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Serum anti-inflammatory interleukin profiles in Nigerian pregnant women infected with *Plasmodium falciparum* malaria

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ABSTRACT

We investigated some anti-inflammatory interleukin profiles in peripheral and placental blood of 96 pregnant women infected with Plasmodium falciparum malaria in Ekpoma, Nigeria. In peripheral blood, interleukin-4 (IL-4) was elevated in mild (10.6 pg/ml) than in moderate (3.7 pg/ml) infection while in placental blood, elevated levels were observed in moderate (11.7 pg/ml) than mild (1.6pg/ml) infection. The depressed levels of interleukin-5 (IL-5) seen in mild than moderate infection in peripheral (331.0 pg/ml versus 419.6 pg/ml) and placental (314.2 pg/ml versus 571.2 pg/ml) blood was statistically significant (χ^2 =10.46 and χ^2 = 74.58; p <0 .05). Interleukin-10 (IL-10) was elevated in mild infection (225 pg/ml) than in moderate infection (56 pg/ml) in peripheral blood and this difference was significant (χ^2 = 101.64; p <0.05) while in placental blood, the elevated levels observed in moderate infection (226 pg/ml) was statistically higher than mild (158.3 pg/ml) infection (χ^2 =11.88; p < 0.05). The volunteers with moderate infection had low haemoglobin level of 7.5g/dl and a mean low birth weight of 2.43kg.

Key words: Anti-inflammatory Interleukin (IL)-4, IL-5, IL-10, Pregnant women, *Plasmodium falciparum*, Nigeria.

INTRODUCTION

Pregnancies in women are characterized by a transient depression of cell-mediated immunity which has been linked to fetal allograft retention and interference of the resistance of mothers to various infectious diseases (1). Malaria parasite sequestration in the intervillous space of the

placenta due to lack of acquired immunity of *Plasmodium falciparum* clones to preferentially bind to the placenta vasculature (2,3,4) place pregnant women at increased risk of malaria infection (5,6) with debilitating outcomes (7). The mechanisms responsible for the increase in malaria susceptibility during pregnancy are associated with cytokines responses (8).

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 (9,10). 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 (11, 12). This Th-1 type shift in malaria infected pregnant women is reflected in depressed levels of Th-2 type cytokines in the placenta (13,14). A Th-2 type cytokine dorminance in the placenta during malaria infection has also been reported (15,16). These controversial reports of Th-2 type cytokine responses to malaria infection in pregnant women have however been associated with poor pregnancy outcomes (15, 13)

In view of the contradictory report on the Th-2 interleukins (anti-inflammatory interleukins) responses to placenta malaria and their role in pregnant women infected with *P. falciparum* malaria, we investigated anti-inflammatory interleukins status namely IL -4, IL – 5, IL - 10 in Nigerian pregnant women with *P. falciparum* malaria; information lacking in our locality. We also established the impact of malarial parasitaemia and cytokines concentrations on haemoglobin level and birth weight.

MATERIALS AND METHODS

Our study area is Ekpoma, Edo State, Nigeria. It lies at latitude 6˚N and longitude 6˚E. Ekpoma is an urban town and it is located in the rainforest zone of southern Nigeria. Here, malaria is endemic and the transmission is perennial with highest transmission occurring during the raining season months of April to October. The dry months are between November and March.

This investigation commenced by obtaining ethical permission from the State Ministry of Health, Benin City, Edo State, Nigeria and FaithDome Medical Center where our volunteers were drawn from. After proper education of the procedures and significance of the investigation, informed consent was obtained from the consenting pregnant women.

Blood samples were collected from 96 *P. falciparum* infected consenting volunteers. At delivery, we also collected their placental blood samples .*P. falciparum* parasitaemia in the peripheral and placental blood smears using Giemsa stain were obtained from these pregnant women. The malaria parasitaemia were grouped as uncomplicated (<1,000 parasite/μL) and moderate (>1,000 parasite/μL). These women were febrile (axillary temperature >37.5˚C) and had other clinical symptoms like headache and vomiting. We excluded volunteers with other overt infections such as measles, respiratory tract infections, salmonellosis and HIV using standard laboratory technique. The blood samples were processed and the serum was subjected to cytokine determination 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, namely, chi-square test using Microsoft Excel statistical package.

RESULTS

Table 1 shows anti-inflammatory interleukins in maternal peripheral blood infected with *P. falciparum* malaria. An increased concentration of IL-4 was observed in moderate (10.6 pg/ml) than mild infection (3.7 pg/ml) parasite level (χ^2 =3.40, p<0.05). A depressed level of IL-5 was seen in mild infection (331.0 pg/ml) than moderate infection (419.6 pg/ml) infection and the difference was statistically different (χ^2= 10.46, p <0.05). IL-10 was elevated in mild (225 pg/ml) than in moderate (56 pg/ml) malaria levels (χ^2 = 101.64, p <0.05).

Table 2 shows anti-inflammatory interleukins profiles in placenta blood infected with *P. falciparum* malaria. Increased concentration of IL-4 was observed in moderate infection (11.7pg/ml) than in mild infection (1.6pg/ml) (χ^2=, 10.0, p< 0.05). A depressed level of IL-5 was seen in mild infection (314.2pg/ml) than in moderate infection (571.2 pg/ml) infection and the difference statistically significant (χ^2 = 74.88, p<0.05). In IL-10, we observed in a higher concentration in moderate infection(226 pg/ml) than mild infection (158.3 pg/ml) infection and this difference was statistically significant (χ^2 =11.88, p<0.05).

The mean birth weight and haemoglobin levels with the intensities (mild and moderate) of infection are presented in table 3. The mean birth weight of those with moderate infection was 2.43kg (2.2kg-3.00kg) while those with mild infection had a normal mean birth weight of 3.00 kg (2.6kg-3.9kg). The mean haemoglobin of mild and moderate parasite levels of infection obtained from peripheral blood of the mothers was 10.2g/dl and 7.5g/dl, respectively.

Table 1: Anti-inflammatory interleukins profiles in maternal peripheral blood infected with *P. falciparum* malaria

Cytokines (pg/ml)	Mild <1000 parasites/µL	Moderate >1000 parasite/µL	Mean	χ^2
IL-4	10.6	3.7	7.2	3.40
IL-5	331.0	419.6	375.3	10.46
IL-10	225	56	140.5	101.64

Table 2: Anti-inflammatory interleukins profiles in placental blood infected with *P. falciparum* malaria

Cytokines (pg/ml)	Mild <1000 parasites/µL	Moderate >1000 parasite/µL	Mean	χ^2
IL-4	2.3	4.7	6.7	10.70
IL-5	314.2	571.2	442.7	74.58
IL-10	158.3	226	192.2	11.88

Table 3: The intensities of infection with the categories of birth weight and haemoglobin

Intensities of infection	Category of birth weight Range (mean)kg	Haemoglobin level (g/dl)
Mild	2.6-3.9 (2.43)	10.2
Moderate	2.2-3.0 (3.00)	7.5

DISCUSSION

Our findings revealed depressed levels of IL-4 in moderate than mild infection in peripheral blood. In contrast, elevated levels of IL-4 were observed in moderate than mild infection in placental blood. This pattern of IL-4 interleukin (Th-2 type interleukin) in response to *P. falciparum* malaria infection in peripheral blood supports the assertion of a shift from a Th-2 type interleukin to a Th1 type interleukin as expressed by the depressed levels of Th-2 interleukin with increased malaria incidence (9,4,13). The result of elevated levels of IL-4 with increased parasitaemia in the placenta corroborates the finding of (17). Report has it that malaria parasite immunization induced the modulation of the development and tissue distribution of memory cell which is critical in ensuring protective immunity owing to an interaction of IL-4/IL-4 receptor on CD8+ T cells (18). Also increased levels of IL-4 were recorded in malaria-infected individuals who received anti-malaria treatment (17). We therefore assert that the increased levels of IL-4 with parasite density in this investigation implicate this cytokine in exhibiting a protective immunity in the placenta of pregnant women and consequently their foetus.

We observed an increased level of IL-5 in moderate than mild malarial levels for both peripheral and placental malaria. This report is consistent with the investigation of (19) where IL-5 concentrations increased with the severity of infection. Malaria parasites contain apical membrane antigen-1 which play a key role in erythrocytic invasion and are also expressed in sporozoites and late stage liver schizonts where it may provide a target of protective cell-mediated immunity (20). *In vitro* study of vaccine apical membrane antigen-1 stimulation of peripheral blood mononuclear cells triggered IL-5 spot forming cells and as a consequence increased production of IL-5 (20). Our result of elevated level of IL-5 in the face of increased malaria incidence thus suggests protective immunity of IL-5 in pregnant women infected with *P. falciparum* malaria.

During pregnancy, the overall immune response of the mother is Th-2 biased to prevent fetal allograft rejection (21). However, in the event of invading malaria parasite, a shift from Th-2 to Th-1 type interleukins have been documented (11,13). Our result of suppressed level of IL-10 with parasite density in peripheral blood aligns with these assertions. We therefore propose a shift from Th-2 to Th-1 type interleukins expressed in depressed level of IL-10 with increased malaria parasitaemia in pregnant women. Furthermore, the result of increased concentration of IL-10 in moderate than mild infection in placenta blood supports the report of (15) and implicates this interleukin in the immunopathology of placental malaria. Maternal monocytes and macrophages are the most likely source of placental IL-10 in malaria infected women (22) of which its massive infiltration characterize placental malaria (23, 24). Elevated levels of IL-10 can increase the acquisition and retention of iron by monocytes and macrophages and increase ferritin synthesis thereby reducing the amount of iron in the plasma and thus contributing to anaemia (25, 26); a known risk factor that could lead to poor pregnancy outcome like low birth weight as observed in this study (16).

In conclusion, IL-5 interleukin can be used as a marker of malaria infection in pregnant women since it was elevated with parasite density. We also deduce that elevated IL-4 in placental blood may be involved in protective immunity against placental malaria and thereby potentiating immunity of their foetus against falciparum malaria . In addition, decreased IL-10 in placenta

blood in moderate Plasmodial infection may implicate this interleukin in the immunopathology of placental malaria associated with poor pregnancy outcome like low birth weight.

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