

# Hematological Parameters and Key Cytokines in Patients With Uncomplicated Falciparum Malaria in Hodeidah, Yemen

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## Research Article

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# Abstract

Immunity to malaria has a major role in controlling disease and pathogenesis with cytokine production being involved in almost each phase of the immune response. The present study aimed to assess hematological variables and to measure plasma levels of TNF $\alpha$ , IFN $\gamma$  and IL10, their ratios, and their relation to parasitemia among patients with uncomplicated falciparum malaria in Hodeidah, Yemen. Forty patients with uncomplicated *P. falciparum* mono-infection and 40 healthy age and sex matched controls were enrolled in the study. Parasitological diagnosis was confirmed, and parasite density estimated. Hematologic parameters, the presence of gametocytes, and plasma cytokine levels were determined. Results revealed lower Hb levels, RBC, lymphocyte and platelet counts and higher neutrophil and reticulocyte counts among patients compared to controls. TNF $\alpha$ , IFN $\gamma$  and IL10 were higher in patients than controls. A relatively higher IL10 production was demonstrated by the significantly lower TNF $\alpha$ /IL10 and IFN $\gamma$ /IL10 ratios in patients than controls. TNF $\alpha$  and IL10 correlated positively with parasite density. Reticulocyte count was higher and IFN $\gamma$  level was lower in the presence of gametocytes. Conclusively, uncomplicated falciparum malaria is associated with the ability to regulate the production of the pro-inflammatory and anti-inflammatory cytokines. This mediates parasite clearance while simultaneously avoiding severe pathology.

## 1. Introduction

Malaria is the most important vector-borne parasitic disease in terms of morbidity and mortality. About 228 million cases and 405,000 annual deaths have been recently estimated. In Yemen, more than 65% of the population are exposed to malaria; endemicity varies with the diversity of the topography and climate (World Health Organization, 2019).

It has been reported that three species of malaria exist in Yemen, *Plasmodium falciparum* (*P. falciparum*), *P. vivax* and *P. malariae* with predominance of *P. falciparum* (more than 90% of cases). Based on active and passive case detection, a malaria prevalence of 16.2% was reported among the population in Hodeidah, west of Yemen (Al-Maktari et al., 2003) and 8% among school children in the same governorate (Alwajeih et al., 2020). Efforts towards malaria control in Yemen have been undertaken since the late seventies (Yemeni National Malaria Control Program, 2010). However, it is estimated that about 38% of the population still lives in high transmission areas (World Health Organization, 2019).

Human malaria infections show a wide clinical spectrum ranging from asymptomatic infection to severe life-threatening disease. Immunity to malaria has a major role in controlling disease and pathogenesis. In high transmission settings, most patients have mild malaria, likely due to pre-existing immune protection (Laishram et al., 2012; Pawar, 2014).

Cytokines are a category of signaling molecules used in cellular communication, they are small proteins 5–20 kDa. They include chemokines, interferons, interleukins, lymphokines and tumor necrosis factor. Based on their functional aspects, they have been classified into pro-inflammatory and anti-inflammatory

cytokines (Wahab and Hussain, 2013). Pro-inflammatory cytokines are important in cell signaling and they up-regulate inflammatory reactions. These are TNF $\alpha$ , IFN $\gamma$  with other interleukins as IL2-IL12 (Zhang and An, 2007). Anti-inflammatory cytokines control the pro-inflammatory cytokine response and participate in regulation of the immune response (Opal and De Palo, 2000). IL10 is the most important anti-inflammatory cytokine. It is a potent de-activator of monocyte/macrophage pro-inflammatory cytokine synthesis (Clarke et al., 1998).

Cytokine production is potentially involved in almost each phase of the immune response to malaria (Pawar, 2014). TNF $\alpha$  is produced by numerous cell types mainly by monocytes and tissue macrophages. TNF $\alpha$  appears to be the most pro-inflammatory cytokine involved in the malaria pathogenesis and cytoadherence of infected erythrocytes (Grau and De Kossodo, 1994).

IFN $\gamma$ , was known as immune interferon, it is a product of human leucocytes and other antigen-stimulated lymphocytes. Functionally, it heightens both the innate and adaptive immune responses against pathogens and tumors and has the ability to maintain homeostasis. IFN $\gamma$  was found to mediate protection against pre-erythrocytic malaria infection in murine models. IFN $\gamma$  level has been linked to reduction of parasitemia and protection from reinfection and complicated disease (McCall and Sauerwein, 2010). However, it is also involved in immunopathology and can exacerbate the malarial disease severity (Dodoo et al., 2002).

IL10 is one of the cytokines that affect malaria infection outcome. IL10 is a fascinating cytokine able to stop immune response by inhibiting the production of a number of cytokines. It suppresses antigen presentation and CD4 + T cell activation and has been linked to high parasitemia. It inhibits the release of pro-inflammatory mediators and thereby inhibits IFN $\gamma$ -induced secretion of TNF $\alpha$  and protects against tissue damage. (Othoro et al., 1999).

The pattern and extent of hematological parameters and cytokines were reported to vary with the level of malaria, its endemicity, demographic factors and different features of the patients. Therefore, the present study was designed to estimate hematological parameters and to determine the plasma levels of TNF $\alpha$ , IFN $\gamma$  and IL10, their ratios and their association with parasitemia among patients with falciparum malaria infection as compared to controls in Hodeidah, Yemen.

## **2. Materials And Methods**

### ***Study area***

The present study was conducted in Hodeidah governorate in the western region of Yemen. Hodeidah has a semi-equatorial climate; warm and humid in summer and moderate in winter. Temperature reaches 40°C during summer and 24°C in winter. The population of the governorate is more than two million people living in rural and urban districts (Yemeni National Malaria Control Program, 2010).

### ***Sample size***

Calculation of the required sample size was based on the detection of an average difference of 50 pg/ml in the key cytokines (TNF $\alpha$ , IFN $\gamma$  & IL10) between patients and control groups, with a SD of 0.0 and 20.0 and with 80% power and 1% significance level using a two-side two-sample t-test (PASS program version 12). Eighty individuals were enrolled in the study and were divided into two groups as follows:

**Group 1:** Forty patients (6-60 years) of both sexes attending governmental and private hospitals who were diagnosed clinically as having malaria and laboratory-confirmed as *P. falciparum* mono-infection. Patients having other chronic diseases, associated infections or vital organ dysfunction were excluded from the study.

**Group 2:** Forty apparently healthy controls, age and sex matched with a negative history of malaria for at least one year. They were selected among persons accompanying the patients and from the workers in the health centers.

### ***Ethical considerations***

Ethical considerations and confidentiality were assured for all participants in the study who gave an informed consent. The study was approved by the Research Ethics Committee of the Medical Research Institute, Alexandria University, Egypt and from the hospital administration in Hodeidah.

### ***Data collection***

Demographic and clinical data were collected through a predesigned structured questionnaire.

### ***Blood sample collection and preservation***

Five milliliters of venous blood were collected into a clean ethylenediaminetetraacetic acid (EDTA) tube. Hematological parameters were studied, and thick and thin blood films were prepared. Subsequently, plasma samples were separated, labeled, and stored at -20°C till cytokines were measured. In the same manner, blood samples were obtained from controls and all procedures done for the patients' samples were applied to the control group.

### ***Laboratory procedures***

#### ***A. Confirmation of diagnosis and estimation of parasitemia***

##### ***Detection of antigen by rapid diagnostic test (RDT)***

Samples from the collected blood of suspected malaria patients were re-screened for *P. falciparum* infection by detecting histidine-rich protein 2 (HRP-2) and plasmodium lactate dehydrogenase antigen (pLDH). In this method, CareStar™ Malaria HRP/pLDH (Pf/PAN) Combo (Access Bio Inc., New Jersey, USA) was used according to the manufacturers' instructions.

### ***Giemsa staining and microscopy***

Positive samples as diagnosed by RDT were confirmed microscopically after Giemsa staining of thick and thin blood films (World Health Organization, 1991). Films were examined for asexual and sexual parasite stages.

Parasite density was estimated by counting asexual parasites against WBCs in thick blood films. One hundred WBCs were counted and parasite density/ $\mu$ l of blood was calculated according to the formula:

$$\text{Parasite density} = \frac{\text{Total WBC count} \times \text{Parasites counted against 100 WBCs}}{100}$$

Parasites per  $\mu$ l were categorized as: low 1-999 parasite/ $\mu$ l, moderate 1000-9999 parasite/ $\mu$ l and high > 10.000 parasite/ $\mu$ l.

### ***B. Hematological parameters***

Hemoglobin (Hb) concentration, RBC, total and differential WBC, and platelet counts were determined using a Sysmex-KN-21 automated hematology analyzer (Block Scientific, Inc., USA).

Relative reticulocyte count was determined using brilliant cresyl blue as a supravital stain (HiMedia<sup>®</sup> Laboratories, India). The number of reticulocytes was expressed as a percentage of the total number of 500 erythrocytes counted (National Committee for Clinical Laboratory Standards, 1997).

$$\text{Reticulocyte count \%} = \frac{\text{Total number of reticulocytes counted}}{\text{Total number of erythrocytes counted (500)}} \times 100$$

### ***C. Measurement of cytokines***

The assays of cytokines were carried out in the laboratory of the University of Science and Technology hospital in Sana'a. Plasma samples were examined for TNF $\alpha$ , IFN $\gamma$ , and IL10 using the Quantikine<sup>®</sup> capture ELISA kits (R&D Systems, Minneapolis, MN, USA). Each sample was examined for the three cytokines on the same day to avoid repeated sample freezing and thawing. All assay procedures were performed according to the manufacturer's instructions and concentrations of cytokines were expressed as pg/ml.

### ***Statistical analysis***

Coded questionnaire and laboratory data were entered, verified and analyzed using Statistical Package for the Social Sciences (SPSS) software, version 23.0 (IBM SPSS Inc., IL, USA). Differences between continuous/interval variables were compared using parametric independent t-test or one-way ANOVA and the non-parametric Mann-Whitney or Kruskal Wallis tests whichever suitable. Differences were considered statistically significant at  $p \leq 0.05$ .

### 3. Results

#### *Characteristics of the study population*

A total of 80 individuals, 40 patients and 40 controls participated in the present study. Their demographic characteristics, parasitological and clinical indices are presented in table (1). Thirty-eight of the 40 patients had fever and 30 had paroxysms. None had complications. The median asexual parasite density of patients was 5,286 parasites/ $\mu\text{l}$  of blood with a range of 412-72,200 parasites/ $\mu\text{l}$ . It is observed that the majority of patients (65%) were of medium grade. Gametocytes were detected in five patients (12.5%).

**Table 1** Characteristics and parasitological indices of *P. falciparum* positive patients and controls

Variable	Patients (n=40)	Controls (n=40)
<b>Gender</b>		
Male	28 (70.0)	28 (70.0)
Female	12 (30.0)	12 (30.0)
<b>Age (years)</b>		
≤10	3 (7.5)	3 (7.5)
>10	37 (92.5)	37 (92.5)
Range	6-60	6-60
Mean±SD	31.23±17.05	32.7±13.63
<b>Residence</b>		
Rural	19 (47.5)	10 (25.0)
Urban	21 (52.5)	30 (75.0)
<b>Temperature (°C)</b>		
Range	36.6-40	36.3-37
Mean±SD	38.31±0.75	36.68±0.20
<b>Malaria history during the last year</b>		
Yes	19 (47.5)	0 (0.0)
No	21 (52.5)	40 (100.0)
<b>Antimalarial intake during the last year</b>		
Yes	14 (35.0)	0 (0.0)
No	26 (65.0)	40 (100.0)
<b>Presence of paroxysms</b>		
Yes	30 (75.0)	0 (0.0)
No	10 (25.0)	40 (100.0)
<b>Gametocytemia</b>		
Yes	5 (12.5)	NA
No	35 (87.5)	NA
<b>Parasite density/μl</b>		
Low (1-999)	4 (10)	NA
Medium (1000-9999)	26 (65)	NA
High (≥ 10000)	10 (25)	NA
Range	412-72,200	NA
Median ± IQR	5286±8924	NA

SD, standard deviation, IQR, interquartile range

### ***Hematologic parameters***

Table (2) presents the values of hematologic parameters among patients and controls. A significant difference was detected in the levels of Hb and RBC counts, lymphocytes and platelet counts which were lower in patients, while neutrophils and reticulocyte counts were significantly higher in patients than controls.

There was no significant difference in total WBCs, monocytes and eosinophil counts. None of the hematological parameters showed significant difference in relation to the grades of parasitemia (Table

3). Patients with gametocytemia had significantly lower Hb levels and RBC counts as well as significantly higher reticulocyte counts (Table 4).

**Table 2** Hematological parameters in falciparum malaria patients as compared to control subjects

Variable	Patients (n=40)	Controls (n=40)	<i>P value</i>
Hb (g/dl)*	11.61±2.10	13.08±1.18	<b>0.001<sup>a</sup></b>
RBC x 10 <sup>12</sup> /L*	4.41±0.85	4.77±0.51	<b>0.021<sup>a</sup></b>
TWBC x 10 <sup>9</sup> /L**	5.2 (3.9-7.0)	5.8 (5.0-6.5)	0.181 <sup>b</sup>
Neutrophils (%)*	57.20±17.41	48.90±11.05)	<b>0.013<sup>a</sup></b>
Lymphocytes (%)*	35.50±14.98	40.78±8.77	<b>0.001<sup>a</sup></b>
Monocytes (%)**	7.0 (5.0-10.0)	7.0 (4.2-8.7)	0.507 <sup>b</sup>
Eosinophils (%)**	3.0 (2.0-4.0)	3.5 (2.0-5.0)	0.281 <sup>b</sup>
Reticulocyte count (%)**	0.8 (0.60-1.75)	0.6 (0.50-0.70)	<b>0.006<sup>b</sup></b>
Platelet count x 10 <sup>9</sup> /l**	108 (86.2-167.7)	295.5 (262-331.5)	<b>0.000<sup>b</sup></b>

Hb; hemoglobin, RBC; red blood cell; TWBC; total white blood cell

\*Mean± standard deviation; \*\*Median (interquartile range)

<sup>a</sup> *P-value* was significant at ≤ 0.05 and calculated for Student's t-test

<sup>b</sup> *P-value* was significant at ≤ 0.05 and calculated for Mann-Whitney test

**Table 3** Hematological parameters in falciparum malaria patients in relation to parasitemia grades

Variable	Parasitemia grades			P value
	Low (n=4)	Medium (n=26)	High (n=10)	
Hb (g/dl)*	11.00±3.68	11.60±1.77	11.89±2.36	0.782 <sup>a</sup>
RBC x 10 <sup>12</sup> /L*	4.05±1.31	4.49±0.81	4.36±0.81	0.617 <sup>a</sup>
TWBC x 10 <sup>9</sup> /L**	3.7 (3.4-4.9)	5.2 (4.0-6.9)	5.6 (4.8-8.6)	0.162 <sup>b</sup>
Neutrophils (%)*	56.75±7.41	56.46±17.39	59.30±21.19	0.912 <sup>a</sup>
Lymphocytes (%)*	22.50±5.50	32.62±14.20	30.60±19.66	0.742 <sup>a</sup>
Monocytes (%)**	8.0 (7.0-19.5)	6.0 (5.0-10)	6.5 (4.75-10.5)	0.331 <sup>b</sup>
Eosinophils (%)**	5.0 (3.2-6.7)	3.0 (2.0-4.0)	3.0 (1.0-4.2)	0.128 <sup>b</sup>
Reticulocyte count (%)**	0.70 (0.50-2.9)	0.8 (0.60-2.7)	0.60 (0.50-1.07)	0.252 <sup>b</sup>
Platelet count x 10 <sup>9</sup> /l**	180.5 (90.7-226)	132 (86.7-186)	94.5 (67.2-114)	0.102 <sup>b</sup>

Hb; hemoglobin, RBC; red blood cell; TWBC; total white blood cell

\*Mean± standard deviation; \*\*Median (interquartile range)

<sup>a</sup> P-value was significant at ≤ 0.05 and calculated for one-way ANOVA test

<sup>b</sup> P-value was significant at ≤ 0.05 and calculated for Kruskal Wallis test

**Table 4** Hematological parameters in falciparum malaria patients in relation to gametocytemia

Variable	Gametocytemia		
	Yes (n=5)	No (n=35)	<i>P</i> value
Hb (g/dl)*	8.64±2.13	12.04±1.74	<b>0.000<sup>a</sup></b>
RBC x 10 <sup>12</sup> /L*	3.32±0.87	4.57±0.73	<b>0.001<sup>a</sup></b>
TWBC x 10 <sup>9</sup> /L**	4.9 (4.3-7.0)	5.2 (3.9-7.1)	0.967 <sup>b</sup>
Neutrophils (%)*	47.80±8.37	58.54±18.02	0.201 <sup>a</sup>
Lymphocytes (%)*	41.60±9.12	30.06±15.18	0.108 <sup>a</sup>
Monocytes (%)**	7.0 (5.5-9.5)	7.0 (5.0-10)	0.805 <sup>b</sup>
Eosinophils (%)**	3.0 (3.2-6.7)	3.0 (2.0-4.0)	0.884 <sup>b</sup>
Reticulocyte count (%)**	3.5 (2.4-3.7)	0.70 (0.50-0.90)	<b>0.001<sup>b</sup></b>
Platelet count x 10 <sup>9</sup> /l**	162 (98-314)	105 (86-164)	0.115 <sup>b</sup>

Hb; hemoglobin, RBC; red blood cell; TWBC; total white blood cell

\*Mean± standard deviation; \*\*Median (interquartile range)

<sup>a</sup> *P*-value was significant at ≤ 0.05 and calculated for Student's t-test

<sup>b</sup> *P*-value was significant at ≤ 0.05 and calculated for Mann-Whitney test

### ***Patterns of key cytokines***

The levels of key cytokines, TNFα, IFNγ, and IL10 were measured in *P. falciparum*-infected participants and compared to controls. There was a significant increase in the three cytokines in patients than in controls. The cytokine ratios, TNFα/ IL 10 and IFNγ/ IL 10 ratios were significantly lower in patients than controls, whereas TNFα/ IFNγ ratio was not significantly different (Table 5).

Regarding parasite density, significantly high TNFα and IL10 levels were found in patients with high parasite density. IFNγ level did not show significant difference in relation to the grade of infection. The ratios TNFα/ IL 10, IFNγ/ IL 10, and TNFα/ IFNγ were not significantly different in infected participants in relation to parasitemia grades (Table 6). Among the three studied cytokines, only IFNγ was significantly low in patients with gametocytemia (Table 7).

**Table 5** Key cytokine levels and ratios in falciparum malaria patients as compared to control subjects

*Key cytokines and ratios	Patients (n=40)	Controls (n=40)	<i>P</i> ** value
TNF $\alpha$	44.95 (25.3-86.7)	2.5 (1.0-3.6)	<b>0.000</b>
IFN $\gamma$	43 (15.7 – 105.4)	1.05 (0.6-1.5)	<b>0.000</b>
IL 10	222.3 (126.7 – 488.7)	1.9 (1.0-3.0)	<b>0.000</b>
TNF $\alpha$ / IL 10	0.17 (0.1-0.4)	1.1 (0.55-2.6)	<b>0.000</b>
IFN $\gamma$ / IL 10	0.15 (0.05 – 0.28)	0.55 (0.39-1.0)	<b>0.000</b>
TNF $\alpha$ / IFN $\gamma$	1.03 (0.47 – 4.0)	2.5(0.95-3.4)	0.073

TNF $\alpha$ , tumor necrosis factor; IFN $\gamma$ , interferon-gamma; and IL10, interleukin-10

\*Median (interquartile range)

\*\**P*-value was significant at  $\leq 0.05$  and calculated for Mann-Whitney test

**Table 6** Key cytokine levels and ratios in falciparum malaria patients in relation to the grade of parasitemia

*Key cytokines and ratios	Parasitemia grade			<i>P</i> ** value
	Low (n=4)	Medium (n=26)	High (n=10)	
TNF $\alpha$ (pg/ml)	21.6 (10.6-231)	42.9 (22.8-125)	54.6 (42.4-97.2)	<b>0.033</b>
Sig. between p. grades	<i>p</i> 1=0.096, <i>p</i> 2= <b>0.028</b> , <i>p</i> 3=0.890			
IFN $\gamma$ (pg/ml)	10.3 (8.7-35.2)	35.5 (10-64.2)	93.3 (31.3-227)	0.188
IL 10 (pg/ml)	100.2 (49.2-148)	216.4 (132-333)	1007 (269-1833)	<b>0.001</b>
Sig. between p. grades	<i>p</i> 1=0.119, <i>p</i> 2= <b>0.001</b> , <i>p</i> 3= <b>0.025</b>			
TNF $\alpha$ / IL 10	0.2 (0.18-0.22)	0.2 (0.11-0.44)	0.06 (0.03-0.33)	0.136
IFN $\gamma$ / IL 10	1.0 (0.16-2.9)	0.14 (0.04-0.27)	0.11 (0.06-0.25)	0.230
TNF $\alpha$ / IFN $\gamma$	0.6 (0.06-1.3)	1.3 (0.61-7.9)	0.76 (0.27-2.7)	0.177

TNF $\alpha$ , tumor necrosis factor; IFN $\gamma$ , interferon-gamma; and IL10, interleukin-10

\*Median (interquartile range)

\*\**P*-value was significant at  $\leq 0.05$  and calculated for Kruskal Wallis test

Sig. between parasitemia grades was done using pairwise Post Hoc test

*p*1: *P*-value for comparing low and medium parasitemia grades

*p*2: *P*-value for comparing low and high parasitemia grades

*p*3: *P*-value for comparing medium and high parasitemia grades

**Table 7** Key cytokine levels and ratios in falciparum malaria patients in relation to gametocytemia

* Key cytokines and ratios	Gametocytemia		<i>P</i> ** value
	Present (n=5)	Absent (n=35)	
TNF $\alpha$ (pg/ml)	52 (14.1-275.9)	44.6 (23.6-75.3)	0.951
IFN $\gamma$ (pg/ml)	7.2 (3.6-24.9)	52.7 (19.4-126)	0.007
IL 10 (pg/ml)	192.3 (112-292)	227.6 (123-709)	0.0526
TNF $\alpha$ / IL 10	0.27 (0.16-0.98)	0.14 (0.06-0.39)	0.212
IFN $\gamma$ / IL 10	0.08 (0.01-0.18)	0.15 (0.06-0.28)	0.158
TNF $\alpha$ / IFN $\gamma$	3.3 (0.8-89)	0.9 (0.43-2.5)	0.098

TNF $\alpha$ , tumor necrosis factor; IFN $\gamma$ , interferon-gamma; and IL10, interleukin-10

\*Median (interquartile range)

\*\**P*-value was significant at  $\leq 0.05$  and calculated for Mann-Whitney test

## 4. Discussion

Malaria is endemic in Yemen with *P. falciparum* being the predominant species. The current study was conducted on forty patients with *P. falciparum* monoinfection and forty healthy age and sex-matched individuals as controls. None of the patients had complications and most of them had a medium parasitemia grade.

The current study revealed that the mean Hb concentration and RBC counts were significantly lower in patients than in controls. This is in agreement with previous studies (Erhabor et al., 2014; Sakzabre et al., 2020). In endemic areas, anemic individuals are more likely to be malaria positive than the non-anemic counterparts (Nlinwe and Nange, 2020). The etiology of anemia in malaria is known to be multifactorial, and due to hemolysis of RBCs, their autoimmune destruction and accelerated removal, depressed erythropoiesis, and splenic phagocytosis or pooling (Bashawri et al., 2002). Removal of non-parasitized RBCs was explained as the most important mechanism of anemia (Imoru et al., 2013).

Considering the leucocytes, there was a significant increase in neutrophils and a significant decrease of lymphocytes in patients with no difference in total WBCs, monocytes, and eosinophils. Malaria-related neutrophilia and/or lymphocytopenia were reported in several studies (Maina et al., 2010; Kotepui et al., 2014; Al-Salahy et al., 2016). Neutrophilia is related to an acute inflammatory response and an early release of neutrophils from the bone marrow in response to infection (Al-Salahy et al., 2016). The decrease in lymphocytes was explained to be due to their sequestration in the spleen (Wickramasinghe and Abdalla, 2000). Unlike the reduction in RBC counts, the changes in WBC total and differential counts were inconsistently reported in malaria patients. Total WBC counts are generally low to normal during malaria, this is widely thought to reflect their localization away from the peripheral circulation (McKenzie et al., 2005). Contrary to the present study findings, leukocytosis, neutropenia, monocytosis were observed in some patients (Maina et al., 2010; Sakzabre et al., 2020). Lymphocytosis was associated with falciparum malaria mortality among children (Ladhani et al., 2002). Increased eosinophil counts were reported in children with asymptomatic falciparum malaria, but low counts and increased eosinophil activity were found in cerebral malaria (Dunyo et al., 1998). A previous study linked the changes in differential WBC counts to the level of host immunity with non-immune patients displaying pronounced changes (Berens-Riha *et al.*, 2014). In the present study, the non-significant changes in monocytes and eosinophils might be attributed to the patients being of uncomplicated malaria.

Regarding reticulocyte counts, this parameter is widely used to evaluate bone marrow erythropoietic activity and it is essential with diagnosis and prognosis of anemia (Peebles et al., 1981). In the present study, although the median reticulocyte count was within the normal range, the count was significantly higher in patients than controls. These findings are in contrast with previous studies (Roberts *et al.*, 2005). Low reticulocyte counts were attributed to the inhibition of erythropoiesis by the malaria parasites and their products (Dormer et al., 1983). It was postulated that sinusoidal obstruction by parasitized RBCs together with kidney involvement may lead to bone marrow hypoxia and consequently to low reticulocyte counts (Abdalla, 1990).

In the present study, platelet counts in parasitized patients were significantly reduced compared to controls. Thrombocytopenia was reported in many previous studies (Erhabor et al., 2014; Sakzabre et al., 2020; Omarine Nlinwe and Nange, 2020). This was explained to be due to sequestration and pooling of the platelets in the spleen as well as to their immune-mediated destruction and coagulation disturbances. There is increasing advocacy for including thrombocytopenia as a severe malaria criterion (Tanwar et al., 2012).

Regarding parasite density in the present study, parasitemia grade was medium in most patients, high in 25% of them and low in 10%. There was no relation between parasitemia grades and any of the hematological parameters. Although this finding is in line with previous reports (Nwanjo and Opara, 2005; Maina et al., 2010), others reported that the alterations of one or more parameters were more obvious in patients with high parasitemia (Leowattana et al., 2008, Erhabor et al., 2014, Kotepui et al., 2015). Discrepant findings may be attributed to the effect of several confounding factors such as malnutrition,

duration of infection, and the degree of adaptive immunity (Berens-Riha *et al.*, 2014; Kotepui *et al.*, 2015; Sakzabre *et al.*, 2020).

Gametocytogenesis may be simply programmed to occur after a number of cycles of asexual replication. Yet it has been suggested that gametocyte production may be influenced by physiological changes that accompany malaria infection as well as by hemolysis of RBCs and specific antibodies (Sinden, 1983). In the present study, low mean values of Hb and RBCs and high reticulocyte counts were observed in the presence of gametocytes.

Regarding the pattern of the key cytokines in *P. falciparum* infection, there was a significant elevation in the levels of TNF $\alpha$ , IFN $\gamma$  as well as IL10 in patients compared to controls. Immune response to *P. falciparum* infection is mediated by the production of pro-inflammatory cytokines and chemokines, followed by the production of anti-inflammatory cytokines (Lyke *et al.*, 2004). Data from animal models have indicated that the pro-inflammatory cytokines are essential to parasite clearance but must be regulated at the appropriate point to prevent pathology (Dodoo *et al.*, 2002). High levels of the pro-inflammatory cytokines TNF $\alpha$  and IFN $\gamma$  have been associated with severe pathology, while low levels of regulatory cytokines such as IL10 were associated with acute malaria (Couper *et al.*, 2008). The balance between the anti-parasitic and the immunopathogenic effects of cytokines is a mark of clinical immunity to malaria (Pawar, 2014).

TNF $\alpha$  is the most famous pro-inflammatory cytokine marker of severe malaria (Armah *et al.*, 2005). In the present study, although its level was high in patients, its effect was probably controlled by the consequent increase of IL10 which led to alleviation of the severity of the disease. TNF $\alpha$  in the present study correlated with the degree of parasitemia. This is in agreement with other studies (Day *et al.*, 1999; Medina *et al.*, 2011).

The early production of IFN $\gamma$  from dendritic cells or monocytes, appears to be a pivotal component of the pro-inflammatory responses leading to upregulation of TNF $\alpha$ . IFN $\gamma$  was reported as an important determinant of the wellbeing of the patients being generally associated with protective mechanisms (Mbengue *et al.*, 2016). In the present study, there was no relation between IFN $\gamma$  and the parasitemia grades, while its level was significantly higher in the absence of gametocytes. Although gametocytes do not cause any clinical manifestation in malaria, it has been shown that immunity against their antigens is elicited early and could reduce the number of gametocytes achieving maturity in the peripheral blood. Late gametocyte immunity may affect their number and infectivity (de Jong *et al.*, 2020). In splenectomized macaques infected with *Plasmodium cynomolgi*, inflammatory cytokines were found to enhance gametocyte destruction through the production of toxic nitric oxides at the peak of infection (Naotunne *et al.*, 1991, 1993).

As to IL10, it is known to down-regulate anti-inflammatory cytokines preventing detrimental immune reactions. In the present study, the level of IL10 was significantly higher in patients than controls and its level was positively related to the parasitemia grades. Yet, there was no relation with the presence of

gametocytes. This is in agreement with previous results (Medina et al., 2011; Goncalves et al., 2012; Moncunill et al., 2013).

Balanced pro-and anti-inflammatory cytokines play a pivotal role in the regulation of malaria. The severity of malaria is related to the balance between IL10 and TNF $\alpha$ -concentration (Othoro et al., 1999). It has been found that the ratios IL10/TNF $\alpha$  and IL10/IFN $\gamma$  returned to the normal range after chemotherapy as found in controls (Goncalves et al., 2012). In the present study, both TNF $\alpha$ /IL10 and IFN $\gamma$ /IL10 were significantly lower in patients than controls, while the ratio TNF $\alpha$  /IFN $\gamma$  was similar to that in controls. This denotes a relatively higher IL10 production in the patients.

All cytokine ratios did not show a relation with parasitemia grade nor with the presence of gametocytes. These findings are supported by some previous reports while they disagree with others (Goncalves et al., 2012; Mbengue et al., 2016).

In conclusion, the main hematological findings in patients with uncomplicated malaria in Hodeidah are low Hb level, low RBC, lymphocyte and platelet counts, and high neutrophil and reticulocyte counts. Individuals displaying such hematological changes should undergo malaria testing. Clinical immunity to malaria is characterized by upregulated levels of the pro-inflammatory cytokines, IFN $\gamma$  and TNF $\alpha$  and the anti-inflammatory cytokine, IL10 with a relatively higher production of the latter. This pattern of cytokine regulation probably mediates parasite clearance while simultaneously avoiding severe pathology.

## Declarations

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