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Serum Levels of Interleukin-10 and Interferon Gamma Among Patients with Plasmodim Falciparum EL-DAMAZIEN - BLUE NILE STATE- SUDAN

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Abstract: *Cytokines play an important role in human immune responses to malarial disease. However, the role of these mediators in disease pathogenesis, and the relationship between host protection and injury remains unclear. In malaria, blood concentrations of cytokines were influent by the infection, such as interleukin (IL-10) and interferon-γ (IFN-γ). In this study serum levels of interleukin-10 (IL-10) and interferon-γ (IFN-γ) were determined in 52 patients and 7 healthy volunteers as controls in El-Damazien, Blue Nile State- Sudan. Blood samples were collected from both patients and control candidates in (EDTA) containers for parasitological and immunological tests. Microscopic examination for Geimsa stained thick and thin blood films were used to detect the positive samples for Plasmodium falciparum. Enzyme linked Immunosorbant Assay (ELISA), was used for the determination of IL-10 and IFN-γ levels. Serum levels of IL-10 and IFN-γ were markedly elevated in patients with malaria (118.99 ± 62.21 ng/ml versus 12.08± 7.77 ng/ml in healthy controls; 40.45 ± 35.31 ng/ml versus 12.33 ± 5.00 ng/ml, respectively; mean ± SD). These results were found to be statistically significant (P= 0.007). Furthermore the levels of these cytokines had significant correlation within parasitemia (0.001 and 0.022 respectively). Thus suggest that stimulatory and inhibitory cytokines for macrophage activation and/or antibody production (i.e., TH1- and TH2-type immunoreaction, respectively) are coexpressed during acute P. falciparum infection and stress the multifactorial network between host and parasite in malaria immunology. Further studies are needed to examine whether serum concentrations of these cytokines also parallel their concentrations at the tissue sites of their production and action.*

Keywords—IL-10; IFN-γ; P.falciparum; Parasitemia; ELISA; El-Damazien

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1. INTRODUCTION

Human malaria is one of the most common causes of morbidity and mortality in tropical and subtropical regions of the world. The World Health Organization (WHO) estimates 300 million to 500 million new cases are still reported each year, about 90% of them in Africa and about half of the world's population (3.3 billion people) is at risk of malaria in more than 100 countries[1]. It's spreading as a result of environmental changes and drug resistance [2]. The most frequent victims are among young children , pregnant women and young adults, of whom at least 2 million die annually due to complications of *P.falciparum* malaria due its high level of mortality and the complications as cerebral malaria and sever malarial anemia [3] [4].

The wide variation in the clinical manifestation ranging from asymptomatic, uncomplicated to life-threading complication which common found in sub-Saharan Africa [5]. While the epidemiology of malaria is depend on the entomological inoculation rate, parasite intensity, level of acquired immunity, host genetic factors, distribution of drug resistance, nutritional status and socioeconomic conditions, standard of health care and education [6].

The association of severity of malaria with the degree of parasitaemia has been known and recently the association between the antigenic diversity and polymorphism of *P.falciparum* with the clinical out-comes of malaria so as to understand the parasite's biology, disease pathogenesis, host immune response and development of effective vaccine [5].

The critical role of antibodies and cytokines released during malaria may play in protection was shown in newborns be protected during the first few months of life by maternal antibodies against surface antigens provide protection against subsequent clinical episodes in children [7]. In addition to that many studies emphasize that cytokines, and particularly the pro-inflammatory cytokines; IFN-γ, IL-1, IL-6, TNF-or anti-inflammatory cytokines, including IL-4, IL-10 and IL-5 can have both detrimental and beneficial effects on the host's ability to cope with infection and combat disease [8].

The balance between pro- and anti-inflammatory cytokines may determine disease severity. IL-10 overproduction in response to IFN-γ may play an important role in severe malarial anemia, but not apparently through down-regulation

of IFN-γ production [9]. Overall, an appropriate production of IL- 10 is required to protect against excessive release of IFN-γ and TNF-α, along with elevated levels of effector molecules such as nitric oxide and reactive oxygen species that can directly inhibit erythropoiesis, also promote enhanced malarial anemia pathogenesis by contributing to bone marrow suppression, dyserythropoiesis, and erythrophagocytosisthe bone marrow, thereby reducing the possibility of severe anemia and mortality induced by the infection with *P. falciparum* malaria [10, 11].

2. Materials and methods

2.1 Study design:

This is a cross sectional study.

2.2 Study area and study period:

The study was conducted in El-Damazien- The Blue Nile state-Sudan during period from October 2017 to October 2018.

2.3 Study population:

A total of 52 febrile patients (cases) and 7 ages and sex matched healthy controls were included in this study.

2.4 Inclusion criteria:

Patients with history of fever proceeding in the past 24 hours with confirmed malaria parasite positive slides and ICT were included study participant's cases and healthy volunteers were included as the study controls.

2.5 Exclusion criteria:

All patients who were negatively diagnosed for malaria parasites. Patients with any known concurrent chronic illness and diseases such as malnutrsion, chronic infection as tuberculosis ,pneumonia or urethritis in addition to known cancer patients.

2.6 Sample size:

The classical statistical method for determining sample size based on an unknown proportion of markers was used at 95 percent confidence level and 10 percent precision. Since there is no known figure for the prevalence of malaria, a prevalence rate of 50 percent was used to calculate the sample size using the formula below as described by Mendenhall *et al*. (1981) [12] .

$n = \frac{Z^2PQ}{d^2}$ Or $N = Z^2 P (100 – P)/ d^2$

Where n = sample size
P = prevalence rate
Z = 1.96 at α = 0.05 (α = desired confidence level)
d = desired width of confidence (precision)
Q = 100-P

There for the sample size (n) was determined as:

$n = \frac{1.96^2 x 50 x 50}{10^2}$

n = 96.04 ≈ 96

3. Methods

3.1 Sample collection and processing:

Intravenous blood for both thick and thin film and for serological tests was collected from each candidate.

3.2 Collection of samples:

Blood was collected aseptically after the forearm was cleaned with 70% alcohol before vein-puncture. 2.5ml venous blood was collected from each to run ELISA for measure cytokines levels into the collection tube containing anticoagulant (EDTA) and then processed to thick and thin film. Blood was transfer as three drops of blood was applied on a clean, dust and grease free glass slide for thick and thin and the rest of blood for serological tests [13,14].

3.3 Preparation of thick film:

After collection of blood on a clean and grease free glass slide, thick film was made by spreading one drop of blood with a spreader evenly on an area about 15×15 mm in diameter. Care was taken to avoid rouleaux formation. Then, the slide was labeled properly and allowed to air-dry by keeping the slide on horizontal position. Precaution was taken during spreading and drying [13].

3.4 Preparation of thin film:

After collection of one drop of blood on a clean grease free slide, thin film was made by spreading the blood using a smooth edged slide or spreader at an angle of 45º from the horizontal plane. A well-prepared thin blood film was judged by having a smooth tail end and free of vertical lines and holes. The slide was then labeled properly and allowed to air-dry [13]. Absolute methanol or ethanol was used to fix the thin film. Following steps were taken for fixing the thin film as described by Cheeshbrough (1999) [13]:

- The slide was placed horizontally on a staining rack.
- A small drop of absolute methanol or ethanol was applied to the thin film.
- Then the slide was allowed to fix for 1-2 minutes.

Staining of the films:

The slide was first placed on a staining rack. Then 10% Giemsa stain having a pH of 7.2 was poured gently on the fixed thin film or de-hemoglobinized thick film until the slide was totally covered. Then the slide was allowed to stain for 30-45 minutes out of the sunlight. Then the stain was washed with clean water. Back of the slide was wiped and placed in a draining rack. The slide was then allowed for air-dry [13].

3.5 Microscopic examination of the stained film

Thick film:

The thick film was examined first by using the 40x objective, a well-stained part of the thick film was selected which was well populated with WBC. Then the selected portion of the film was examined with 100x oil immersion objective by moving along the width of the slide. At least 100 fields were examined before a slide was considered as negative for malaria parasite [13].

Thin film:

The thin film was examined by the 40x objective first followed by 100 x oil immersion objectives. After applying immersion oil the film was examined by moving along the edge of the film. Then moving the slide inward by one field, returning in a lateral movement and so on. At least 100 fields were examined before a slide was considered as negative for malaria parasite [13].

3.6 Determination of malaria parasitaemia:

Parasites density determination:

Semi quantitative count (thick film) was used the following semiquantitative scale:

+ 1-10 asexual parasites per 100 thick film fields
++ 11-100 asexual parasites per 100 thick film fields
+++ 1-10 asexual parasites per single thick film field
++++ more than 10 asexual parasites per single thick film field [15]

3.7 Detection of Antigen:

Antigen was detected by immunochromatographic (ICT) method with Malaria *P. falciparum and P.vivax Plasmodium.* Antigen onsite Rapid Screening Kit. The kit was manufactured by OnSite Malaria P.f/P./v Ag Rapid test, CTK Biotech. Inc. USA. Lot no: F0323N9102. Test procedure performed according to manufactories instructions.

Principle of the test:

The Malaria onsite Rapid test is a one step chromatograhic immunoassay which specifically detects the Antigens of *P. falciparum and Pan Plasmodium* in human whole blood. The test utilizes *P .falciparum* specific antigen HRP-II and *Pan* Antigen aldolase to ensure test specificity and sensitivity.

Sample collection for serology:

Two and half milliliters (2.5 mls) of venous blood were collected in EDTA containers at the time of recruitment of participants for determination of cytokines. The blood was then centrifuged immediately and plasma was collected and stored at – 20^{o} C until analysis.

3.8 Determination of IL-10:

INF-γ and IL-10 levels expression were determined using specific monoclonal antibodies for both INF-γ and IL-10 using sandwich ELISA at 450 nm wavelength. The sets were manufactured by BioLegend's ELISA MAX™ Deluxe Sets Cat. No. 430104 and Cat. No. 430604 respectively. Test procedure performed according to manufactories instructions.

3.9 Ethical consideration and permission:

Ethical approval was sought from the Sudan University of Science and technology, in addition to an individual orientation for patients about the purpose and benefit of the study were explained.

3.10 Data analysis:

Data was first entered in Microsoft excel, then converted and analyzed using SPSS 15.0, by using one way Anova and Chi square test, the significant consider when the *P*.value <0.05.

4. Results

The results showed that IL-10 levels in malaria patients was 118.9 and SD was 62.21, while the levels in healthy participants was 12.08 and SD was 7.77. The difference was found to be statistically significant (P= 0.0001) (Table 1). While results showed that INF-γ levels in malaria patients was 40.45 and SD was 35.31, while the levels in healthy participants was 12.33 and SD was 5.00.The difference was found to be statistically significant (P= 0.0001) (Table 2).

The statistical correlation within density of parasite and desired cytokines IL-10 and INF-γ was significant at the 0.001 and 0.022 respectively (Table 3) (Figure 1,2).

Concerning the gender the results showed insignificant difference was be found statistically at *P*=0.862 for IL-10 and *P*=0.567 for INF-γ (Table 4).

Table 1: Mean and SD of IL- 10 in patients and control

Group Sample		IL-10 (ng/ml)	*P*. value
Patients	**Mean**	118.99	0.000
	SD	62.21	
Control	**Mean**	12.08	
	SD	7.77	

Table 2: Mean and SD of INF-γ in patients and control

Group Sample		INF-γ (ng/ml)	*P*. value
Patients	**Mean**	40.45	0.000
	SD	35.31	
Control	**Mean**	12.33	
	SD	5.00	

Table 3: Correlation between parasitemia and cytokines

Parasitemia		IL-10 (ng/ml)	INF-γ (ng/ml)
Control	Mean	12.33	12.08
	SD	5.00	7.77
(+)	Mean	30.75	109.28
	S. D	14.058	73.458
(++)	Mean	34.85	126.07
	S. D	26.10	58.157
(+++)	Mean	61.04	124.07

	S. D	62.84	59.36
(++++)	Mean	63.134	97.36
	S. D	41.31	66.45

Table 4: Correlation between Gender and cytokines (IL-10 and INF-γ)

Cytokine	Gender	N	Mean	S.D	*P*. value
IL-10	**Male**	25	104.48	69.32	0.567
	Female	34	107.64	68.05	
INF-γ	**Male**	25	40.14	42.16	0.862
	Female	34	34.89	27.84	

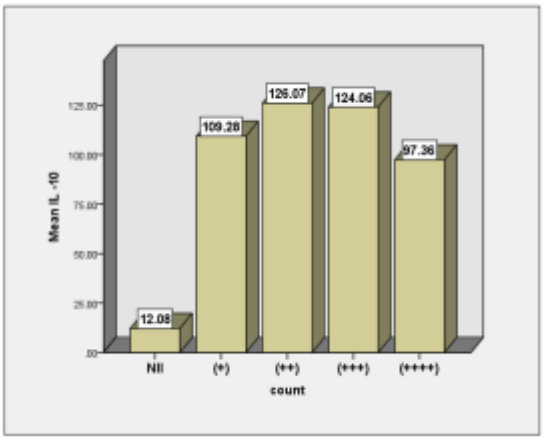

Figure 1: Correlation between Parasitemia and IL-10

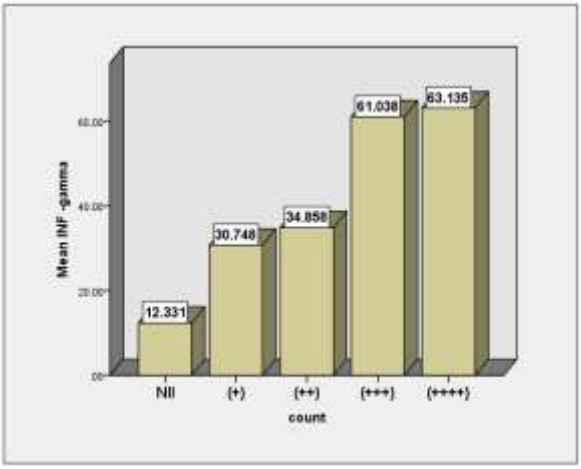

Figure 2: Correlation between parasitemia and INF-γ

5. Discussion

The analysis of results obtained from our study demonstrates correlation between IL-10 and INF-γ cytokine and malaria infection in endemic area. The patterns of cytokines during infection with malaria play an important role in pathogenesis, clinical manifestation and complications which has been clearly confirmed. Furthermore variations in their secretion affected by many epidemiological and socio-economic factors that latter on determine the status of immunity against malaria.

Both cytokines IL-10 and INF-γ levels were elevated in patients with malaria compared to healthy controls; IL-10 in all patients had elevated levels of parasitemia compared to the control groups. The regulatory IL-10 is known to control inflammation during malaria infections and thus protect against immunopathology, but, in so doing, it reduces the effectiveness of other immune mechanisms which assist in increases of parasitemia [16].The high levels of IL-10 possibly regulate type1cytokines and the action of TNF-α, IL-6 and IL-12[17]. In addition to counter-regulatory roles during the pregnant who were susceptible to malaria infection, resulting in maternal anemia and its consequences [18].

Consistent with the present results, study carried by Peyron *et al.* (1994) [19] showed that IL-10 elevated in infection with *P. falciparum* return to virtually normal levels 7 days after antimalarial chemotherapy. Anthers study carried by Wilson *et al.* (2010) [20] showed that associated with Th1 and Th2 cytokine homeostasis. IL-10 and G-CSF levels were elevated in the asymptomatic pregnant when compared with the healthy group. Collectively, these findings support the hypothesis that IL-10 is a critical factor in down-regulating the pathogenesis of severe malaria.

Our study IL-10 levels appear not to affect by gender in response to inflammatory process.

Conversely the result showed elevated IL-10 levels among different parasitemia, while INF-γ is remarkable increases with parasitemia suggesting the effective role of INF- γ in protection against malaria and this finding consistent with previous data showing that IFN-γ controls parasite multiplication, promotes parasite clearance and protects against the severity of malarial anemia and severe malaria), This is also consistent to previous studies have shown a protective effect of IFN-γ against clinical malarial anemia among young children living in a holoendemic area [21].

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