**Brief Report**

Cytokine Profiles in Children with Severe *Plasmodium falciparum* Malaria in an Area of Unstable Malaria Transmission in Central Sudan

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**Summary**

Background: Few data exist concerning pathogenesis of severe malaria in areas of unstable malaria transmission. Objectives: The study was conducted in Senga hospital, central Sudan, which is characterized by unstable malaria transmission to investigate the cytokine profiles in children with severe *Plasmodium falciparum* malaria. Methods: Enzyme-linked immunosorbent assay was used to measure the concentrations of three cytokines, interferon gamma (IFN-γ), interleukin-4 (IL-4) and IL-10, in sera of three groups of children (31 in each arm): those with one or more manifestations of severe malaria, those children with uncomplicated *P. falciparum* malaria and healthy controls. Results: The levels of both IFN-γ and IL-10 were significantly higher in patients with severe *P. falciparum* malaria. Medium positive correlations were observed between IFN-γ and IL-10. Conclusion: Thus, the high levels of both IFN-γ and IL-10 indicated their role in the pathogenesis of severe *P. falciparum* malaria.

Key words: Malaria, severe, children, cytokines, pathogenesis, Sudan.

**Introduction**

Malaria continues to be a major global public health burden, causing 250 million clinical cases and nearly one million deaths annually, of which 85% are children under the age of 5 years. About 86% of malaria cases and >91% of malaria deaths worldwide occur in sub-Saharan Africa [1, 2]. Severe malaria is a medical emergency with high mortality especially when there is multiple organ dysfunction [3]. Cerebral malaria and severe malarial anaemia are two major syndromes causing malaria-related mortality in children. The pattern of these two severe forms varies depending on the intensity of transmission: cerebral malaria is more common in older children in areas with lower intensity of transmission, whereas severe malarial anaemia is often seen in children <2 years of age in areas with intense transmission [4]. Understanding the interactions that underlie both control and disease should be helpful when investigating the pathogenesis of severe malaria. There is some evidence suggesting that immune responses and cytokines are thought to play an important role in the pathogenesis of severe malaria [5]. The role of cytokines in malaria severity remains controversial, because different studies have reported different cytokines as being associated either with severe malaria or with protection against severity of the disease in humans [6–9]. In addition, it has been shown that the balance between cytokine concentrations determines the severity of malaria in infected children [10]. Indeed, the outcome of *Plasmodium falciparum* infection may depend on a fine balance between appropriate and inappropriate induction of these immune-regulatory factors. Thus, the current study was conducted in Senga in central Sudan, which is characterized by unstable malaria transmission [11].

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to investigate the cytokine profiles [interferon gamma (IFN-γ), interleukin-4 (IL-4) and IL-10] in children with severe *P. falciparum* malaria so as to add to our previous research on severe malaria and the role of these cytokines in the pathogenesis of malaria [12, 13].

**Materials and Methods**

The study was conducted at Senga hospital, central Sudan during the period of August through September 2009. Children presented with one of World Health Organization manifestations of severe *P. falciparum* were approached to participate in the study [3]. Two groups were the control: children with uncomplicated *P. falciparum* and healthy (free of malaria) children volunteers that matched for age and weight. After taking an informed consent from the parents or guardians, structured questionnaires were administered to collect information about socio-demographic characteristics. Blood films were prepared, the slides were Giemsa stained and the number of asexual *P. falciparum* parasites per 200 white blood cells were counted and double checked blindly by an expert microscopist. Haemoglobin concentrations were estimated by Hemocue Haemoglobinometer (HemoCue AB, Angelhom, Sweden).

**Cytokines measurements**

Five millilitres of blood were withdrawn in plain tube, centrifuged and kept at −20°C until processed in the laboratory for cytokines. Standard sandwich enzyme-linked immunosorbent assay (ELISA) was used for IFN-γ, IL-4 and L-10 using pairs of cytokine-specific, monoclonal antibodies according to the manufacturer’s instructions (BD Biosciences, San Diego, CA, 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.

**Statistics**

Data were entered in computer using SPSS for windows version 13.0 and double-checked before analysis. Data were checked for normality. Socio-demographic data were normally distributed and one way analysis of the variance (ANOVA) was used for comparing the mean (SD). Cytokines data were found to be not normally distributed; Kruskal–Wallis tests were used to compare the significance of differences between the three groups. *P* < 0.05 was considered as statistically significant. Correlations between continuous variables were assessed by the Spearman’s rank test. *R*-value of 0.30–0.49 indicates medium correlation and value of 0.50–1.0 large correlation.

**Ethics**

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

**Results**

While the three groups were (31 children in each arm) well matched in their age, weight and height; the temperature was significantly higher and haemoglobin was significantly lower in patient with severe *P. falciparum* malaria (Table 1). Thirty-one children presented with different manifestations of severe *P. falciparum* namely: repeated convulsions (13; 41.9%), cerebral malaria (3; 9.6%), severe anaemia (12; 38.7%), hyperparasitaemia (20; 64.5%) and more than one manifestation (10; 32.2%).

The levels of both IFN-γ and IL-10 were significantly higher in patients with severe *P. falciparum* malaria (Table 2). There was no significant difference in the levels of these cytokines in the different manifestations of severe malaria (data not shown).

Medium positive correlations were observed between IFN-γ and IL-10. Likewise, there was a significant positive correlation between parasite count and IL-10 (Table 3).

**Discussion**

The main findings of the current study were: significantly higher levels of both IFN-γ and IL-10 in children with severe malaria, significant positive correlations between IFN-γ and IL-10 and medium positive correlations between parasite count and IL-10. This is in agreement with previous observations from south East Asia indicating that serum levels of

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<td><strong>Basic clinical and biochemical characteristics of the three groups of the children</strong></td>
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<td><strong>Variables</strong></td>
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<td>Age, years</td>
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IL-10 and IFN-γ were markedly increased in patients with severe malaria [5, 14]. During the acute phase of uncomplicated P. falciparum malaria, increase of the Th1 cytokine IFN-γ may play a role in limiting progression from uncomplicated malaria to severe and life-threatening complications [15]. IFN-γ is a critical mediator of immunity to malaria and is generally associated with protective mechanisms [16, 17].

In the current study, we found the highest levels of IL-10 in the group of severe malaria. These results are in line with earlier findings showing increased levels of IL-10 in plasma from patients with severe malaria [5–9]. In Malawian children with severe malaria, elevated levels of IL-10 have been observed [18]. The medium positive correlations between IFN-γ and IL-10 in the current study may indicate the balance between these cytokines. Many studies proved that the severity of malarial disease is affected by the balance of pro-inflammatory to anti-inflammatory cytokines in plasma [18]. However, the role of IL-10 in malaria is still controversial, because high levels have been shown to be associated with both severe disease and protection against P. falciparum infections [6, 19, 20]. In the current study, there were medium positive correlations between parasite count and IL-10. Previously, IL-10 also showed the best evidence of a direct relationship with parasitaemia, as has been demonstrated in children with high-density parasitaemia [19] that reflects functionally less-effective parasite clearance [21].

The limitations inherent in examining cytokine production at a single time point and in circulation rather than in the local microenvironments complicate the interpretation of our results. Furthermore, due to fund constrain we conducted this small-sample-sized study that might lack the power. Interestingly, we recently observed high levels of these cytokines in pregnant women with uncomplicated P. falciparum malaria in central Sudan [13]. Perhaps because 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 [15].

References


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