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RESEARCH ARTICLE

THE EFFECTS OF MALARIA IN THE COURSE OF PREGNANCY ON NEONATAL BIRTH WEIGHT AND CYTOKINES IN ELDWIEM, SUDAN

[1,*]Emad Abd Elhalim Nour Elgalil, [2,3]Ali Mahmoud Mohammed Edris, [4]Fathelrahman M. Hassan, [5]Eltaib Mohammed and [6]Babiker A. Mohammed

[1]Faculty of Medical Laboratory Science, Omdurman Islamic University, Sudan
[2]Faculty of Medical Laboratory Science, University of Khartoum, Sudan
[3]college of Applied Medical Science, University of Bisha, Saudi Arabia
[4]College of applied Medical Science, Imam Abdulrahman Bin Faisal University, Saudi Arabia
[5]College of Medicine, Bakht-Alruda University, Sudan
[6]College of Medicine, Karary University, Sudan

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ABSTRACT

Background: Inflammatory cytokines play an integral role in human immune responses to malarial disease. However, the role of these mediators in disease pathogenesis, as well as relationship between host protection and injury remains unclear; so this study aimed at assessing the neonatal cytokines accompanying maternal malaria **Methods:** A prospective cross-sectional hospital based study that enrolled a total of 180 pregnant women, among whom (150 with confirmed maternal malaria as cases and 30 without malaria as controls). Socio-demographic data were collected using a structured questionnaire. Plasmodium infection was microscopically diagnosed using Giemsa-stained blood smear. The birth weights (BW) of the newborns were recorded soon after delivery. Cord blood cytokines were examined via ELISA technique. **Results:** An association was noted between maternal malaria and birth weight reduction (2592g vs. 3101g). The present study, revealed a significantly elevation in Tumor necrosis factor TNF-α and Interleukin 8 IL-8 in neonates born to women infected with malaria parasite (P. value = 0.016, 0.047 respectively) compared to healthy controls. Nonetheless, our findings noted no statistical difference in IL-10 between cases and control groups (p. value= 0.25). **Conclusion:** There is a relationship between elevation of neonatal inflammatory cytokines, birth weight reduction and malaria infection during pregnancy. The findings might explain some of the adverse effects on the health of neonates born to women infected with malaria parasite.

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INTRODUCTION

In Sub-Saharan African countries, encompassing Sudan, malaria in the course of pregnancy is a major public health threat which results in significant morbidities and mortalities among pregnant women and their fetuses (Nega *et al.,* 2015). Consequently, maternal malaria presents a significant impact on the neonates, being the primary cause of abortion, stillbirth, premature delivery, fetal death, low birth weight (LBW) and fetal/child development retardation in malaria-endemic countries (Dombrowski *et al.,* 2018). Not surprisingly, increased susceptibility to malaria in pregnancy is well recognized, and has generally been assumed to be due to hormonal changes resulting in altered immunity (Tian *et al.,* 1998). Just as cerebral malaria lead to parasite sequestration in the brain, maternal malaria results in parasite sequestration in the placenta (Adam *et al.,* 2012). Sequestration is a phenomenon whereby malaria-infected erythrocytes accumulate in the microvasculature of placenta causing a serious deleterious to the fetus because it induces some specific changes in the placenta via two mechanisms (Okamgba *et al.,* 2018). First, it attracts some leucocytes in the organ, leading to pathological changes that alter the materno-fetal exchange system and results into intra-uterine growth retardation and low birth weight (Djontu *et al.,* 2016). Second, it stimulates placental cells to secrete substances that recruit inflammatory cells may also lead to placental damage and negatively impacting fetal growth (De Moraes *et al.,* 2013). Moreover, exposure of fetus to malaria antigens due to damage of the placental barrier may make the newborn more susceptible to immunologically mediated hemolysis or to dyserythropoiesis resulting in fetal anemia (Uneke, 2007). Interestingly, in two separate evolving body of studies conducted in southern Malawi, by Brabin *et al.,* 2004 and

***Corresponding author:* Emad Abd Elhalim Nour Elgalil,**
Faculty of Medical Laboratory Science, Omdurman Islamic University, Sudan.

Cessie *et al.,* 2002, who recorded prevalence of fetal anemia 23.4 per cent and 23.3 per cent correspondingly (Brabin *et al.,* 2013; Cessie *et al.,* 2002), whereas in Maputo Mozambique, up to 93 per cent of newborns were found to have fetal anemia (Bergstrom *et al.,* 1993). However, a statistically significant link was established between fetal anemia and maternal malaria infection (Uneke *et al.,* 2007). Additionally, placental infection is characterized by an inflammatory response wherein monocytes infiltrated in the intervillous space (IVS) along with cytokines production, such as the tumour necrosis factor (TNF) and gamma interferon (IFNγ) which are immune cellular response mediators that have adverse effects during gestation (Vásquez *et al.,* 2013). Because, Cytokines have been shown to play vital roles in normal pregnancy both in the maintenance of placental growth and in the modulation of maternal immune reactivity to prevent rejection of the conceptus (Raghupathy *et al.,* 2012). Accordingly, A growing body of study by Ann *et al*., 1999 noted that, increased TNF-α or IL-8 expression in the placenta was associated with intrauterine growth retardation but not with preterm delivery (Ann *et al.,* 1999). The results suggest that malaria infections induce a potentially harmful proinflammatory response in the placenta. Therefore, this study was carried out to better understand the association between malaria infection during pregnancy and neonatal cytokine levels; furthermore it can help in identifying maternal malaria consequences on birth weight.

PATIENTS AND METHODS

Study population: One hundred and eighty (180) pregnant women in their second and third trimester attending at Eldweim teaching hospital, Sudan, were recruited from April to October, 2016 as a part of a prospective cross sectional hospital based study investigating the adverse consequence of maternal malaria on neonates via studying neonatal cytokines. Of the 180 studied participants 150 had peripheral malaria smear positive were enrolled and 30 matched women with negative blood smear were ascertained as control. Women with eclampsia, diabetes mellitus, HIV infection, chronic disease such as liver, heart, kidney and lung were excluded from this study. Detailed history and physical examination was done to mother pre-entry to labour or section room for delivery. Following birth neonates were excluded if they were born before 32 weeks' gestation, diagnosed with serious illness at birth or had genetically determined disease or major malformation. At birth, information was gleaned from medical record, including the neonate's gender and birth weight.

Collection of cord blood samples: Blood samples were aseptically withdrawn initially from the maternal peripheral circulation for parasitological examination and secondly from the umbilical cord at delivery for cytokines measurement. A needle was inserted into the umbilical vein above the clamp and the cord blood samples were collected into plain vacationers. Then, the blood samples allowed to clot and centrifuged for 10 minutes at 3000 rpm and the serum was harvested and stored at -80 C^o till used for cytokines study. The mother ages, weight and gender of newborn were recorded.

Parasitological examination: Thick and thin blood films were prepared from maternal blood, then stained with Giemsa then examined by ×100 oil immersion, all the slides were blindly double-checked.

Cytokines enzyme-linked immunosorbent assay (ELISA): Quantitative (ELISA) was performed with commercially available assay to determine serum level of cytokines IL-10-, TNF- α, IL-8 using same set of reagents provided by (Biolegends ELISA MAX Deluxe). The (Biolegand ^{TM}) set of cytokines contains the components necessary to develop ELISA for natural or recombinant cytokines in serum, plasma and cell culture supernatants. The assay of all cytokines measured was similar in the procedure; the difference is confined to the concentration of standards.

Statistical analysis: The data were exported to a Microsoft Excel worksheet .Cytokines concentrations were analyzed in relation to the relevant clinical data by comparing means of the different levels among cases using unpaired t-test. Data were presented as mean ± standard error (SE) or median and range.

RESULTS

Cord blood Serum for cytokine measurements was available from 180 enrolled pregnant women. One hundred and fifty had *P. falciparum* malaria whereas the remaining was negative and used as controls. Table 1 shows the characteristics at enrollment of these pregnant women; of whom 41(23%) primigravidae and 139(77%) multigravidae were studied. Furthermore, a total 21(12%) were normally delivered while 159(88%) were delivered by C/S. Of the 150 pregnant women 43(29%) had malaria infection in second trimester while 107(71%) were infected in third trimester.

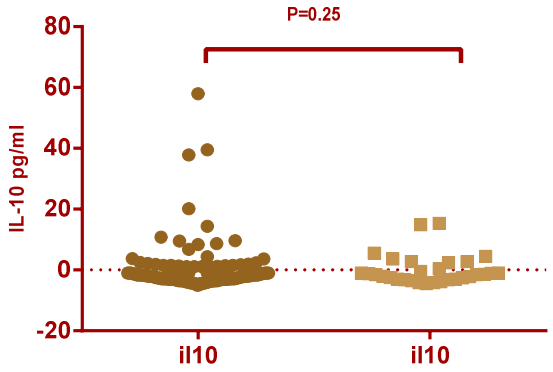
Table 1. Prevalence of malaria infection in relation to demographic/obstetric data among women with and without peripheral malaria infections

Characteristic	Malaria-infected(n=150)	Malaria-uninfected (n=30)	Total
Parity:			
Primipara	33(22%)	8(27%)	41
Multipara	117(78%)	22(73%)	139
Delivery:			
Normal labor	18 (12%)	3(10%)	21
C/S	132 (88%)	27(90%)	159
Baby gender:			
Male	71(47%)	15(50%)	86
Female	79(53%)	15(50%)	94
Malaria Infection:			
In 2nd trimester	43(29%)	0	43
In 3rd trimester	107(71%)	0	107

Table 2 shows a significant higher concentration of IL-8 and TNF- α in neonates born to women infected with malaria parasite (P value, 0.047, 0.016, respectively) compared to control group. Maternal malaria was associated with a reduction in birth weight (2668g vs. 3120g) compared to uninfected counterparts while women with and without peripheral malaria infection were similar in terms of age.

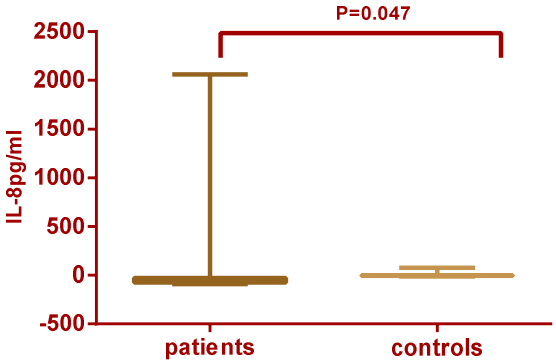
Table 2. Cytokine concentration in patients and control

Variables	Group	Mean	Std. deviatin	P-value
IL-10 (pg/ml)	Patients	5.758	7.478	0.25
	Controls	4.839	4.899	
IL-8 (pg/ml)	Patients	99.91	278.0	0.047
	Controls	14.55	16.36	
TNF-α (pg/ml)	Patients	23.26	18.59	0.016
	Controls	12.96	6.59	
Weight (g)	Patients	2592.04	512.42	0.507
	Controls	3101.17	557.7	
Age (years)	Patients	25.86	5.238	0.947
	Controls	25.11	5.315	



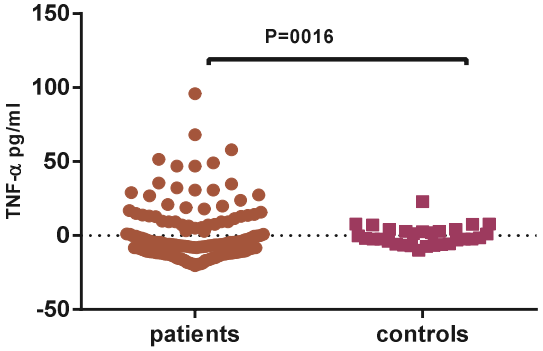
P-value of unpaired t-test (0.25)

Figure 1. Comparison between descriptive statistics of IL-10 concentration between patient and control group



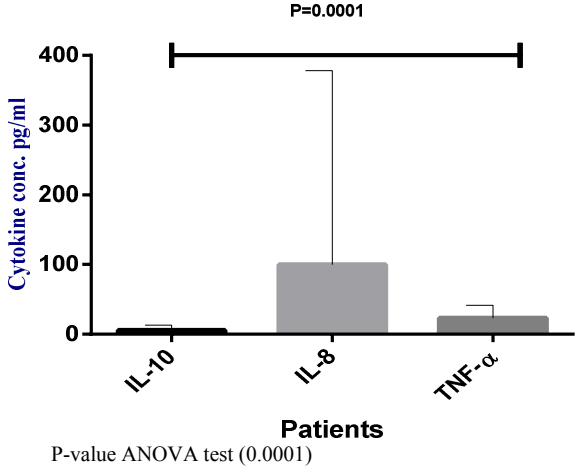
P-value of unpaired t-test (0.047)

Figure 2. Comparison between descriptive statistics of IL-8 concentration between patient and control group



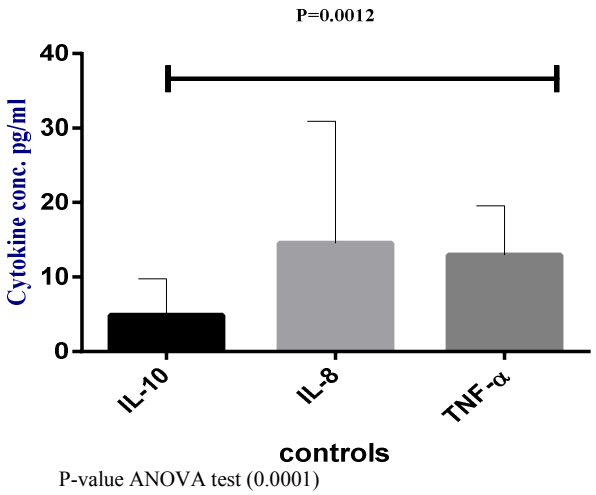
P-value of unpaired t-test (0.016)

Figure 3. Comparison between descriptive statistics of TNF- α concentration between patient and control group



P-value ANOVA test (0.0001)

Figure 2. Patients cytokines (IL-10, IL-8 & TNF- α)



P-value ANOVA test (0.0001)

Figure 5. Control cytokines (IL-10, IL-8 & TNF- α)

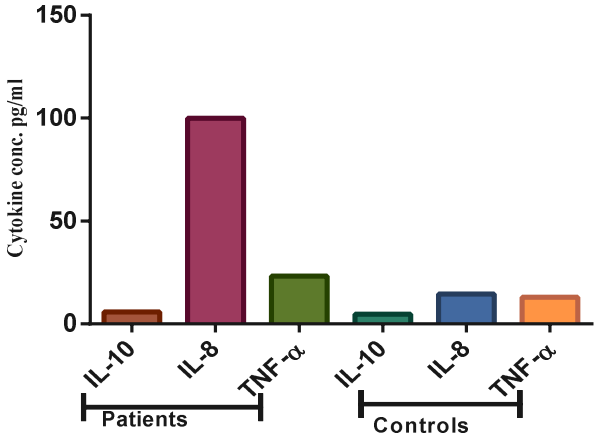


Figure 4. Patients and control cytokines (IL-10, IL-8 & TNF- α)

DISCUSSION

Malaria during pregnancy is associated with a proinflammatory immune response characterized by increased levels of cytokines and chemokines (Michal *et al.,* 2017). The impact of this infection on the plasma levels of some maternal and neonate cytokines known to regulate T cells differentiation and function and how this could affect birth weight remain undefined (Djontu *et al.,* 2016). Accordingly, this study aimed to investigate the effect of maternal malaria on the levels of IL-8, IL-10, and TNF-α in Sudanese neonatal cord blood in relation with pregnancy outcomes (birth weight). Our study showed a reduction in mean birth weight in newborns of malaria-positive mothers (2592.04 vs. 3101.17) when compared to those of uninfected mothers. Our findings were in agreement with results of many researchers who reported that the maternal malaria had been affected on birth outcomes, particularly low birth weight (Guyatt and Snow, 2004; Tiono *et al.,* 2009). Additionally, a lower birth weight and elevated in serum TNF-α were noted by Rogerson *et al.,* 2003 in the study done in Malawian, suggesting, a placental production of TNF-α may be implicated in impaired fetal growth in Malawian women (Rogerson *et al.,* 2013). As well as, the striking finding of this study was reported that the levels of IL-8 and TNF-α were increased in cord serum of neonate born from women infected with malaria parasite (p value= 0.047, 0.016 respectively) compared to those born from uninfected women. However, a relative increased expression of IL-10 was noted, in infants born to mothers with peripheral parasitaemia, albeit not statistically significant.

The result is similar to the findings of Moormann *et al.,* 1999 who, reported a positive correlation between pregnancy-associated malaria and increased expression of placental interleukin -8 (IL-8), and tumor necrosis factor (TNF) α in Malawi (Moormann *et al.,* 1999). The same is also true when compared with Flanagan et al, 2010 who, demonstrated that exposure to malarial Ag *in utero* results in increased Th1 pro-inflammatory responses (IFN-γ, TNF-α, IFN-γ:IL-10) in resolved Placental malaria infection[20]. These observations demonstrate a clear effect of exposure to malarial Ag in foetal life on the immune environment at birth, with a regulatory response dominating in the newborns with ongoing chronic Placental malaria (Flanagan *et al.,* 2010). On the other hand, the discordance with the results of Bayoumi et al, 2009, which showed that IL-10 was decreased in cord sera of, infected women (Bayoumi *et al.,* 2009). Accordingly, Fried *et al.,* 1988, we noted that maternal malaria decreases IL-10 concentrations and elicits IFN-g, IL-2, and TNF-a in the placenta, shifting the balance toward type 1 cytokines (Fried *et al.,* 1998). This is the first demonstration that these placental cytokine changes are associated with poor pregnancy outcomes in humans.

Conclusion

The data presented in this study demonstrated the relationships between the elevation of neonatal inflammatory cytokines, birth weight reduction and malaria infection during pregnancy. The findings might explain some of the adverse effects on the health of babies born to women infected with malaria parasite.

Notwithstanding, these findings, a deep understanding of the immune response to malaria remains elusive. However, further work is required to characterize the relationships between maternal malaria, neonatal cytokines, birth outcome and subsequent long-term offspring health.

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Consent and ethical approval: Permission and informed consent was taken in advance from mothers and their husbands for participating their babies in the study. The study received ethical approval from the Ethical committee at the Faculty of Medicine, University of Khartoum, Sudan.

Conflict of interest: We have no conflict of interest related to this work.

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