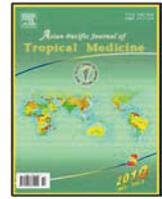


Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Medicine

journal homepage: www.elsevier.com/locate/apjtm



## Document heading

Pro-inflammatory cytokines profiles in Nigerian pregnant women infected with *Plasmodium falciparum* malariaNmorsi OPG<sup>1\*</sup>, Isaac C<sup>1</sup>, Ohaneme BA<sup>1</sup>, Obiazi HAK<sup>2</sup><sup>1</sup>Tropical Diseases Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria<sup>2</sup>Department of Medical Laboratory Sciences, Ambrose Alli University, Ekpoma, Nigeria

## ARTICLE INFO

## Article history:

Received 2 April 2010

Received in revised form 17 May 2010

Accepted 12 July 2010

Available online 20 September 2010

## Keywords:

Pro-inflammatory cytokines

Pregnant women

*Plasmodium falciparum*

Nigeria

## ABSTRACT

**Objective:** To investigate the pro-inflammatory cytokines profiles in Nigerian pregnant women infected with *Plasmodium falciparum* (*P. falciparum*) malaria. **Methods:** Peripheral, and placental blood samples were collected from 96 consenting volunteers comprising 76 *P. falciparum* infected pregnant women and 20 healthy uninfected pregnant women in Ekpoma, Nigeria, and subjected to ELISA for cytokines evaluation. **Results:** Increased serum concentrations of interferon-gamma (IFN- $\gamma$ ) was observed in infected pregnant women than their uninfected counterparts [(31.2 $\pm$ 20.9) pg/mL vs (1.8 $\pm$ 0.9) pg/mL] and these differences were statistically significant ( $\chi^2 = 26.18, P < 0.05$ ). The depressed levels of interleukin-12 (IL-12) seen in peripheral blood of the infected pregnant women than the uninfected women [(13.9 $\pm$ 3.6) pg/mL vs (28.4 $\pm$ 5.28) pg/mL] respectively was not statistically significant ( $\chi^2 = 4.96, P > 0.05$ ). The interleukin-6 (IL-6) was significantly elevated in infected pregnant women (81.0 $\pm$ 26.1 pg/mL) than in the uninfected pregnant women [(25.0 $\pm$ 5.0) pg/mL] ( $\chi^2 = 29.58, P < 0.05$ ). In all, mean cytokines concentration of IL-6, IL-12 and IFN- $\gamma$  in the placental blood from infected pregnant women were (53.5 $\pm$ 23.4) pg/mL, (8.7 $\pm$ 6.9) pg/mL and (16.4 $\pm$ 4.0) pg/mL, respectively. The multigravidae had a higher haemoglobin level of 10.2 g/dL and birth weight of 3 000 g than the primigravidae with lower haemoglobin level of 7.5 g/dL and birth weight of 2 430 g. **Conclusions:** The elevated IFN- $\gamma$  among the malarious pregnant women implicates it as the major cytokine mediator in the host responses to systematic *P. falciparum* malaria in our locality.

## 1. Introduction

*Plasmodium falciparum* (*P. falciparum*), the most lethal of the *Plasmodium* species infecting man, incapacitates and kills million each year. Pregnant women are highly vulnerable to this infection where they are at high risk of being infected with malaria owing to the ability of the parasite to adhere to trophoblastic villous epithelium and sequester in the placenta which could eventually lead to poor pregnancy outcomes<sup>[1]</sup>. It is estimated that over 200 000 infants die annually in sub-Saharan Africa as a result of their mothers becoming infected with malaria during pregnancy<sup>[2]</sup>. Malaria infections in the placenta and peripheral blood of pregnant women have been reported to influence immune responses<sup>[3]</sup>.

During successful pregnancies, fetal trophoblasts and maternal leukocytes secretes predominantly Th-2 type

cytokines to prevent initiation of inflammatory and cytolytic-type responses that might damage the integrity of the materno-fetal placental barrier<sup>[4]</sup>. In response to invading malaria parasites, however, it has been documented that Th-1 type cytokines are produced to reverse the Th-2 type bias within the placenta<sup>[5,6]</sup>. Inconsistent reports on the response of some pro-inflammatory interleukins (Th-1 type cytokines) to peripheral and placental malaria have been documented<sup>[7,8]</sup>.

In spite of the role pro-inflammatory cytokines play in cases of malaria infection in pregnant women<sup>[8]</sup>, especially in being crucial in the establishment and maintenance of pregnancy as well as protecting the foetus from infections<sup>[9,10]</sup>, information on pro-inflammatory cytokines status of malaria-infected pregnant women is lacking in our locality. This study therefore investigated the profile of some pro-inflammatory cytokines, namely IFN- $\gamma$ , IL-12 and IL-6, in both peripheral and placental blood of pregnant women infected with *P. falciparum* malaria. We reported the impact of malarial parasitaemia, cytokines concentrations on haemoglobin level and birth weight.

\*Corresponding author: Prof. Nmorsi OPG, Tropical Diseases Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria.  
E-mail: nmorsi@yahoo.com

## 2. Materials and methods

Our study area is Ekpoma, Edo State, Nigeria. Here, malaria is endemic and the transmission is perennial with highest transmission occurring during the raining season of April to October. The dry season is between November and March.

At the onset of our study, we obtained ethical permission from the State Ministry of Health, Benin City, Edo State, Nigeria and FaithDome Medical Center, Ekpoma, Nigeria where our volunteers were selected from. After introduction of the procedures and significance of the investigation, informed consent was obtained from the consenting pregnant women in the labour ward of Faith Dome Medical Center, Ekpoma, Nigeria. Medical histories were obtained from these volunteers to ascertain those with febrile (axillary temperature  $>37.5^{\circ}\text{C}$ ) and other clinical symptoms like headache and vomiting conditions as well as their parity status. We excluded volunteers with other overt infections such as measles, respiratory tract infections, salmonellosis and HIV by using the standard laboratory technique.

Blood samples were collected from 96 consenting volunteers comprising 76 *P. falciparum* infected pregnant women and 20 uninfected pregnant women which were used as the control subjects. Peripheral blood samples were obtained by venipuncture. The placental blood samples were obtained by compressing fresh tissue of the placenta in a tissue grinder after removal of the umbilical cord and fetal tissues immediately after delivery. The neonates were weighed immediately after delivery.

*P. falciparum* parasitaemia in the peripheral and placental blood smears using Giemsa stain were obtained from these pregnant women. The serum samples were immediately obtained from both the infected and uninfected pregnant women and these samples were subjected to cytokine determination by using commercial standard enzyme linked immunosorbent assay (ELISA) obtained from Abcam, UK according to the manufacturer's instruction.

We subjected the data obtained from this investigation to statistical analysis. Chi-square test was used by Microsoft Excel statistical package.

## 3. Results

A significant increase in IFN- $\gamma$  concentrations were observed in infected pregnant women [(31.2 $\pm$ 20.9) pg/mL] than uninfected pregnant women [(1.8 $\pm$ 0.9) pg/mL] parasite levels ( $\chi^2=26.18$ ,  $P<0.05$ ). Depressed level of IL-12 was seen in sera of pregnant women with *P. falciparum* [(13.9 $\pm$ 3.6) pg/mL] than their counterparts without malaria infection [(28.4 $\pm$ 5.3) pg/mL] and the difference was not statistically significant ( $\chi^2=4.96$ ,  $P>0.05$ ). IL-6 was significantly elevated among the non malarious pregnant women [(81.0 $\pm$ 26.1) pg/mL] than malarious pregnant women [(25.0 $\pm$ 5.0) pg/mL] ( $\chi^2=29.58$ ,  $P<0.05$ ). The IFN- $\gamma$  concentration in infected placental blood was (16.4 $\pm$ 4.0) pg/

mL, IL-12 was (8.7 $\pm$ 6.9) pg/mL, and IL-6 was (53.5 $\pm$ 23.4) pg/mL.

In the sera from peripheral blood, the mean concentration of IFN- $\gamma$  from the multigravidae [(20.9 $\pm$ 19.7) pg/mL] was statistically different from their primigravidae counterparts [(40.2 $\pm$ 10.7) pg/mL] ( $\chi^2=6.00$ ,  $P<0.05$ ). The mean concentration of IL-12 among the infected multigravidae pregnant [(17.5 $\pm$ 3.6) pg/mL] was not significantly different from the IL-12 obtained from infected primigravidae women [(8.5 $\pm$ 2.2) pg/mL] ( $\chi^2=3.12$ ,  $P>0.05$ ). The multigravidae had a mean sera IL-6 concentration of (52.5 $\pm$ 39.6) pg/mL while the primigravidae had mean sera concentration level of (109.5 $\pm$ 38.8) pg/mL and this difference was statistically different ( $\chi^2=20.4$ ,  $P>0.05$ ). The mean birth weight of neonates were (3 041.0 $\pm$ 174.4) g in multigravidae infected group, and (2 421.0 $\pm$ 131.1) g in primigravidae infected group. While haemoglobin level were (10.2 $\pm$ 2.3) g/dL in multigravidae infected group, and (7.5 $\pm$ 1.7) g/dL in primigravidae infected group.

## 4. Discussion

We observed an increased level of IFN- $\gamma$  in infected pregnant women as well as the primigravidae. We assert that increased level of systemic IFN- $\gamma$  in response to increased parasitaemia led to increased IFN- $\gamma$  cytokine levels in the placenta. This increased level has been suggested to be associated with predominance of CD8 $^+$  cells (IFN- $\gamma$ -producing T-cells) in response to an attempt to ensure parasite clearance, hence, exhibiting a level of protective immunity in pregnant women[11,12]. Also our high IFN- $\gamma$  concentration confirms the assertion of that IFN- $\gamma$  is potently active in gametocyte killing and their high IFN- $\gamma$  could be protective towards gametocytaemia[13]. However, our investigation contradicts the report of kabyemela *et al*[14] where the levels of IFN- $\gamma$  was unchanged between the malarious and nonmalarious patients.

It was reported that IL-12 concentrations in maternal peripherals blood and placentas with malaria infection were suppressed than in the infected pregnant women. This assertion is proved valid by an in vitro study in which lipopolysaccharide added to schizont extracts of *P. falciparum* resulted in the activation of antigen presenting cells with increased production of IFN- $\gamma$  and a corresponding decrease in IL-12[8]. *P. falciparum* infected erythrocytes have been implicated to exhibit lipopolysaccharide-like effects by mobilizing CD4 $^+$  and CD8 $^+$  T cells in eliciting IFN- $\gamma$  [7]. We thus hypothesize a synergy of protective immune mechanism of IFN- $\gamma$  and IL-12 cytokines in which increased incidence of malaria infection results in depressed levels of IL-12 in peripheral and placental systems. This observation had been documented earlier[15].

We reported an elevated level of IL-6 in the sera from the peripheral blood of uninfected pregnant women than their infected counterparts. This therefore implicates IL-6 in the

immunopathogenesis of malaria infection in the peripheral blood of pregnant women in our locality. It has been documented that low-density parasitaemia and its treatment induced a mild increase in IL-6 concentration with a sharp fall of haemoglobin content of reticulocyte, implying reduced-capacity of the haemoglobin to incorporate iron which could result in anaemia<sup>[16]</sup>. Malarial anaemia has been associated with poor pregnancy outcomes<sup>[17]</sup>.

We observed low birth weight among infected volunteers with relatively higher mean concentration of IFN- $\gamma$  and low concentration of IL-12 and IL-6. Generally, pro inflammatory cytokines have been implicated in parasitaemia control during the course of malaria<sup>[10]</sup>. This assertion is partly valid by the relatively higher IL-12 and IL-6 concentrations observed among the multigravidae which ascribe them as being involved in the immune responses in these categories of our volunteers with relatively low parasitemia. However, their depressed concentration among the primigravidae indicated their failure to mediate their immune system. We therefore deduce that the relatively low concentration of IL-6 and IL-12 implicates them in the immunopathogenesis of the maternal anaemia and low birth weight which we observed in our investigation. This investigation confirms the earlier report of Moormann et al<sup>[18]</sup> where they documented that malaria infections during pregnancy lead to the delivery of lower birth weight infants. Conclusively, the elevated IFN- $\gamma$  among the malarious pregnant women implicate this cytokine as the major mediator in the host responses to systematic *P. falciparum* malaria in our locality.

### Conflict of interest statement

We declare that we have no conflict of interest.

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