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Full Length Article

Alterations in T-helper cell type 1 and blood cell parameters in malaria-infected patients

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ABSTRACT

Malaria is a global public health disease. Haematological and cytokine alterations are the major sources of its pathological conditions. Therefore, blood and serum of patients attending health centres were screened to investigate the effects of *Plasmodium falciparum* on the T-helper cell type 1 and blood cell parameters using Enzyme linked immunosorbent assay and automatic hematology analyzer respectively. Approximately 55% of malaria-infected patients with average parasitaemia of 2523.64 parasite/μl of blood concurrently suffered anaemia, thrombocytopenia, leucopenia, microcytosis and hypochromasia. However, thrombocytopenia and leucopenia were age-specific and their prevalences in children within ≤10 years were higher. These disease conditions significantly vary with severity of malaria infection ($p < 0.05$). Blood parameters with the exception of RBC and MCHC were significantly lower in the infected patients ($p < 0.05$) with 12.9% and 41.2% reduction in haemoglobin and platelet counts respectively. A high plasma concentration of IL-10, IL-12, INF-γ and TNF-α, ratio of IL-10/TNF-α (1.86) and IL-10/INF-γ (1.55) were recorded among the malaria-infected groups. This study revealed that unregulated interaction of the parasite with host immune response has important consequences in disease progression and thus relevant for therapeutic and vaccine development.

1. Introduction

Malaria is still a life-threatening parasitic disease in many countries worldwide, causing between 80 and90% morbidity and mortality in sub-Saharan Africa [1,2]. In this region, *Plasmodium falciparum* and *P. vivax* are common human malaria parasites and the prognosis is severe [3]. The activities of their erythrocytic asexual stages in asymptomatic and symptomatic patients are important foci in the pathogenesis [4,5]. Infections in less immuned individuals, especially young children and pregnant women are always severe, resulting in anaemia, thrombocytopenia, leucopenia and other haematological disorders [4]. Most infected individuals are incapacitated for days due to recurrent clinical episodes attributed to the release of malaria pigment (hemozoin) and other toxic factors such as glycosylphosphatidylinositol (GPI) when the infected erythrocytes rupture [5].

Previous studies [6,7] showed that *P. falciparum*, upon invasion of red blood cell, interferes with rigidity and deformability properties of the cell to permit the direct exchange of metabolites. Consequently, inflammatory cytokines (TNF-α, IL-1, IL-10 and IFN-γ), cascade coagulation and sequestration of parasitized RBCs increase, and thus the manifestations of malaria infection ensued [8–10].

However, a steady balance between pro- and anti-inflammatory responses is obligatory to elicit anti-parasitic activities and enhance parasite clearance; otherwise an uncontrolled production of pro-inflammatory cytokines, such as IL-6, TNF and IFN-γ may lead to severe malaria syndromes thereby suppressing T-cell response [11,12]. The resulting protective Th1-dependent immune responses to blood-stage malaria infection are mediated by IFN-γ and TNF-α to optimize nitric oxide production [13,14].

Report [15] has shown that infections due to *P. falciparum* result in progressive structural, biochemical, and mechanical modifications of the host cells that lead to life-threatening complications. Information on the mechanism of alteration in infected host cell required in predicting the severity of malaria is limited. This study therefore investigated the effect of *P. falciparum* on the type 1 T-helper cell and blood cell parameter amongst the malaria infected patients in Ilorin, Kwara state, Nigeria for better understanding of

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host-parasite interaction which can be exploited in the effective management of malaria infection.

2. Materials and methods

2.1. Study design, sample and data collection

The blood and serum of patients attending health centres in Ilorin, Kwara State (located at Lat. 8° 30′N and Long. 8° 50′N and Long. 4° 20′E and 4° 35′E), Nigeria were used for this study. Thick and thin blood films were prepared and stained with Giemsa and *P. falciparum* was identified under oil immersion microscope. The estimation of parasitaemia was done by counting the number of parasites per 200 white blood cells (WBC) on blood films. Complete blood cell counts was conducted using an automatic hematology analyzer (Pentra ABX) and peripheral blood smears were performed for routine differential blood cellular quantification.

In accordance with manufacturer's recommendations, the levels of TNF-α, IL-I0 and IL-12 in the serum were determined using Enzyme linked immunosorbent assay, (ELISA) Kit procured from Abcam, Cambridge, United Kingdom. The test kits were sensitive detecting cytokines levels at less than 0.8 pg/ml and cross reactivity was not observed for any other proteins tested.

2.2. Ethical clearance and procedure for data collection

The study protocol was approved by University of Ilorin ethical review committee and the Medical and Information Management units of selected health centres in Ilorin, Kwara state. The approval detail of the study was contained in the letter with reference number KW/REF/GT0126.

2.3. Statistical analysis

Statistical analysis was performed with SPSS version 20; (SPS Inc., Chicago IL). Descriptive statistics were expressed as means and standard deviations while the student's t-test and Chi square analysis were applied to determine the differences in haematological parameters and cytokines levels. Comparison of associations between the haematological parameters and parasite density were assessed using the Pearson's linear regression. The level of statistical difference was set at $p < 0.05$.

3. Results

Of the total 223 out-patients (100 males and 123 females) with average age of 21.41 years enrolled in the study, 55.2% was infected with *Plasmodium falciparum* with average parasitaemia of 2523.64 parasite/μl of blood (Table 1). Approximately, 25%, 26% and 5% of malaria-infected individuals had low, moderate and severe infection respectively. Similarly, 55.2% and 41.7% of infected patient were anaemic and thrombocytopenic respectively. Table 2 shows that the prevalences of haematological disorder among the malaria-infected patients was not sex related ($P > 0.05$) but thrombocytopenia and leucopenia were age –specific with high prevalences in children within age-group of $\leq$10 years. However, these disease conditions significantly vary with degree of malaria infection ($p < 0.05$). For instance, 12.9% and 41.2% reduction were observed in haemoglobin and platelet counts among infected participants.

Table 3 revealed that lower values of red blood cell, packed cell volume, haemoglobin and differential counts of neutrophil, platelets and white blood cells were recorded among malaria patients. High plasma concentrations of IL-10, IL-12, INF-γ and TNF-α, also ratio of IL-10/TNF-α (1.86) and IL-10/INF-γ (1.55) were obtained among the infected patients (Table 3). Further analysis indicated that haematological indices significantly decreased ($p < 0.05$) whereas immunological indices increased with increasing parasitaemia (Table 4).

Table 1
Demographical and clinical characteristics of study population.

	N (%)
Sex (Male/Female)	100/123
Mean Age + Std.dev.	21.41 ± 10.27
Number infected with malaria	123(55.2)
Mean intensity + Std.dev	2523.64 ± 4.47
Degree of malaria infection	
No Infection	100(44.8)
Low	55(24.7)
Moderate	57(25.6)
Severe	11(4.9)
Anaemia	
Malaria patient	123(55.2)
Uninfected patient	7(3.1)
Thrombocytopenia	
Malaria patient	93(41.7)
Uninfected patient	21(9.4)

4. Discussion

Pronounced haematological and cytokine alterations are trademarks in malaria infection and other infectious diseases [4,13,14]. The current study confirms that anaemia, thrombocytopenia, hypochromasia and leucopenia were common disorders in malaria infected patients. This is similar to reports in many malaria-endemic settings worldwide [16–19]. However, the relative severity of the haematological disorders varied, similar in infected male and female but higher in young children. This observed age and sex relative pattern of morbidity is a common characteristic in endemic areas in Africa [20,21]. The severity of these disorders is observed to be increasing with parasite density in infected individuals except in the case of hypochromasia which is more prevalent among individual with low parasite density. This observation is concordant with reports of Erhart et al. [22] and Saravu et al. [23].

Anaemia is a common clinical indicator of severe malaria in children [24,25], a phenomenon that follows inhibition of erythropoiesis and massive destruction of both parasitized and non-parasitized red blood cells. The low mean haemoglobin concentration (Hb) and packed cell volume (PCV) observed in our infected patient indicate anaemic condition. This is similar to many reports [26–28]. The reduction in Hb and PCV increases with the intensity of infection in malaria [29]. Thus, individual with severe malaria are more susceptible to severe anaemia.

Thrombocytopenia and platelet dysfunction are another important pathological conditions observed in 76.4% malaria infected group in this study. This agrees with previous findings [30–33]. Other studies [34,35] reported that 95% of falciparum malaria patient had thrombocytopenia. The mechanism of this disease condition has been well described [36–38]. The authors postulated that immune mediated lysis, sequestration in the spleen and a dyspoietic process in the bone marrow diminish platelet production which may probably lead to reduced platelets subpopulation in the circulation. However, the pathogenic mechanisms by which platelets mediate disease severity in patients with falciparum malaria remain to be delineated. Previous study [39] revealed that platelets in patients with *P. falciparum* expressed Toll-like receptors (TLRs), which release prepackaged inflammatory mediators such as Nitric oxide (NO), a key mediator of platelet homeostasis. A decreased bioavailability of NO in patients with severe malaria may contribute to increased platelet activation and consumption

Table 2
Prevalence of haematological disorders with respect to age, sex and infection status of the malaria infected participants.

		Haematological conditions				
Variables	No. examined	Anaemia (%)	Thrombocytopenia (%)	Leucopenia (%)	Microcytosis (%)	Hypochromasia (%)
Overall	123	100(81.3)	94(76.4)	93(75.6)	26 (21.1)	56 (45.5)
Sex						
Male	55	44 (80.0)	42 (76.4)	44(80.0)	12 (21.8)	22(40.0)
Female	68	56 (82.4)	52 (76.4)	49(72.1)	14 (20.6)	34(50.0)
P. value		0.739	0.350	0.308	0.818	0.517
Age group (years)						
≤10	23	21 (91.3)	20 (86.9)	20(86.9)	4 (17.4)	11(47.8)
11–20	25	19 (76.0)	16 (64.0)	19 (76.0)	7 (28.0)	10 (40.0)
21–30	59	49 (83.1)	46 (77.9)	14(23.7)	11 (18.6)	27 (45.8)
≥31	16	11 (68.8)	12 (75.0)	7(43.8)	4 (25.0)	8 (50.0)
P. value		0.289	0.005	0.028	0.791	0.531
Malaria infection						
Low	55	38 (69.1)	42 (76.4)	37(67.3)	9 (16.4)	29 (52.7)
Moderate	57	54 (94.7)	44 (77.2)	46(80.7)	16 (28.1)	23(40.4)
Severe	11	8 (72.7)	8 (72.7)	10(90.9)	1 (9.1)	4 (36.4)
P. value		0.002	0.002	0.018	0.174	0.047

Table 3
Comparison of haematological and immunological indices among infected and uninfected individuals in the study populations.

	Uninfected patients		Infected patients		P. value
	Mean ± Std.dev.	95% CI	Mean ± Std.dev.	95% CI	
Hematological indices					
RBC (10^3/μl)	3.68 ± 0.88	3.17–3.79	3.46 ± 0.63	3.55–3.77	0.724
Haemoglobin (g/dl)	14.44 ± 0.63	10.22–15.66	9.09 ± 1.54	6.82–9.37	<0.001
PCV (%)	33.96 ± 4.92	32.22–35.71	26.45 ± 4.07	25.73–27.18	<0.001
MCHC (g/dl)	32.53 ± 2.55	31.63–33.44	32.17 ± 5.37	31.22–33.13	0.712
MCV (pg)	89.83 ± 15.20	84.44–95.22	71.90 ± 8.03	70.46–73.33	<0.001
MCH (pg)	31.01 ± 4.66	29.36–32.66	26.81 ± 2.85	26.29–27.31	<0.001
WBC (10^3/μl)	3.82 ± 0.69	36.09–41.75	3.21 ± 0.85	31.18–34.13	0.007
Neutrophil (10^3/μl)	2.03 ± 0.42	19.11–22.77	1.21 ± 0.57	11.41–13.48	<0.001
Basophil (10^3/μl)	0.02 ± 0.003	22.25–23.18	0.04 ± 0.02	38.63–44.88	<0.001
Eosinophil (10^3/μl)	1.43 ± 0.18	14.66–15.56	3.26 ± 0.51	30.33–35.15	<0.001
Platelet count (10^3/μl)	80.27 ± 10.07	76.70–83.84	47.16 ± 26.31	42.46–51.85	<0.001
Immunological indices					
INF-γ (pg/ml)	41.43 ± 9.81	39.27–4.32	51.04 ± 8.57	43.63–54.74	<0.001
TNF-α (pg/ml)	27.65 ± 8.28	26.10–2.91	42.37 ± 5.52	40.18–44.35	<0.001
IL-10 (pg/ml)	25.18 ± 13.18	27.32–3.10	79.01 ± 10.07	20.19–30.17	0.278
IL-12 (pg/ml)	10.82 ± 10.13	6.07–11.21	23.11 ± 0.20	20.02–20.70	0.282
IL-10/TNF-α ratio	0.91 ± 1.52	0.32–0.96	1.86 ± 1.31	0.31–2.12	0.001
IL-10/INF-γ ratio	0.61 ± 0.84	0.47–0.80	1.55 ± 0.26	0.61–1.98	0.001

Table 4
Variation of haematological and immunological indices with intensity of infection.

	Intensity of infection			P. value
	Low	Moderate	Severe	
Haematological indices				
RBC (10^3/μl)	3.77 ± 0.64	3.63 ± 0.63	3.31 ± 0.45	0.208
Haemoglobin (g/dl)	9.46 ± 1.49	8.98 ± 1.44	7.7 ± 1.49	<0.001
PCV (%)	27.78 ± 4.47	25.74 ± 5.19	25.30 ± 2.99	<0.001
MCHC (g/dl)	31.59 ± 6.27	32.32 ± 4.70	34.37 ± 2.93	0.416
MCV	72.01 ± 5.62	71.05 ± 9.16	75.70 ± 11.28	<0.001
MCH (pg)	27.30 ± 3.20	26.66 ± 2.31	24.96 ± 2.80	<0.001
WBC (10^3/μl)	3.45 ± 0.85	2.99 ± 0.86	3.21 ± 0.48	<0.001
Neutrophil (10^3/μl)	1.12 ± 0.53	1.22 ± 0.58	1.67 ± 0.46	<0.001
Basophil (10^3/μl)	0.05 ± 0.001	0.04 ± 0.001	0.01 ± 0.002	<0.001
Eosinophil (10^3/μl)	3.50 ± 1.33	3.41 ± 1.62	1.52 ± 0.62	<0.001
Platelet count (10^3/μl)	51.00 ± 19.70	47.18 ± 26.42	46.36 ± 27.65	<0.001
Immunological indices				
INF-α (pg/mL)	43.5 ± 15.07	49.04 ± 19.31	52.92 ± 12.22	<0.001
TNF-γ (pg/mL)	40.3 ± 11.28	42.35 ± 17.63	42.71 ± 18.84	<0.001
IL-10 (pg/mL)	48.93 ± 17.39	69.37 ± 13.90	80.60 ± 23.18	0.008
IL-12 (pg/mL)	20.5 ± 21.08	30.81 ± 14.93	27.61 ± 13.09	<0.001
IL-10/TNF-α	1.21 ± 0.05	1.64 ± 0.56	1.89 ± 0.92	0.061
IL-10/INF-γ	1.12 ± 0.23	1.41 ± 0.24	1.52 ± 0.73	0.024

[39–41]. The trend of decreasing platelets with increasing levels of parasitaemia observed in this study has been previously noted for malaria infection due to *P. falciparum* [22,23].

We further observed low value of differential WBC counts among infected malaria patient as compared to uninfected ones. This finding is in direct contrast with report of Ladhani et al. [42] but consistent with many studies [30,22,43]. The association of *P. falciparum* malaria to low WBC count has been suggested to be attributable to sequestration and accelerated destruction of leukocytes [44]. Further studies have shown that the production of proinflammatory cytokines by parasite immunogenic antigen (Gly cosylphosphatidylinositol) increase phagocytosis of RBC and WBC, thus causing TNF-α, causing altered hemopoiesis [45,46].

Cytokines are considered to act as double-edged swords in the pathogenesis of malaria, especially in *P. falciparum* infections [47,48]. The high levels of innate immune response in malaria infection is driven by malarial pigment (hemozoin, Hz), and glycosylphosphatidylinositols (GPIs) in the circulating monocytes and neutrophils, and resident macrophages [49,50,44]. In this study, plasma concentrations of TNF-α, IFN-γ, IL-10 and IL-12 increased with increasing parasite density and it is in accordance with the report of Mugweru and his colleagues [51]. However, T-helper cell type 1 cytokines is essential to achieve elimination of infection with less damage to the host. Walther et al. [52] noted that expression of these proinflammatory cytokines may have far reaching consequences on disease outcome such as fever, paroxysms, anaemia, cerebral malaria and many other systemic infection symptoms [51,53–55]. It has been postulated that induction of TNF-α delocalizes ferroportin in the reticuloendothelial system that mediates stimulation of macrophage and intestinal iron absorption. This, however, decreases iron absorption and enhances the release of hepcidin to the circulation, which its increased serum level is often associated with severe malaria anaemia [56,57]. Development of Th1 response can be antagonized indirectly by IL-10, which inhibits the production of proinflammatory cytokines. IL-10 induces B-cell proliferation. The inhibition of interferon and TNF-α secretion by IL-10 synthesis has been reported to be important in counteracting the pathological role of macrophages in cerebral malaria [58]. This accounted for high level of this cytokine and its relative smaller ratio with TNF-α in the infected group and it is consistent with many other studies [59,12]. Much consistent evidence based on experimental models and human *P. falciparum* infection suggests increased IL-10 levels may favor parasite multiplication by inhibiting parasite-killing effector mechanisms in humans and mice [60,61].

In conclusion, outcome of this study underscores haematological alterations as the hallmark pathological conditions of malaria infection and their significance in the prediction of severe infection. It also revealed that unregulated interaction of the parasite with host immune response can have important consequences for disease progression. Therefore, it becomes pertinent to incorporate findings of this study in the on-going malaria vaccine and effective therapeutic development.

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