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CYTOKINES AND CLINICAL MANIFESTATIONS OF MALARIA IN ADULTS WITH SEVERE AND UNCOMPLICATED DISEASE

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ABSTRACT

Pro- and anti-inflammatory cytokines are supposed to be involved in malaria pathogenesis. Their relationship with clinical manifestations of the disease, however, is rarely studied in adults from non-endemic countries with imported disease, particularly with severe malaria. In this study we compared serum levels of gamma interferon (IFNγ) and interleukins: IL-12, IL-18, IL-10 in healthy adults and patients with severe or uncomplicated imported malaria, with predominance of *Plasmodium falciparum* and *Plasmodium vivax* infections within studied group. Severe malaria was shown to be associated with elevated serum levels of IFNγ and IL-18 as well as with relative deficiency of IL-12 mediated response in comparison to uncomplicated malaria cases, while IL-10 was found to be higher in all malaria patients compared to the controls.

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Overall, the results of our study are consistent with the observations from the regions with holoendemic malaria transmission, suggesting a pivotal role of impaired IL-12 expression in severe malaria. On the contrary, patients with severe malaria included into our study presented with the pattern of excessive production of inflammatory IFNγ and IL-18, what seems to be an unusual finding compared to the results of the studies on African children and may be the feature of severe malaria in non-immune adults.

BACKGROUND

As a major cause of morbidity and mortality in many tropical regions of the world, malaria still remains an important global public health concern. In non-endemic countries imported malaria is a rare, but serious clinical problem, with non-immune adults returning from travel in endemic areas being susceptible to severe manifestations of *Plasmodium falciparum* infection [1, 2]. Growing problem of antimalarial drug resistance and lack of an effective vaccine makes the insight into the complex pathogenesis of malaria vital for the development of new therapeutic tools and control of the disease.

Cytokines seem to be involved both in protection and pathology in malaria infection. Early and effective inflammatory response, mediated by gamma interferon (IFN-γ) in the interleukin 12 (IL-12) and 18 (IL-18) dependent manner, seems to be crucial for the control of parasitaemia and resolution of malaria infection through the mechanisms of the tumor necrosis factor α (TNFα) induction and enhanced release of the antiparasitic reactive nitrogen and oxygen radicals [3-4]. On the other hand, severe malaria has long been associated with high circulating levels of proinflammatory cytokines such as TNFα, IFN-γ, interleukin 1 (IL-1) and interleukin 6 (IL-6) [4-5]. Their excessive production may affect the disease outcome through their direct systemic effect and by increasing cytoadherence of parasitized erthrocytes to the endothelium via upregulation of adhesion molecules in *Plasmodium falciparum* infections [6]. The expression of cytokines in general as well as the balance of pro- and antiinflammatory response are supposed to be involved in malaria pathogensis, but their relationship with the pattern and extent of vital organ dysfunction in malaria infection has not been well defined yet. Severe malarial anaemia has been associated with low serum levels of IL-12 and low interleukin 10 (IL-10) to TNFα serum concentrations ratio in a few studies of childhood malaria in holoendemic areas [7-9]. However, the manifestations of severe malaria vary with geographic location and malaria transmission intensity as well as with the age of the patient [1, 6, 10]. In non-immune adults severe malaria often presents as a

multiorgan disorder with renal failure, hepatic dysfunction with jaundice and shock while in African children cerebral malaria and severe anaemia predominate [2, 6]. Recent *in vitro* experiments have shown that peripheral blood mononuclear cells from clinically immune individuals from areas of high endemicity produce lower amounts of IFN-γ in response to *P. falciparum* schizont antigens than those from previously unexposed donors, indicating that the control of clinical symptoms may depend on the host ability to regulate strictly the inflammatory response [11]. Studies in mice undergoing primary malaria infection have suggested that the profile of cytokines, including IFN-γ, released early in the course of the infection, may predict the final outcome of the disease [3, 12]. Data concerning involvement of cytokines in naturally acquired malaria infection in non-immune adults, particularly with severe manifestations of the disease, are scarce.

OBJECTIVE

The aim of this study was to evaluate the relationship between clinical manifestations of malaria infection and the serum levels of pro- and anti-inflammatory cytokines in adult patients with uncomplicated and severe malaria.

MATERIAL AND METHODS

Thirty four adult patients with a diagnosis of malaria acquired during touristic or professional stay in endemic areas, admitted in the years 1995 to 2004 to the Clinic of Tropical and Parasitic Diseases of the Interfaculty Institute of Maritime and Tropical Medicine of the Medical University of Gdańsk were included in the study.

Malaria was confirmed either by examination of thick and thin peripheral blood smears or by polymerase chain reaction (PCR) method. Quantitive parasite counts were reported from thin blood films as the percents of parasitized erythrocytes in 500 red blood cells, and the level of antimalarial antibodies in serum of all patients was also measured with the use of indirect immunofluorescence antibody test (IFA) [13]. A full history was taken from each patient and a detailed physical examination was performed on admission. Peripheral venous blood samples were taken from all patients for biochemical and hematological assessment as well as for serum cytokines concentration measurement.

Patients received adequate antimalarial treatment and were followed up until discharge from hospital or death. Basing on the results of laboratory and clinical findings during hospitalization patients were divided into two groups according to the severity of the disease. Severe and uncomplicated malaria cases were distinguished with the use of criteria based on World Health Organisation description of severe manifestations and complications of malaria, as shown in the table 1 [1, 10].

Table 1. Entry criteria of severe malaria group (see references 1 and 10)

Impaired consciousness
Prostration – inability to sit unassisted
Acidosis – arterial pH < 7,25 or venous lactate level > 15 mmol/L
Multiple convulsions
Circulatory collapse
Pulmonary oedema or Acute Respiratory Distress Syndrome
Abnormal bleeding
Jaundice – serum bilirubin concentration of > 3 mg/dL
Macroscopic haemoglobinuria
Severe anaemia – haemoglobin of < 7 g/dL or haematocrit <20%
Renal failure – urine output <400 mL/24hrs or serum creatinine level >3 mg/dL
Hypoglycaemia – serum glucose level of < 40 mg/dL
Hyperparasitaemia – > 5%
Hyperpyrexia – temperature >40^{o}C

Eight healthy Polish adults with no history of travel to malaria endemic areas were recruited to the study as a control group for cytokines determination.

Blood samples for cytokines measurement were collected within twenty four hours from admission, centrifuged immediately at 4000 g for 5 minutes and stored at –70^{o}C until analysis. Serum concentrations of IFN-γ, IL-12, IL-18, IL-10 were determined using enzyme-linked immunosorbent assays obtained commercially (ELISA, BenderMedSystems, Austria). All specimens were measured in duplicate and the mean of the two values was taken for the analysis. The sensitivity of these assays, as defined by manufacturer, are 0.27 pg/ml, 19.1 pg/ml, 55 pg/ml, 0.05 pg/ml respectively.

Comparisons between two groups were performed using the nonparametric Mann-Whitney U-test. Correlations between continuous variables were assessed with the use of Spearman rank test. The level of significance in all cases was set at P of <0.05.

RESULTS

Among 34 adult patients included into the study, 22 persons were diagnosed as *Plasmodium falciparum* cases, 8 as *P. vivax* cases and one had mixed infection (*P. falciparum* and *P. vivax*). Moreover, 2 cases of *P. ovale* infection and one of *P. malariae* were found in the studied group. Twenty-seven patients had uncomplicated malaria, while 7 developed severe malaria (all of them *P. falciparum* infection). The titre of antimalarial antibodies measured with the IFA test was higher than 1:80 in 21 patients, in 10 patients it was equal or lower than 1:80 and in 3 patients it was not examined. The results of the laboratory blood examinations of 34 patients are summarized in Table 2.

The following clinical manifestations of severe form of the disease were observed in the studied group: impairment of consciousness including cerebral malaria, renal failure, acidosis, severe prostration, hyperparasitaemia, jaundice and hyperpyrexia, as shown in Table 3. One patient died in the result of multi-organ failure.

Table 2. Results of the laboratory blood examinations of 34 malaria patients

Characteristic	Uncomplicated malaria n=27	Severe malaria n=7
Age (yr) *	39,6 ± 11,5	35,7 ± 9,9
№ male/ № female	24 / 3	7 / 0
Temp. (^{o}C) *	37,9 ± 1,11	38,7 ± 0,82
Parasitaemia (%) *	0,34 ± 0,47	3,6 ± 3,02
Haemoglobin (g/dL) *	11,55 ± 1,76	11,05 ± 1,79
Platelet count (10^6/L) *	130,8 ± 79,87	50,4 ± 18,62
Bilirubin serum level (mg/dL) *	1,2 ± 0,65	2,7 ± 2,47
Glucose serum level (mg/dL) *	97,5 ± 18,62	97 ± 7,62
Creatinine serum level (mg/dL) *	1,1 ± 0,23	2,39 ± 3,1

* mean value ± SD is presented

Table 3. Manifestations of severe malaria in the studied group of 34 patients

Clinical feature	№ of cases*
Impaired consciousness	2
Renal failure	1
Acidosis	1
Hyperparasitaemia	3
Jaundice	3
Hyperpyrexia	1
Prostration	1

* Total number exceeds 7 as some patients presented with more than one feature of severe malaria.

No significant differences between mean serum concentrations of any measured cytokine were observed in patients divided into groups according to different species of Plasmodium they were infected with. However, several associations between both pro- and anti-inflammatory cytokines serum levels and the severity of the disease were noted.

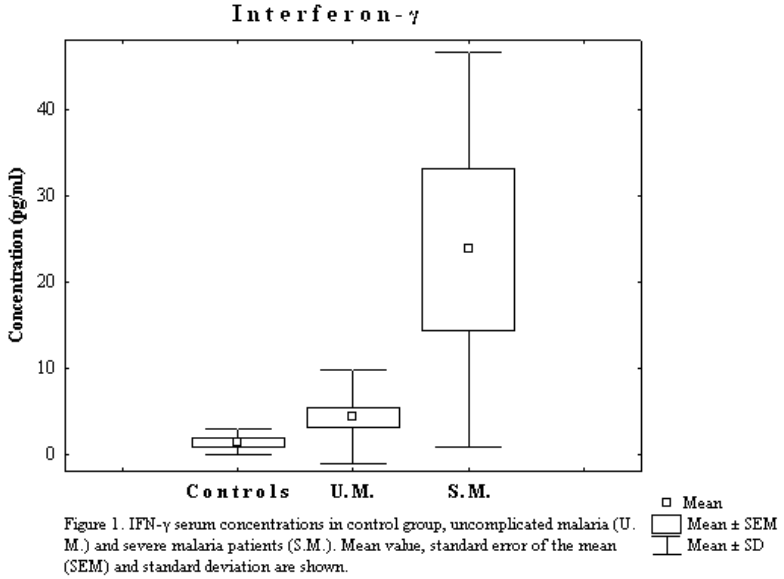

Figure 1. IFN-γ serum concentrations in control group, uncomplicated malaria (U. M.) and severe malaria patients (S.M.). Mean value, standard error of the mean (SEM) and standard deviation are shown.

Mean serum level of IFN-γ was found to be significantly higher in severe as well as uncomplicated malaria group compared to the controls (23,71 pg/ml vs 1,40 pg/ml;

p<0,01 and 4,23 pg/ml vs 1,40 pg/ml; p<0,05 – respectively, as in the fig. 1). Moreover, the mean serum concentration of IFN-γ was significantly higher in patients reporting their primary symptomatic Plasmodium infection, than in those with the history of more than one malaria infections (13,73 pg/ml vs 3,13 pg/ml; p<0,05 - respectively).

As far as IL-12 is concerned (fig. 2), a significant difference was observed between its mean serum concentration in uncomplicated malaria patients compared to the controls (191,99 pg/ml vs 6,84 pg/ml with p<0,05), but its level was lower in patients with severe manifestation of the disease in comparison to those with uncomplicated malaria (102,49 pg/ml vs 191,99 pg/ml with p<0,05).

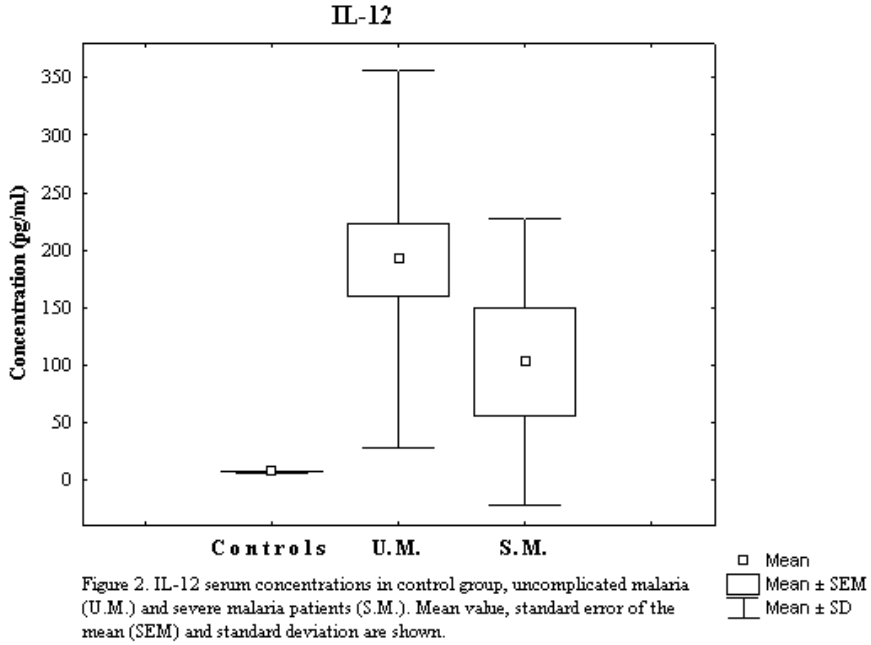

Figure 2. IL-12 serum concentrations in control group, uncomplicated malaria (U.M.) and severe malaria patients (S.M.). Mean value, standard error of the mean (SEM) and standard deviation are shown.

On the contrary, mean serum level of IL-18 was higher in severe malaria patients than in uncomplicated disease group and the control group (1255,22 pg/ml vs 390,30;p<0,05 and 1255,22 pg/ml vs 299,25 pg/ml; p<0,05, respectively). In addition, association was found between IL-12/IL-18 serum concentrations ratio and the disease severity (0,12 in severe vs 1,08 in mild malaria, p<0,01).

Mean serum level of IL-10 was significantly higher in both severe and uncomplicated malaria patients than in the controls (45,19 pg/ml vs 1,65 pg/ml; p<0,01 and 28,69 pg/ml vs 1,65 pg/ml; p<0,0001, respectively).

Similar relations regarding all mentioned cytokines were seen between severe and uncomplicated malaria patients within groups confined to Plasmodium falciparum infected individuals.

No significant correlation of cytokines levels and parasitaemia or the level of malarial antibodies was found in the studied group.

DISCUSSION

In this study we demonstrated the relations between a number of both pro- and anti-inflammatory type cytokines expression and the degree of severity and outcome pattern of malaria infection in adult patients. Detailed analysis showed uncomplicated manifestation of the disease is associated with elevated serum levels of IL-12 as well as IFN-γ in comparison with the values measured in the sera of the healthy controls. As a potent immunomodulatory cytokine, IL-12, released by macrophages in response to infectious agents, is involved in cell-mediated immune response via Th1 cells induction. The latter produce IFN-γ, which in turn is supposed to enhance TNFα, reactive nitrogen and oxygen radicals production what seems to be essential for the resolution of malaria infection [4]. IL-12 serum level turned out to be significantly lower in severe compared to mild malaria patients in our observations, what is consistent with most of the studies carried out in endemic regions [7, 9, 14, 15]. Several mechanisms of IL-12 low activity in severe malaria had been previously suggested i.e. suppressive activity of anti-inflammatory cytokines such as IL-10 or macrophage activity impairment due to malaria pigments [9, 14]. Genetic factors were also proposed to explain that phenomenon basing on the results of animal model studies. Overall, our study is consistent with the hypothesis of the pivotal role of IL-12 for the control of the disease severity. Studies carried out in Africa associated defective IL-12 production with severe malarial anaemia, which is a common feature of Plasmodium falciparum infection in children there. Either a direct role of IL-12 defective production in erythropoiesis or its influence on protective immune responses in general, were suggested to be involved in the development of severe anaemia during malaria infection. However, we did not observe any case of profound anaemia among our patients with low IL-12 serum level, what seems to support rather the latter mechanism of IL-12 participation in severe manifestations of the disease. In addition, several studies on African children associated severe malaria with reduced IFN-γ activity [14,16]. This is in contrast to the results of our study, since we demonstrated that high IFN-γ serum level is clearly linked to the disease severity. Overproduction of IFN-γ leading to severe pathology may be the feature of malaria infection in non-immune adults [3]. This seems to be supported by

recent in vitro experiment, in which peripheral blood mononuclear cells from clinically immune residents of high malaria transmission region produce less IFN-γ in response to P. falciparum antigens, than those from unexposed donors [11]. Interestingly, in our study patients with the history of previous malaria infections had significantly lower serum IFN-γ concentrations than those reporting the disease as their primary.

Significant elevation of IFN-γ level in severe malaria patients of our study was accompanied by high IL-18 concentrations, what is also an unusual pattern regarding the results of the studies on childhood malaria from endemic areas, demonstrating in most cases the opposite relation between IL-18 level and the disease severity there [7,17-19]. In rodent studies treatment with IL-18 was shown to increase IFN-γ levels in mice infected with P. yoelli and P. berghei leading to a delay in the onset of parasitaemia and better survival rate [20]. In humans, proinflammatory role of IL-18 in Plasmodium spp. infection as well as the capability of IFN-γ induction had been suggested to play role in protective responses in a few studies concerning children. In our observations, excessive production of both of these molecules was associated with severe forms of malaria infections, what is consistent with the results of one study on adult patients with severe and uncomplicated malaria carried out in Thailand [21]. Moreover, we found that IL-12/IL-18 serum levels ratio is much lower in patients with severe clinical manifestations than in individuals with uncomplicated malaria, what supports our observation that severity of the infection is related to IL-18 dependent IFN-γ overproduction, linked with impaired IL-12 activity. The reasons for this phenomenon are not clear, however. High levels of IL-10 found in the sera of severe malaria patients may contribute to IL-12 suppression as suggested before, but on the other hand this may be insufficient to control the inflammatory cascade mediated by IL-18 along with IFN-γ. Down-regulation of human monocyte IL-12 production, in the absence of similar effects on other proinflammatory cytokines, is a well documented phenomenon [14].

In all but one of the patients included in our study, other coexistent parasitic infections were excluded basing on detailed, routine diagnostic examinations. Most of the studies carried out in malaria endemic areas do not take this circumstance into consideration. However, children from rural African regions are likely to be infected simultaneously with more than one pathogen prevalent in tropical countries and coincidental parasitic infections may interfere with cytokines released in Plasmodium spp. infection. This may partially contribute to differences in the results of studies on populations of malaria endemic countries and our results.

In our study, the peripheral parasite count was not associated with any cytokine serum concentration, neither were the parasite species. Thus, apart from parasite-related factors and mechanisms acquired with previous exposures, conceivable host factors may

be supposed to affect the regulation of cytokines expression. Similarly as in the case of other cytokines involved in malaria infection, i.e. TNFα, their production may be controlled at gene level [4]. Other molecules, which were not assessed in our study as well as the influence of soluble cytokine receptors could also be taken into consideration, while explaining the mechanisms of our observations.

CONCLUSIONS

In summary, the results of our study support the hypothesis that effective Plasmodium spp. infection resolution along with uncomplicated clinical manifestation of the disease and the favorable outcome depend on strict regulation of pro- and anti-inflammatory responses mediated by relevant cytokines. The excessive inflammatory response pattern demonstrated by high IFN-γ and IL-18 expression and relative IL-12 deficiency in the individuals included in our study may be the common feature of severe malaria in adults with imported disease. However, the results of these study should be considered as preliminary, therefore further studies are needed on larger group of patients to determine the cytokines involvement in particular clinical manifestations of the disease.

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