with malaria which was 50.76 ng/mL and pregnant women without malaria which was

44.94ng/mL groups, respectively. The significantly higher concentration of CSA estimated in the pregnant with malaria group gives an indication of susceptibility to *Plasmodium* parasitaemia due to placental sequestrated parasites as stated by Elliott *et al*. (2005).

The significantly low CSA concentration estimated in the non-pregnant women with malaria group suggests generally low circulating antibodies to placental and CSA-binding infected erythrocytes which suggest parasite adhesion to other molecules and non-immune immunoglobulins (Fried and Duffy, 1996; Beeson *et al.*, 2000). Fried *et al.,* (1998a), Beeson *et al.,* (1999) and Ricke *et al.,* (2000) in separate studies suggested that women who are not pregnant and men generally lack antibodies to placental and CSA-binding infected erythrocytes, thus representing the fact that these parasites have novel variants to which the men and women have not been exposed to previously.Fried and Duffy,(1996) Beeson *et al.,*(1999) reported that isolates from non-pregnant adults and children typically bind to molecules in the vasculature, such as CD36 and intercellular adhesion molecules. Ricke *et al.,* (2000) reported the CSA-binding parasitized erythrocytes are rarely observed outside pregnancy and non-pregnant and primigravid women generally lack acquired immunity to these parasites. This is because primigravid women lack antibodies to CSA-binding parasitized erythrocytes and as such rely on innate immune mechanisms to control and clear pregnancy-associated parasite variants. They further stated that the reasons are not fully understood but innate responses are inadequate to prevent parasite accumulation in the placenta.

5.4 MALARIA PARASITE BURDEN

Parasites may be present in the placenta and contribute to maternal anaemia in the absence of documented peripheral parasitaemia (Steketee *et al.*, 2001). The mean parasite count estimated for the pregnant women with malaria group(1532 parasites/μL) in this study compared with the non-pregnant women with malaria group (4077 parasites/μL) was significantly lower . This

finding could be explained by the fact that isolates from non-pregnant adults typically bind to molecules in the vasculature, such as CD36 and intercellular adhesion molecules and rely on innate immune mechanisms to control and clear such parasite variants. Studies have demonstrated that innate recognition and phagocytosis of parasitized erythrocytes occurs primarily through the engagement of pattern-recognition receptor CD36 on monocytes and macrophages (Serghides *et al.,* 2006). The argument could therefore be advanced that the binding of parasitized erythrocytes to molecules in the vasculature makes such parasitized erythrocytes more bioavailable for detection through testing using peripheral blood compared to the pregnant with malaria group where the accumulation of large numbers of parasitized erythrocytes and monocytes in the placenta sequester such parasitized erythrocytes from the peripheral smear and as such the detection of a low count in peripheral smears.

Chapter 6 CONCLUSION

6.1 CONCLUSION

Malaria infection was associated with reduction in haemoglobin concentration which was further worsened when associated with pregnancy. Findings from this study establishes evidence to the fact that chondroitin sulphate concentration is increased in pregnancy and slightly rises in the presence of malaria. Higher chondroitin sulphate A concentration was associated with lower malaria peripheral parasite count and could suggest the possibility of ruling out malaria in the pregnant without malaria group when indeed parasites could be sequestered. In conclusion, CSA is elicited in pregnant women but has inverse correlation with peripheral malaria parasitaemia.

6.2 RECOMMENDATIONS

In the light of the conclusions drawn from the findings of the study, It is recommend that

1. Malaria parasite count as estimated from microscopy should not be the sole basis for ruling out malaria or monitoring malaria in pregnancy.
2. Further studies should be conducted to establish the correlation between chondroitin sulphate A concentration and placental malaria sequestration.

REFERENCES

Abrams E.T., Brown H., Chensue S.W., Turner G.D., Tadesse E., Lema V.M., Molyneux M.E., Rochford R., Meshnick S.R. and Rogerson S.J. (2003) Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. *J Immunol* 170, 2759-2764.

Achidi E.A., Kuoh A.J., Minang J.T., Ngum B., Achimbom B.M., Motaze S.C., Ahmadou M.J. and Troye-Blomberg M. (2005) Malaria infection in pregnancy and its effects on haemoglobin levels in women from a malaria endemic area of Fako Division, South West Province, Cameroon. *J Obstet Gynaecol* 25, 235-240.

Achur R.N., Valiyaveettil M., Alkhalil A., Ockenhouse C.F. and Gowda D.C. (2000) Characterization of proteoglycans of human placenta and identification of unique chondroitin sulfate proteoglycans of the intervillous spaces that mediate the adherence of Plasmodium falciparum-infected erythrocytes to the placenta. *J Biol Chem* 275, 4034440356.

Adam I., Nour B.Y., Almahi W.A., Omer E.S. and Ali N.I. (2007) Malaria susceptibility and cortisol levels in pregnant women of eastern Sudan. *Int J Gynaecol Obstet* 98, 260-261.

Adams J.H., Blair P.L., Kaneko O. and Peterson D.S. (2001) An expanding ebl family of Plasmodium falciparum. *Trends Parasitol* 17, 297-299.

African Medical and Research Foundation (2012) Malaria.

Akindele J.A., Sowunmi A. and Abohweyere A.E. (1993) Congenital malaria in a hyperendemic area: a preliminary study. *Ann Trop Paediatr* 13, 273-276.

Anagnos D., Lanoie L.O., Palmieri J.R., Ziefer A. and Connor D.H. (1986) Effects of placental malaria on mothers and neonates from Zaire. *Z Parasitenkd* 72, 57-64.

Ansell J., Hamilton K.A., Pinder M., Walraven G.E. and Lindsay S.W. (2002) Short-range attractiveness of pregnant women to Anopheles gambiae mosquitoes. *Trans R Soc Trop Med Hyg* 96, 113-116.

Antelman G., Msamanga G.I., Spiegelman D., Urassa E.J., Narh R., Hunter D.J. and Fawzi W.W. (2000) Nutritional factors and infectious disease contribute to anemia among pregnant women with human immunodeficiency virus in Tanzania. *J Nutr* 130, 1950-1957.

Baeurle S.A., Kiselev M.G., Makarova E.S. and Nogovitsin E.A. (2009) Effect of the counterion behavior on the frictional–compressive properties of chondroitin sulfate solutions. *Polymer* 50, 1805 - 1813.

Baird J.K. (1995) Host age as a determinant of naturally acquired immunity to Plasmodium falciparum. *Parasitol Today* 11, 105-111.

Barfod L., Dobrilovic T., Magistrado P., Khunrae P., Viwami F., Bruun J., Dahlback M., Bernasconi N.L., Fried M., John D., Duffy P.E., Salanti A., Lanzavecchia A., Lim C.T., Ndam N.T., Higgins M.K. and Hviid L. (2010) Chondroitin sulfate A-adhering Plasmodium falciparum-infected erythrocytes express functionally important antibody epitopes shared by multiple variants. *J Immunol* 185, 7553-7561.

Barnhill J.G., Fye C.L., Williams D.W., Reda D.J., Harris C.L. and Clegg D.O. (2006) Chondroitin product selection for the glucosamine/chondroitin arthritis intervention trial. *J Am Pharm Assoc (2003)* 46, 14-24.

Barnwell J.W., Asch A.S., Nachman R.L., Yamaya M., Aikawa M. and Ingravallo P. (1989) A human 88-kD membrane glycoprotein (CD36) functions in vitro as a receptor for a cytoadherence ligand on Plasmodium falciparum-infected erythrocytes. *J Clin Invest* 84, 765-772.

Baruch D.I., Pasloske B.L., Singh H.B., Bi X., Ma X.C., Feldman M., Taraschi T.F. and Howard R.J. (1995) Cloning the P. falciparum gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell* 82, 77-87.

Bashiri A., Burstein E., Sheiner E. and Mazor M. (2003) Anemia during pregnancy and treatment with intravenous iron: review of the literature. *Eur J Obstet Gynecol Reprod Biol* 110, 2-7.

Beeson J.G., Brown G.V., Molyneux M.E., Mhango C., Dzinjalamala F. and Rogerson S.J. (1999) Plasmodium falciparum isolates from infected pregnant women and children are associated with distinct adhesive and antigenic properties. *J Infect Dis* 180, 464-472.

Beeson J.G., Rogerson S.J., Cooke B.M., Reeder J.C., Chai W., Lawson A.M., Molyneux M.E. and Brown G.V. (2000) Adhesion of Plasmodium falciparum-infected erythrocytes to hyaluronic acid in placental malaria. *Nat Med* 6, 86-90.

Berendt A.R., Ferguson D.J. and Newbold C.I. (1990) Sequestration in Plasmodium falciparum malaria: sticky cells and sticky problems. *Parasitol Today* 6, 247-254.

Berendt A.R., Simmons D.L., Tansey J., Newbold C.I. and Marsh K. (1989) Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for Plasmodium falciparum. *Nature* 341, 57-59.

Blackman M.J., Heidrich H.G., Donachie S., McBride J.S. and Holder A.A. (1990) A single fragment of a malaria merozoite surface protein remains on the parasite during red cell invasion and is the target of invasioninhibiting antibodies. *J Exp Med* 172, 379-382.

Boel M.E., Rijken M.J., Brabin B.J., Nosten F. and McGready R. (2012) The epidemiology of postpartum malaria: a systematic review. *Malar J* 11, 114.

Bonner P.C., Zhou Z., Mirel L.B., Ayisi J.G., Shi Y.P., van Eijk A.M., Otieno J.A., Nahlen B.L., Steketee R.W. and Udhayakumar V. (2005) Placental

malaria diminishes development of antibody responses to Plasmodium falciparum epitopes in infants residing in an area of western Kenya where P. falciparum is endemic. *Clin Diagn Lab Immunol* 12, 375-379.

Bouharoun-Tayoun H., Oeuvray C., Lunel F. and Druilhe P. (1995) Mechanisms underlying the monocyte-mediated antibody-dependent killing of Plasmodium falciparum asexual blood stages. *J Exp Med* 182, 409-418.

Bouyou-Akotet M.K., Adegnika A.A., Agnandji S.T., Ngou-Milama E., Kombila M., Kremsner P.G. and Mavoungou E. (2005) Cortisol and susceptibility to malaria during pregnancy. *Microbes Infect* 7, 1217-1223.

Bouyou-Akotet M.K., Ionete-Collard D.E., Mabika-Manfoumbi M., Kendjo E., Matsiegui P.B., Mavoungou E. and Kombila M. (2003) Prevalence of Plasmodium falciparum infection in pregnant women in Gabon. *Malar J* 2, 18.

Brabin B. and Piper C. (1997) Anaemia- and malaria-attributable low birthweight in two populations in Papua New Guinea. *Ann Hum Biol* 24, 547-555.

Brabin B. and Rogerson S.J. (2001) The epidemiology and outcomes of maternal malaria. In *Malaria in pregnancy: deadly parasite, susceptible host*, pp. 27 - 52 [E. Duffy and M. Fried, editors]. New York: Taylor & Francis.

Brabin B.J. (1983) An analysis of malaria in pregnancy in Africa. *Bull World Health Organ* 61, 1005-1016.

Brabin B.J. and Johnson P.M. (2005) Placental malaria and pre-eclampsia through the looking glass backwards? *J Reprod Immunol* 65, 1-15.

Brabin B.J., Kalanda B.F., Verhoeff F.H., Chimsuku L.H. and Broadhead R.L. (2004) Risk factors for fetal anaemia in a malarious area of Malawi. *Ann Trop Paediatr* 24, 311-321.

Brair M.E., Brabin B.J., Milligan P., Maxwell S. and Hart C.A. (1994) Reduced transfer of tetanus antibodies with placental malaria. *Lancet* 343, 208-209.

Brentlinger P.E., Behrens C.B. and Micek M.A. (2006) Challenges in the concurrent management of malaria and HIV in pregnancy in sub-Saharan Africa. *Lancet Infect Dis* 6, 100-111.

Brustoski K., Moller U., Kramer M., Hartgers F.C., Kremsner P.G., Krzych U. and Luty A.J. (2006) Reduced cord blood immune effector-cell responsiveness mediated by CD4+ cells induced in utero as a consequence of placental Plasmodium falciparum infection. *J Infect Dis* 193, 146-154.

Buffet P.A., Gamain B., Scheidig C., Baruch D., Smith J.D., Hernandez-Rivas R., Pouvelle B., Oishi S., Fujii N., Fusai T., Parzy D., Miller L.H., Gysin J. and Scherf A. (1999) Plasmodium falciparum domain mediating adhesion to chondroitin sulfate A: a receptor for human placental infection. *Proc Natl Acad Sci U S A* 96, 12743-12748.

Bull P.C., Lowe B.S., Kortok M., Molyneux C.S., Newbold C.I. and Marsh K. (1998) Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nat Med* 4, 358-360.

Bulmer J.N., Rasheed F.N., Morrison L., Francis N. and Greenwood B.M. (1993) Placental malaria. II. A semi-quantitative investigation of the pathological features. *Histopathology* 22, 219-225.

Caraballo H. and King K. (2014) Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. *Emerg Med Pract* 16, 1-23; quiz 23-24.

Chan J.A., Fowkes F.J. and Beeson J.G. (2014) Surface antigens of Plasmodium falciparum-infected erythrocytes as immune targets and malaria vaccine candidates. *Cell Mol Life Sci* 71, 3633-3657.

Chen Q., Fernandez V., Sundstrom A., Schlichtherle M., Datta S., Hagblom P. and Wahlgren M. (1998) Developmental selection of var gene expression in Plasmodium falciparum. *Nature* 394, 392-395.

Christophers S.R. (1924) The mechanism of immunity against malaria in communities living under hyper-endemic conditions. *Ind J Med R* 12, 273-294.

Cohen S., Mc G.I. and Carrington S. (1961) Gamma-globulin and acquired immunity to human malaria. *Nature* 192, 733-737.

Colomer J., Colomer C., Gutierrez D., Jubert A., Nolasco A., Donat J., Fernandez-Delgado R., Donat F. and Alvarez-Dardet C. (1990) Anaemia during pregnancy as a risk factor for infant iron deficiency: report from the Valencia Infant Anaemia Cohort (VIAC) study. *Paediatr Perinat Epidemiol* 4, 196-204.

Cornet M., Le Hesran J.Y., Fievet N., Cot M., Personne P., Gounoue R., Beyeme M. and Deloron P. (1998) Prevalence of and risk factors for anemia in young children in southern Cameroon. *Am J Trop Med Hyg* 58, 606-611.

Covell G. (1950) Congenital malaria. *Trop Dis Bull* 47, 1147-1167.

Currie G.A. (1968) Immunology of pregnancy: the foeto-maternal barrier. *Proc R Soc Med* 61, 1206-1212.

Dahlback M., Nielsen M.A. and Salanti A. (2010) Can any lessons be learned from the ambiguous glycan binding of PfEMP1 domains? *Trends Parasitol* 26, 230-235.

Daneshvar C., Davis T.M., Cox-Singh J., Rafa'ee M.Z., Zakaria S.K., Divis P.C. and Singh B. (2009) Clinical and laboratory features of human Plasmodium knowlesi infection. *Clin Infect Dis* 49, 852-860.

David P.H., Hommel M., Miller L.H., Udeinya I.J. and Oligino L.D. (1983) Parasite sequestration in Plasmodium falciparum malaria: spleen and antibody modulation of cytoadherence of infected erythrocytes. *Proc Natl Acad Sci U S A* 80, 5075-5079.

De Beaudrap P., Turyakira E., White L.J., Nabasumba C., Tumwebaze B., Muehlenbachs A., Guerin P.J., Boum Y., McGready R. and Piola P. (2013) Impact of malaria during pregnancy on pregnancy outcomes in a Ugandan prospective cohort with intensive malaria screening and prompt treatment. *Malar J* 12, 139.

de Moraes-Pinto M.I., Verhoeff F., Chimsuku L., Milligan P.J., Wesumperuma L., Broadhead R.L., Brabin B.J., Johnson P.M. and Hart C.A. (1998) Placental antibody transfer: influence of maternal HIV infection and placental malaria. *Arch Dis Child Fetal Neonatal Ed* 79, F202-205.

Degen R., Weiss N. and Beck H.P. (2000) Plasmodium falciparum: cloned and expressed CIDR domains of PfEMP1 bind to chondroitin sulfate A. *Exp Parasitol* 95, 113-121.

Desai M., ter Kuile F.O., Nosten F., McGready R., Asamoa K., Brabin B. and Newman R.D. (2007) Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 7, 93-104.

Diagne N., Rogier C., Sokhna C.S., Tall A., Fontenille D., Roussilhon C., Spiegel A. and Trape J.F. (2000) Increased susceptibility to malaria during the early postpartum period. *N Engl J Med* 343, 598-603.

Diakite O.S., Kayentao K., Traore B.T., Djimde A., Traore B., Diallo M., Ongoiba A., Doumtabe D., Doumbo S., Traore M.S., Dara A., Guindo O., Karim D.M., Coulibaly S., Bougoudogo F., Ter Kuile F.O., Danis M. and Doumbo O.K. (2011) Superiority of 3 over 2 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in mali: a randomized controlled trial. *Clin Infect Dis* 53, 215-223.

Dicko A., Mantel C., Thera M.A., Doumbia S., Diallo M., Diakite M., Sagara I. and Doumbo O.K. (2003) Risk factors for malaria infection and anemia for pregnant women in the Sahel area of Bandiagara, Mali. *Acta Trop* 89, 17-23.

Dondorp A.M., Ince C., Charunwatthana P., Hanson J., van Kuijen A., Faiz M.A., Rahman M.R., Hasan M., Bin Yunus E., Ghose A., Ruangveerayut R., Limmathurotsakul D., Mathura K., White N.J. and Day N.P. (2008) Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. *J Infect Dis* 197, 79-84.

Dondorp A.M., Pongponratn E. and White N.J. (2004) Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. *Acta Trop* 89, 309-317.

Doolan D.L., Dobano C. and Baird J.K. (2009) Acquired immunity to malaria. *Clin Microbiol Rev* 22, 13-36.

Dorman E. and Shulman C. (2000) Malaria in pregnancy. *Curr Obstet Gynecol* 10, 183 - 189.

Duffy P.E. (2001) Immunity to malaria during pregnancy: different host, different parasite. In *Malaria in pregnancy: deadly parasite, susceptible host*, pp. 70 - 126 [P.E. Duffy and M. Fried, editors]. London: Taylor & Francis.

Duffy P.E. and Fried M. (1999) Malaria during pregnancy: parasites, antibodies and chondroitin sulphate A. *Biochem Soc Trans* 27, 478-482.

Duffy P.E. and Fried M. (2003) Antibodies that inhibit plasmodium falciparum adhesion to Chondroitin Sulfate A are associated with increased birth weight and the gestational age of newborns. *Infect Immun* 81, 12.

Egan T.J. (2008) Haemozoin formation. *Mol Biochem Parasitol* 157, 127-136.

Egwunyenga O.A., Ajayi J.A., Popova-Duhlinska D.D. and Nmorsi O.P. (1996) Malaria infection of the cord and birthweights in Nigerians. *Cent Afr J Med* 42, 265-268.

Elenkov I.J. (2004) Glucocorticoids and the Th1/Th2 balance. *Ann N Y Acad Sci* 1024, 138-146.

Elgari M.M. (2013) Evaluation of hematological parameters of Sudanese pregnant women attending Omdurman Al Saudi Maternity Hospital. *Egypt. Acad. J. Biol. Sci.* 5.

Elliott S.R., Brennan A.K., Beeson J.G., Tadesse E., Molyneux M.E., Brown G.V. and Rogerson S.J. (2005) Placental malaria induces variant-specific antibodies of the cytophilic subtypes immunoglobulin G1 (IgG1) and IgG3 that correlate with adhesion inhibitory activity. *Infect Immun* 73, 5903-5907.

Etard J.F., Kodio B. and Ronsmans C. (2003) Seasonal variation in direct obstetric mortality in rural Senegal: role of malaria? *Am J Trop Med Hyg* 68, 503-504.

Fievet N., Cot M., Chougnet C., Maubert B., Bickii J., Dubois B., Le Hesran J.Y., Frobert Y., Migot F. and Romain F. (1995) Malaria and pregnancy in Cameroonian primigravidae: humoral and cellular immune responses to Plasmodium falciparum blood-stage antigens. *Am J Trop Med Hyg* 53, 612-617.

Fleming A.F. (1989a) The aetiology of severe anaemia in pregnancy in Ndola, Zambia. *Ann Trop Med Parasitol* 83, 37-49.

Fleming A.F. (1989b) Tropical obstetrics and gynaecology. 1. Anaemia in pregnancy in tropical Africa. *Trans R Soc Trop Med Hyg* 83, 441-448.

Forsyth R., Brigden C. and Northrop A. (2006) Double blind investigation of the effects of oral supplementation of combined glucosamine hydrochloride (GHCL) and chondroitin sulfate (CS) on stride characteristics of veteran horses. *Equine veterinary journal* Supplement 38, 622 - 625.

Fried M. and Duffy P.E. (1996) Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta. *Science* 272, 1502-1504.

Fried M., Muga R.O., Misore A.O. and Duffy P.E. (1998a) Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes. *J Immunol* 160, 2523-2530.

Fried M., Nosten F., Brockman A., Brabin B.J. and Duffy P.E. (1998b) Maternal antibodies block malaria. *Nature* 395, 851-852.

Gardner M.J., Hall N., Fung E., White O., Berriman M., Hyman R.W., Carlton J.M., Pain A., Nelson K.E., Bowman S., Paulsen I.T., James K., Eisen J.A., Rutherford K., Salzberg S.L., Craig A., Kyes S., Chan M.S., Nene V., Shallom S.J., Suh B., Peterson J., Angiuoli S., Pertea M., Allen J., Selengut J., Haft D., Mather M.W., Vaidya A.B., Martin D.M., Fairlamb A.H., Fraunholz M.J., Roos D.S., Ralph S.A., McFadden G.I., Cummings L.M., Subramanian G.M., Mungall C., Venter J.C., Carucci D.J., Hoffman S.L., Newbold C., Davis R.W., Fraser C.M. and Barrell B. (2002) Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature* 419, 498-511.

Garner P. and Gulmezoglu A.M. (2006) Drugs for preventing malaria in pregnant women. *Cochrane Database Syst Rev*, CD000169.

Greenwood A.M., Armstrong J.R., Byass P., Snow R.W. and Greenwood B.M. (1992) Malaria chemoprophylaxis, birth weight and child survival. *Trans R Soc Trop Med Hyg* 86, 483-485.

Gupta S., Snow R.W., Donnelly C. and Newbold C. (1999a) Acquired immunity and postnatal clinical protection in childhood cerebral malaria. *Proc Biol Sci* 266, 33-38.

Gupta S., Snow R.W., Donnelly C.A., Marsh K. and Newbold C. (1999b) Immunity to non-cerebral severe malaria is acquired after one or two infections. *Nat Med* 5, 340-343.

Guyatt H.L. and Snow R.W. (2001) The epidemiology and burden of Plasmodium falciparum-related anemia among pregnant women in subSaharan Africa. *Am J Trop Med Hyg* 64, 36-44.

Guyatt H.L. and Snow R.W. (2004) Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. *Clin Microbiol Rev* 17, 760-769, table of contents.

Hay S.I., Guerra C.A., Tatem A.J., Noor A.M. and Snow R.W. (2004) The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect Dis* 4, 327-336.

Health Protection Agency (2011) Health Protection Report.

Hempelmann E. and Marques H.M. (1994) Analysis of malaria pigment from Plasmodium falciparum. *J Pharmacol Toxicol Methods* 32, 25-30.

Higgins M.K. (2008) The structure of a chondroitin sulfate-binding domain important in placental malaria. *J Biol Chem* 283, 21842-21846.

Hoestermann C.F., Ogbaselassie G., Wacker J. and Bastert G. (1996) Maternal mortality in the main referral hospital in The Gambia, west Africa. *Trop Med Int Health* 1, 710-717.

Hommel M., Elliott S.R., Soma V., Kelly G., Fowkes F.J., Chesson J.M., Duffy M.F., Bockhorst J., Avril M., Mueller I., Raiko A., Stanisic D.I., Rogerson S.J., Smith J.D. and Beeson J.G. (2010) Evaluation of the antigenic diversity of placenta-binding Plasmodium falciparum variants and the antibody repertoire among pregnant women. *Infect Immun* 78, 1963-1978.

Ismail M.R., Ordi J., Menendez C., Ventura P.J., Aponte J.J., Kahigwa E., Hirt R., Cardesa A. and Alonso P.L. (2000) Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. *Hum Pathol* 31, 85-93.

Jordan K.M., Arden N.K., Doherty M., Bannwarth B., Bijlsma J.W., Dieppe P., Gunther K., Hauselmann H., Herrero-Beaumont G., Kaklamanis P., Lohmander S., Leeb B., Lequesne M., Mazieres B., Martin-Mola E., Pavelka K., Pendleton A., Punzi L., Serni U., Swoboda B., Verbruggen G., Zimmerman-Gorska I., Dougados M. and Standing Committee for International Clinical Studies Including Therapeutic Trials E. (2003) EULAR Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). *Ann Rheum Dis* 62, 1145-1155.

Kane E.G. and Taylor-Robinson A.W. (2011) Prospects and Pitfalls of Pregnancy-Associated Malaria Vaccination Based on the Natural Immune Response to Plasmodium falciparum VAR2CSA-Expressing Parasites. *Malar Res Treat* 2011, 764845.

Khattab A., Kun J., Deloron P., Kremsner P.G. and Klinkert M.Q. (2001) Variants of Plasmodium falciparum erythrocyte membrane protein 1 expressed by different placental parasites are closely related and adhere to chondroitin sulfate A. *J Infect Dis* 183, 1165-1169.

Kriek N., Tilley L., Horrocks P., Pinches R., Elford B.C., Ferguson D.J., Lingelbach K. and Newbold C.I. (2003) Characterization of the pathway for transport of the cytoadherence-mediating protein, PfEMP1, to the host cell surface in malaria parasite-infected erythrocytes. *Mol Microbiol* 50, 1215-1227.

Kwak-Kim J.Y., Gilman-Sachs A. and Kim C.E. (2005) T helper 1 and 2 immune responses in relationship to pregnancy, nonpregnancy, recurrent spontaneous abortions and infertility of repeated implantation failures. *Chem Immunol Allergy* 88, 64-79.

Kyes S., Horrocks P. and Newbold C. (2001) Antigenic variation at the infected red cell surface in malaria. *Annu Rev Microbiol* 55, 673-707.

Kyes S., Pinches R. and Newbold C. (2000) A simple RNA analysis method shows var and rif multigene family expression patterns in Plasmodium falciparum. *Mol Biochem Parasitol* 105, 311-315.

Lavstsen T., Salanti A., Jensen A.T., Arnot D.E. and Theander T.G. (2003) Subgrouping of Plasmodium falciparum 3D7 var genes based on sequence analysis of coding and non-coding regions. *Malar J* 2, 27.

Lee G.H., Benner D., Regidor D.L. and Kalantar-Zadeh K. (2007) Impact of kidney bone disease and its management on survival of patients on dialysis. *Journal of Renal Nutrition* 17, 38-44.

Leenstra T., Phillips-Howard P.A., Kariuki S.K., Hawley W.A., Alaii J.A., Rosen D.H., Oloo A.J., Nahlen B.L., Kager P.A. and ter Kuile F.O. (2003) Permethrin-treated bed nets in the prevention of malaria and anemia in adolescent schoolgirls in western Kenya. *Am J Trop Med Hyg* 68, 86-93.

Leopardi O., Naughten W., Salvia L., Colecchia M., Matteelli A., Zucchi A., Shein A., Muchi J.A., Carosi G. and Ghione M. (1996) Malaric placentas. A quantitative study and clinico-pathological correlations. *Pathol Res Pract* 192, 892-898.

Lindsay S., Ansell J., Selman C., Cox V., Hamilton K. and Walraven G. (2000) Effect of pregnancy on exposure to malaria mosquitoes. *Lancet* 355, 1972.

Lucchi N.W., Koopman R., Peterson D.S. and Moore J.M. (2006) Plasmodium falciparum-infected red blood cells selected for binding to cultured syncytiotrophoblast bind to chondroitin sulfate A and induce tyrosine phosphorylation in the syncytiotrophoblast. *Placenta* 27, 384-394.

Lucchi N.W., Peterson D.S. and Moore J.M. (2008) Immunologic activation of human syncytiotrophoblast by Plasmodium falciparum. *Malar J* 7, 42.

Luxemburger C., McGready R., Kham A., Morison L., Cho T., Chongsuphajaisiddhi T., White N.J. and Nosten F. (2001) Effects of malaria during pregnancy on infant mortality in an area of low malaria transmission. *Am J Epidemiol* 154, 459-465.

MacLeod J. and Rhode R. (1998) Retrospective follow-up of maternal deaths and their associated risk factors in a rural district of Tanzania. *Trop Med Int Health* 3, 130-137.

Maier A.G., Cooke B.M., Cowman A.F. and Tilley L. (2009) Malaria parasite proteins that remodel the host erythrocyte. *Nat Rev Micro* 7, 341-354.

Mankhambo L., Kanjala M., Rudman S., Lema V.M. and Rogerson S.J. (2002) Evaluation of the OptiMAL rapid antigen test and species-specific PCR to detect placental Plasmodium falciparum infection at delivery. *J Clin Microbiol* 40, 155-158.

Marsh K., Forster D., Waruiru C., Mwangi I., Winstanley M., Marsh V., Newton C., Winstanley P., Warn P. and Peshu N. (1995) Indicators of lifethreatening malaria in African children. *N Engl J Med* 332, 1399-1404.

Marsh K. and Howard R.J. (1986) Antigens induced on erythrocytes by P. falciparum: expression of diverse and conserved determinants. *Science* 231, 150-153.

Matejevic D., Neudeck H., Graf R., Muller T. and Dietl J. (2001) Localization of hyaluronan with a hyaluronan-specific hyaluronic acid binding protein in the placenta in pre-eclampsia. *Gynecol Obstet Invest* 52, 257-259.

Matteelli A., Caligaris S., Castelli F. and Carosi G. (1997) The placenta and malaria. *Ann Trop Med Parasitol* 91, 803-810.

Matteelli A., Donato F., Shein A., Muchi J.A., Leopardi O., Astori L. and Carosi G. (1994) Malaria and anaemia in pregnant women in urban Zanzibar, Tanzania. *Ann Trop Med Parasitol* 88, 475-483.

Matthews J.H., Benjamin S., Gill D.S. and Smith N.A. (1990) Pregnancyassociated thrombocytopenia: definition, incidence and natural history. *Acta Haematol* 84, 24-29.

McGready R., Davison B.B., Stepniewska K., Cho T., Shee H., Brockman A., Udomsangpetch R., Looareesuwan S., White N.J., Meshnick S.R. and Nosten F. (2004) The effects of Plasmodium falciparum and P. vivax infections on placental histopathology in an area of low malaria transmission. *Am J Trop Med Hyg* 70, 398-407.

McGregor I.A., Wilson M.E. and Billewicz W.Z. (1983) Malaria infection of the placenta in The Gambia, West Africa; its incidence and relationship to stillbirth, birthweight and placental weight. *Trans R Soc Trop Med Hyg* 77, 232-244.

Mebius R.E. and Kraal G. (2005) Structure and function of the spleen. *Nat Rev Immunol* 5, 606-616.

Meeusen E.N., Bischof R.J. and Lee C.S. (2001) Comparative T-cell responses during pregnancy in large animals and humans. *Am J Reprod Immunol* 46, 169-179.

Megnekou R., Hviid L. and Staalsoe T. (2009) Variant-specific immunity to Plasmodium berghei in pregnant mice. *Infect Immun* 77, 1827-1834.

Menendez C. (2006) Malaria during pregnancy. *Curr Mol Med* 6, 269-273.

Menendez C., Ordi J., Ismail M.R., Ventura P.J., Aponte J.J., Kahigwa E., Font F. and Alonso P.L. (2000) The impact of placental malaria on gestational age and birth weight. *J Infect Dis* 181, 1740-1745.

Miller L.H., Baruch D.I., Marsh K. and Doumbo O.K. (2002) The pathogenic basis of malaria. *Nature* 415, 673-679.

Mockenhaupt F.P., Rong B., Till H., Eggelte T.A., Beck S., Gyasi-Sarpong C., Thompson W.N. and Bienzle U. (2000) Submicroscopic Plasmodium falciparum infections in pregnancy in Ghana. *Trop Med Int Health* 5, 167173.

Monif G.R.G. and Baker D.A. (2004) *Infectious disease in Obstetrics and Gynaecology*, 6th ed. New York: Parthenon.

Moore J.M., Nahlen B.L., Misore A., Lal A.A. and Udhayakumar V. (1999) Immunity to placental malaria. I. Elevated production of interferongamma by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. *J Infect Dis* 179, 1218-1225.

Moormann A.M., Sullivan A.D., Rochford R.A., Chensue S.W., Bock P.J., Nyirenda T. and Meshnick S.R. (1999) Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation. *J Infect Dis* 180, 1987-1993.

Murphy S.C. and Breman J.G. (2001) Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *Am J Trop Med Hyg* 64, 57-67.

Murray C.J.L., Ortblad K.F., Guinovart C. and Lim S.S. (2014) Global, regional and national incidence and mortality for HIV, tuberculosis and malaria during 1990-2013: a systemic analysis for the Global Burden of Disease Study 2013. *Lancet* 384, 1005-1070.

Mutabingwa T.K., Bolla M.C., Li J.L., Domingo G.J., Li X., Fried M. and Duffy P.E. (2005) Maternal malaria and gravidity interact to modify infant susceptibility to malaria. *PLoS Med* 2, e407.

Muthusamy A., Achur R.N., Bhavanandan V.P., Fouda G.G., Taylor D.W. and Gowda D.C. (2004) Plasmodium falciparum-infected erythrocytes adhere both in the intervillous space and on the villous surface of human placenta by binding to the low-sulfated chondroitin sulfate proteoglycan receptor. *Am J Pathol* 164, 2013-2025.

Nahlen B.L. (2000) Rolling back malaria in pregnancy. *N Engl J Med* 343, 651652.

Nathwani D., Currie P.F., Douglas J.G., Green S.T. and Smith N.C. (1992) Plasmodium falciparum malaria in pregnancy: a review. *Br J Obstet Gynaecol* 99, 118-121.

Nguyen-Dinh P., Steketee R.W., Greenberg A.E., Wirima J.J., Mulenda O. and Williams S.B. (2008) Rapid spontaneous postpartum clearance of Plasmodium falciparum parasitaemia in African women. *Lancet* 2, 751 - 752.

Nielsen M.A., Staalsoe T., Kurtzhals J.A., Goka B.Q., Dodoo D., Alifrangis M., Theander T.G., Akanmori B.D. and Hviid L. (2002) Plasmodium falciparum variant surface antigen expression varies between isolates causing severe and nonsevere malaria and is modified by acquired immunity. *J Immunol* 168, 3444-3450.

Nosten F., McGready R. and Mutabingwa T. (2007) Case management of malaria in pregnancy. *Lancet Infect Dis* 7, 118-125.

Nosten F., McGready R., Simpson J.A., Thwai K.L., Balkan S., Cho T., Hkirijaroen L., Looareesuwan S. and White N.J. (1999) Effects of Plasmodium vivax malaria in pregnancy. *Lancet* 354, 546-549.

Nwagha U.I., Ugwu V.O., Nwagha T.U. and Anyaehie B.U. (2009) Asymptomatic Plasmodium parasitaemia in pregnant Nigerian women: almost a decade after Roll Back Malaria. *Trans R Soc Trop Med Hyg* 103, 16-20.

Ockenhouse C.F., Tegoshi T., Maeno Y., Benjamin C., Ho M., Kan K.E., Thway Y., Win K., Aikawa M. and Lobb R.R. (1992) Human vascular endothelial cell adhesion receptors for Plasmodium falciparum-infected erythrocytes: roles for endothelial leukocyte adhesion molecule 1 and vascular cell adhesion molecule 1. *J Exp Med* 176, 1183-1189.

Ofori M.F., Dodoo D., Staalsoe T., Kurtzhals J.A., Koram K., Theander T.G., Akanmori B.D. and Hviid L. (2002) Malaria-induced acquisition of antibodies to Plasmodium falciparum variant surface antigens. *Infect Immun* 70, 2982-2988.

Ogbodo S.O., Nwagha U.I., Okaka A.N., Ogenyi S.C. and Okoko R.O. (2009) Malaria parasitaemia among pregnant women in a rural community of eastern Nigeria; need for combined measures. *Niger J Physiol Sci* 24, 95100.

Ordi J., Ismail M.R., Ventura P.J., Kahigwa E., Hirt R., Cardesa A., Alonso P.L. and Menendez C. (1998) Massive chronic intervillositis of the placenta associated with malaria infection. *Am J Surg Pathol* 22, 1006-1011.

Owusu-Agyei S., Koram K.A., Baird J.K., Utz G.C., Binka F.N., Nkrumah F.K., Fryauff D.J. and Hoffman S.L. (2001) Incidence of symptomatic and asymptomatic Plasmodium falciparum infection following curative therapy in adult residents of northern Ghana. *Am J Trop Med Hyg* 65, 197-203.

Philippe E. and Walter P. (1985) Placental lesions in malaria. *Arch Fr Pediatr* 42 Suppl 2, 921-923.

Pitkin R.M. and Witte D.L. (1979) Platelet and leukocyte counts in pregnancy. *JAMA* 242, 2696-2698.

Planche T. and Krishna S. (2006) Severe malaria: metabolic complications. *Curr Mol Med* 6, 141-153.

Quinn T.C., Jacobs R.F., Mertz G.J., Hook E.W., 3rd and Locklsey R.M. (1982) Congenital malaria: a report of four cases and a review. *J Pediatr* 101, 229-232.

Ramharter M., Grobusch M.P., Kiessling G., Adegnika A.A., Moller U., Agnandji S.T., Kramer M., Schwarz N., Kun J.F., Oyakhirome S., Issifou

S., Borrmann S., Lell B., Mordmuller B. and Kremsner P.G. (2005) Clinical and parasitological characteristics of puerperal malaria. *J Infect Dis* 191, 1005-1009.

Recker M., Bouma M.J., Bamford P., Gupta S. and Dobson A.P. (2009) Assessing the burden of pregnancy-associated malaria under changing transmission settings. *Malar J* 8, 245.

Reeder J.C., Cowman A.F., Davern K.M., Beeson J.G., Thompson J.K., Rogerson S.J. and Brown G.V. (1999) The adhesion of Plasmodium falciparuminfected erythrocytes to chondroitin sulfate A is mediated by P. falciparum erythrocyte membrane protein 1. *Proc Natl Acad Sci U S A* 96, 5198-5202.

Ricke C.H., Staalsoe T., Koram K., Akanmori B.D., Riley E.M., Theander T.G. and Hviid L. (2000) Plasma antibodies from malaria-exposed pregnant women recognize variant surface antigens on Plasmodium falciparuminfected erythrocytes in a parity-dependent manner and block parasite adhesion to chondroitin sulfate A. *J Immunol* 165, 3309-3316.

Riley E.M., Hviid L. and Theander T.G. (1994) Malaria. In *Parasitic infections and the immune system*, pp. 119-143 [F. Kierszenbaum, editor]. San Diego: Academic Press.

Riley E.M., Schneider G., Sambou I. and Greenwood B.M. (1989) Suppression of cell-mediated immune responses to malaria antigens in pregnant Gambian women. *Am J Trop Med Hyg* 40, 141-144.

Robert C., Pouvelle B., Meyer P., Muanza K., Fujioka H., Aikawa M., Scherf A. and Gysin J. (1995) Chondroitin-4-sulphate (proteoglycan), a receptor for Plasmodium falciparum-infected erythrocyte adherence on brain microvascular endothelial cells. *Res Immunol* 146, 383-393.

Roberts D.D., Sherwood J.A., Spitalnik S.L., Panton L.J., Howard R.J., Dixit V.M., Frazier W.A., Miller L.H. and Ginsburg V. (1985)
Thrombospondin binds falciparum malaria parasitized erythrocytes and may mediate cytoadherence. *Nature* 318, 64-66.

Rogerson S.J., Brown H.C., Pollina E., Abrams E.T., Tadesse E., Lema V.M. and Molyneux M.E. (2003a) Placental tumor necrosis factor alpha but not gamma interferon is associated with placental malaria and low birth weight in Malawian women. *Infect Immun* 71, 267-270.

Rogerson S.J., Chaiyaroj S.C., Ng K., Reeder J.C. and Brown G.V. (1995) Chondroitin sulfate A is a cell surface receptor for Plasmodium falciparum-infected erythrocytes. *J Exp Med* 182, 15-20.

Rogerson S.J., Mkundika P. and Kanjala M.K. (2003b) Diagnosis of Plasmodium falciparum malaria at delivery: comparison of blood film preparation methods

and of blood films with histology. *J Clin Microbiol* 41, 1370-1374. Rogerson S.J., van den Broek N.R., Chaluluka E., Qongwane C., Mhango C.G. and Molyneux M.E. (2000) Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey. *Am J Trop Med Hyg* 62, 335340.

Rowe J.A., Claessens A., Corrigan R.A. and Arman M. (2009) Adhesion of Plasmodium falciparum-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. *Expert Reviews in Molecular Medicine* 11, e16.

Ruchi K., Pradeep B. and Varun K. (2013) A study of erythron status in pregnant and non-pregnant age matched females of Jodhpur. *IOSR J. Pharmacy* 3.

Salem H.H., Maruyama I., Ishii H. and Majerus P.W. (1984) Isolation and characterization of thrombomodulin from human placenta. *J Biol Chem* 259, 12246-12251.

Sartelet H., Milko-Sartelet I., Garraud O. and Picot S. (1997) Plasmodium falciparum persists in the placenta after three days' treatment with quinine. *Trans R Soc Trop Med Hyg* 91, 431.

Schaefer L. and Schaefer R.M. (2010) Proteoglycans: from structural compounds to signaling molecules. *Cell Tissue Res* 339, 237-246.

Schantz-Dunn J. and Nour N.M. (2009) Malaria and pregnancy: a global health perspective. *Rev Obstet Gynecol* 2, 186-192.

Scherf A., Hernandez-Rivas R., Buffet P., Bottius E., Benatar C., Pouvelle B., Gysin J. and Lanzer M. (1998) Antigenic variation in malaria: in situ switching, relaxed and mutually exclusive transcription of var genes during intra-erythrocytic development in Plasmodium falciparum. *EMBO J* 17, 5418-5426.

Schofield L. (2007) Intravascular infiltrates and organ-specific inflammation in malaria pathogenesis. *Immunol Cell Biol* 85, 130-137.

Serghides L., Patel S.N., Ayi K. and Kain K.C. (2006) Placental chondroitin sulfate A-binding malarial isolates evade innate phagocytic clearance. *J Infect Dis* 194, 133-139.

Sevene E., Gonzalez R. and Menendez C. (2010) Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. *Expert Opin Pharmacother* 11, 1277-1293.

Sharma J.B. (2003) Nutritional anemia during pregnancy in non industrial countries. *Progress in Obst. & Gynae (Studd)* 15, 103 - 122.

Shulman C.E. (1999) Malaria in pregnancy: its relevance to safe-motherhood programmes. *Ann Trop Med Parasitol* 93 Suppl 1, S59-66.

Shulman C.E., Graham W.J., Jilo H., Lowe B.S., New L., Obiero J., Snow R.W. and Marsh K. (1996) Malaria is an important cause of anaemia in primigravidae: evidence from a district hospital in coastal Kenya. *Trans R Soc Trop Med Hyg* 90, 535-539.

Singh S.K., Hora R., Belrhali H., Chitnis C.E. and Sharma A. (2006) Structural basis for Duffy recognition by the malaria parasite Duffy-binding-like domain. *Nature* 439, 741-744.

Smith J.D., Chitnis C.E., Craig A.G., Roberts D.J., Hudson-Taylor D.E., Peterson D.S., Pinches R., Newbold C.I. and Miller L.H. (1995) Switches in expression of Plasmodium falciparum var genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell* 82, 101-110.

Smith J.D., Gamain B., Baruch D.I. and Kyes S. (2001) Decoding the language of var genes and Plasmodium falciparum sequestration. *Trends Parasitol* 17, 538-545.

Smith J.D., Subramanian G., Gamain B., Baruch D.I. and Miller L.H. (2000) Classification of adhesive domains in the Plasmodium falciparum erythrocyte membrane protein 1 family. *Mol Biochem Parasitol* 110, 293310.

Srivastava A., Gangnard S., Round A., Dechavanne S., Juillerat A., Raynal B., Faure G., Baron B., Ramboarina S., Singh S.K., Belrhali H., England P., Lewit-Bentley A., Scherf A., Bentley G.A. and Gamain B. (2010) Fulllength extracellular region of the var2CSA variant of PfEMP1 is required
for specific, high-affinity binding to CSA. *Proc Natl Acad Sci U S A* 107, 4884-4889.

Staalsoe T., Megnekou R., Fievet N., Ricke C.H., Zornig H.D., Leke R., Taylor D.W., Deloron P. and Hviid L. (2001) Acquisition and decay of antibodies to pregnancy-associated variant antigens on the surface of Plasmodium falciparum-infected erythrocytes that protect against placental parasitemia. *J Infect Dis* 184, 618-626.

Steketee R.W., Nahlen B.L., Parise M.E. and Menendez C. (2001) The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg* 64, 28-35.

Steketee R.W., Wirima J.J., Bloland P.B., Chilima B., Mermin J.H., Chitsulo L. and Breman J.G. (1996) Impairment of a pregnant woman's acquired ability to limit Plasmodium falciparum by infection with human immunodeficiency virus type-1. *Am J Trop Med Hyg* 55, 42-49.

Su X.Z., Heatwole V.M., Wertheimer S.P., Guinet F., Herrfeldt J.A., Peterson D.S., Ravetch J.A. and Wellems T.E. (1995) The large diverse gene family var encodes proteins involved in cytoadherence and antigenic variation of Plasmodium falciparum-infected erythrocytes. *Cell* 82, 89-100.

Suh K.N., Kain K.C. and Keystone J.S. (2004) Malaria. *CMAJ : Canadian Medical Association Journal* 170, 1693-1702.

Tako E.A., Zhou A., Lohoue J., Leke R., Taylor D.W. and Leke R.F. (2005) Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. *Am J Trop Med Hyg* 72, 236-242.

Taylor-Robinson A.W. and Smith E.C. (1999) A dichotomous role for nitric oxide in protection against blood stage malaria infection. *Immunol Lett* 67, 1-9.

Taylor H.M., Kyes S.A., Harris D., Kriek N. and Newbold C.I. (2000) A study of var gene transcription in vitro using universal var gene primers. *Mol Biochem Parasitol* 105, 13-23.

ter Kuile F.O., Parise M.E., Verhoeff F.H., Udhayakumar V., Newman R.D., van Eijk A.M., Rogerson S.J. and Steketee R.W. (2004) The burden of coinfection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. *Am J Trop Med Hyg* 71, 41-54.

Tolia N.H., Enemark E.J., Sim B.K. and Joshua-Tor L. (2005) Structural basis for the EBA-175 erythrocyte invasion pathway of the malaria parasite Plasmodium falciparum. *Cell* 122, 183-193.

Tongo O.O., Orimadegun A.E. and Akinyinka O.O. (2011) Utilisation of malaria preventive measures during pregnancy and birth outcomes in Ibadan, Nigeria. *BMC Pregnancy Childbirth* 11, 60.

Treutiger C.J., Heddini A., Fernandez V., Muller W.A. and Wahlgren M. (1997) PECAM-1/CD31, an endothelial receptor for binding Plasmodium falciparum-infected erythrocytes. *Nat Med* 3, 1405-1408.

Udeinya I.J., Schmidt J.A., Aikawa M., Miller L.H. and Green I. (1981) Falciparum malaria-infected erythrocytes specifically bind to cultured human endothelial cells. *Science* 213, 555-557.

Uneke C.J. (2008) Impact of placental Plasmodium falciparum malaria on pregnancy and perinatal outcome in sub-Saharan Africa: part III: placental malaria, maternal health, and public health. *Yale J Biol Med* 81, 1-7.

UNICEF (2012) Malaria.

van der Heyde H.C., Nolan J., Combes V., Gramaglia I. and Grau G.E. (2006) A unified hypothesis for the genesis of cerebral malaria: sequestration, inflammation and hemostasis leading to microcirculatory dysfunction. *Trends Parasitol* 22, 503-508.

van Eijk A.M., Ayisi J.G., Ter Kuile F.O., Misore A.O., Otieno J.A., Kolczak M.S., Kager P.A., Steketee R.W. and Nahlen B.L. (2002) Malaria and human immunodeficiency virus infection as risk factors for anemia in infants in Kisumu, western Kenya. *Am J Trop Med Hyg* 67, 44-53.

van Geertruyden J.P., Thomas F., Erhart A. and D'Alessandro U. (2004) The contribution of malaria in pregnancy to perinatal mortality. *Am J Trop Med Hyg* 71, 35-40.

Verdy E., Bessous V., Dreyfus M., Kaplan C., Tchernia G. and Uzan S. (1997) Longitudinal analysis of platelet count and volume in normal pregnancy. *Thromb Haemost* 77, 806-807.

Walker-Abbey A., Djokam R.R., Eno A., Leke R.F., Titanji V.P., Fogako J., Sama G., Thuita L.H., Beardslee E., Snounou G., Zhou A. and Taylor D.W. (2005) Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. *Am J Trop Med Hyg* 72, 229-235.

Walter P.R., Garin Y. and Blot P. (1982) Placental pathologic changes in malaria. A histologic and ultrastructural study. *Am J Pathol* 109, 330-342.

Wegmann T.G., Lin H., Guilbert L. and Mosmann T.R. (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 14, 353-356.

WHO (1986) WHO expert committee on malaria 18th report. In *WHO Technical Report Series*: World Health Organization.

WHO (2013) Malaria: World Health Organization.

Yamada M., Steketee R., Abramowsky C., Kida M., Wirima J., Heymann D., Rabbege J., Breman J. and Aikawa M. (1989) Plasmodium falciparum associated placental pathology: a light and electron microscopic and immunohistologic study. *Am J Trop Med Hyg* 41, 161-168.