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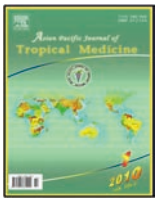

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# Pro-and anti-inflammatory cytokines profiles among Nigerian children infected with *Plasmodium falciparum* malaria

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ABSTRACT

**Objective**: To examine array of some pro- and anti-inflammatory cytokines, namely, interleukin-4 (IL-4), interleukin-10 (IL-10), interferon-γ (IFN-γ), interleukin-5(IL-5), interleukin-6 (IL-6), interleukin-12 (IL-12) and tumor necrosis factor-α (TNF-α) concentrations in some Nigerians with falciparum malaria. **Methods**: Sera were obtained from the blood samples of 96 Nigerian children with *Plasmodium falciparum* infection. The sera were subjected to cytokine evaluation using commercial standard enzyme linked immunosorbent assay kits (Abcam, UK). **Results**: Mean pro-inflammatory cytokines in serum of children with uncomplicated and complicated malaria were IL-5 482.2 pg/mL versus 526.7 pg/mL, IL-6 98.8 pg/mL versus 82.6 pg/mL, IL-12 24.1 pg/mL versus 15.9 pg/mL, TNF-α 107 pg/mL versus 511.7 pg/mL and IFN-γ 2.1 pg/mL versus 2.5 pg/mL. The anti-inflammatory cytokines status of IL-4 were 4.7 pg/mL versus 20.3 pg/mL, and IL-10 were 216 pg/mL versus 143.8 pg/mL in uncomplicated versus complicated/severe malaria cases. Participants with uncomplicated malaria had mean parasitaemia level of 3 158.9 parasites/μL while mean parasitaemia level for participants with complicated malaria was 12 550.5 parasite/μL and this difference was statistically significant ($\chi^2$ =5 614.6, $P$<0.05). The difference between mean haemoglobin level for uncomplicated malaria (9.6 g/dL) and severe malaria (3.9 g/dL) was statistically significant ($\chi^2$ = 2.3, $P$<0.05). The relationship between serum level of IL-6, IL-12, IFN-γ, IL-10 and IL-4 and ages showed positive correlation at $r$=0.92, 0.99, 0.86, 0.95 and 0.85, respectively; while IL-5 and TNF-α had negative correlation at $r$=-0.99 and -0.99, respectively. **Conclusion**: IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ are involved in the immunopathology and immunoregulation of uncomplicated and complicated malaria infections. IL-6, IL-12, IFN-γ and IL-10 depressed in complicated/severe malaria may not provide any protective immunity and may be indicators of poor prognosis in *Plasmodium falciparum* infected Nigerian children.

## 1. Introduction

*Plasmodium falciparum*, the most lethal of the malarial species infecting humans, incapacitates and kills millions each year[1]. Of these, children especially those below five years are more vulnerable and are at increased risk of acquiring this infection. Malaria like other human diseases is driven by the production of the pro-inflammatory cytokines. These proinflammatory stimulates the production of additional cytokines which in totality generate tissue pathology[2]. The anti-inflammatory cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10) mainly act by inhibition of the production of pro-inflammatory cytokines or counteracting many biological effects of the pro-inflammatory mediators. It has been documented that systemic malarial infection elicits an array of immune responses[3]. This manifests production of several cytokines such as IL-10, interleukin-12 (IL-12), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α)[4-7].

Cytokines have been implicated in the immunopathology of malarial infection which predicts the prognosis and outcome of malaria infection. For instance during acute malaria attacks large amounts of pro-inflammatory cytokines such as TNF-α and interferon-γ (IFN-γ) are released into circulation[8,9]. Also a relative deficiency of anti-inflammatory IL-10 has been linked to a poor outcome in adult Vietnamese cerebral malaria patients and increased risk of malaria induced severe anaemia in Africa[10,11].

In our locality, information in this regard appears lacking. Therefore, in this study we examine array of some pro-

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and anti-inflammatory cytokines, namely, IL-4, IL-10, IFN-γ, IL-5, IL-6, IL-12 and TNF-α concentrations in some Nigerians with falciparum malaria. We showed the relationship between age, malarial parasitaemia, haemoglobin concentration and the cytokines concentration.

## 2. Materials and methods

This investigation was carried out in Ekpoma, Edo State, Nigeria.

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 where our patients and their blood samples were obtained. After proper education of the procedures and significance of the investigation and informed consent obtained from parents/caregivers the informed consenting children were selected.

Blood samples were collected from 96 volunteers including 47 cases without *Plasmodium falciparum* infection and 49 cases with confirmed *Plasmodium falciparum* infection between 1 and 15 years old. The determination of positivity of these children for malaria was based on *Plasmodium falciparum* parasitaemia in their blood smears using Giemsa stain. The malaria parasitaemia was grouped as uncomplicated (<10 000 parasite/μL) and complicated/severe (>10 000 parasite/μL). These children were febrile (axillary temperature >37.5 ℃) and had other clinical symptoms like headache, vomiting, diarrhea, respiratory distress, prostration, jaundice associated with complicated malaria. We excluded volunteers with other overt infections such as measles, respiratory tract infections and HIV using standard laboratory technique. The blood samples were processed and the serum was subjected to cytokine evaluation using commercial standard enzyme linked immunosorbent assay (ELISA) obtained from Abcam, UK according to the manufacturer's instruction.

The data obtained from this study were subjected to statistical analysis, namely, chi-square test and correlation using Microsoft Excel statistical package.

## 3. Results

Pro-inflammatory cytokines of the children with uncomplicated ($n$=47) and complicated malaria ($n$=49) were IL-5 482.2 pg/mL versus 526.7 pg/mL, IL-6 98.8 pg/mL versus 82.6 pg/mL, IL-12 24.1 pg/mL versus 15.9 pg/mL, TNF-α 107 pg/mL versus 511.7 pg/mL and IFN-γ 2.1 pg/mL versus 2.5 pg/mL. Of these cytokines, difference in the TNF-α status of the uncomplicated and complicated cases were statistically significant ($\chi^2$ = 264.6, $P$<0.05). Participants with uncomplicated malaria had mean parasitaemia level of 3 158.8 parasites/μL while mean parasitaemia level for patients with complicated malaria was 12 550.5 parasite/μL and this difference was statistically significant ($\chi^2$ =5 614.6, $P$<0.05). The difference between mean haemoglobin level for uncomplicated malaria (9.6 g/dL) and severe malaria (3.9 g/dL) was statistically significant at ($\chi^2$ = 2.3, $P$<0.05).

Anti-inflammatory cytokines of IL-4 were 4.7 pg/mL versus 20.3 pg/mL, and IL-10 were 216 pg/mL versus 143.8 pg/mL in uncomplicated cases ($n$=47) versus complicated/severe malaria cases ($n$=49) and these differences were statistically significant ($\chi^2$ = 9.74, 14.52, respectively).

The profile of pro-and anti-inflammatory cytokines with age is reported in Table 1. The highest serum IL-5 (937.5 pg/mL) and TNF-α (520.7 pg/mL) were obtained from children between 1-5 years while the children above 11 years had the highest serum concentration of IL-6 (162.0 pg/mL), IL-12 (33.0 pg/mL), IFN-γ (4.6 pg/mL), IL-4 (15.6 pg/mL) and IL-10 (450.0 pg/mL). The Serum IL-6, IL-12, IFN-γ, IL-10 and IL-4 with age showed positive correlation at $r$=0.92, 0.99, 0.86, 0.95 and 0.85, respectively while IL-5 and TNF-α had negative correlation both at $r$=-0.99.

**Table 1**
The pro- and anti-inflammatory cytokine profiles of Nigerian children according to their age groups(pg/mL).

| Age (years) | IL-5 | IL-6 | IL-12 | TNF-α | IFN-γ | IL-4 | IL-10 |
|---|---|---|---|---|---|---|---|
| 1-5 ($n$=40) | 937.5 | 59.0 | 15.9 | 520.7 | 2.6 | 3.4 | 26.0 |
| 6-10 ($n$=31) | 510.0 | 81.0 | 26.4 | 271.0 | 2.8 | 4.7 | 146.0 |
| >11 ($n$=25) | 310.0 | 162.0 | 33.0 | 90.1 | 4.6 | 15.6 | 450.0 |
| Correlation | -0.99 | 0.92 | 0.99 | -0.99 | 0.86 | 0.85 | 0.95 |

## 4. Discussion

It demonstrated an increased pro-inflammatory cytokines of TNF-α, IL-5 and IFN-γ in patients with severe malaria compared to patients suffering from uncomplicated malaria. Also, patients with severe malaria had depressed levels of IL-6 and IL-12 compared to those with uncomplicated malaria. These results support the findings of Jackobsen *et al*, Perkins *et al* and Akanmori *et al*[4,5,7]. Excessive production of IL-6 has been suggested to be involved in the prognosis of severe malaria[4], therefore it demonstrates a protective role of this pro-inflammatory cytokine where IL-6 concentration decreased with increased parasitaemia. Furthermore, as observed in our study, severe malaria has been associated with deficiency of IL-12 and increased production of IFN-γ[12]. Also, IL-12 has been critically linked to or act through IFN-γ production[13-15]. Higher levels of parasitaemia during acute infection and severe mortality were reported after infection of mouse C57BL/6GKO (IFN-γ -/-) with *Plasmodium*, and these conditions were associated with reduced amounts of IL-12[16]. hence the association between IL-12 and IFN-γ suggested a mechanism of immunoregulation between IFN-γ and IL-12 in resolving malaria infection in our study.

Our investigation of anti-inflammatory cytokines showed an increased level of IL-10 in patients with uncomplicated malaria compared to individuals suffering from severe malaria. Conversely, the concentration of IL-4 was increased in individuals suffering from complicated malaria compared to patients with uncomplicated malaria. Our finding for IL-10 contradicts the report of Akanmori *et al*[7] where IL-10 production was associated with hyperparasitaemia. Early IL-10 production (Th2 response) has been associated with susceptibility to malaria infection[17,18] and it is thought that this cytokine has a prominent anti-inflammatory effect, limiting in some way the damage inflicted on normal tissues by an excessive Th1 (pro-inflammatory) response[19]. To further illustrate this fact, it has been reported that in

malarous individuals, an increased level of IL-10 resulted in depressed level of TNF-α or vice versa[20], supporting our finding of interaction of a protective role of the balance between IL-10 and TNF-α in individuals suffering from *Plasmodium falciparum* malaria. The response of Th1 and Th2 (IL-4) seems to be required in the control of malaria infection but need to be adequately tuned in intensity and time[21,22]. The shift from Th1 to Th2 response during peak parasitaemia has been documented to play important role in parasite clearance[23], which probably explains the increased level of IL-4 among participants with complicated malaria.

This investigation demonstrated low haemoglobin level among participants diagnosed with severe malaria. This observation is corroborated by the reports of Kurtzhals *et al* and Premji *et al*[24,25]. Our findings,which indicated that higher levels of IL-12 are associated with uncomplicated malaria, and that lower levels of IL -12 are found in children with severe malaria and severe anaemia are consistent with Torre *et al*[21]. Furthermore, as observed in this investigation, high TNF-α concentrations strongly correlate with increasing severity of malaria disease[26,27], supporting the assertion from an in vitro study that after rupture of parasitized red blood cells, the malaria pigment and other soluble antigens may stimulate production of TNF-α in human monocytes[28]. It is important to note that the balance between IL-10 and TNF-α has been proposed to probably modulate severe malarial anaemia in children and may therefore be a consequence of immunologic inflammation[27,29].

We observed levels of IFN, IL-4, IL-10, IL-6 and IL-12 incerased as aging while level of IL-5 and TNF-α decreased ras aging. The increased levels of IL-10 and decreased levels of TNF-α with age contradicts the report of Pettiford *et al*[30]. However, it is widely accepted that Th2 down regulates Th1 derived cytokines. Elevated levels of IL-10 have been reported to play protective role among children with severe malaria[31,32]. Furthermore, TNF-α has been implicated in the inhibition of parasitaemia[33, 34]. In vitro study has revealed ability of IL-10 to inhibit TNF-α in response to malaria antigens[35]. We therefore hypothesize that the increased level of IL-10 influenced the decreased concentration of TNF-α which confers a level of protective immunity with age. Reduced IFN-γ level has been reported in African children with severe malaria[36]. In another finding, overproduction of IFN-γ, a feature of malaria in non-immune adults has been documented to probably lead to severe pathology[37]. Our result supports these findings in which IFN-γ concentration increased with age which probably elucidates the improved immunity to malaria infection with age. It has been advanced that malaria tends to be more severe in children than in adults, presumably because partial immunity develops over time[38,25]. Our result of increased IL4 with age could be as a result of lower IL-4-making-lymphocytes in children than in adults[25], thereby conferring protective immunity with increasing age. IL-6 and IL-12 in this study were elevated with age, although the concentration of IL-6 was more than IL-12. This pattern may be due to a type 1 shift from IL-12 to IL-6 dominance as documented for some other pro-inflammatory cytokines[31].

In conclusion, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ in this investigation have been demonstrated to be involved in the immunopathology and immunoregulation of uncomplicated and complicated malaria infections. IL-6, IL-12, IFN-γ and IL-10 depressed in complicated/severe malaria may not offer any protective immunity and may be indicators of poor prognosis in *Plasmodium falciparum* infected Nigerian children.

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