CHAPTER 1
INTRODUCTION

1. Overview of malaria

1.1 Malaria and Pregnancy

Pregnant women are more susceptible to the adverse effects of malaria. They are more prone to *P. falciparum* infection as compared to non-pregnant women and, once infected, there is a tendency towards increased severity of disease (1, 2) caused in part by the transient down regulation of cell-mediated immunity that occurs during pregnancy (3). The effects of malaria on pregnant women varies according to the woman’s level of immunity, her gravidity, the trimester of pregnancy, and the presence or absence of co–morbidity (4, 5). The main factors, influencing the epidemiology of falciparum malaria and malaria of other *Plasmodium* species, are the intensity of transmission and the level of immune response (6, 7). Malaria transmission in an area may be stable or unstable. Immunity is achieved through continued exposure to malarial parasite(s). In areas of stable malaria transmission, most malaria infections are asymptomatic (8).

In areas of unstable, nonendemic transmission, adult women having no significant level of immunity are more likely to be symptomatic when
parasitaemic, and are at a greater risk of developing severe complications (2).

1.2 Malaria and Maternal Health

Malaria endemicity and parity are two of the factors influencing the outcome of malaria on a mother and her baby (9). Primigravidae are more susceptible than multigravidae and have higher incidence of placental malaria (9, 10). In areas where malaria is endemic, even the babies of asymptomatic pregnant women may suffer intrauterine foetal growth retardation and low birthweight (10). The evidence for malaria infection in pregnancy can be obtained from either the intensity of peripheral parasitaemia during pregnancy or placental infection at the time of delivery (9). Parasite densities in placental infections are sometimes difficult to assess with accuracy because there seems to be no correlation between parasite density in peripheral blood and in the placenta in pregnant women with well-developed immunity in malaria-holoendemic regions (9). Thus, there may be large numbers of infected RBCs in the placenta, (as many as 65%), whereas the peripheral blood is free from parasites (9).

1.3 Pathophysiology of Malaria

When an Anopheles mosquito penetrates human skin with its proboscis to obtain blood, it injects saliva mixed with an anticoagulant. If the mosquito is infected with Plasmodium cells known as sporozoites (a stage in its life cycle) it injects these into the bloodstream of its victim, the parasite makes its way to the liver where it rapidly grows, develops and multiplies mitotically into schizonts (pre-erythrocytic, PE, schizonts) in the liver. In Plasmodium falciparum the PE schizonts take 5½ - 7 days to develop. After maturation, each measures about 60mm in diameter and contains up to 30,000 merozoites. Merozoites, which are the next stage of division phase of the life cycle, either invade other liver cells (hepatocytes) or enter the host’s bloodstream. In the bloodstream the merozoites invade the red cells
through the sinusoids of the liver. These merozoites become attached to the red cells by special organelles, which bind to specific glycophorin protein receptors on the red cell membranes. The membranes become indented and the parasites enter the red cells. Thus, the merozoites in the bloodstream develop into trophozoites within a vacuole formed by the internal membrane of the host cell. The trophozoites feed on haemoglobin by ingesting small amounts of red cell cytoplasm. Malaria pigment, haemoglobin, is formed as an end product of haemoglobin breakdown. The trophozoite is fully developed through nuclear and cytoplasmic division in the process of schizogony to form schizonts. Mature schizonts rupture from the cells releasing merozoites, haemoglobin, and toxins into the host’s plasma. The entry of toxic metabolites into the blood circulation brings about the well-known fever and chills that is clinical manifestation of malaria (6, 7).

In *P. falciparum*, the incubation time from infection to an attack is 9-14 days. Those merozoites which are not destroyed by the host’s immune system invade new red cells and develop into trophozoites and schizonts thereby causing further red cells to be destroyed. After several erythrocytic cycles, some merozoites enter red cells and instead of developing into schizonts they follow a sexual development and become gametocytes (these are thought to form in response to a developing immunity, lack of nutrients, or an accumulation of metabolites or parasite debris) [6,7]. These gametocytes (gamete producing) are ingested by a female *Anopheles* mosquito in a blood meal or they die if not taken by a mosquito vector. In the stomach of the mosquito, the male gametocyte rapidly divides into a number of male gametes each with a flagellum, which becomes free and highly motile, that on contact and entry into female gamete fertilization occurs to result in a zygote.
The zygote develops into a motile oökinete, which penetrates the stomach wall of the mosquito, forming oöcyst (which contains large number of sporozoites). The sporozoites when mature leave the oöcyst and spread to all parts of the mosquito, particularly to the salivary glands and can then be injected into a subsequent victim, starting the cycle again. In *P. vivax*, *P. ovale* and probably *P. malariae*, all stages of development subsequent to the liver cycle can be observed in the peripheral blood. However, in the case of *P. falciparum* only ring forms and gametocytes are usually present in the peripheral blood; developing forms appear to stick to the blood vessels of the large organs such as the brain and cause restriction to the blood flow with severe complications (11, 12, 13).

Figure 1: Life cycle of Malaria Parasite, *Plasmodium vivax* highlighting infective and diagnostic check points.
Figure 2: - Structure of Sporozoite.

Figure 3: - Parasite invasion of erythrocytes.
1.4. The *Plasmodium* species causing malaria.

*Plasmodium falciparum*. This species causes falciparum malaria (formerly called malignant tertian malaria), manifest severe complications through cerebral malaria and renal failure. Fever occurs about every 48 hours but this periodicity is often masked because the stages are not always synchronous. This periodicity is termed tertian because of fever on the first day, no fever on the second and then a return of fever on the third day. *Plasmodium falciparum* requires an average ambient temperature of at least 20°C so is found mainly in the hotter and humid regions of the world. Its main species are found in tropical and subtropical Africa and parts of Central America and South America, Bangladesh, Pakistan, Afghanistan,
Nepal, Sri Lanka, East Asia, Indonesia, Philippines, Haiti, Solomon Islands, Papua New Guinea and many islands in Melanesia. It is also found in parts of India, the Mediterranean and countries of North Africa (6, 11, 13).

Again, malaria caused by *P. falciparum* (falciparum malaria) is the most severe form of the malarial disease and is globally widespread, representing up to 80% of malaria cases worldwide (14).

**Plasmodium vivax.** This species causes vivax malaria (formerly called benign tertian malaria) which rarely kills. This species is not found in tropical Africa mainly because black Africans lack the red cell surface Duffy antigen required by *P. vivax* cell invasion (11, 12, 13). It usually occurs at places with an average summer temperature of only 16ºC. *Plasmodium vivax* is mainly found in South America (occurring as far south as northern Argentina), Mexico, Sri Lanka, Papua New Guinea, and the Solomon Islands. It is also found in parts of South East Asia, Indonesia, Philippines, Madagascar, tropical and subtropical Africa, Korea and China (11). Together with *P. ovale, P. vivax* is considered as causing relapsing malaria, so named because it can remain in a dormant hypnozoite stage for very long periods (years) in the liver.

**Plasmodium ovale.** This species causes rare ovale malaria (formerly called tertian malaria) with a long incubation period and relapses at three-month intervals. It is found mainly in West Africa where it accounts for up to 10% of malaria infections (11). There have been sporadic reports of infections from other parts of Africa and from the Philippines, Indonesia, China, and parts of the Far East, South East Asia and South America (11). Like *P. vivax*, it is recurrent with a dormant liver stage.
*Plasmodium malariae*. This species causes quartan malaria with fever returning every 72 hours. It is remarkable in that it can persist in the blood of a host for decades at very low densities, but it does not have a dormant stage in the liver. Relapses can sometimes occur half a century after being infected (6, 13). *Plasmodium malariae* accounts for up to 25% of malaria infections in tropical Africa and it is also found in Guyana, India, Sri Lanka and Malaysia of which it accounts for less than 10% of malaria infections (11).

1.5 MOSQUITO VECTORS

Mosquitoes belong to the Order Diptera of the Class Insecta in the Phylum Arthropoda. Their life span is generally 3-4 weeks. They go through complete metamorphosis stages in their development i.e. from the egg, larval and pupal stages to a mature adult. Anopheline mosquitoes are the exclusive vectors of human malaria:

The *Anopheles* vectors

There are about 422 species of *Anopheles* widespread globally, many of them are sibling species that can only be identified using genetic techniques. Of these, about 70 are malaria vectors but only about 40 are important (15). The ability to spread *Plasmodium* parasites depends on the mosquito living long enough for the parasite to complete its development, the mosquito's propensity to feed on humans; and whether or not the mosquito is physiologically suitable for the parasite. However, most *Anopheles* are believed to support normal development of at least one of the *Plasmodium* species. Their breeding sites vary and include permanent or temporary pools, swamps, seepages, rice fields, tree holes, ditches and reservoirs with some requiring sunlight and others shade for breeding (6).
1.6 Pathogenesis, treatment, prevention and complications.

Malaria is an acute febrile illness with incubation period of 7 days or longer. Thus, a febrile illness developing less than one week after the first possible exposure cannot be considered malaria (16).

The different malarial parasites produce fevers of different frequency, depending on how long it takes to complete schizogony in erythrocytes. With the first attack of *P. falciparum*, fever is usually irregular rather than occurring with a regular, repeating pattern as seen with a tertian fever in subsequent attacks, and there are usually no relapses unlike with *P. ovale* and *P. vivax* where hypnozoites are formed (16). For *P. falciparum* the temperature may rise at 48 hour intervals. Again, *P. falciparum* has the potential to cause infected red blood cells (RBCs) to adhere to the linings of small blood vessels (Figure 6). Such sequestrations of the parasites cause considerable obstruction to tissue perfusion which may be responsible for adverse outcome of malaria (12).

Malaria is especially dangerous to pregnant women and small children. Severe and complicated malaria is usually caused by delaying treatment of an uncomplicated attack of *P. falciparum*. The patient complains of headache, fever, aches and pains all over the body, diarrhoea and abdominal pain are sometimes present. Spleen and liver are often palpable on clinical examination. This may be misdiagnosed as influenza in nonendemic areas, and unless treated promptly, the clinical picture can deteriorate rapidly (17). Clinical expositions such as impaired consciousness, weakness, and jaundice may be prominent in patients with severe and complicated malaria. Other complications are cerebral malaria (non-rousable coma), generalized convulsions, normocytic anaemia, renal failure, hypoglycaemia, fluid, electrolyte and acid-base disturbances, pulmonary oedema, circulatory collapse, shock, disseminated intravascular coagulation, hyperpyrexia, hyperparasitaemia, and malarial
haemoglobulinuria. These features may occur singly or in combinations (6).

As to whether the *Plasmodium* parasites cause their mosquito hosts such discomfort and morbidity is a matter of debate, with several contrasting opinions stressed. It does seem that mosquitoes that are carrying the malaria parasite do experience decreased life expectancy and higher mortality rates than their non-infected counterparts (15). Cell-mediated and humoral immunity plays a crucial role in immune-competent individuals living in malaria endemic areas. More immunoglobulins especially IgG is raised when the immune cells are activated by specific antigens, for instance, circumsporozoite proteins found on sporozoites. Vaccines are currently being developed against three stages of the parasite: gametocytes, sporozoites, and intra-erythrocytic merozoites (16, 18).

1.6.1. Prevention of Malaria Infection
Prevention may be carried out either by interrupting transmission in vector control, or by giving the patient prophylactic drugs. As yet, there is no widespread effective vaccination scheme. Vector control effectiveness has declined in recent years; due to lack of personnel, inefficient insecticide usage, and mass population movements (19) and other factors alluded. However, simple means such as insecticide impregnated mosquito nets remain effective. The use of prophylactic drugs has been proved effective, for both travellers and people living in endemic areas.

1.7. Malaria and Socioeconomic factors
The low social status of women in developing countries limits their access to economic resources and basic education and thus their ability to make decisions related to their health and nutrition. Some women are denied access to care when it is needed either because of cultural practices of seclusion or because decision-making is the responsibility of other family members. Lack of access to, and use of essential obstetric services
including malaria prophylaxis, are crucial factors that contribute to high maternal mortality (20, 21, 22, 23) Excessive physical work coupled with poor diet are factors contributing to poor maternal outcomes.

Most of these deaths could be avoided if preventive measures were taken and adequate care was available. Poverty, ignorance and malnutrition are the factors which equally contribute to the enormous burden of malaria in pregnancy. Around half a billion cases of malaria each year result in well over one million deaths, and over ninety percent of all these deaths occur in sub-Saharan Africa (14).

1.8. Malaria transmission and Maternal Immunity in high transmission areas.

In areas of stable malaria transmission, infection during pregnancy has been estimated to cause 75,000–200,000 infant deaths each year (24). Women in stable transmission areas have greatest risks of developing these complications during their first and second pregnancies (24). The level of immunity to malaria infection depends on the intensity of transmission, the number of previous pregnancies and the presence of other conditions not excepting HIV which increasingly impair the efficacy of immune response during pregnancy (25). The prevalence and intensity of malarial infection during pregnancy is higher among HIV-positive women and the risk to the woman and her newborn exists regardless of the number of times the woman has given birth (26).

Women in areas of unstable transmission and whose immunity has been diminished by HIV or other factors are more prone to severe illness as a result of malaria infection than non-pregnant women and may also experience poor pregnancy outcomes.

These women are at risk of developing malaria–related complications during every pregnancy. Hence, there is a need to find effective strategies to minimize the impact of malaria during pregnancy, thus preventing
illness in asymptomatic pregnant women and managing disease in women with clinical illness.

1.8. Clinical features of malaria infection

In areas of malaria endemicity malaria such as Africa or Asia, malaria is often the most common cause of fever. The first symptoms of the disease are non-specific, and include absence of wellbeing, headache, fatigue, muscle aches (pain), and abdominal discomfort, which are followed by irregular fever. Nausea, vomiting, and orthostatic occur customarily [27]. Generalized seizures are linked with *falciparum* malaria and might be followed by coma (cerebral malaria). Most patients with uncomplicated infections have few abnormal physical features other than fever, mild anaemia, and, after several days, a palpable spleen. The liver can become enlarged, especially in young children, whereas mild jaundice is more common in adults. Young children living in areas of stable malaria transmission, encounters repetitive infections that causes chronic anaemia and splenomegaly [27].

The exposition of severe *falciparum* malaria depends on age [28]. Severe anaemia and hypoglycemia commonly occur in children, whereas acute pulmonary oedema, acute kidney injury, and jaundice are more frequent in adults; coma (cerebral malaria) and acidosis occur in all age groups. Mortality rate peaks when the proportion of infected erythrocytes (parasitaemia) exceeds 2%, although the relation between parasite density and prognosis in falciparum malaria is very uneven.

1.9. Malaria burden worldwide

Malaria accounts for a significant economic burden on Africa which has leading malaria cases across the globe [29]. Estimates of the annual incidence of malaria vary widely. According to the estimates of The World Malaria Report, 2011, there were 216 million episodes of malaria in 2010,
of which approximately 81%, or 174 million cases, were in the African Region [World Malaria Report, 2011], about 91% being due to *P. falciparum* [World Malaria Report, 2011]. However, the actual number of cases may be much higher and the number of confirmed cases reported by national malaria control programmes was only 11% of the estimated number of cases [World Malaria Report, 2011]. Hay et al. have estimated the number of clinical cases of *P. falciparum* malaria in 2007 at 451 million (95% credible interval 349-552) (30). According to the estimates of World Malaria Report, 2011, Africa accounts for the vast majority of malaria cases (81%) [World Malaria Report, 2011]. According to the World Malaria Report, 2011, there were 655 000 malaria deaths worldwide in 2010, compared to 781,000 in 2009 [World Malaria Report, 2011; 2010]. Children under the age of 5 years accounted for about 86% of deaths globally. A recent systematic investigation by Murray et al. has estimated that the global malaria deaths is increased from 995 000 in 1980 to a peak of 1 817 000 in 2004, then decreasing to 1 238 000 (929 000 - 1 685 000) in 2010 (almost double of the WHO estimate for the same year) [31]. This study demonstrated more deaths in individuals aged 5 years or older than has been estimated in previous studies: 435 000 (307 000 - 658 000) deaths in Africa and 89 000 (33 000 - 177 000) deaths outside of Africa in 2010 [31].

In 2012, worldwide, there were an estimated 207 million cases of malaria (95 % uncertainty interval, 135-287 million), with 80 % of these cases reported in the African Region [World Malaria Report, 2013]. In 2012, there seems to be a significant decrease in the occurrence of malaria.

With regard to deaths due to malaria, in 2012, there was a reduction compared to the previous year, with 627 000 worldwide (95 % uncertainty interval, 473 000 – 789 000); in which 90 % of the cases were covered by the African Region [World Malaria Report, 2013].
Figure 5: - Malaria Transmission Worldwide.
Figure 6: - Malaria death rate per 100’000 population.
Figure 7. Malaria cases per 100,000
2.0. Cytokines

Cells of the immune system require a communication network that can, act locally or at a distance, precisely or globally, and momentarily or in a sustained manner. Among the networks that allow such sophistication in the mammalian immune response and that utilizes an extraordinary variety of cell membrane-bound and soluble messengers, the cytokines are the best identified of these groups of messengers [32]. The term "cytokine" refers to a large group of non-enzymatic low molecular weight hormone-like proteins whose actions are both diverse and overlapping and which affect diverse and overlapping target cell populations [32]. Cytokines have been studied broadly for the past 3 decades. Previously the terms interleukins, interferons, growth factors, and TNFs were used to designate them, among other designations. These molecules arbitrate their effect in essentially every important biological process, from cell proliferation to inflammation, immunity, migration, fibrosis, repair, and angiogenesis [33]. Cytokines are regulatory proteins that are secreted by white blood cells and several other cell types in the body. Their pleiotropic action involves the regulation of both innate and adaptive immune responses and the modulation of inflammatory responses, in addition to many other activities. Some cytokines were reported for the first time approximately 50 years ago, but their molecular characterization occurred much later. Pyrogenic endogenous was among the factors recognised in the 1950s, which is probably similar to the protein now known as interleukin 1 (IL-1), nerve growth factor and interferon (IFN). In the 1960s, researchers discovered that lymphocytes produce soluble mediators, termed lymphokines, involved in the regulation of both humoral and cell mediated immune response [34] including the expression of MHC antigens and the regulation of the Th1–Th2 balance [35]. TNF, another important, pleiotropic cytokine, was reported originally in the 1970s as a mediator of LPS-induced necrosis of transplantable tumors [36]. The number of well
recognized cytokine genes and proteins nowadays has reached a couple of hundreds, and new ones are still being discovered [37]. Significant differences have been noted in the immensity and profile of the cytokine response to a number of immunogens among strains of experimental animals and among individuals in human populations (38). The underlying mechanisms are likely to be multiple, but one important level of variation is seen in the cytokine genes themselves. Polymorphisms have been identified in coding regions, promoters and introns, which have been linked to altered cytokine production or activity and with susceptibility to various infectious agents, autoimmune diseases and allergies in humans. Polymorphisms in regulatory regions in particular can be expected to affect the level of gene expression, therefore, cytokine gene polymorphisms can act both as markers of diseases susceptibility or severity and clues to pathogenesis [39].
2.1. Immune response to malaria

The immune response to malaria parasite is a complex process depending on the parasite species and the stage of the infection [40]. In areas with holoendemic malaria transmission, it takes nearly a decade to develop immunity to the parasite [41]. The immunity achieved is not sterile and the infected individual still suffers from infection, though the symptoms tend to be less severe [42]. The incidence of the most severe exposition of *P. falciparum* infection (i.e. cerebral malaria and severe malarial anaemia)
declines radically after the age of five, while the anti-parasitic immunity develops slower [43]. It is quite possible to find children in the age of 7-8 years old with high parasitaemias but without any obvious symptoms. This gradually acquired immunity is evidenced by lower parasite densities, less clinical complications and enhanced parasite specific immune responses [44].

The ability of serum of individuals residing in endemic areas to agglutinate parasite-infected red blood cells (iRBC) is considered to reflect the development of immunity (45). Variation in the antigens expressed on the surface of infected RBCs may be one of the possible explanation of the long delay of the immune development, but there could be other possible explanation, for example successful masking of critical *P. falciparum* antigens. It has been shown that passive transfer of immunoglobulins from semi-immune adults to malaria infected children lead to clearance of parasitaemia [46]. Further, it has been demonstrated that immunoglobulins from Africa semi-immune donors given to Thai patients results in the reduction of parasitaemia [47]. However, the transferred immunoglobulins very seldom cleared parasitaemia completely, in most instances parasites subsequently persisted at low density.

*In vitro*, the passively transferred immunoglobulins efficiently clear the parasites when acting together with monocytes, revealing that the protective role of antibodies may be to mediate antibody-dependent phagocytosis or cell-mediated killing of parasites or both [47]. It has also been reported that *in vitro* activated neutrophils and monocyte-derived macrophages can efficiently kill intracellular blood stage parasites in the absence of antibodies, an effect that is also seen with human NK and γδ T cells, arguing for a parasite control mechanism independent of antibodies (48).
The innate resistance to malaria infection is demonstrated by the fact that a wide spectrum of genetic disorders of the hemoglobin and erythrocytes (i.e. sickle cell anaemia, thalassemia) or enzymes deficiencies (G6PD) have been selected in areas that have been or are endemic to malaria(44).

2.2. Clinical immunity
The immune response to malaria is not completely understood. Non-specific host defence mechanisms control the infection initially. Both humoral and cell mediated immune response contributes to protection. Exposure to sufficient parasite strains confers protection from clinical exposition, but not from infection (premunition). Asymptomatic parasitaemia is prevalent in adults and older children living in high transmission areas (27).

2.3. Innate immunity to blood stages
TLR2 functions as a heterodimer with TLR1 or TLR6 and recognizes a wide variety of ligands, one of which is *P. falciparum* glycosylphosphatidylinositol (GPI) [49]. The *P. falciparum* GPI is also one of the ligands recognized by TLR4 [49]. TLR9, which acts as a receptor for double-stranded DNA (CpG DNA), is activated by *Plasmodium* DNA [50].

2.4. DCs
DCs plays a crucial role during malaria infection. Their maturation during malaria course have been documented in lab experiments using monocytes in human as well as in mouse [51].

It has been shown, previously that the function of DC is compromised during malaria. This phenomenon is based on an in vitro observation. It has been established that binding of *P. falciparum* iRBC to DC derived from human monocytes inhibits DCs maturation. Thus their capacity to act as an
APC could be reduced [52]. In African children encountered with severe 
*P. falciparum* episodes, the frequency of myeloid-derived CD11c+BDCA3+ DC was significantly high during the acute phase of the infection [53]. Furthermore, when the DC subpopulation is compared of individuals residing in similar endemic areas and infected with either *P. falciparum* or *P. vivax*, there was an increase in plasmacytoid CD123+ DC. The same study reported a decrease in the ratio of myeloid to plasmacytoid DC during malaria infection regardless of the malaria species [54].

At an early stages of malaria infection, DCs efficiently produce proinflammatory cytokines, and, as the infection progresses, their ability to produce proinflammatory cytokines is gradually reduced, and they acquire increased capacity to produce anti-inflammatory responses. Additionally, DCs stimulate NK cells, direct T cells to induce programmed Th1/Th2 responses, and commences the development of cell-mediated and humoral adaptive immunity [55]. Thus, DCs provide a critical link between the innate and adaptive immune responses and aids in framing the pathogen-specific adaptive immune responses.

These reports suggest that DC subpopulations are differently affected in humans during blood-stage malaria. This variability in the effects of malaria infection on DC function could be due to many factors including the *Plasmodium* species, the extremity of the infection, and the patient population examined [56].

### 2.5. γδ T cells

In the early stages of malaria infection, γδ T cells directly recognize the pathogen through MHC-independent mechanisms that involve the γδ T cells (57). γδ T cells are known to be an important IFN-γ producers during the blood stage malaria infection and are associated with the clearance of malaria parasites [48]. The proliferation of γδ T cells depends on IL-2. (58). γδ T cells have shown to play a protective role in malaria
Studies do report that activated γδ T cells and clones obtained from healthy malaria-naïve donors inhibit the replication of the erythrocytic stages *P. falciparum* in vitro. Cell to cell contact and the presence of intact granulae is required for the effective clearance of the parasite (59). Granulysin and perforin have been implicated in the antimicrobial activity of γδ T cells. In malaria, granulysin seems to play a major role in the effective clearance of the parasites and perforin a minor role [60].

In East Africa, in individuals living in malaria endemic area, γδ T cells were found to be significantly elevated in patients infected with *P. falciparum* alone or in mixed infection with *P. falciparum* and *P. vivax* [61].

### 2.6. NK cells

During malaria infection, NK cells are also considered to be an important IFN-γ producers and like the γδT cells are associated with effective killing of malarial parasites (62). A study of experimental *P. falciparum* infection showed that NK cells were minor IFN-γ producers in response to iRBCs before and after *P. falciparum* infection [63]. In contrast, several *in vitro* studies have demonstrated that NK cells rapidly induce IFN-γ production in response to *P. falciparum*-iRBCs via IL-12 and IL-18 signaling [48].

### 2.7. Malaria during Pregnancy

Currently, 25 million pregnant women are at an increased risk to develop malaria and malaria during pregnancy constitutes of about 10,000 maternal and 200,000 neonatal deaths each year (64). However, these figures might be underrated when considering the significant impact of malaria on perinatal morbidity and mortality as well as exaggeration by co-infections, such as HIV.

A number of mechanisms have been evolved by the malarial parasite to evade the immune system and particularly the complex placental
environment. That the pregnant women suppress her cell-mediated immunity is known, but the only evidence for increased vulnerability to infections during pregnancy is for intracellular parasites, such as *Plasmodium*. This suppressive state of Th1 immunity and up-regulation of Th2 responses could be the possible explanation for the women’s increased susceptibility. However, this explanation could not justify some of the features associated with malaria during pregnancy. For example, why primigravidae is more susceptible compared to secundigravidae or multigravidae or why the gravity of the disease including symptoms and other complications tends to decrease over successful pregnancies is not well understood. The understanding of why pregnant women are at an increased risk to develop malaria is still incomplete, but one possible explanation is that *P. falciparum* infected parasites are able to bind to special proteins in the intervillous spaces of the placenta, creating the condition known as placental malaria (PM) 65. This condition is probably one of the most complex interactions between a host and a pathogen, taking place in the placenta known today.

*P. falciparum*’s ability to invade and infect the host so efficiently is mainly due to its remarkable potential to adhere and sequester. During the erythrocytic stage, young ring-stage parasites circulate in the blood and mature into trophozoites, which than produces adhesion molecules that are expressed on the surface of erythrocyte. These adhesive ligands aid in the adherence of parasite to the endothelial tissue. One of the major family of proteins that have been recognised as one of these adhesive ligands on the erythrocyte is the *P. falciparum* erythrocyte membrane protein 1 (*PfEMP1*), encoded by the *var* multigene family (66). In areas of malaria endemicity, an individual acquires a vast range of antibodies against these proteins after multiple exposures and are therefore said to be semi-immune. However, the development of a new organ, the placenta, in the pregnant women provides a new niche to which infected erythrocytes (iE) can and
will adhere (67). These parasites express a unique repertoire of \( P f \)EMP1 proteins that are not previously encountered by the primigravidae. A particular variant of \( P f \)EMP1 known as var2CSA has the capacity to bind to chondroitin sulphate A (CSA) expressed on the surface of trophoblasts in the IVS (68). Binding of iE in the placental tissue causes physiological changes, such as thickening of the trophoblast membrane, intervillous infiltrates of immune cells, 69-71 and deposition of malarial pigment, called hemozoin, in phagocytic cells.72 As a result of placental malaria, infants are usually born with a low birth weight (LBW)72 and the infants have been shown to have lesser chances of surviving their first 2 years of life.73, 74 Possible cause of LBW is intrauterine growth retardation or pre-term delivery as a result of placental insufficiency and impaired blood flow,75 but the exact mechanism is still not fully understood. What really occurs inside the IVS once the parasites adhere is not completely clear. The binding of iE to CSA in the placental is believed to stimulate syncytiotrophoblasts and cells in the placenta to release various chemokines. Elevated levels of chemokines have been reported, especially levels of MCP-1, MIP-\( \alpha \) and \( \beta \) as well as IP-10 in placentas from infected women. 76, 77 MIP-1\( \alpha \) and \( \beta \) recruits additional macrophages and MCP-1 plays a crucial role in recruiting monocytes to the infected placenta.76 Overall these factors lead to massive infiltration of inflammatory cells into the placenta. The inflammatory environment with an increased plasma cytokine levels causes a shift in the placental cytokine balance which is required for the clearance of parasites.78 On the other hand, overproduction of cytokines may also contribute to pathology, particularly high TNF levels have been found associated with LBW.79 Thus, the balance between pro and anti-inflammatory factors appears to be important.
2.8. Factors associated with the susceptibility of pregnant women to malaria.

Pregnancy leads to a number of physiological alterations that affect the way the *Plasmodium* parasite invades its host. A number of assumptions have attempted to explain the increased vulnerability of pregnant women to malaria and the high frequency of placental infection. It has been propounded that pregnancy malaria is eased by hormone-dependent, depression of the immune system or non-specific effector mechanisms (The immunology and pathogenesis of malaria, 2005). However, it has been suggested that despite this susceptibility, the maternal immune system continues to respond positively to the parasite leading to better fetus consequences (80). In addition, the fact that women who have had multiple pregnancies (multigravids) are at a reduced risk than those in their first pregnancy (primigravids) (24) indicates that immune build-up is acquired after several pregnancies and infections.

2.8.1 Increased Cortisol Concentrations.

Pregnancy has been regarded as a stage of generalized immunosuppression which is mainly sustained by increased blood levels of cortisol (81). It has been demonstrated that cortisol levels are higher in pregnant women with malarial infection than in those without (82), advocating that a sustained increase in cortisol levels underlies the increased susceptibility of pregnant women, specifically primigravidae women, to malaria. This postulate has been validated by the discovery of remarkable association between cortisol concentration and *P. falciparum* infection, on one hand, and a strong connection between parasite load in *P. falciparum*-infected primigravidae women, on the other hand (83). While cortisol levels would describe an
increased susceptibility to malaria, they do not explain why parasites have a predisposition for growing and multiplying in the placenta.

2.8.2. Decreased Cell-Mediated Immunity.

Studies of immune function have indicated a brief depression of cell-mediated immunity in pregnant women, particularly in the second and third trimesters. This impairment of cell-mediated immunity permits fetal allograft retention and allows pregnancy to continue without rejection (84). However, there is interruption with resistance to various infectious diseases including malaria. Cell-mediated immune responses to malarial antigens are more noticeably suppressed in first pregnancies than in following ones (85, 86).

2.8.3 Inhibition of Type-1 cytokine Responses.

Th1-type responses are of parasitological significance. For example, significant increase in tumour necrosis factor (TNF)-α (87) interferon-γ (87), interleukin 1β (88), and interleukin-2 (89) have been found in placental blood or tissue in response to malaria parasite infection. These cytokines are known to assist in the clearance of parasites from the placenta by easing phagocytic activity of macrophages, generating reactive oxygen intermediates and L-arginine-derived nitric oxide, and inducing the proliferation of T cells (90). However, overproduction of Th-1 cytokines can compromise pregnancy by leading to maternal anaemia, spontaneous abortions, and premature deliveries (91). Hence, during pregnancy the immune system is biased towards type-2 humoral defense mechanisms and away from type-1 Cellular responses. Pregnant women would therefore be more vulnerable to malaria because of the inhibition of type-1 cytokine responses (92).