Cytokine Profiles in Peripheral, Placental and Cord Blood in an Area of Unstable Malaria Transmission in Eastern Sudan

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Summary

Background: Understanding the cytokine interactions that underlie both control and disease should be helpful when investigating the pathogenesis of malaria during pregnancy. Few data exists concerning pathogenesis of malaria during pregnancy in areas of unstable malaria transmission. Objectives: The study was conducted in New Halfa hospital, eastern Sudan, which is characterized by unstable malaria transmission to investigate the cytokine profiles in peripheral, placental and cord blood in parturient women. Methods: Enzyme-linked immunosorbent assay was used to measure the concentrations of three cytokines, interferon-γ (IFN-γ), interleukin-4 (IL-4) and IL-10, in sera from peripheral, placental and cord blood of 87 Sudanese women. Results: The concentrations of these cytokines were significantly higher in peripheral, placental sera from uninfected women than in sera from infected women. IFN-γ concentrations were significantly lower in the cord sera from uninfected women in comparison to the infected ones. The levels of these cytokines were not significantly different between the primiparae and multipare. Cord sera in all groups showed lower levels of these cytokines. Strong positive correlations were observed between peripheral and placental cytokines. Conclusion: The immune responses that occur in placental, peripheral and cord blood were influenced by the malaria infections, irrespective of the parity. The immune response during Plasmodium falciparum infection is not different in the peripheral and placental compartments, further studies are required.

Key words: malaria, pregnancy, cytokines, cord, placenta, Sudan.

Introduction

It has been estimated that 90% of the global malaria burden occurs in sub-Saharan Africa, where during pregnancy 40% of the women are exposed to malaria infections [1]. Malaria during pregnancy poses a substantial risk to the mother, her fetus and the neonate [2]. Malaria during pregnancy is a major health problem in Sudan, where it has been reported to be associated with maternal anaemia, low birth weight infants and as the main cause of maternal mortality [3–6].

During pregnancy, the immune system may be biased towards Type 2 humoral defense mechanisms rather than towards Type 1 cellular responses, this may be fundamental for fetal well-being [7]. The systemic suppression of pro-inflammatory responses from T helper 1 (Th1) cells, i.e. increased circulating levels of interferon-γ (IFN-γ) and tumor necrosis factor α, along with increased local expression of anti-inflammatory cytokines such as interleukin-4 (IL-4), IL-6 and IL-10, has been reported [8].

Placental malaria is associated with cell mediated inflammatory responses and alters the cytokine balance in favor of Th1 types (i.e. pro-inflammatory) [9, 10]. The placental production of chemokines may be an important trigger for monocytes accumulation in the placenta [11]. Understanding the cytokine interactions that underlie both control and disease
should be helpful when investigating the pathogenesis of malaria during pregnancy.

The current study was conducted in an area that is characterized by unstable malaria transmission in eastern Sudan [12], where malaria is substantial burden affecting pregnant women irrespective to their age or parity [3]. The study aimed to investigate the cytokine profiles of IFN-γ, IL-4 and IL-10 in peripheral, placental and cord blood from parturient women so as to add to on-going data on the pathogenesis of malaria during pregnancy in the area [13, 14].

Materials and Methods

The study was conducted between October 2006 and March 2007 at the labour ward of New Halfa teaching hospital, eastern Sudan. The detail of the study design has been mentioned elsewhere [14]. In summary, after taking an informed consent, women with a singleton baby were approached to participate in the study. Those with antepartum hemorrhage, hypertensive disorder of pregnancy (diastolic blood pressure > 90 mmHg) and diabetes mellitus were excluded.

Structured questionnaires were administered to these women to collect information about socio-demographic characteristics and parity.

Maternal, placental and cord blood films were prepared, the slides were Giemsa stained and the number of asexual *Plasmodium falciparum* parasites per 200 white blood cells were counted and double checked blindly by an expert microscopist. Maternal hemoglobin concentrations were estimated by Hemocue haemoglobinometer (HemoCue AB, Angelhom, Sweden).

Immediately after delivery, 5 ml of maternal, placental and cord blood were collected using the biopsy-pool method (for the placental) and direct collection for the peripheral and cord blood. Briefly, a block of tissue (5 cm × 5 cm × 5 cm) was excised from the basal side of the placenta, resulting in the formation of a large pool of intervillous blood at the excision site. Blood was quickly withdrawn in plain tube and centrifuged and kept at −20°C until processed in the laboratory for cytokines.

Full thickness placental blocks of around 2–3 cm were taken from the placentae, kept in neutral buffer formalin for histopathology examinations. The presence of placental malaria infection was based on the pathological classification of Bulmer et al. [15]; uninfected (no parasites or pigment), acute (parasites in intervillous spaces), chronic (parasites in maternal erythrocytes and pigment in fibrin or cells within fibrin and/or chorionic villous syncytiotrophoblast or stroma) and past (no parasites and pigment confined to fibrin or cells within fibrin).

Sera samples obtained at enrollment were analyzed by standard sandwich enzyme-linked immunosorbent assay (ELISA) for IFN-γ, IL-4 and L-10 using pairs of cytokine-specific, monoclonal antibodies according to the manufacturer’s instructions (eBioscience Inc., 6042 Cornerstone Court West, San Diego, CA 92121, USA). Each plate included standard of recombinant human cytokine run in parallel with samples. All samples were run in duplicates and the mean value was used in all analyses.

Data were entered in computer using SPSS for windows and double-checked before analysis. Data (cytokines) were not normally distributed; Mann-Whitney test U (2-group comparisons) or Kruskal-Wallis (more than 2-group comparisons) tests were used to determine the significance of differences between the variables. Post hoc test for multiple means of comparisons was used for multivariate analysis. Correlations between continuous variables were assessed by the Spearman rank test. *p* < 0.05 was regarded as significant.

The study received ethical clearance from the Research Board at the Faculty of Medicine, University of Khartoum.

Results

The triplet samples; maternal peripheral, placenta and cord sera were analyzed in 87 parturient women. While 53 women had past placental malaria infections, 34 showed no infections, according to placental histopathological examinations. Among them 33 and 54 were primiparae and multiparae, respectively.

Table 1 shows the concentrations of IFN-γ, IL-4 and IL10 in the peripheral, placental and cord sera from all the recruited women. The concentrations of these cytokines were significantly higher in peripheral, placental sera from uninfected women than in sera from infected women. Cord concentrations of IL-4 and IL-10 were slightly—IFN-γ concentrations were significantly lower—lower in the cord sera from uninfected women in comparison to the infected ones.

Cord sera contained significantly less concentrations of these cytokines than the peripheral and placental sera. The difference was not significant when the peripheral and placental sera concentrations were compared.

When comparisons were made according to the parity, similar pattern was observed. The levels of these cytokines were not different when the primiparae were compared to multiparae (Table 2). The same findings were observed (no difference between the cytokines levels between primiparae and multiparae) when data of the infected women were analyzed separately (data not shown).

Strong positive correlations were observed between peripheral and placental samples (*r* = 0.89, *p* < 0.001), between peripheral and cord samples (*r* = 0.82, *p* < 0.001) and between placental and cord samples (*r* = 0.66, *p* < 0.001) for IFN-γ. Likewise, strong positive correlations were observed between peripheral and placental samples for IL-4 (*r* = 0.82, *p* < 0.001) and for IL-10 (*r* = 0.15, *p* < 0.001).
There was no correlation between peripheral and cord samples \((r = 0.2, p = 0.06)\) or placental and cord samples \((r = 0.13, p = 0.2)\) for IL-4. This was true with regard to peripheral and cord samples \((r = 0.12, p = 0.02)\) or placental and the cord samples \((r = 0.1, p = 0.3)\) for IL-10. The same findings were observed when the data of infected women were analyzed separately (data not shown).

### Discussion

In the current study, IFN-\(\gamma\), IL-4 and IL-10 concentrations (mainly peripheral and placental) were higher in uninfected women than in the infected women. There were no differences in the levels of these cytokines in primiparae as compared with multiparae. Furthermore, cord sera had lower levels of these cytokines in comparison to sera from placenta and maternal peripheral blood. The high levels of these cytokines in sera of uninfected women might suggest that these cytokines are involved in the control of parasitemia in peripheral blood and in the placenta. The low concentration of these cytokines found in \(P. falciparum\)-infected women further supports this idea. The anti-inflammatory cytokine environment is thought to be maintained, in part, by the high progesterone levels in pregnancy, which induces Th0 to Th2 conversion [16].

IFN-\(\gamma\) production by intervillous blood cells was associated with protection from malaria, and impaired IFN-\(\gamma\) production was mooted as a cause for the increased susceptibility to placental malaria [17]. However, previously malaria-infected placentas had been reported to have a higher IFN-\(\gamma\) levels than did uninfected placentas [18]. On the contrary,

**TABLE 1**

*The median (interquartile range) of sera cytokine levels in infected \((n = 53)\) and uninfected \((n = 34)\) parturient Sudanese women*

<table>
<thead>
<tr>
<th>Cytokines, (pg ml(^{-1}))</th>
<th>Mother</th>
<th>Placenta</th>
<th>Cord</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-(\gamma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (\text{Infected})</td>
<td>215.4 (112.3–375.8)</td>
<td>226.8 (135.2–387.2)</td>
<td>123.8 (80.8–224.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>(\text{Non-infected})</td>
<td>358.6 (201.1–662.4)</td>
<td>278.4 (203.9–470.2)</td>
<td>89.4 (32.1–169.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (\text{Infected})</td>
<td>25.0 (15.6–41.0)</td>
<td>26.3 (15.6–39.6)</td>
<td>5.0 (1.0–13.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Non-infected})</td>
<td>22.3 (10.3–30.3)</td>
<td>21.0 (11.6–34.3)</td>
<td>5.7 (2.6–13.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (\text{Infected})</td>
<td>121.4 (82.3–254.3)</td>
<td>148.7 (86.2–276.3)</td>
<td>54.9 (34.4–101.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Non-infected})</td>
<td>162.4 (97.0–469.1)</td>
<td>203.5 (110.6–367.6)</td>
<td>54.2 (27.6–86.2)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**TABLE 2**

*The median (interquartile range) of sera cytokine levels in primiparae \((n = 33)\) and multiparae \((n = 54)\) parturient Sudanese women*

<table>
<thead>
<tr>
<th>Cytokines, (pg ml(^{-1}))</th>
<th>Mother</th>
<th>Placenta</th>
<th>Cord</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-(\gamma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparae (\text{Infected})</td>
<td>230.3 (175.3–438.7)</td>
<td>272.7 (140.9–387.2)</td>
<td>152.4 (89.4–218.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Multiparae (\text{Infected})</td>
<td>278.4 (146.7–467.4)</td>
<td>249.8 (181.0–415.8)</td>
<td>100.9 (49.3–165.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>(p)</td>
<td>0.6</td>
<td>0.2</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparae (\text{Infected})</td>
<td>23.0 (16.3–35.6)</td>
<td>23.6 (12.3–37.6)</td>
<td>5.0 (1.0–9.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiparae (\text{Infected})</td>
<td>26.3 (13.6–48.0)</td>
<td>27.6 (17.3–43.0)</td>
<td>6.3 (1.0–17.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(p)</td>
<td>0.6</td>
<td>0.2</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparae (\text{Infected})</td>
<td>125.3 (84.2–271.1)</td>
<td>172.2 (86.2–334.4)</td>
<td>54.9 (45.2–113.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiparae (\text{Infected})</td>
<td>119.4 (82.3–240.7)</td>
<td>137.0 (85.2–234.7)</td>
<td>54.9 (27.6–96.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>(p)</td>
<td>0.6</td>
<td>0.6</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>
Moorman et al. [19] found no detectable IFN-γ in placental biopsy specimens from primigravid specimens from Malawian women. Differences in IFN-γ responses to malaria infection during pregnancy were reported between different African settings. In neighboring Kenya, IFN-γ levels were found in about 40% of placental plasma samples and were associated with malaria infection and poor fetal outcome [20].

The enhanced IL-4 and IL-10 expression, perhaps in concert with other anti-inflammatory immunomodulatory cytokines, curtails the potentially hazardous effects of Th1-related cytokine production on systemic immunity during pregnancy, thus ensuring the retention of the fetal allograft [21]. Perhaps, IL-4 blocks NK activity of the decidua which may have potentially deleterious effect on the fetus like thrombosis, inflammation and miscarriage [22].

Our finding of higher peripheral blood IL-10 levels in P. falciparum-infected mothers is in concert to the previous recent observations [23]. IL-10 characterizes normal human pregnancy and is thought to prevent inflammatory responses that might damage the integrity of the materno-fetal placental barrier [20, 24]. During placental malaria, despite the placental shift toward Th1-type cytokines, IL-10 concentrations are elevated compared with healthy placentas [25]. IL-10 has a major role in controlling inflammatory responses and preventing materno-fetal placental barrier damages [9, 19, 25].

Likewise, high levels of IL-12 have been reported in uninfected women [26]. However, these studies should be compared cautiously, because of the difference in the endemicity. Furthermore, in the later study malaria infections were diagnosed by microscopy (current infections), while in our study the placental histopathology was the tool used to diagnose malaria placental infections which were past infection.

The influence of endemicity on the results of our study is obvious as there were no significant differences in the levels of these cytokines between the primiparae and multiparae. Thus, in this area of eastern Sudan, the pathogenesis of malaria is the same irrespective of the parity. Previously we have observed that, pregnant women of eastern Sudan are susceptible to peripheral malaria as well as placental malaria irrespective to their age and parity [3, 14].

Yet, gravidity-based differences in cytokine responses to malaria have been proposed to explain the difference in susceptibility to malaria between primigravid and multigravid women [17].

In contrast to the previous findings [26] our study showed that, cord sera had the lower cytokines levels. This might support the previous assumption, as we investigated these cytokines in past malarial infections mainly. Yet, we investigated IFN-γ, IL-4 and IL-10, while the former study investigated IL-12 and IL-15 and the difference in their passage through placental barrier and neonatal antigenicity may be varied in various cytokines.

Unlike Bouyou-Akotet et al. [26] reports, we found strong positive correlations between the peripheral and placental sera concentrations of cytokines, suggesting that anti-malaria immune responses occurring in the placenta are influenced by the cytokines from mother’s blood, and that the immune response during P. falciparum infection is not different in the peripheral and placental compartments.

**Conclusions**

The patterns of the immune responses that occur in placental, peripheral and cord blood were influenced by the malaria infections, irrespective of the parity. IFN-γ, IL-4 and IL-10 are key mediators in the host response to *P. falciparum* infection during pregnancy in women living in unstable malaria transmission. Immune response during *P. falciparum* infection is not different in the peripheral and placental compartments, further studies are required.

**References**