CHAPTER - 2
REVIEW OF LITERATURE

REVIEW

2.1 Malaria in Pregnancy

Each year, 25-30 million women become pregnant in malaria. Endemic areas of Africa, and similar numbers are exposed to malaria in Asia, Oceania and South America (93). Malaria is an important cause leading to severe anemia in pregnant African women, and by this mechanism malaria causes an estimated 10,000 maternal deaths each year (94). Moreover, malaria infections result in 75,000-2,000,000 low birth weight (LBW) babies each year, due to...
combinations of preterm delivery (PTD) and fetal growth restriction (FGR) (93, 95). Effects on miscarriage and stillbirth are unknown, but adequate malaria control strategies could alone prevent 3-8% of infant deaths (93). Despite numerous studies conducted over the last decades, malaria in pregnancy (MIP) remains an important public health problem that has proved difficult to tackle. The consequences of MIP on both maternal health and birth outcomes have been explored by several studies from areas with different malaria transmission patterns. Anaemia is the dominating consequence of MIP on maternal health, data on malaria-related maternal mortality are sparse (96). For the foetus, the most commonly reported adverse effect of MIP is an increased risk of low birth weight (LBW) (97).

MIP is thought to affect birth outcomes through two mechanisms, intrauterine growth restriction (IUGR) and preterm delivery, which might at least partially explain these discordant findings. It has been estimated that MIP in settings with stable malaria transmission in Africa is potentially responsible for up to 70% of IUGR and 36% of preterm delivery (98). In recent years control of MIP has relied partly on intermittent preventive treatment (IPT), with WHO. Currently recommending at least two doses with sulphadoxine-pyrimethamine (SP) (99) however, growing resistance of malaria parasites to SP in many regions (100), combined with changing epidemiology of malaria, indicate that other prevention approaches must be strengthened.

Limited epidemiological data exist for MIP in India. A prospective hospital-based study conducted in Chandigarh, northern India from 1984-1985 showed that severity of clinical illness were significantly higher in pregnant patients for both P. vivax and P. falciparum (101). A community-based study in Orissa, East India, found that primigravidae were more
likely to be parasitaemic than multigravidae and that newborn infants of infected mothers were more likely to be infected with malaria (102).

Pregnant women were more likely to be parasitaemic than non-pregnant women, and pregnant women with severe *P. falciparum* infection experienced complication, such as cerebral malaria, abortion and intrauterine foetal death. Malaria infection was more prevalent in primigravidae than multigravidae with the highest prevalence of infection seen in the second trimester, irrespective of parity. Analysis of data from 1992-1995 shows that pregnant women with malaria were more anemic than uninfected pregnant women or infected non-pregnant women (103).

In India, the majority of malaria episodes in pregnant women (approx. 2/3) is accounted to *P. falciparum* with the remainder due to *P. vivax* (100, 102). The detrimental effects of *P. falciparum* in pregnancy for both mother and child are well known, however, little is known about MIP due to *P. vivax*. Studies from Thailand (103) and central India (102) have demonstrated that *P. vivax* malaria was more common in primigravidae than in multigravidae, and was associated with mild anaemia and an increased risk of LBW.

### 2.1.1 Susceptibility to Malaria in Pregnancy

Malaria is dangerous to both the mother and foetus. Pregnant women are at a greater risk of malaria infection and of symptomatic malaria disease than non-pregnant adults (104). They are more attractive to mosquitoes (105). Parasite densities are higher in pregnant women than in non-pregnant adults. In two studies, complexity of infections did not differ (106) whereas a third study showed an increase in young pregnant women (107). These studies together infer that the ability to limit parasite replication is impaired in pregnancy.
2.2 Pregnancy-associated malaria and malaria endemicity
Almost all our knowledge about pathogenesis of, and immunity to, pregnancy-associated malaria comes from areas of high transmission. In low transmission areas, women of all gravidities are susceptible to symptomatic and severe maternal disease, miscarriage, stillbirth and congenital malaria are common complications, and malaria is an important cause of low birth weight (108).

2.3 Pathogenesis of Plasmodium falciparum
Adhesion of *Plasmodium falciparum* infected erythrocytes to human cells has a central role in the development of severe malaria. Parasite adhesion interactions involves binding to endothelial cells (cytoadhesion), rosetting with uninfected erythrocytes and platelet-mediated clumping of infected erythrocytes (109).

High fevers and associated ‘flu’-like symptoms are the consequences of parasite presence and the resulting host inflammatory responses (110). The incubation period before the appearance of symptoms fluctuates from 12 days to over a year. In some cases of infections a life-threatening illness evolves, characterized by various clinical manifestations, including impaired consciousness, coma, breathing difficulties, severe anaemia and multi-organ failure (110, 111). A combination of a high parasite burden and the sequestration of mature *P. falciparum*-infected erythrocytes (IEs) in microvascular beds throughout the body are responsible for the clinical manifestations of severe malaria (112). The sequestered mass of IEs causes microvascular obstruction (113, 111) metabolic disruptions, such as acidosis (114) and liberation of damaging inflammatory mediators (115, 116) which altogether can cause severe disease and death of the human host. Sequestration allows the parasite to evade the host’s normal splenic
clearance mechanisms that remove aged or damaged erythrocytes, thus playing advantageous role for the parasite (117).

2.4 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 inspite of this susceptibility, the maternal immune system continues to respond positively to the parasite leading to better fetus consequences (118). In addition, the fact that women who have had multiple pregnancies (multigravids) are at a reduced risk than those in their first pregnancy (primigravids) (93) indicates that immune build-up is acquired after several pregnancies and infections.

2.4.1 Increased Cortisol Concentrations

Pregnancy has been regarded as a stage of generalized immunosuppression which is mainly sustained by increased blood levels of cortisol (119). It has been demonstrated that cortisol levels are higher in pregnant women with malarial infection than in those without (120) 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 (121). 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.

2.4.2 Decreased Cell-mediated Immunity

A brief depression of cell-mediated immunity in pregnant women is indicated in studies of immune function, particularly in the second and third trimesters. This impairment of cell-mediated immunity permits fetal allograft retention and allows pregnancy to continue without rejection (122). 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 (123, 124).

2.4.3 Inhibition of Type-1 Cytokine Responses

Th1-type responses are of parasitological significance. For example, in response to malaria parasite infection, a significant increase in tumour necrosis factor (TNF)-α (125) interferon-γ (125) interleukin 1β (126) and interleukin-2 (127) have been found in placental blood or tissue. 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 (128). However, overproduction of Th-1 cytokines can compromise pregnancy by leading to maternal anaemia, spontaneous abortions, and premature deliveries (129). 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 (130).

2.5 CONSEQUENCES OF MALARIA IN PREGNANCY

2.5.1 Maternal Health and Malaria

Although pregnant women residing in malaria endemic areas have an increased rates of parasitaemia and parasite density compared to non-pregnant women, but as some degree of pre-existing immunity is retained during pregnancy, infection remains principally asymptomatic (131). However, even malaria-immune women (i.e., those who have evolved some level of immunity against severe infection as a consequence of long residence in areas of malaria endemicity) are vulnerable to placental malaria (132). Because of the placental sequestration of large number of parasites, peripheral blood smears often fail to diagnose evidence of infection (131).

Following lack of appropriate or prompt treatment may cause adverse pregnancy outcome, including severe anemia, which is the major maternal consequence of malaria and can be life-threatening (131). Aside from anemia, malaria may accord to maternal mortality by increasing the probability and gravity of obstetric conditions such as pre-eclampsia/eclampsia and postpartum hemorrhage by as much as 50% (133).

2.5.2 Effects on Birth Outcome

The effect of malaria in pregnancy on the new born is very disastrous. Low birth weight (defined as birth weight <2500g) is associated with a remarkable increase in infant mortality (134). In areas of high malaria transmission in Africa, the probability of low birth weight approximately
doubles if women have placental malaria with the pronounced effect in primigravidae (135).

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 (135). Malaria induced low birth weight is evaluated 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 (136). Another estimate indicates 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 (135). Not surprisingly, this effect is highest in infants born to primigravidae at 17.6% of neonatal deaths and 9.8% of infant deaths (135).

The relative contribution of intrauterine growth retardation (IUGR) or preterm delivery in causing low birth weight differs by the level of malaria endemicity as well as other factors, such as access to prompt treatment and spread of HIV (96). In areas of high malaria endemicity where women are exposed to a greater frequency of antenatal infections and may have acquired immunity to avoid most febrile episodes that cause preterm delivery, IUGR is probably to be the principal cause of malaria associated low birth weight (137).

2.6. **Effect on Infant Outcomes**

In areas of high malaria endemicity, the prevalence of foetal anaemia at birth is high, and an increased threat is associated with the presence of high-density parasitaemia in the mother at delivery (138). Few studies have reported the effect of malaria in the pregnant mother on anaemia or malaria in the infant (Epidemiology and Burden of malaria in pregnancy, 2007). Some studies have demonstrated that the risk of all-cause anaemia is
evaluated to be three times higher among infants born to mothers with placental parasitaemia, even after altering environmental and ecological confounders (139).

Current evidence also specifies an association between placental malaria and minimized development of cellular and antibody responses to *P. falciparum* epitopes in infants (140). A birth cohort study from Tanzania described a 41% increased risk of malaria infection in infants born to mothers with placental malaria (141). This study demonstrated that multigravidae are also at an increased risk, inferring that offspring of multigravid women with malaria may have greater clinical manifestations than previously acknowledged, even after altering for the effect of HIV on malaria in pregnancy, which is more evident in multigravidae (142).

These are important review, because if placental malaria indeed affects infant morbidity in multigravidae, then the burden of malaria in pregnancy in Africa expands beyond that observed in paucigravidae, and the total burden may have been vastly underrated. This is essential to establish in prospective studies. Placental malaria also minimizes infant transplacental transfer of maternal antibodies and cellular immune responses in the infant to several other infectious diseases, including measles, Streptococcus pneumonia and tetanus (143).

Studies have established that congenital malaria in the native populations of malaria-endemic areas usually have rare occurrence (144) and more recurrent in offspring of non-immune mothers with malaria (145). Moreover, more recent studies from both malaria-endemic and non-endemic areas have demonstrated higher prevalences of congenital malaria ranging from 8% to 33% (Epidemiology of malaria in pregnancy, 2007).

2.7. Maternal Anaemia
The most common consequence of *P. falciparum* malaria infection is anemia. In sub-Saharan Africa, it is evaluated that between 200,000 and 500,000 pregnant women are affected with severe anemia as a result of malaria (93) and *P. falciparum* malaria in pregnancy is the root cause of up to 10,000 maternal anemia-related deaths in sub-Saharan Africa yearly (146).

However, there have been contradictory reports from parts of sub-Saharan Africa on the correlation between placental malaria and maternal anemia. From the earlier report of the Ubangi district of Zaire, it was postulated that malarious placentas had no consistent connectivity to maternal anemia (146). In other studies, maternal anemia and placental malaria were linked in all gravidity and age groups, with maternal anemia highest among women with placental malaria than those without placental malaria (147). In most areas of stable malaria transmission, many other causes of anemia have been recognised, including both nutritional (iron, folate and protein deficiency) and non-nutritional (hookworm or HIV infection, hemoglobinopathy) factors (148). Since many of these factors related to anemia occur simultaneously in pregnancy and no distinctive hallmarks of malaria-driven anemia have been discovered, it is inappropriate to assess the contribution of placental malaria infection in causing anemia in pregnancy (149).

Apart from its remarkable contribution to maternal mortality and both maternal and fetal morbidity, anemia in pregnancy is the probable risk factor for infant iron deficiency anemia (150) that, if left untreated, can be associated with adverse behavioral and cognitive development. Severe anemia in pregnancy is an important factor contributing directly and indirectly to maternal death. During pregnancy, severe anemia may cause circulatory changes related to an increased risk of heart failure and acute onset of anemia due to quick cardiac deterioration and remarkable reduction in hemoglobin (Hb) concentration to < 80g/L (151).
Such alterations can lead to the failure of compensatory mechanisms, accumulation of lactic acid and breathlessness at rest (151). In addition, during labor, women with severe anemia are less able to endure even moderate blood loss and, as a result, are at an increased risk of blood transfusion during delivery (152). For the fetus, severe maternal anemia may cause intrauterine growth retardation, still birth, and low birth weight (153). The mechanism of malaria-driven anemia can be explained in association with iron status in pregnancy. The iron status in pregnancy is affected by malaria parasites, which effect the anemia noticed in pregnancy (154). *P. falciparum* may affect iron status by minimizing intestinal iron absorption, sequestrating iron within the malarial pigment haemegozoin, consuming iron for its own metabolism, promoting/stimulating the mobilization of iron to body stores, and releasing iron into the circulation during intravascular haemolysis.

### 2.8 Malaria transmission and maternal immunity in high transmission areas

In areas, of high (stable) malaria transmission, such as in many parts of sub-Saharan Africa; many adults have developed immunity to malaria. They do not develop symptoms after an infection. However, when a pregnant woman has been infected with malaria, even if she displays no clinical symptoms, there are chances for her to develop placental parasitaemia, which can contribute to maternal anaemia and impaired foetal growth, two of the leading causes of LBW and poor survival of newborns and infants in Africa (135). In areas of stable malaria transmission, infection during pregnancy has been estimated to cause 75,000-200,000 infant deaths each year (93). Women in stable transmission areas have greatest risks of developing these complications during their first and second pregnancies (93). 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 (155). 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 (156). 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.

2.9 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 [149]. In areas with holoendemic malaria transmission, it takes nearly a decade to develop immunity to the parasite [150]. The immunity achieved is not sterile and the infected individual still suffers from infection, though the symptoms tend to be less severe (151) 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 [157] 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 (158).
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 (159). 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.

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 (158).

### 2.9.1 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 inhabiting high transmission areas (160).

### 2.9.2 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) (161). The *P. falciparum* GPI is also one of the ligands recognized by TLR4 (161) TLR9, which acts as a receptor for double-stranded DNA (CpG DNA), is activated by *Plasmodium* DNA (162).

#### 2.9.2.1 Dendritic cells
The role played by DCs during malaria infection is crucial. Their maturation during malaria course have been reported in lab experiments using monocytes in human as well as in mouse (163). It has been shown, previously that the function of DC is compromised during malaria. This phenomenon is based on an \textit{in vitro} observation. It has been established that binding of \textit{P. falciparum} iRBC to DC derived from human monocytes inhibits DCs maturation. Thus their capacity to act as an APC could be reduced (164). Furthermore, when the DC subpopulation is compared of individuals residing in similar endemic areas and infected with either \textit{P. falciparum} or \textit{P. vivax}, there was an increase in plasmacytoid CD123+ DC. The same study reported a decrease in the ratio of myeloid to plasmocytoid DC during malaria infection regardless of the malaria species (165).

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 (166). 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 \textit{Plasmodium} species, the extremity of the infection, and the patient population examined [167].

\textbf{2.9.2.2 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 (168). The proliferation of γδ T cells depends on IL-2 (169). γδ T cells have shown to play a protective role in malaria (168). 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 (170). 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 (171).

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* (172).

**2.9.2.3 NK cells**

During malaria infection, NK cells are also considered to be an important IFN-γ producers and like the γδ T cells are associated with effective killing of malarial parasites (173). 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 (174).

**3. Acquired immunity to blood stages**

**3.1 Role of antibodies in *P. falciparum* immunity**

Most antibody responses are T-cell dependant and hence require interaction between B cells and CD4+ T helper cells.
Several studies have reported the different roles played by each of the four IgG subclasses in the acquisition of naturally acquired immunity to malaria. Significant differences in the distribution of these IgG subclasses between clinically protected and nonprotected individuals have been reported. The cytophilic isotypes, IgG1 and IgG3, is prevalent in protected adults having low parasite and reduced risk of malaria pathology, while the non-cytolytic antibodies (IgG2 and IgG4) predominate in non-protected children and adults with primary attack (175). The role of cytophilic antibodies was further supported by the finding that parasite-specific IgG3, but not total IgG, was inversely correlated with susceptibility to clinical malaria (176). These observations have led to the conclusion that the development of naturally acquired immunity in individuals residing in malaria endemic areas may be associated with an age-dependant switch from IgG2 and IgG4 to IgG1 and IgG3 subclasses (177). The cytophilic antibodies have been shown to bind to Fc receptors on monocytes and mediate antibody-dependant cellular inhibition in African adults (177). Other studies have shown that IgG2 levels to ring-erythrocyte surface antigen (RESA) and to merozoite surface antigen 2 (MSP2) are associated with resistance to malaria (178) while, IgG4 levels to the same antigens were shown to be lower and positively correlated with the risk of infection.

IgE and IgM classes have been proved to play different roles in the protection and/or pathogenesis of malaria. The role of IgE antibodies in malaria pathogenesis/immunity remains unclear due to conflict in the studied area. Some studies correlate IgE antibodies to severe pathology, while others have shown that IgE antibodies were associated with protection. Higher levels of total and anti-malaria IgE were detected in patients suffering from cerebral malaria than individuals with uncomplicated malaria (179). In Sub-Saharan Africa, in different endemics conditions, two studies have been reported to play a protective role for IgE
against malaria, where high levels of *P. falciparum*-specific IgE were shown to reduce the risk of subsequent malaria attack (180).

### 3.2 CD4+ T lymphocytes

CD4+ T cells have been shown to play a crucial role in the immune response against malaria parasite; by activating B cells to produce high level of antimalarial antibodies, by enhancing the induction of CD8+ T cell responses, thereby restricting the development of liver stage parasites (181).

CD4+ T cells also restrict growth of erythrocyte *Plasmodium* parasites through cytokine secretion and macrophage activation (182).

Previous studies involving human individuals, who were vaccinated with radiation-attenuated sporozoites (IrSp) of *P. falciparum* have shown that they were protected from infection to malaria. They were shown to raise circumsporozoite (CS) antigen-specific CD4+ T cells with cytolytic activity (183). Th1-type CD4+ T cell producing high amount of IFN-γ were prevalent during the immunization of human volunteers with synthetic peptide containing universal CD4+ T cell epitope (184). The primary role of CD4+ T cells is to aid in the development of CD8+ and B cell responses, it is not completely apparent that memory CD4+ T cells do play a role in protective immunity against malaria (185). Nevertheless, it has been shown, using mice lacking B cell or with T cell lines in normal mice that memory CD4+ T cells alone have the capacity to protect against some rodent malarials (186). From the fact that individual never develop sterilizing immunity to malaria and that this immunity is rapidly lost if an individual moves away from an endemic area, it is evident that CD4+ memory to malaria is short-lived (187). In a recent review, Struik and Riley propounded a different explanation. They suggested that, immunity to symptomatic disease may be lost, but immunological memory by both T
and B cells is retained, that is why individuals who were previously exposed rapidly regain immunity on re-exposure to the parasite (188).

4. Pregnancy and the Immune System

4.1 Immune Tolerance

The central role of the immune system is to protect the host from pathogens. Maternal immunity during pregnancy is a complex process since fetal allograft has to be accepted by the immune system and at the same time maintain protection against microbes and other pathogens. In other words, the maternal immune system needs substantial regulation for the survival of the foetus but not to such an extent that it compromises protection of the mother. The mechanisms involved in tolerance of the fetus by the maternal immune system is still difficult to trace out. Medawar was the first to validate the concept of fetal-maternal tolerance and put forward the concept of the fetal allograft to elucidate the special immune relationship between the mother and the fetus (189). The idea propounded by him was that, the survival of semi-allogeneic fetus was because of the suppression of the immunological interaction between the mother and the fetus, possibly due to lack of exposure of fetal antigens to maternal cells due to physical separation of maternal and fetal tissue by the placenta. Although justifications proposed by him were not precise, the hypothesis had a profound influence in the research field during the following years. These ideas were later enlarged and reviewed by Wegmann (190) who suggested that pregnancy is purely a Th2 phenomenon in which the Th1 cells that mediate cytotoxic responses are suppressed, and the pregnant women is increasingly reliant on Th2 response for the production of antibodies and sustained pregnancy. The Th2 hypothesis is currently under argument (191, 192). However, it has been evident that the Th1/Th2 ratio is changed in the decidua during
pregnancy, which is apparently supported by the increase in local progesterone levels (a potent Th2 inducer) and the secretion of Th2 cytokines, such as IL-4, IL-5 and IL-10 (193). However, a firm Th2 dominance throughout pregnancy does not seem to be crucial and recent data suggests that a successful early pregnancy is aided by the cytokines which typically are associated with Th1 responses. Th1 cytokines are believed to be deleterious for a sustained pregnancy since administration of IL-2, IFN-γ and TNF-α to pregnant mice have been shown to cause fetal resorption (194) and high levels of Th1 cytokines have been found in women with spontaneous abortions (195, 196, 197). Thus, anti-inflammatory factors such as IL-10 and TGF-β are required for a sustained pregnancy. In addition, studies do report that the Th1 cytokines might not be related to pregnancy and that the secretion of Th1 cytokines may be due to the cellular immunity that is activated as a result of pregnancy complications, such as pre-eclampsia and spontaneous fetal loss (198). Therefore the actual prognosis that pregnancy is a Th2 phenomenon might have been too simplified. A recent report by Mor et al, (199) suggested that pregnancy is both a pro- and anti-inflammatory condition, depending upon the stage of gestation. Furthermore, the absence of classical MHC class I (HLA-A and HLA-B) and class II molecules on the trophoblast is one of the important immune evasion strategy by the fetus. Other factors contributing to maternal tolerance of the fetus include expression of Fas ligand on fetal cells to eliminate activated T cells (200) and local production of the T-cell suppressive substance indoleamine 2,3-dioxygenase (IDO) expressed by fetal syncytiotrophoblasts and decidual macrophages (201). Also, placental exosomes, which express proteins that suppresses the cytotoxicity of T and NK cells, have been found in maternal circulation (202, 203).

4.2 Immune activation during pregnancy
Extensive studies have authenticated that both the innate and the adaptive immune system are crucial for a successful pregnancy. The maternal immune system changes both locally in the placental environment and in the peripheral circulation. Adaptive maternal immunity is generally suppressed, while the innate components of the immune system are activated systemically (204). In a normal pregnancy, the human decidua, or implantation site, is inhabited by large numbers of immune-competent cells such as macrophages, NK cells (205,206,207,208) but B cells are not present. These cells (macrophages, NK) have shown to play a crucial role during the first trimester since their depletion results in termination of the pregnancy (209) which proves that they play a significant role in placental development, implantation or decidual formation. A special NK-cell subset known as uterine NK (uNK) cell, which is the major immune cell type in the decidua accounts for upto 70% of all decidual leucocytes. uNK cells differs both phenotypically and functionally from peripheral NK cells (210) and have been shown to play a crucial role in the vascularization during early pregnancy (211) and decreased numbers of uNK cells have been linked to intrauterine growth restriction (212). Macrophages accounts for upto 20-25% of the decidual leucocytes but their numbers may differ depending on hormones affecting the influx of leucocytes and monocytes into the decidua. Also, the migration of macrophages may be stimulated by the local production of MIP-1 by cytotrophoblasts (213). Since there is a scarcity of immune defense systems within the placental tissue, it is assumed that the macrophages inhabiting decidua plays a crucial role in non-specific host defense within the placenta. They could enhance the clearance of apoptotic cells and debris as well as secreting cytokines and immunosuppressive prostaglandins, which may hamper the function of Tc cells and uNK cells.