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Interleukin 8 and Transforming Growth Factor-Beta in Patients With Malaria in Lagos State, Nigeria.

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ABSTRACT

INTRODUCTION

The interaction between pro- and anti-inflammatory cytokines such as interleukin-8 (IL-8) and transforming growth factor beta (TGF-β) plays an important role in malaria pathogenesis and outcome. TGFβ, produced by a wide range of cells, has a pivotal role in the control of the transition between pro-

inflammatory (Th1-type) and anti-inflammatory (Th2-type) response during the acute and resolving phases of malaria infection. The role of IL-8 in *P. falciparum* malaria is unknown although studies indicate its likely use as a biomarker of intensity of malaria. The aim of this study was to measure the plasma levels of IL-8 and TGF- βin 136 individuals with malaria and correlate the production of these cytokines with the severity of the disease

METHOD

IL-8 and TGF- βlevels were determined using enzyme-linked immunosorbent assay. The severity of malaria was established by parasitemia, clinical symptoms and haematological parameters.

RESULTS

Patients with malaria had a mean IL-8 level of 508.8±755.1pg/mlcompared to controls who had no detectable levels of plasma IL-8.The difference in the mean between both groups was statistically significant ($p<0.05$). The level of parasitemia among patients with high IL-8 concentration and low IL-8 concentrations given as 21,097±24,340.4parasites/µL and 43,200±75,819.1parasites/µL, respectively, was observed and the difference in the mean levels of parasitemia between high and low chemokine levels was statistically significant ($p<0.05$). Amongst patients with low levels of TGF-β mean packed cell volume were within normal average ranges (34.55±8.0%) whereas patients with high TGF-β levels had mean packed cell volume below normal

average ranges (29.20±12.5%). This difference in mean packed cell volumes between high and low TGF-β levels was observed to be statistically significant ($p<0.05$).The relationship between TGF-β levels and packed cell volume was negatively correlated ($r = -0.27$).

CONCLUSION

These findings suggest that fine mechanisms regulate the interaction between TGF-β and IL-8 in the immune response to *Plasmodium falciparum* infection, seemingly directing*in vivo* modulations in red cell population, andindicating a likely pointer to malaria disease and levels of parasitemia.

Keywords: Interleukin-8 (IL-8); Transforming growth factor beta (TGF-β); *Plasmodium falciparum*

INTRODUCTION

Half the world's population of about 3.4 billion people are at risk of malaria. Current figures estimate that about 135-287 million people suffer from malaria and 0.5-0.8 million die annually[1]. In tropical countries especially sub-Saharan Africa, malaria wields great influence on human health where it poses the greatest impact of morbidity and mortality among infectious diseases[2]. In this region of the world severe malaria anemia happens to be a frequent complication of *Plasmodium falciparum* infections in young children and is one of the main causes of severe anemia, with a case-fatality rate reaching 23% in malaria

holoendemic areas[3]. In a country like Nigeria which still remains one of the two countries accounting for 40% of global malaria deaths[1] (WHO, 2013), there still remains a high prevalence of anemia and malaria parasitemia in the general population especially in malaria endemic areas[4].

Malaria is highly endemic in Lagos state posing a major challenge to the state as it impedes human development. The cosmopolitan nature of the state, coupled with the abundant distribution of coastal areas, encourages the development of stagnant water which fosters the breeding of insect vectors and the rise in malaria[5]. The Ikorodu area currently has a prevalence rate of 13% compared to 0.5% prevalence on the Lagos Island[6].

Complications of severe anemia and cerebral malaria are the major cause of morbidity and mortality, although evidence suggests that the host's immunological response plays a vital role in the pathophysiology of this disease in humans[7, 8]. Immunity to malaria is dependent on both the innate and adaptive arms (both cell- and antibody mediated) of the immune system, which are required for adequate protection[9]. Regulation of the host-immune response to invading pathogens depends largely on the development of acquired immunity mediated by pro- and anti-inflammatory cytokines[10].The pathogenesis of the complications of anaemia and parasitemia in *P. falciparum* infections appears to involve a dysregulation of this

immune system[11]. The balance between pro- and anti-inflammatory cytokines and the pathogenic effects that result from dysregulation still however remain poorly understood[12].

The pathogenesis of malaria is a complex process in which a common outcome might be reached by different routes[11]. This idea emphasizes the relevance of diagnostic and prognostic parameters in predicting the specific risks associated with different clinical characteristics. Of relevance to diagnosis and prognosis is the perception that specific clinical conditions might have distinguishable immunological features. It has been suggested that a marked imbalance in cytokines found in serum might be used as a marker of progression to fatal outcome[13].

The pathology associated with malaria, being immune mediated and capable of leading to adverse systemic effects, further engenders the quest in the determination of the roles and relationships of associated cytokines which may be linked with heightened disease severity during the presentation of various malaria parasites stages[14].

The goal of improving malaria therapy and furthering advancements in the design of effective vaccines may thus be achieved with a better understanding of the workings of these cytokines produced *in vivo* during parasite killing. Attention has also focused on blocking cytokines which are harmful to the host, particularly during overwhelming infection.

Interleukin-8 (IL-8) belongs to a class of cytokines referred to as chemokines. Chemokines are small pro-inflammatory peptides (8 to 17 KDa) which play important roles in bridging the innate and adaptive immune systems[15] (Luster, 2002). IL-8 is secreted by many cells including monocytes, macrophages, and endothelial cells. It is chemotactic for T cells and is a chemoattractant and an activator of neutrophils, facilitating the passage of these leukocytes and other cells from the circulation into the tissues[16, 17]. They do this by activating corresponding receptors on responsive cells, thereby inducing chemotaxis of immune cells to sites of infection; activated neutrophils in tissues degranulate and cause tissue damage.

Several studies implicate IL-8 among other cytokines in the pathogenesis of severe malaria cases compared to uncomplicated and matched healthy controls[12, 18]. Some chemokines, including IL-8 have also been identified as biomarkers of cerebral malaria mortality in Ghanaian children[19]. Furthermore, reports from Thailand have revealed elevated IL-8 levels in falciparum-infected patients[16]. IL-8 serum concentration among patients suffering from severe *P. falciparum* malaria have been shown to be highest at a time when no parasite was detected in the blood smear[20], indicating a continued production of bioactive IL-8 during and after clinical recovery from *P. falciparum* infection, which may suggest that this cytokine is involved as well in the healing process[16]. There is however still a

dearth of *in vivo* data on IL-8 in clinical infections in our locality.[18]

Transforming growth factor (TGF-β) is a multifunctional growth factor peptide reported to be involved in many physiological and pathological processes such as vascular remodeling and atherogenesis[21]. TGF-β, produced by a wide range of cells such as macrophages, NK, T, and B cells, has a pivotal role in the control of the transition between pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type) response during the acute and resolving phases of malaria infection[22]. The balance between Th1 and Th2 immune response is important in determining the level of malaria parasitemia, disease outcome and rates of recovery, the over production of both pro-inflammatory and anti-inflammatory cytokines can be responsible for disease severity and mortality[23].

TGF-β appears to be an important cytokine for maintaining the balance between protection and progression towards disease, depending on the plasma concentration. At low levels, TGF-β has pro-inflammatory properties, whereas high levels of TGF-β are associated anti-inflammatory effects[24]. Although pro-inflammatroy responses often are associated with protective cell-mediated immunity, and anti-inflammatory responses are associated with susceptibility to malaria, the balance of pro- and anti-inflammatory cytokines appears to be an important determinant of whether a protective or a pathogenic response develops.[25]

In *P. falciparum* malaria the involvement of TGF-β becomes necessary since it inhibits IFN-γ and TNF-α production, cytokines which support the inflammatory process, at the same time up-regulating IL-10 and down-regulating the expression of adhesion molecules. Two important roles of TGF-β in malaria infection are thus identified depending on the phase of the infection; promoting Th1-mediated mechanisms that control parasite growth early in the infection and down regulating Th1-like responses to limit inflammation associated pathology later in the infection[24]. The overall outcome of both phases being likely periods of an experienced pathological condition during the course of the malaria disease. It is thus apparent that the timing and magnitude of the TGF-β response is crucially important in determining the outcome of infection[26].

In a longitudinal study of the relationship between pro- and anti- inflammatory cytokines production and clinical immunity to malaria in Ghana, it was shown that high ratios of IFN-γ, IL-12 or TNF-α to TGF-β were associated with reduced risk of parasitemia but increased risk of febrile illness[27]. These data support the notion that anti-inflammatory cytokines are required to down-regulate the pathological effects of high concentrations of pro-inflammatory cytokines. A dynamic equilibrium seems to be required with pro-inflammatory effector mechanisms targeting and controlling the parasite, and anti-inflammatory cytokines suppressing immunopathology.

In Lagos, few studies have examined the immune response in *P. falciparum* malaria to determine the relationship between plasma cytokine levels and parameters relating to disease severity in the host, especially in areas where malaria is highly endemic, such as Ikorodu coastal areas of Lagos state. The main goal of his study therefore, was to measure the plasma levels of IL-8 and TGF-β and to assess the relationships between these cytokines and various host factors pertaining to malaria disease severity in our environment.

MATERIALS AND METHODS

Study area

This study was conducted at a General hospital and four primary health centres at Ikorodu Local Government Area, located approximately 18.5 km from the state capital Ikeja, in Lagos State Nigeria. Ikorodu, a city with an area of 394 sq.km and one of 57 Local Government areas in Lagos state, is located in the southwestern corner of Nigeria close to the Gulf of Guinea, on the Northern edge of the Lagos lagoon; the city shares a boundary with Ogun state.

The General Hospital was at Ijede and the primary health centres were at Imota, Bayeku, Agura and St. Kizito Health Post Oreta.The geographical coordinates of the sites visited are in close contiguity, with Ijede 6° 34' 0" North, 3° 36' 0" East; Agura 6° 34' 0" North, 3° 38' 0" East; Imota 6° 40' 0" North, 3° 40' 0" East; Bayeku 6° 44' 0"

North, 3° 41' 0" East; and Oreta 6° 53' 0" North, 3° 52' 0"East.Agriculture mainly farming and fishing are their major occupations, although with economic expansion, these communities now boast of a variety of vocational enterprises, besides salary earning jobs. This Local Government area had previously been identified as highly endemic for malaria transmission in the state[28]. The location of these communities by the shore of Lagos lagoon and around coastal areas encourages the development of stagnant water responsible for the breeding of anopheles mosquito, a situation contributing to the stable pattern and continuous transmission of malaria all year round

Study design

An analytical, cross-sectional study design was utilized.

Study population

A hundred and thirty six individuals who had malaria attack based on *P. falciparum* parasitaemia in their thick blood smears stained by Giemsa stain participated in the study. They also had fever (axillary temperature of >37,5ºC) and clinical symptoms such as headache vomiting, prostration and other symptoms and signs of malaria. They were recruited after informed consent was obtained following a thorough explanation of all procedures and the objectives of the investigation.

Ethical consideration/issues

Institutional: Prior to the commencement of sample collection, ethical approval from the College of Medicine Ethics Committee of University of Lagos and Lagos State Health management Board was obtained.

Individual: Patients were evaluated and enrolled at the health centers. A written informed consent for participation in the study was obtained, while an assent form was obtained from parents or guardians of individuals considered as minors prior to inclusion in the study.

Patients were informed of the procedures involved in the study; with regards to risk/harm they were informed of the discomfort associated with the collection of blood samples, no other adverse effects or risk was expected to be associated with participation in this study.

Patients were free to consent or decline from the study at any time during the study period. Patients found positive for malaria were given adequate treatments, according to the national malaria treatment guidelines.

Sample collection

Whole venous blood (3 ml) was collected from a peripheral vein by venipuncture into sterile EDTA vacutainer tubes. The blood was processed by centrifugation and afterwards the plasma was stored at -70℃.

Parasitological assessments

Blood samples were stained with 3% Giemsa's solution for 45minutes. Diagnoses were established by standard light

microscopy. The thick blood smears for the initial screening were examined for the presence of parasitemia in a limited number of microscopic fields. A second blood smear was used to calculate the parasite burden. The level of parasitemia (asexual parasites/µL blood) was estimated from the thick smears by counting the number of asexual parasites against leucocytes, assuming each patient had 8000 leucocytes/µL. Parasitemia per µL was calculated by using the formula: (Parasitemia (per µL) = number of parasites × 8000/number of leucocytes).

Cytokine assays

For cytokine assays, venous blood samples were drawn aseptically into Vacutainer® tubes (Beckton Dickinson and Company). Plasma samples were separated and aliquots were frozen at -70^{o}C until assayed. Plasma samples were analysed for IL-8 and TGF-β by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions (MABTECH AB, Sweden). Each plate included a standard curve of recombinant human cytokine. All specimens were measured in duplicate using an ELISA microplate reader (TECAN sunrise ELISA reader), and the means of the values were used in all analyses.

Statistical analysis

Data was presented as mean ± standard deviation. Student's t-test was used to test the difference between two means, while one-way ANOVA was used to compare means of three or more groups (multiple comparisons

of means). Pearson correlation (for parametric data) and Spearman correlation (for non-parametric) were used to test relationship between two variables. Data were analysed with Excel 2007 and SPSS statistical package (SPSS version 17.0, SPSS INC Chicago, IL, USA).A two-tailed *P*value less than 0.05 was considered statistically significant.

RESULTS

Patients

Between 5th August and 20th of November 2013, 1,414 subjects from the primary health centres at Ikorodu Local Government Area of Lagos State, Nigeria were screened for malaria infection. Of these, 136 (9.6%) were smear positive for *P. falciparum* infection. All smear-positive cases met the inclusion criteria for the study and were enrolled in the study. Table 1 shows the baseline characteristics of the malaria patients.

The mean age of the malaria patients was 16.1 years (range: 3-63). At study entry, the mean axillary temperature was 37.3ºC (33.5-40.4), while mean parasite density was 35,057.5 (19-245,647) parasites/µL, mean white blood cell count was 5.8 $\times 10^3/mm^3$ (1.5-24.2 $\times 10^3/mm^3$), mean PCV was 34.2% (12-53%), mean TGF-β was 23,672pg/ml (0-93,675.2pg/ml) while mean IL-8 was 508.8pg/ml (0-3406.6pg/ml).

Difference in plasma cytokine levels and host characteristics

Plasma IL-8 at low levels (< 675.7pg/ml), showed elevated mean parasite densities (43,200±75,819.1parasites/µL) when

compared to high levels of IL-8 (> 675.7pg/ml), which had a reduced mean parasite densities (21,097±24,340.4parasites/µL). The difference in mean parasite densities between high and low values of IL-8 was statistically significant ($P < 0.05$). TGF-β at high and low levels did not show any significant difference with respect to parasite densities of the malaria patients (Table 3).

Among malaria patients with low levels of TGF-β (< 9,393.6pg/ml), mean values of packed cell volume were seen to be raised within average normal ranges (34.5±8.0), whereas patients with high levels of TGF-β (> 9,393.6pg/ml), had mean values of packed cell volume being lowered (29.2±12.5). The difference in mean values of packed cell volume between high and low levels of TGF-β was observed to be statistically significant ($P < 0.05$). There was a negative correlation between TGF-β levels and packed cell volume ($r = -0.27$, $P = 0.001$). IL-8 did not show any correlation with this parameter (Table 4).

DISCUSSION

In a bid to developing new approaches and methods of controlling severe disease due to malaria a better understanding of malaria pathogenesis is of vital importance.Cytokines play a significant role in the progression and outcome of malaria[22, 29-31]. Certain complications such as that of anemia and cerebral malaria, predominantly seen in African children are known to be major causes of morbidity and mortality in malaria

disease[29]. In this study, the packed cell volume, a test used to screen for anemia[30], was found to be negatively correlated with TGF-β.

The pathogenesis of malaria anemia is not well understood, although destruction of infected erythrocytes accompanied by clearance of uninfected erythrocytes, erythropoietic suppression and dyserythropoiesis, can all contribute to anemia[3]. Various cytokines have been implicated in these effects although their role in the development of anemia is not well understood[12]. Earlier research had reported malaria-induced anemia as being multifactorial, with hemolysis occurring more frequently in nonimmune children and dyserythropoiesis occurring more often in regions with frequent and recurrent infections[29, 12]. Other research have also implicated TGF-β as being a powerful inhibitor of erythropoiesis, acting essentially by decreasing recruitment of hematopoietic progenitors from quiescence into active cell-cycling status and by increasing the length of the G1 phase of cycling progenitors[31-33].

Murine studies have now demonstrated that elevated TNF-α levels contribute to bone marrow suppression and red cell destruction whereas elevated IL-10 is thought to stimulate hematopoiesis[12]. TNF-α elevation has been associated with anemia and high density *P. falciparum* infection in children[34], whereas reduced IL-10 [35], and IL-10/TNF-α ratios have been demonstrated in African

children with severe malaria-induced anemia [36-39].

TGF-β is known to down-regulate the production of TNF-α and IL-10 and has been attributed to both positive and negative effects on erythropoiesis[38, 31, 40]. Recent studies have however made it clear that an important cause of reduced erythropoiesis in children with severe malaria anemia is due to an imbalance in inflammatory mediatior[39].

TGF-β, a protein that controls proliferation, cellular differentiation, and other functions in most cells, has a crucial role in controlling the transition between pro-inflammatory (Th1-type) and anti-inflamatory (Th2-type) responses during the acute and resolving phases of malaria infection[41, 42].

A successful type 1 response to malaria requires a well-timed and proportional release of interleukin IL-12, IFN-γ, and TNF-α to minimize parasitemia and preserve erythropoiesis[39]. IL-12 stimulates production of IFN-γ and TNF-α from T-cells and natural killer (NK) cells, thereby further augmenting type 1 responses. A number of cytokines and chemokines can promote IL-12 [e.g, granulocyte macrophage-colony stimulating factor (GM-CSF) and IFN-γ], while others decrease IL-12 production [e.g, TGF-β, IL-13, monocyte chemotactic protein (MCP)-1/CCL2]. As such, the overall ability of the innate immune response to generate IL-12 a condition largely determined by the timing of TGF-β production is an important event that mediates the development of malaria anemia[39].

In our study, low mean volumes of packed red cells, conditions indicative of anemia, were associated with TGF-β at high concentrations. Earlier reports postulated that the anti-inflammatory response of TGF-β may suppress IL-12 but may be insufficient for preventing excessive TNF-α production, which could promote anemia in children with acute malaria[10]. Also malaria patients with slightly raised and normal volumes of packed red cells, a state deviating from an anemic conditionwere associated withlow TGF-β concentrations.This was in agreement with studies done in Gabon and Thailand where severe malaria, described as being characterized by high-density parasitemia and severe anemia, had reduced TGF-β levels being linked to severe malaria[23, 43, 10]. However, in contrast, a study conducted in the city of Ouagadougou (Burkina Faso) found elevated TGF-β levels in children with severe malaria[22]. These variances in results were explained as being due to the differences in malaria endemicity in both rural and urban regions of Gabon and Burkina Faso respectively where studies were conducted. The rural area of Lambaréné, Gabon had a high level of *P. falciparum* transmission, whereas the urban region of Ouagadougou is mesoendemic for *P. falciparum*[39]. This close similarity in endemicity shared between our study site at Ikorodu, with its proximity to coastal areas and all year round transmission, and that seen in Gabon, explains the similarity in results between these two sites.This further

buttresses the contention by Lyke et al, which asserts the frequency of malaria-induced dyserythropoiesis occurring more often in regions with frequent and recurrent infections[12]. In our study, TGF-β levels were however not associated with the number of parasites; this is in accord with previous research done where no correlation between parasitemia and TGF-β levels could be found[43].

IL-8 being a neutrophil chemoattractant, performs pro-inflammatory functions and thus should play a significant role in malaria disease outcome. Little is however known about its role in the pathogenesis of malaria[44], with insinuated opinions about this role being unlikely[12]. We reported mean parasitemia levels to be elevated in patients with low levels of IL-8 and reduced mean parasitemia levels in patients who had high IL-8 plasma concentrations, these findings however diverge from previous reports, where higher levels of this cytokine were reported to be found mainly among malaria patients with severe parasite burden[45, 18]. Studies in Thailand provided reports where IL-8 was seen to be elevated in severe non-fatal malaria patients[16]; similar results were also observed in Malian children [12], although children with severe malaria in this study had ten-fold higher concentrations of IL-8 compared to either healthy controls or individuals with uncomplicated malaria, suggesting that geographical placements may have an influence on cytokine levels in relation to parasitemia. Furthermore, in

contrast to studies reporting a correlation between IL-8 and parasite number, we found no such relationship between these two parameters[20]. We however observed initial mean plasma IL-8 concentrations being raised at 508.8 pg/ml in malaria positive patients tested, whereas in subjects without malaria, no plasma IL-8 was detectable, this result has been found to be in line with previous studies where levels of endogenous pyrogens such as IL-8 were reported to have been elevated in malaria, with patients devoid of malaria, having no plasma IL-8 detectable[16]. An interesting find in the study by Friedland et al, was that plasma IL-8 levels remained elevated throughout the period of investigation[16]; the continued production of IL-8 during and after clinical recovery from *P. falciparum* infection was believed to indicate that the cytokine is involved in the healing process, which may explain the increased IL-8 levels having a mean reduced parasitemia in our study, indicating a likely curative function attributed to this cytokine. Nonetheless, additional studies in Gabonese children and adults did however illustrate that higher plasma IL-8 levels were associated with acute malaria and a slow cure after malaria chemotherapy[45]. Consequently, we postulate that elevation in plasma concentration of this chemokine in *in vivo* circulation is dependent on the stage of the malaria disease in the host, whether at initial onset or at a future state towards resolution of infection.

CONCLUSION

Studies outlined here support a model in which the pathogenesis of malaria disease is largely driven by an over production or an underproduction of anti-inflammatory cytokines and chemokines and suppression of erythropoiesis that is driven by dysregulation of innate anti-inflammatory mediators. With no definite end in sight to having the upper hand to challenges posed by malaria infections, it is our confident desire that advancing in malaria disease prognoses be continually improved on particularly in areas directed to anemia and parasite burden as these are major concerns in morbidity and mortality amongst African children.

Further work in different geographic areas is needed to confirm whether these cytokines have similar effects on the levels of parasitemia and packed cell volumes in different populations.

The design of intervention strategies will boarder around interests directed towards cells and cytokines possibly implicated in dyserythropoiesis or erythropoietic suppressionin malaria mediated anemia, as well as biomarkers for indications for parasitemia of different degrees.

Since there is a dearth of information, it is therefore recommended that data in this direction be investigated in order to establish their roles in malaria disease pathogenicity, especially at varying stages of malaria disease states. Further studies are therefore, needed to better understand the associations

between parasitic burden and balancing effects of pro- and anti-inflammatory cytokines in determining resolution or persistence and worsening of *P. falciparum* malaria infection.

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Table 1: Baseline characteristics of patients

Characteristics	Frequency	Percentage (%)
Age (Years)		
3-17 years	94	69.1
18-32 years	27	19.9
33-47 years	11	8.1
48-63 years	4	2.9
Sex		
Male	58	42.7
Female	78	57.4
Temperature(°C)		
<36.5	42	30.9
36.5-37.5	36	26.5
>37.5	58	42.7
White blood cell count (× 10^3/mm^3)		
<4.0	37	27.2
4.0-10.0	88	64.7
>10.0	11	8.1
Packed cell volume (%)		
<35	71	52.2
35-55	65	47.8
Parasite density (parasites/µL)		
<10,000	54	39.7
10,000-19,999	24	17.6
20,000-39,999	22	16.2
40,000-59,999	15	11.2
60,000-99,999	11	8.1
>100,000	10	7.3
Cytokines (pg/ml)		
IL-8 low levels	86	63.2
IL-8 high levels	50	36.8
TGF-β low levels	77	56.6
TGF-β high levels	59	43.4

Table 3: Mean comparisons of variable host factors at low and high cytokine levels using student t-Test

	Host factors	Cytokine level	N	Mean	P –value	- value
	PCV (%)	Low TGFβ level	77	34.5	0.007	0.46
		High TGFβ level	59	29.2		0.00
	Temperature (℃)	Low TGF-β level	77	37.3	0.937	).743
TGF-β		High TGF-β level	59	37.3		).112
	Parasite density (Parasites/µl)	Low TGF-β level	77	33,213	0.253	).633
		High TGF-β level	59	37,502		).108
	PCV (%)	Low IL-8 level	86	30.7	0.209	).773
		High IL-8 level	50	34.8		
IL-8	**Temperature (℃)**	Low IL-8 level	86	37.3	0.512	).147
		High IL-8 level	50	37.3		
	Parasite density (Parasites/µl)	Low IL-8 level	86	43,200.0	0.007	).001
		High IL-8 level	50	21,097.0		).932

Significant at $P \leq 0.05$ (95% confidence Interval)

Table 4: Correlation of white cell counts, packed cell volume and cytokine concentration

Cytokines	WBC count		Packed cell volume		Parasite count	
	r-value	p-value	r-value	p-value	r-value	p-value
IL-8	0.003	0.974	0.171	0.044	-0.13	0.125
TGF-β	-0.128	0.131	-0.270**	0.001	0.095	0.263

** Correlation is significant at the 99% confidence interval (CI) $P \leq 0.01$