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THE BURDEN OF MALARIA IN PREGNANCY AND INTERVENTION STRATEGIES – A REVIEW

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Abstract
Malaria infection during pregnancy results in poor maternal and foetal outcomes, especially maternal anaemia and low birth weight infant. Anaemia results from depletion of erythrocytes which are attacked by the malaria parasites. Low birth weight is primarily a consequence of intrauterine growth restriction (IUGR). The pathologic disarray of placental basal structure following intense infiltration of leucocytes into the placenta is known to mediate IUGR. Identifying other pathogenic factors which may be present would greatly improve the intervention strategies.
In this review, the burden of malaria infection and malaria in pregnancy (MIP), histological and pathologic changes in parasitized placenta and protection against MiP are discussed with reference to infection with *Plasmodium falciparum*. The level of utilization of intervention measures, the need for urgent assessment of new anti-malarial drugs (e.g. artemisinin-based combination therapies, ACTs) in pregnancy and the problem posed by poor detection of placental parasitaemia are highlighted.

Key words: anaemia, intrauterine growth restriction, low birth weight, malaria, *Plasmodium falciparum*.

INTRODUCTION

Malaria is one of the infectious diseases of clinical importance, particularly in sub-Saharan Africa. It is caused by infection of erythrocytes with protozoan parasites of the genus plasmodium. The four Plasmodial species capable of causing infection in humans are *P. falciparum, P. vivax, P. ovale* and *P. malariae*. Rarely, infection with monkey malaria parasite, *P. knowlesi* may occur (WHO, 2006)]. In this review, the burden of malaria, malaria in pregnancy, placental malaria, histological and pathologic changes in discussed with reference to infection with *Plasmodium falciparum*.

Diagnosis of malaria in pregnancy is usually underestimated, because of parasite sequestration in the developing placenta, thus resulting in low peripheral parasitaemia (Mockenhaupt, *et al*, 2002). Placental malaria (PM) resulting from heavy accumulation of parasites in the placenta accounts for poor pregnancy outcomes seen in PM-positive mothers. Malaria is known to be associated with acquired immunity; thus young children are more susceptible to malaria infection than adults (Tako *et*

al, 2005). Pregnancy tends to increase this susceptibility to malaria Gilles *et al*, 1969), with primigravidae experiencing most severe complications (Brabin, 1991). Some of the components of acquired immunity to malaria infection have been identified.

Malaria Infection

Malaria is a major public health problem in Africa, where up to 45 countries are tagged as malaria-endemic and about 588 million people are at risk of the disease (WHO, 2008). Infection with *P. falciparum* gives the most severe form of malaria disease. The level of transmission in an area is determined by the annual entomological inoculation rate (EIR), defined as the number of inoculations of malaria parasites received by one person in one year (WHO, 2006).

Malaria-endemic areas or areas of high malaria transmission have EIR values > 10/year while areas of low malaria transmission or areas of unstable malaria transmission have EIR < 5/year [1]. EIR as high as 500 – 1000 can be reached in a few parts of tropical Africa (Hary *et al*, 2000). Adults living in malaria-endemic areas acquire partial immunity to malaria due to series of exposure to the parasite during childhood (Baird, 1995; Jensen, *et al*, 2004), and as such they do not experience acute clinical malaria. Consequently, in malaria-endemic areas, only young children who have not substantially developed sufficient immunity suffer from an acute manifestation of malaria. Lack of immunity is also seen in people (both adults and children) living in areas of low malaria transmission and they have a high risk of progression to severe malaria if untreated (WHO, 2006).

Adults living in malaria-endemic areas have self-limiting clinical symptoms of malaria, and treatment with partially-effective antimalarial drugs paradoxically appears clinically effective, whereas the use of such ineffective drugs in young children may be fatal (WHO,2006).

The rapid emergence of parasite resistance to existing anti-malarial drugs constitutes a great problem in the management of malaria infection. Resistance to anti-malarials as defined by WHO is “the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a medicine given in doses equal to – or higher than – those usually recommended but within the tolerance of the subject, with the *caveat* that the form of the drug active against the parasite must be able to gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action” (WHO, 2006).

Delayed and/or ineffective treatment of falciparum malaria causes overwhelming increase in parasite density and severe malaria may ensue. Symptoms of severe malaria include cerebral malaria, metabolic acidosis, hypoglycaemia, severe anaemia, and acute renal failure or acute pulmonary oedema in adults (WHO, 2006). Jaundice and respiratory distress are also seen in severe malaria in children. Uncomplicated malaria is a symptomatic infection with malaria parasitemia but without signs of severity and/or evidence of vital organ dysfunction (WHO, 2006).Several studies have reported that the severity of falciparum malaria is due to accumulation of falciparum-infected erythrocytes (IEs) in the micro vascular capillaries of vital organs whereby the parasite avoids immune mechanisms present in the spleen (Macpherson *et al*, 1985; Pongponratn *et al*, 1991). This constitutes, in part, to the virulent factor of the parasite. Adults in malaria-endemic areas often have asymptomatic malaria infection which might be contributory to frequent misdiagnosis of the disease.

In addition to lack of natural immunity in children, malaria infection in this group is made worse by resetting and cytoadherence, which largely contributes to the severe form of malaria (Macpherson *et al,* 1985; Wahlgren *et al,* 1994). Clinical diagnostic approach alone often results in over diagnosis of malaria, due to its non-specificity. Need, therefore, arises for parasite-based diagnosis in patients with clinical suspicion of malaria.

Malaria In Pregnancy

In Africa, 42% of women have malaria at some point during their pregnancy [ter Kuile *et al,* 2004]. The severe manifestation of malaria in pregnancy is due to the accumulation of trophozoite- or schizont-infected erythrocytes (IEs) in the placenta (Bulmer *et al,* 1993; Beeson *et al,* 2002). This causes maternal anaemia, low birth weight (LBW) and increased perinatal and infant mortality in supposedly clinically immune women living in malaria-endemic areas (Brabin, 1983; Steketee *et al,* 2001). Gametocytes are rarely found in placenta (Desowitz and Buchbinder, 1992). It is estimated that malaria infection during pregnancy accounts for low birth weight (birth weight less than 2500 g) in 75,000 – 200,000 newborns yearly (Steketee *et al,* 2001) and severe anaemia in 400,000 women, of whom an estimated 10,000 may die directly of anaemia (Guyatt and Snow, 2001). In Nigeria, 11 % of maternal deaths are attributed to malaria (FMoH, 2000).

Malaria in pregnancy is associated with certain levels of acquired immunity. The acquisition of this anti-disease immunity contributes to the asymptomatic nature of malaria in pregnancy in endemic areas (Staalsoe *et al,* 2001). An important component of the acquired immunity seems to be due to production of anti-IE adhesive antibodies, which inhibit the adhesion of IEs to the placental receptors. Presence of these inhibitory antibodies is pregnancy- associated and gravidity-dependent, such that primigravidae exhibit the least immunity and are at higher risk of placental parasitemia than are multigravidae (Maubert *et al,* 1999; Ricke *et al,* 2000). Similarly, young women of child-bearing age may also be more susceptible to malaria than older women because they are still in the process of acquiring immunity. In a report by O'Neil-Dunne *et al.* (2001) it was demonstrated that a majority of pregnant women irrespective of gravity lack anti-adhesive antibodies prior to first trimester. However, the antibodies levels in both multigravidae and primigravidae increase incessantly from 12 weeks and 20 weeks respectively, then reaching similar levels at term (O'Neil-Dunne *et al.* 2001). The reported greater susceptibility to placental infection in primigravidae than in the multigravidae may probably result from the lag in antibody production in the former. The period of onset of pregnancy-associated immune response in both groups of women correlates with the peak prevalence of *P. falciparum* infection in placenta (O'Neil-Dunne *et al.* 2001). This view was supported in a later study by Jauniaux *et al*. (2003), which reported onset of placental blood flow at 10 – 12 weeks of gestation (O'Neil-dunne *et al,* 2001). Thus, sequestration in the placenta of IEs might likely not occur prior to 10 – 12 weeks of gestation.

Although malaria in pregnancy commonly manifests with febrile symptoms; however, these symptoms have poor prognostic importance for malaria, thus prompting the need for parasite-based diagnostic tests. The diagnosis of pregnancy-associated malaria is usually delayed and there are insufficient data on safety profile of effective anti-malarial drugs in pregnancy. Consequently, the protection of pregnant women residing

in endemic areas remains a challenge for national malaria control programmes.

Placental Malaria

An approximate 25 million pregnant women living in sub-Saharan Africa are at risk of *P. falciparum* infection every year, and one in four women has evidence of placental infection at the time of delivery (Desai *et al,* 2007). Placental malaria (PM) is often characterized by poor pregnancy outcomes – maternal morbidity, low birth weight, and preterm delivery (Menendez *et al,* 2000; Sullivan *et al,* 1999). Upon invasion of human erythrocytes, the parasite inserts its antigenic protein in knob-like protrusions present on the surfaces of the cells (Aikawa *et al,* 1983). PM ensues due to the accumulation in the placenta of these infected erythrocytes (IEs). The IEs bind tenaciously to placental receptors called Chondroitin Sulphate A and hyaluronic acid molecules located on the syncytiotrophoblast lining of the placenta or may adhere to secreted Chondroitin Sulphate A (CSA) in the intervillous space (Fried *et al,* 1996; Beeson *et al,* 1999; Beeson *et al,* 2000; Muthusamy *et al,* 2004). The binding is facilitated via the antigenic parasite ligand called *P. falciparum* erythrocyte membrane protein I (PfEMPI) (Newbold *et al,* 1997; Reeder *et al,* 1999). Cytoadherence to the placental adhesive molecules is specific for parasitized cells and maximal adhesion to hyaluronic acid (HA) has been shown to occur at physiologic pH of 7.2 – 7.6 (Beeson *et al,* 2000); while placental Chondroitin Sulphate proteoglycan (CSPG) is saturable at coating concentration of 100 – 200 ng of CSPG/ml (Achur *et al,* 2000). The cytoadherence of IEs may result in capillary obstruction and induction of pro-inflammatory cytokines in response to harmful parasite factors resulting in tissue damage and clinical manifestation (Newbold *et al,* 1997; Miller *et al,* 1994). The findings of Fried *et al.* (1998) showed an elevation of T-cell derived cytokines– IFN-gamma and TNF-alpha in PM, and an association between the cytokines and poor pregnancy outcomes.

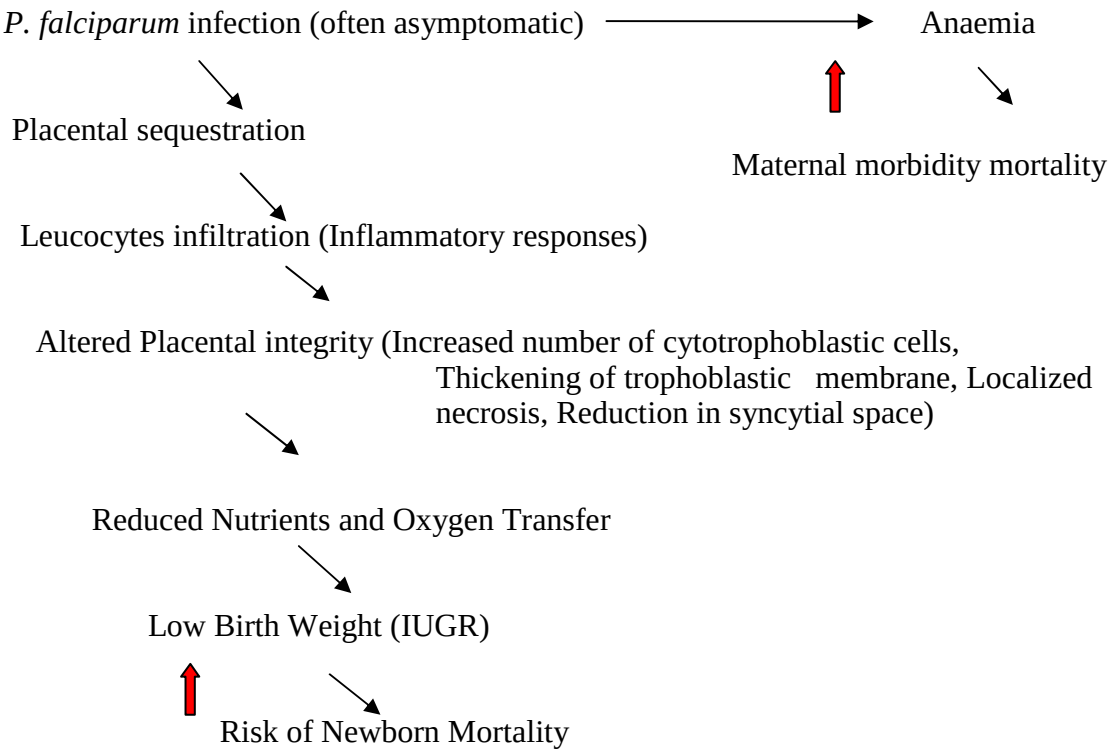
However, these cytokines were reported as a component of immune responses as they occurred at a higher level in uninfected placentae from multigravidae than in infected placentae (Moore *et al,* 1999).

Leucocytes infiltration (mostly mononuclear cells) of the intervillous spaces, mediated by chemoattractant proteins occurs in response to the inflammatory processes (Ordi *et al,* 1998). In their study, Abrams *et al.* (2003) reported that three beta chemokines namely macrophage-inflammatory protein 1 (MIP-1) alpha, monocyte chemoattractant protein 1 (MCP-1) and I-309, and one alpha chemokine interleukin (IL) -8 are associated with malaria infection during pregnancy. The intense infiltration of the villous cytotrophoblastic cells in heavily parasitized placenta usually is associated with irregular thickening of the trophoblastic basement (Galbraith *et al,* 1980) or 'massive chronic intervillousitis (Ordi *et al,* 1998). Bulmer *et al.* (1993 a&b) in two separate studies had earlier delineated the histopathologic changes of parasitized placenta, including increase in the number of cytotrophoblastic cells, syncytiotrophoblastic necrosis at localized sites, thickening of the trophoblastic membrane in an irregular fashion and reduction in syncytium. These structural alterations could interfere with the normal physiologic materno-foetal transfer of molecules such as antibodies and oxygen.

The changes probably affect the receptors for the immunoglobulin (Ig) G present on the syncytiotrophoblast

and which mediate the transfer of the IgG across the placenta (Saji *et al*, 1999). In another study, Okoko *et al.* (2001) reported that the transfer of antibodies against herpes simplex virus type 1 (HSV-1), respiratory syncytial virus (RSV) and varicella zoster virus (VZV) and transfer of IgG 1 and IgG 2 were significantly impaired in parasitized placenta. Similar impairment in antibody transfer had earlier been reported with the transfer of tetanus toxoid antibody (Brair *et al*, 1994). The 'victim' of impairment of these essential molecules, the foetus, is then left unprotected and susceptible to infectious diseases including malaria.

Sequence of pathologic changes in malaria-endemic area



In placental malaria, there is selective accumulation of *P. falciparum* IEs that express an antigenically distinct form of PfEMPI (Fried *et al*, 1996), not encountered during childhood disease and so escape previously acquired immune responses. Placental parasites and parasite lines selected for CSA binding in vitro almost always express a distinct var gene named *var2CSA* (Salanti *et al*, 2003; Tuikue *et al*, 2005). The gene expression determines cytoadhesion of PfEMPI antigen to the placental chondroitin sulphate A receptor (Viebig *et al*, 2005). High anti-var2CSA immunoglobin G levels correlated with protection against poor outcomes of malaria in pregnancy (Salanti *et al*, 2004). Earlier studies in late 90s suggested future research on identification of the regions of the protein antigen which have greatest binding activity and which might form a component of malaria vaccine, specifically targeting malaria in pregnancy. Few studies have elucidated the composition of gene *var2CSA* and identified several regions of the gene called Duffy binding-like (DBL) domains. DBL-alpha domain is implicated in rosette formation (Udomsangpetch *et al*, 1989). Few of these domains have been shown to be capable of inducing antibodies against parasite adhesion. In an attempt to produce vaccines, small constructs of

these large proteins are developed and used in immunization in animal studies. DBL-FCR3 (construct) has recently been shown to induce IE adhesion antibodies (Nielsen *et al*, 2009). Salanti *et al.* (2010) in a very recent study reported inhibition of parasite binding by immunization with DBL3-HB3T1 and DBL1-3D7 but production of antibodies was not sustained during the entire immunization process. Previously, Fernandez *et al.* (2008) showed that immunization with DBL6-FCR3 gives similar results as with DBL3-HB3TI and DBL1-3D7. This clearly indicates that various domains in the antigen partake in the adhesion process and as such, an effective vaccine against placental malaria would be a multidomain vaccine.

Placental malaria poses daunting problems in its diagnosis prior to delivery. This is because microscopy of peripheral thick blood film often fails to identify a considerable portion of the placental parasites (Mockenhaupt *et al*, 2002). The possibility of existence of placental parasitemia in the absence of peripheral blood parasitemia, and even the persistence of parasites in the placenta after initiation of anti-malarial treatment had been documented by Sartelet *et al.* (1997).

Intervention Strategies For Malaria In Pregnancy

Malaria in pregnancy (MIP) continues to be a major public health problem in many endemic countries. Due to the immense health burden, several national and international organizations have recommended intervention strategies. When fully implemented, these strategies are expected to drastically reduce the burden of MIP.

The main components of prevention and control of MIP include the following:

- Focused antenatal care (ANC) and health education
- Insecticide treated nets, including long-lasting insecticide nets (LLINs)
- Intermittent preventive treatment in pregnancy with the use of Sulphadoxine-Pyrimethamine (S-P – based IPTp)
- Case management of malaria infection with appropriate anti-malarial drug

Focused ANC is a personalized care provided to a pregnant woman, which emphasizes on the woman's overall health, her preparation for childbirth and readiness for complication (emergency) preparation. Focused ANC is timely, friendly, simple and safe services to a pregnant woman. It provides opportunity for early detection and treatment of certain pregnancy-related medical conditions such as malaria, anaemia, pre-eclampsia /eclampsia, sexually transmitted infections (including HIV/AIDS) (JHPIEGO, 2008). WHO recommends a schedule of 4 antenatal visits with 3 visits occurring after quickening (i.e after 16weeks of gestation). In a study conducted by Eckert *et al*. (2005) in 20 African countries, good antenatal clinic registration was observed among pregnant women; however, attendance at second visit was poor. In Nigeria, antenatal clinic registration is reported to be about 60 %, while second visitation is found to be less than 50 % (Eckert *et al*, 2005).

Insecticide treated nets (ITNs) protect pregnant women from mosquito sting, especially at night (Enato, 2010). However, proper care in handling the net is very important to prevent tear. Instruction on how to properly use net to give the maximum protection to pregnant women is very important, and this should be emphasized during ANC.

Intermittent preventive treatment in

pregnancy involves giving full treatment course of an effective antimalarial drug to women during pregnancy. The assumption for this is based on the fact that every pregnant woman in malaria endemic areas is infected with *P. falciparum* and many of which do not experience any clinical symptoms (van Ejik *et al*, 2004). The drug of choice for IPTp in most malaria-endemic countries is sulfadoxine-pyrimethamine (SP) of which at least 2 treatment doses are used as preventive measure. The main challenges to the effective utilization of IPTp are decrease in attendance at second and subsequent ANC visits and 'missed doses'. There is also the problem of emergence of resistant parasite strains to the SP.

Case management is treatment of symptomatic malaria with an effective anti-malarial agent. In accordance with the Nigerian anti-malarial treatment guideline, oral quinine is the first line of drugs in the treatment of acute uncomplicated *P. falciparum* malaria in pregnancy (FMoH, 2004). It is recommended that irrespective of IPTp adherence, any pregnant woman with symptoms of the illness should receive appropriate and effective case management (WHO, 2004). In the view of reserving the use of quinine to treatment of only complicated malaria infection, there is need to urgently evaluate safety and effectiveness of ACTs in pregnant women. Already, there are epidemiological reports of *in utero* exposure to arthemisinins during pregnancy without congenital malformation (McGready *et al*, 2008). Notwithstanding, assessment of birth outcomes, including pre-term delivery, LBW, abortion, and other congenital abnormalities in women treated with ACT through a systematic study is imperative.

In conclusion, it should be noted that research efforts to improve our understanding of the pathogenic mechanism of MiP is urgently needed. In particular, understanding of the clinical importance of these cytokines in PM may lead to a better management of the infection. Similarly, development of malaria vaccine will see to the protection of young infants whom levels of immunity is determined by antibodies acquired via materno-foetal transfer. Future research should be directed towards improving the diagnosis of MiP followed by prompt therapy. Partnership between the national malaria control programmes and community will aid efficiently and timely delivery anti-malarial intervention tools (e.g.ITNs and IPTp) to the target groups. In view of persistent placental parasitemia after initiation of chemotherapy, the development of site-targeted anti-malarial drugs that are safe and effective during pregnancy for chemotherapy and IPT should be considered.

REFERENCES

Abrams ET, Brown H, Chensue SW, *et al.* (2003) Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated *beta* chemokine expression. J. Immun. 170:2759-2764.

Achur RN, Valiyaveettil M, Alkhalil A, Ockenhouse CF, and Gowda DC. (2000) Characterization of proteoglycans of human placenta and identification of unique chondroitin sulphate proteoglycans of the intervillous spaces that mediate the adherence of *Plasmodium falciparum*-infected erythrocytes to the placenta. J. Biol. Chem. 275:40344-40356.

Aikawa M, Rabbege JR, Udeinya I, Miller LH. (1983) Electron microscopy of knobs in *Plasmodium falciparum*-infected erythrocytes. J Parasitol. 69:435–437.

Baird JK. (1995) Host age as a determinant of naturally acquired immunity to *Plasmodium falciparum* malaria. Parasitol. today.11:105-111.

Beeson JG, Amin N, Kanjala M and Rogerson SJ. (2002) Selective accumulation of mature

asexual stages of *plasmodium falciparum* infected erythrocytes in the placenta. Infect. Immun. 70:5412-5415.

Beeson JG, Brown GV, Molyneux ME, Mhango C, Dzinjalamala F, and Rogerson SJ.(1999) *Plasmodium falciparum* isolates from infected pregnant women and children are each associated with distinct adhesive properties. J. Infect. Dis. 180:465-472.

Beeson JG, Rogerson SJ, Cooke BM, *et al*. (2000) Adhesion of *Plasmodium falciparum*-infected erythrocyte to hyaluronic acid in placenta malaria. Nature Med. 6:86-90.

Brabin B. (1991) An assessment of low birth weight risk in primiparae as an indicator of malaria control in pregnancy. Int. J. Epidemiol. 20:276-283.

Brabin BJ. (1983) An analysis of malaria in pregnancy in Africa. Bull. World. Health. Org. 61:1005-1016.

Brair ME, Brabin BJ, Miligan P, Maxwell S, and Hart CA. (1994) Reduced transfer of tetanus antibodies with placental malaria. Lancet. 343:208-209.

Bulmer JN, Rasheed FN, Francis N, Morrison L, and Greenwood BM. (1993a) Placental malaria. I. pathological classification. Histopathol. 22:211-218.

Bulmer JN, Rasheed FN, Francis N, Morrison L, and Greenwood BM. (1993b) Placental malaria. II. A semi quantitative investigation of the pathological features. Histopathol. 221:219-223.

Desai M, ter Kuile FO, Nosten T, et al. (2007) Epidemiology and burden of malaria in pregnancy (Review). Lancet Infect. Dis. 7:93-104.

Desowitz RS, and Buchbinder G. (1992) The absence of *Plasmodium falciparum* gametocytes in the placental blood of a woman with a peripheral parasitaemia. Annals Trop. Med. Pathol. 86:191-192.

Eckert E, Hyslop A, Snow R. (2005) MEASURE Evaluation. Macro International APHA.

Enato E. F. O.(2010) Pharmaceutical care in malaria. In: Oparah CA (editor). Essentials of Pharmaceutical Care, Acybex publication, Lagos. 393 – 411.

Federal Ministry of Health. (2000) Malaria situation analysis document. Federal Ministry of Health, Nigeria: 14.

Federal Ministry of Health. (2004) National Malaria Control Policy for Nigeria. National Malaria and Vector Control Division, Lagos, Nigeria.

Fernandez P, Kviebig N, Dechavanne S, *et al*. (2008) Var2CSA DBL6-episolon domain expressed in HEK 293 induces limited cross reactive blocking antibodies CSA binding parasites Malar. J 7:10.

Fried M and Duffy PE. (1996) Adherence of plasmodium falciparum to chondroitin sulphate A in the human placenta. Science. 272:1502-1504.

Fried M, Muga RO, Misore AO, and Duffy PE. (1998) Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes. J. Immun. 160:2523-2530.

Galbraith RM, Fauk WP, Galbraith GM, Holbrook TM, and Bray RS. (1980) The human materno-fetal relationship in malaria. I. Identification of pigment and parasites in the placenta. Trans. R. Soc. Trop. Med. Hyg. 74:52-60.

Gilles HM, Lawson JB, Sibelas M, Voller A, and Allan N. (1969) Malaria, anaemia and pregnancy. Annals Trop. Med. Parasitol. 63:245-263.

Guyatt HL, and Snow RW. (2001) The epidemiology and burden of plasmodium falciparum-related anaemia among pregnant women in sub-Saharan Africa. Am. J. Trop. Med. Hyg. 64(suppl):36-44.

Hary SI, Roggers DJ, Toomer JF, Smow RW. (2000) Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: Literature Survey, internet access and review. Trans. R. Soc. Trop. Med. Hyg. 94:113-127.

Jauniaux E, Gulbis B, and Burton GJ. (2003) The human first semester gestational sac limits rather than facilitates oxygen transfer to the foetus - a review. Placenta. 24(suppl A):586-593.

Jensen ATR, Magistrado P, Sharp S, et al. (2004) Plasmodium Falciparum associated with severe childhood malaria preferentially express PFEMPI encoded by group A var genes. J. Exp. Med. 199:1179-1190.

JHPIEGO. (2008) Prevention and Control of malaria in pregnancy. Reference manual for health care providers, 2nd edition.

Macpherson GG, Warrel MJ, White NJ, Looreesuwan S, and Warrel DA. (1985) Human cerebral malaria. A quantitative ultra structural analysis of parasitized erythrocyte sequestration.

Am. J. Pathol. 119:385-407.

Maubert B, Fievert N, Tami G, Cot M, Boudin C, and Deloron P. (1999) Development of antibodies against chondroitin sulphate A-adherent *Plasmodium falciparum* in pregnant women. Infect. Immun. 67:5367-5371.

McGready R, Tan SO, Ashley EA, Pimanpanarak M, Viladpai-nguen J, et al. (2008) A randomised controlled trial of artemether-lumefantrine versus artesunate for uncomplicated Plasmodium falciparum treatment in pregnancy. *PLoS Med.* 2008, 5(12): e253. doi:10.1371/journal.pmed.0050253.

Menendez C, Ordi J, Ismail MR, et al. (2000) The impact of placental malaria on gestational age and birth weight. J. Infect. Dis. 181:1740-1745.

Miller LH, Good MF, and Milon G. (1994) Malaria pathogenesis. Science. 264:1878-1883.

Mockenhaupt FP, Ulmen U, von Gaertner C, Bedu-Addo G, and Bienzle U. (2002) Diagnosis of placental malaria. J. Clin. Microb. 40:306-308.

Moore JM, Nahlen BL, Misore A, Lal AA, and Udhayakumar V. (1999) Immunity to placental malaria. I. Elevated production of interferon-gamma by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. J. Infect. Dis. 179:1218-1225.

Muthusamy A, Achur RN, Bhavanandan VP, Fouda GG, Taylor DW and Gowda DC. (2004) *Plasmodium falciparum*-infected erythrocytes adhere both in the intervillous space and on the villous surface of human placenta by binding to the low sulphated chondroitin sulphate proteoglycan receptor. Am J Pathol. 164:2013-20.

Newbold CI, Craig AG, Kyes S. PfEMP-1, polymorphism and pathogenesis. (1997) Annals Trop. Med. Parasitol. 91:551-557.

Nielsen MA, Pinto VV, Resende M, et al. (2009) Induction of adhesion-inhibitory antibodies against placental *Plasmodium falciparum* parasites by using single domains of VAR2CSA. Infect. Immun. 77:2482-2487.

O'Neil-dunne, Achur RN, Agbor-Enoh ST, et al. (2001) Gravidity-dependent production of antibodies that inhibit binding of *plasmodium falciparum*-infected erythrocytes to placental chondroitin sulphate proteoglycan. Infect. Immun. 69:7487-7492.

Okoko BJ, Wesumperuma LH, Ota MOC, *et al.* (2001) The influence of placental malaria infection and maternal hypergammaglobulinemia on transplacental transfer of antibodies and IgG subclasses in a rural West African population. J. Infect. Dis. 184:627-632.

Ordi J, Ismail MR, Ventura PJ, *et al*. (1998) Massive chronic intervillositis of the placenta associated with malaria infection. Am. J. Surg. Pathol. 22:1006-1011.

Pongponratn E, Riganti M, Punpoowong B, and Aikawa M. (1991) Microvascular sequestration of parasitized erythyrocytes in human falciparum malaria: a pathological study. Am. J. Tropl. Med. Hyg. 44:168-175.

Reeder JC, Cowman AF, Davern KM et al (1999). The adhesion of plasmodium falciparum-infected erythrocyte to Chondroitin Sulphate A is mediated by *P. falciparum* erythrocyte membrane protein-1. Proc. Ntl Acad. Sci. 96: 5198-5202.

Ricke CH, Staalsoe T, Koram K, et al. (2000) Plasma antibodies from malaria-exposed pregnant women recognize variant surface antigens on *plasmodium falciparum*-infected erythrocytes in a parity-dependent manner and block parasite adhesion to chondroitin sulphate A. J. Immun. 165:3309-3316.

Saji F, Samejima Y, Kamiura S, and Koyama M. (1999) Dynamics of immunoglobulins at the feto-maternal interface. Rev Reprod. 4:81-89.

Salanti A, Dahlback M, Turner L, et al. (2004) Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. J Exp Med. 200:1197-1203.

Salanti A, Resende M, Ditlev SB, et al. (2010) Several domains from VAR2CSA can induce *Plasmodium falciparum* adhesion-blocking antibodies. Malar. J. 9:11.

Salanti A, Staalsoe T, Lavstsen T, *et al.* 2003) Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering *Plasmodium falciparum* involved in pregnancy-associated malaria. Mol. Microbiol. 49:179-191.

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

Staalsoe T, Shulmar CE, Buhner JN, Kawuondu K, Marsh K, Hviid I. (2001) Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated *plasmodium falciparum* malaria. Lancet. 363:283-289.

Steketee RW, Nahlen BL, Parise ME, and Menendez C. (2001) The burden of malaria in pregnancy in malaria-endemic areas. Am. J. Trop. Med. Hyg. 64(suppl):28-35

Sullivan AD, Nyirenda T, Cullinan T, Taylor T, Harlow SD, James SA and Meshnik SR. (1999) Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. J. Infect. Dis. 179:1580-1583.

Tako EA, Zhou A, Lohoue J, et al. (2005) Risk factors for placental malaria and its effect on pregnancy outcome in Yaoundé, Cameroon. Am. J. Trop. Med. Hyg. 2005, 72:236-242.

ter Kuile FO, Parise ME, Verhoeff FH, et al. (2004) The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa. Am. J. Trop. Med. Hyg. 71:41-54.

Tuikue Ndam NG, Salanti A, Bertin G, *et al*. (2005) High level of var2csa transcription by *Plasmodium falciparum* isolated from the placenta. J. Infect. Dis. 192:331-335.

Udomsangpetch R, Wahlin B, Carlson J, *et al*. (1989) *Plasmodium falciparum*-infected erythrocytes form spontaneous erythrocyte rosettes. J. Exp. Med. 169:1835–1840.

van Ejik AM, Ayisi JG, ter Kuile FO. (2004) Effectiveness of intermittent preventive treatment with sulphadoxine-pyrimethamine for control of malaria in pregnancy in Western Kenya: a hospital-based study. Trop. Med. Int. Health. 9(3): 351 – 360.

Viebig NK, Gamain B, Scheidig C, *et al*. (2005) A single member of *Plasmodium falciparum* var multigene family determines cytoadhesion to the placental receptor chondroitin sulphate A. EMBO Rep 6:775-781.

Wahlgren M, Fernandez V, Scholander C, and Carlson J. (1994) Rosetting. Parasitol today. 10:73-79.

World Health Organization. (2004) A strategic framework for malaria prevention and control during pregnancy in the African region. World Health Organization Regional Office for Africa, Brazzaville AFR/MAL/04.01.

World Health Organization. (2006) WHO guidelines for the treatment of malaria. World Health Organization, Geneva.

World Health Organization. (2008) World Malaria Report 2008. World Health Organization, Geneva: 99-101.