Research Article

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Journal of Medical Microbiology and Immunology

2019
Vol.3 No.1:12

Immune-based Investigation of Tumour Necrosis Factor-Alpha (TNF-α), Interleukin-1 Beta (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10) among Pregnant Women Infested with *Plasmodium falciparum* in Port Harcourt

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Abstract

This cross-sectional study investigated some inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) concentrations in pregnant women with *Plasmodium falciparum* infection in Port Harcourt, Rivers State, South-South Nigeria. A total of 162 samples were randomly collected from pregnant women who are attending Braithwaite Memorial Hospital and College of Health and Technology Health Services Centre Antenatal clinics between May 2017-August 2018, all in Port Harcourt, through convenient sampling technique. The average mean age of the parasitized pregnant women stood at 30.3 ± 47 years while the control subjects stood at 30.3 ± 51 years. The plasma obtained from the blood samples after centrifugation at 1500 rpm were subjected to cytokine laboratory based analysis using commercial standard Enzyme Linked Immunosorbent Assay kit (ELISA-Elabscience Biotechnology Inc, USA). Malaria diagnosis and blood parameters were carried out using standard parasitological and haematological Standard techniques. The result from the study revealed that pregnant women with *Plasmodium falciparum* infection within the age group of 29-33 years had the highest prevalence and mean parasitaemia level of 41.2% and 7955.11 ± 1225.75 parasites/µL (χ^2=0.641, p=0.962; F=1.173, p=0.328) respectively. In terms of gravidity and occupation, the primigravidae and traders had the highest prevalence of 40.2% and 30.4% (χ^2=4.004, p>0.05; χ^2=1.732, p=0.500) respectively. The mean concentrations of IL-1β, IL-6, and IL-10 (176.32 ± 27.34 pg/ml, 329.09 ± 19.11 pg/ml, and 376.78 ± 24.95 pg/ml) were highly elevated in pregnant women with severe parasitaemia (p=0.1609; 0.0000; and 0.0511) when compared with mild and moderate parasitaemia. The mean cytokine levels of TNF-α, IL-β, IL-6, and IL-10, were significantly elevated in pregnant women with *P. falciparum* infection when compared to respective apparently healthy matched controls *(*p<0.0001). However, the correlations between plasma levels of IL-6 and *P. falciparum* parasite density in pregnant women showed a strong positive relationship (p<0.0001). Nonetheless, it is strongly believed that inflammatory cytokines are involved in immune-pathogenesis and immune-regulation of *Plasmodium falciparum* infection. Nevertheless, cytokines as found in this study could be used as promising prognostic and diagnostic biomarkers for *falciparum* malaria progression or regression outcome, especially in developing communities such as Sub-Sahara Africa where the burden of malaria incidence is always increasing in an alarming proportion.

Keywords: Inflammatory cytokines; *Plasmodium falciparum;* Parasitaemia; Pregnant women; Niger delta; Endemic zone; Necrosis factors

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Citation: Barikuura DF, Ngozika WG, Obioma A (2019) Immune-based Investigation of Tumour Necrosis Factor-Alpha (TNF-α), Interleukin-1 Beta (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10) among Pregnant Women Infested with *Plasmodium falciparum* in Port Harcourt . J Med Microbiol Immunol Res Vol.3 No.1:12

Received: April 23, 2019; **Accepted:** May 13, 2019; **Published:** May 20, 2019

Introduction

Malaria is an acute or chronic disease caused by an obligate intracellular protozoan parasite of the genus *Plasmodium*. *Plasmodium falciparum* is one of the most virulent species which causes illnesses and deaths worldwide, with an estimated 300 million clinical cases annually [1,2]. According to World Health Organization (WHO), more than 216 million clinical cases of malaria were reported in 91 countries globally in 2016, with an estimated 445,000 deaths [3]. In sub-Saharan Africa, *P. falciparum* was implicated for 99.7% cases of malaria in 2017 [4]. In the year 2017, Nigeria had the highest (25%) malaria cases among the five countries reported to have half of all malaria cases globally [4]. Pregnant women, children and naïve adults are more vulnerable to *P. falciparum* malaria in malaria endemic areas [5,6]. Pregnant women are more prone to severe *P. falciparum* malaria than non-pregnant women. The effects of malaria during pregnancy range from spontaneous abortion, low birth weight, still birth, congenital infection to maternal death as reported by Azuonwu et al. [7]. Okpere et al. [8] reported that a minimum of 6 million women worldwide are susceptible to malaria infection in pregnancy, while at least 10,000 maternal deaths and 200,000 newborn deaths are attributed to malaria annually. According to Perkins et al. [9] over 80% of deaths reportedly caused by *P. falciparum* infection occur in sub-Saharan Africa. Several studies suggest that many progressive attempts made to develop malaria vaccine to effectively control malaria parasites and its devastating impact to the immune system have yielded unsatisfactory results because of the massive complexities of the parasite and its multiple phases' life cycle which seems dynamic especially in the tropical regions that favours its high fecundity rate [10]. Nonetheless, it is strongly believed that some inflammatory cytokines are potentially implicated in the immune-pathogenesis of *falciparum* malaria. However, research has it that toxigenic glycosylphosphatidylinositols (GPIs) from *P. falciparum* could induce production of tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon gamma (IFN-γ) in macrophages [11,12]. Furthermore, Ayimba et al. [13] in their study suggested that cytokine and chemokine production profiles and their dynamics may prove useful in evaluating either the progression or the retrogression of *P. falciparum* malarial disease. Nevertheless, it has been reported that excessive production of proinflammatory cytokine, such as tumor necrosis factor (TNF) can cause pathological disorder, while early production of proinflammatory T helper type 1 (Th1) cytokines, including tumor necrosis factor (TNF), interleukin-12 (IL-12) and possibly interferon-gamma (IFN-γ) may limit the progression from uncomplicated malaria to severe and life-threatening complications [13]. Furthermore, several studies agree that Th1 responses are important for clearance of *falciparum* malaria, and elevated levels of interleukin-6 (IL-6) and interleukin-10 (IL-10) were observed in serum of patients with severe *P. falciparum* malaria [13,14]. In many parts of Africa, interventions such as the use of long-lasting insecticides treated bed nets, access to WHO-recommended antimalarial therapies and improved disease prevention efforts have dramatically reduced the burden of malaria [15]. However, several studies incriminated this prevention strategy for the upsurge of *P. falciparum* drug-resistance and insecticide resistance of female *Anopheles* mosquito [16-18]. Malaria burden may steadily rise, particularly in sub-Saharan Africa, unless newer prevention and treatment strategies are developed. One approach towards achieving this prospect would be to gain more robust scientific insight into the process involved in malaria pathogenesis and immune response. However, there is visible paucity of research information on the use of immune based laboratory technique to assay for the presence of necrosis factors in malaria parasitized pregnant women in Port Harcourt, Rivers State, as most of the Hospitals and Health centres adopt the conventional methods of film preparation and staining technique and of course the use of rapid screening methods, with a lot of complications and conflicting results, based on low specificity and sensitivity of the methods. However, this study was designed and aimed to evaluate the levels of inflammatory cytokines (IL-1β, IL-6, TNF-α, and IL-10) *in falciparum* malaria-infected pregnant women in Port Harcourt, Rivers State, South-South Nigeria. It is strongly expected that data generated from this study would strengthen the real time prognosis, diagnosis and managements of malaria infected pregnant women in the region who are supposedly regarded as the vulnerable group. It is further believed that the gains of the study would also underpin the need for our Health management policies to change and adopt the outcome of this study as a viable method of assaying for malaria parasite, especially among vulnerable groups in malarial prone or endemic regions across the globe.

Materials and Methods

Study area

This study was performed in Port Harcourt, a malaria holoendemic cosmopolitan city, the capital city of Rivers State, South-South geopolitical zone of Nigeria. The climate of the area is marked by two distinct seasons, rainy and dry. The rainy season starts in May and last till October, with July and August as Months of peak rainfall. The dry season which starts from December to March are characterized with periodic harmattan. The vegetation in South-South Nigeria reflects that of the mangrove rainforest zones. Port Harcourt is within the Niger Delta area, very close to the Atlantic Ocean. The major occupations of the people include fishing, farming, trading, and public service. *Anopheles gambiae* complex is the main malaria vector in Nigeria while *P. falciparum* is the most prevalent malaria species (95% cases) [19-21].

Ethics statement

Prior to the study, ethical permission was obtained from the State Ministry of Health and Rivers State University Teaching Hospital, Port Harcourt. Also, informed consent was obtained from participants or their respective parents or guardians before sample collection was initiated.

Study design and laboratory experimental

Participants with mixed infection of malaria parasites and those with overt chronic infections were excluded while those who met the inclusion criteria were selected for this study. A total of 162 samples were collected for the study. One hundred and two (102) samples from pregnant women (n=102) which were positive for

P. falciparum parasites while 60 samples (n=60) were negative for *P. falciparum* parasites, which also served as a healthy controls. Pre-tested structured questionnaires were used to obtain socio-demographic data from the pregnant women. Blood samples were collected by venepuncture aseptically into EDTA container, labelled with barcode and taken to laboratory for malaria microscopy and total leucocyte count for absolute parasite density determination. Thick and thin malaria blood films were made as described by Cheesbrough [22]. The slides were stained with 3% solution of Giemsa stain as described by World Health Organisation [23]. The presence of *P. falciparum* parasitaemia was determined by microscopic examination of thick and thin smears using 100x oil immersion objective field. Also, Rapid Diagnostic Tests (RDTs) were carried out on positive slides using CareStart™ Malaria Histidine Rich Protein 2 (HRP2) (*Pf*) kits (Access Bio, Inc. USA) according to the manufacturers' instructions.

Determination of absolute parasite density

The absolute parasite density of participants infected with *P. falciparum* parasites was determined and calculated as described by Agomo et al. [24]. The total leucocyte count determination for each subject was done using automated haematology analyzer (Sysmex Kx-21N, USA) according to the manufacturers' instructions.

Determination of degree of parasitaemia

The degree of parasitaemia was graded as described by Adesina et al. [25]. Parasitaemia with absolute parasite density of less than 1000 per microliter of blood (<1000 parasites/µl) was graded as low parasitaemia; however, parasitaemia with absolute parasite density of greater than 1000 per microlitre of blood but less than 10,000 per microlitre of blood (>1000 to 9,999 parasites/µl) was graded as moderate parasitaemia while parasitaemia with absolute parasite density of greater than 10,000 per microlitre of blood (>10,000 parasites/µl) was graded as high parasitaemia.

Determination of cytokine concentration in plasma

TNF-α, IL-1β, IL-6, and IL-10 concentrations in plasma were measured using the sandwich enzyme-linked immunosorbent assays (ELISAs) technique with human IL-6, IL-1β,TNF-α and IL-10 Enzyme Linked Immunosorbent Assay Kits according the manufacturers' instructions (Catalog Numbers: E-EL-H0102; E-EL-H0149; E-EL-H0109; and E-EL-H0103 Elabscience®, USA). 100 µl of standards of various concentrations, samples and controls were dispensed into appropriate well and incubated for 90 minutes at 37°C. The liquid was removed, and immediately 100 µl of biotinylated detection antibody was added to each well, covered with the plate sealer, gently mixed up and incubated for 60 minutes at 37°C. After incubation, the solution was aspirated from each well, 350 µl of wash buffer was added to each well, soaked for one to two minutes and was further aspirated from each well. Aspiration and washing was repeated thrice, while the remaining liquid from the wells were removed by striking the wells sharply with the plate face down on absorbent paper at the end of washing. Then, 100 µl of HRP conjugate working solution was added into each well. It was covered with the plate sealer and gently mixed and incubated for 30 minutes at 37°C. Then, the plate was aspirated and washed thoroughly for five times. Ninety microlitres (90 µl) of substrate reagent was added to each well, covered with new plate sealer, and incubated for 15 minutes at 37°C in the dark. The reaction was stopped by adding 50 µl of stop solution to each well. A qualitative result was observed with respect to sample colour changed from blue to yellow completely. The optical density value of the coloured solution in each well at once, was determined using a micro-plate reader (Rayto Microplate Reader RT-2100C, China) at 450 nm wavelength. Analysis was performed in duplicates. Cytokines concentrations (pg/mL) were determined by using the standard curve and multiplied by the dilution factor. The detection range of cytokine assayed was 7.81-500 pg/mL for IL-1β, IL-6, TNF-α, and IL-10.

Statistical analysis

The data generated from this study were analyzed using GraphPad Prism version 5.0.3. Frequencies, percentages, means and standard deviations were calculated for appropriate variables. Comparisons between groups for categorical variables were done using Chi-square (χ^2) test. Student's t-test and analysis of variance (ANOVA) were used to evaluate differences between means of two or more groups. Pearson product moment correlation coefficients were used to test relationship between independent and dependent variables. The level of statistical significance was set as $p<0.05$.

Results

Characteristics of the study participants

Table 1 shows the socio-demographic characteristics of pregnant women studied. Their mean (±SD) age was 30.3 ± 4.7 years and 30.3 ± 5.1 years for the non-infected and infected pregnant women respectively. Pregnant women within the age group of 29-33 years had the highest prevalence of infection (41.2%) with *P. falciparum* malaria while those within the age group 39-43 years had the lowest prevalence (2.9%). Though, there was no statistical significant difference in their age group (χ^2=0.641, p=0.962). Gestational age of pregnancy of study subjects shows that those in their second trimester had the highest prevalence (48%) infection with the *P. falciparum* while those in their third trimester had the lowest prevalence (20.6%). However, this was not statistically significant (χ^2=0.059, p=0.963). Characteristics on gravidity shows that the primigravidae had a prevalence of 25.5%, secundigravidae had a prevalence of 34.3%, while the multigravidae had a prevalence of 40.2%. The difference was statistically significant (χ^2=7.000, p=0.037). The socio-demographics of the pregnant women on education and occupation show no significant difference (χ^2=1.732, p=0.500).

Table 2 shows the various mean parasitaemia of *P. falciparum* malaria-infected pregnant women according to different age groups studied. Those within the age group of 29-33 years had the highest mean ± SE parasite density of 7955.11 ± 1225.75 parasites/µl, while those within the age group of 19-23 years had the lowest parasite density of 5042.00 ± 900.18 parasites/µl. Comparisons of the mean parasitaemia showed no statistical

Table 1 Socio-demographic characteristics of pregnant women.

Characteristics	Non-Infected Pregnant Women (n=60) Frequency (%)	Infected Pregnant Women (n=102) Frequency (%)
Age group (years)		
19-23	6 (10.0)	8 (7.8)
24-28	15 (25.0)	27 (26.5)
29-33	22 (36.7)	42 (41.2)
34-38	15 (25.0)	22 (21.6)
39-43	2 (3.3)	22 (21.6)
Mean age (years) + SD	30.3 ± 4.7	22 (21.6)
Gestational age		
1st Trimester	20 (33.3)	32 (31.4)
2nd Trimester	28 (46.7)	32 (31.4)
3rd Trimester	12 (20.0)	32 (31.4)
Gravidity		
Primigravidae	15 (25.0)	41 (40.2)
Secundigravidae	24 (40.0)	35 (34.3)
Multigravidae	21 (35.0)	26 (25.5)
Education		
Primary	2 (3.3)	3 (2.9)
Secondary	27 (45.0)	35 (34.3)
Secondary	31 (51.7)	64 (62.8)
Occupation		
Civil Servants	14 (23.3)	25 (24.5)
Traders	22 (36.7)	31 (30.4)
Student	4 (6.7)	8 (7.8)
Self-employed	9 (15.0)	15 (14.7)
Un-employed	11 (18.3)	23 (22.6)

Table 2 Comparisons of mean Parasitaemia of *P. falciparum* malaria-infected pregnant women by age group.

Age Group	Age Group	Mean ± SE Parasitaemia (Parasites/µl)
19-23	8 (7.84)	5042.00 ± 900.18
24-28	26 (25.49)	5269.34 ± 1381.18
29-33	42 (41.17)	7955.11 ± 1225.75
34-38	23 (22.55)	7102.51 ± 1606.66
39-43	3 (2.95)	762.67 ± 178.92
F	2.465	1.173
P-value	0.05	0.328

significant difference among the different age groups of pregnant women (F=1.173, p=0.328).

Plasma cytokine levels in different degree of parasitaemia

Table 3 shows comparisons of mean parasitaemia of proinflammatory cytokines (TNF-α, IL-1β, and IL-6), anti-inflammatory cytokine (IL-10) and degrees of parasitaemia such as mild, moderate and severe among *P. falciparum* infected pregnant women. The mean concentration of tumor necrosis factor-alpha (TNF-α) evaluated in moderate parasitaemia (109.33 ± 14.36 pg/ml) was higher compared with that of mild parasitaemia (83.16 ± 15.08 pg/ml) and severe parasitaemia (85.49 ± 18.70 pg/ml). However, the difference among them was not statistically significant (p=0.3143). Interleukin-1 beta (IL-1β) had a higher mean concentration in severe parasitaemia (176.32 ± 27.34 pg/ml) compared with that in moderate and mild parasitaemias, however, the differences among them was not statistically significant (p=0.1609). The mean concentration of IL-6 was higher in severe parasitaemia (329.09 ± 19.11 pg/ml) compared with moderate and mild parasitaemia, and comparisons of the means showed a statistical significant difference among them (p<0.05). The mean concentration of IL-10, was higher in severe parasitaemia (376.78 ± 24.95 pg/ml) compared with moderate and mild parasitaemia. However, comparisons of the mean parasitaemia was not statistically significant (p=0.0511).

Plasma levels of cytokines in *P. falciparum* infected and un-infected participants

Table 4 shows comparisons of the mean concentrations of the

Table 3 Comparisons of mean SEM of inflammatory cytokine levels and Parasitaemia among *P. falciparum* malaria-infected pregnant women.

Parameters	*P. falciparum* Parasitaemia			p Value
	Mild (1-999 Parasites/µl) n=23	Moderate (1000-9999 Parasites/µl) n=58	Severe (>10,000 Parasites/µl) n=21	
TNF-∝(pg/ml)	83.16 ± 15.08	109.33 ± 14.36	85.49 ± 18.70	0.3143
IL-Iβ(pg/ml)	118.40 ± 12.48	159.73 ± 14.67	176.32 ± 27.34	0.1609
IL-6 (pg/ml)	160.74 ± 22.50a	250.34 ± 15.67a	329.09 ± 19.11b	0.0000
IL-10(pg/ml)	314.12 ± 23.62	370.56 ± 11.49	376.78 ± 24.95	0.0511

Table 4 Comparisons of mean SEM cytokines level of *P. falciparum* malaria-infected pregnant women and un-infected pregnant women.

Parameters	TNF-∝ (pg/ml)	IL-Iβ (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)
Un-Infected n=60	40.23 ± 5.28	44.63 ± 6.14	124.00 ± 24.78	106.70 ± 12.82
Infected n=102	96.61 ± 8.01	153.10 ± 10.59	247.60 ± 12.30	357.60 ± 10.06
p value	<0.0001	<0.0001	<0.0001	<0.0001

various cytokines evaluated in *P. falciparum* malaria-infected pregnant women and uninfected pregnant women used as matched controls. The mean concentration of IL-10 was higher in *P. falciparum* malaria-infected pregnant women compared with the mean concentrations of proinflammatory cytokines, TNF-α, IL-6, IL-1β, and IL-6. Also, the mean concentrations of cytokines in *P. falciparum* malaria treated pregnant women were generally low compared with those infected. Comparisons of the mean concentrations of cytokines among them showed a statistical significant difference (p<0.0001).

Relationship between plasma cytokine levels and *P. falciparum* parasite density

Figures 1-4 shows Pearson correlations between malaria parasite densities and TNF-α, IL-Iβ, IL-6, IL-10 plasma levels in pregnant women infected with *P. falciparum* malaria. There was a weak linear relationship between malaria parasite density and TNF-α plasma levels (r=0.003; p=0.9791) as shown in **Figure 1**. Correlation between malaria parasite density and IL-1β, IL-6, and IL-10 showed positive linear relationship (r=0.12, p=0.230; r=0.42, p<0.0001; r=0.07; p=0.50) as shown in **Figures 2-4** respectively.

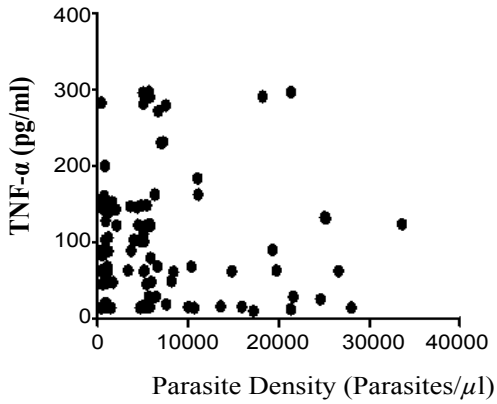

Figure 1 Zero correlation between parasite density and TNF-α plasma levels in pregnant women infected with *P. falciparum* (r=0.00; p=0.98).

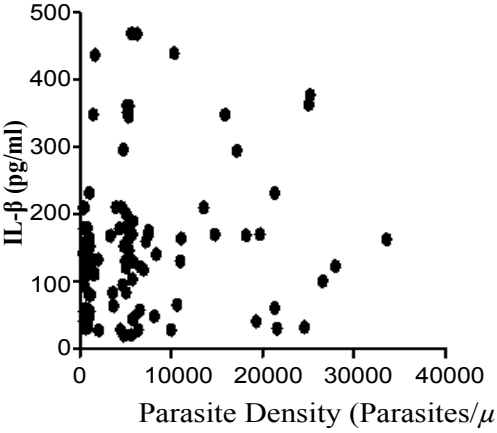

Figure 2 Positive correlation between parasite density and IL-1β plasma levels in pregnant women infected with *P. falciparum* (r=0.12; p=0.230).

Discussion

This study investigated the level of some inflammatory cytokines release during infection with *P. falciparum* in pregnant women. Previous studies had reported that immune responses contribute to disease pathogenesis and immune protection of the host [12]. The age-related prevalence of *P. falciparum* in this study showed a sharp increase in infection with increase in age from 7.8% among pregnant women for age group 19-23 years and peaked at 41.2% for age group 29-33 years before a sharp decline to 2.9% at age group 39-43 years. This difference however, was not statistically significant (p=0.963). This finding agrees with the previous work of Ohalete et al. [26], which reported that age and gender have no influence on the incidence of malaria among pregnant women in Imo State, Nigeria. It was observed in this study that malaria prevalence among pregnant women decreased with increasing age. This is also consistent with findings of Agomo et al. which reported the highest malaria prevalence of 20.5% among pregnant women in the age group 15-19 years and 7.1% in those that are above 34 years, in Lagos, South-West Nigeria [24]. The differences in the reported prevalence rate of malaria may be attributed to the different regions and seasons the studies were

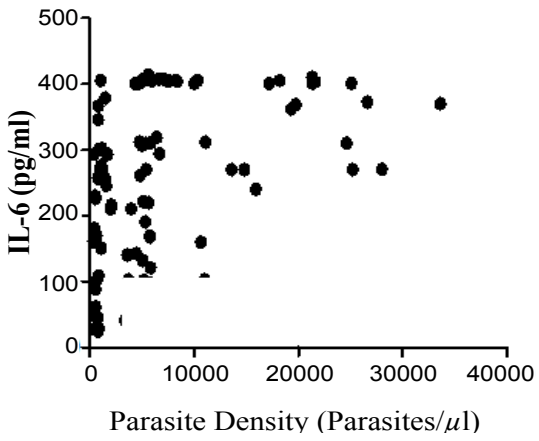

Figure 3 Positive correlation between parasite density and IL-6 plasma levels in pregnant women infected with *P. falciparum* (r=0.42; p<0.0001).

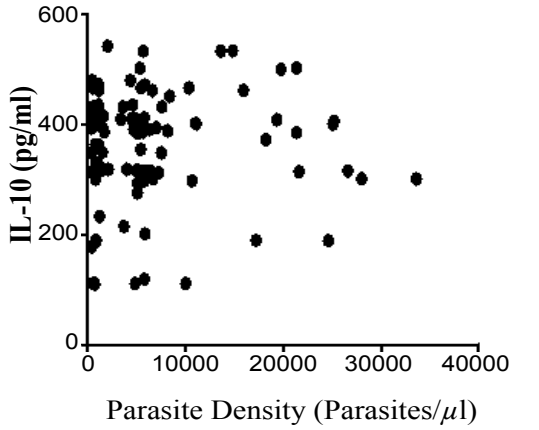

Figure 4 Positive correlation between parasite density and IL-10 in plasma levels in pregnant women infected with *P. falciparum* (r=0.07; p=0.50).

carried out and different levels of prophylaxis to mosquito bites. In terms of gestational age of the study subjects, it was observed that pregnant women in their first trimester (33.3%) had lower prevalence than those in their second trimester (46.7%), while those in the third trimester had the lowest (20.0%) **(Table 1)**. This corroborates the fact that in pregnancy intraplacental parasitaemia increases with gestational age with the highest risk of infection being in the second trimester [27]. Enato et al. in their study showed that most pregnant women in malaria endemic settings have asymptomatic parasitaemia which agrees with a report of a survey carried out in Benin City Nigeria, which showed that about 80% of pregnant women presented symptoms suggestive of malaria infection, although many of them were never confirmed positive through laboratory test [28,29]. In relation to parity the prevalence of parasitaemia was higher among the primidigravidae (40.2%) than the secundigravidae (34.3%) and multigravidae of (25.5%). This is in agreement with the findings of Agomo et al. [24] which showed that primigravidas (9.1%) were more infected than the secundigravidas and multigravidas respectively. Reports of several studies showed that primigravidas are more at risk to falciparum malaria in pregnancy as immunity against malaria in pregnancy increases with advancing in parities, [8] which implied that primigravidas are more susceptible because of lack of pre exposure to malaria parasite, thereby lacking specific immunity to placental malaria characterized by sequestration of *Plasmodium falciparum* infected red blood cells [30,31]. There was difference in the prevalence of falciparum malaria infection between the different occupational groups studied with the highest prevalence among pregnant women who were traders (30.4%), while those of them who were student had the lowest prevalence (7.8%). These observed differences might be due to the nature of their work which makes them more expose to female Anopheles mosquitoe bites. However, occupation according to this study did not influence infection with *P. falciparum* malaria among pregnant women (χ^2=1.732; p>0.05).

Table 2 shows mean parasitaemia of pregnant women infected with *P. falciparum* malaria. The average value of malaria parasite density (5226 parasites/µl) in the peripheral blood of *P. falciparum* infected pregnant women in this study was slightly higher than that obtained by Agomo et al. [24] in Lagos, South East Nigeria, with a mean parasite density of 4550 parasites/µl, and significantly higher than that reported by Akinboro et al. [32], among pregnant women (461.33 parasites/µl) in both secondary and tertiary hospital in Osogho, South-West Nigeria. Enato et al. [28] in Edo state, Southern Nigeria reported a geometric mean value of malaria of 636.96 ± 1450.11 parasites/µl and 4250.36 ± 1386.01 parasites/µl in peripheral and placental blood respectively. In another similar work by Achidi et al. [33], 565 parasites/µl was reported as the geometric mean malaria parasite density, of which the trend of parasitaemia was observed to be higher in younger pregnant women than older pregnant women. However, this finding is in contrast with the finding of this study. The variation in densities of parasitaemia as reported by various researchers might suggest different techniques of malaria parasite density determination and various levels of malaria endemicity in the affected geographical settings where the studies were carried out. The present study was carried out during the wet season, a period that favours the vector of malaria parasites due to stagnant waters for bleeding in various locations thereby creating more bleeding sites and enhancing transmission of *Plasmodium falciparum* parasites. This observation supports the position that in malaria endemic areas, pregnant women are more at risk to malaria infection due to reduced maternal immunity from increased steroid levels in pregnancy, increased attractiveness of the pregnant women to female anopheles mosquito bites and increased adherence of parasitized erythrocytes to chondroitin sulphate A expressed in the placentae [8]. Sequestration and adherence of malaria parasite, especially *P. falciparum* to endothelial cells specific organs tissue such as spleen, brain, kidney and placental may be attributed to the reduction in malaria parasite load in peripheral blood [34,35].

This study is consistent with earlier studies which demonstrated that *P. falciparum* infection triggers immune responses in pregnant women [35], leading to production of inflammatory cytokines. Several studies have shown that pathogen-associated molecular patterns (PAMPs) of *P. falciparum*, glycosylphosphatidylinositols

(GPIs) activates signal-transducing proteins called toll-like receptor (TLR2 and TLR4) of innate immune cells to induce proinflammatory cytokines such as TNF-α, IL-Iβ, IL-6, which can contribute to malaria parasite pathogenesis [1,36]. Haemozoin, parasites membrane-derived particles, and uric acids have been identified as potent mediators of *P. falciparum*-induced inflammatory response that also contributes in the secretion of cytokines in malaria [12].

Findings in the present study **(Table 4)** show significant increase in the concentrations of TNF-α, IL-Iβ, IL-6 and IL-10 in *P. falciparum* malaria-infected pregnant women compared with their uninfected pregnant counterparts. This finding corroborates that of Nmorsi et al. [37] which showed that IL-6 was significantly elevated in infected pregnant women (81.0+26.1 pg/ml) than in the uninfected pregnant women (25.0+5.0 pg/ml) ($p<0.05$), and Bostrom e*t al*. [38] who reported that IL-10 was steadily elevated among *P. falciparum* infected pregnant women than their matched uninfected controls and therefore, might be useful as one of the strong potential predictive biomarkers of *P. falciparum* infection in pregnancy. Ifeanyichukwu et al. [35] reported significantly increased concentrations of TNF-α, IL-6, and IL-10 in malaria-infected pregnant women than the uninfected matched controls in Aba, South-eastern Nigeria, which agrees with the present study. In contrast, Bayoumi et al. [39] reported that IFN-γ, IL-4, and IL-10 were significantly increased in the peripheral blood of uninfected pregnant women than the infected counterparts. The differences in results could probably be the gap in sample size, the limitation of ex-*vivo* cytokines measurements, and the geographical location where the research was carried out such as unstable malaria transmission area.

P. falciparum infection has long been associated with high circulating levels of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-1β, and IL-6. Previous studies have demonstrated a link between TNF-α, IL-6, IL-10 and the severity of the disease in human malaria [40,41]. Anti-inflammatory cytokine, IL-10 has been incriminated as an immunoregulator during *P. falciparum* Infection, thereby neutralizing the effect of other pro-inflammatory cytokine, TNF-α, IL-16, produced by T helper 1 (Th1) cells [42].

Interestingly, IL-10 is produced by monocytes, Th2 cells, and B cells, and it inhibits producing inflammatory cytokine in Th1 and CD8+ cells interleukin-10 (IL-10) also induce the proliferation of B cells, and synthesis of immunoglobulin, necessary for the development and maturation of antimalarial antibodies [43].

In terms of parasitaemia **(Table 3)**, this study showed that inflammatory cytokines, IL-1β, IL-6, and IL-10, evaluated were more expressed in moderate parasitaemia than in mild parasitaemia. These findings confirm the work of Ifeanyichukwu et al. [35] which reported increased levels of proinflammatory cytokines and anti-inflammatory cytokines, IFN-γ, TNF-α, IL-4, IL-6, and IL-10 among malaria-infected pregnant women in Aba, South-eastern Nigeria.

However, the finding did not agree completely with that of Szobo et al. [44] who reported significantly increased concentrations of IL-4 and IL-10 in mild infection than moderate infection. This could account to the fact that the innate immunity plays a critical role in controlling the effect of malaria parasite on human leading to increased expression of anti-inflammatory cytokines, IL-10, in mild infection which indicates early and effective immunity, but as infection progresses, more cytokines of interest are released.

Furthermore, on determining the relationship between parasite density and inflammatory cytokines in pregnant women infected with *P. falciparum* malaria, the present study shows a strong positive relationship between malaria parasite density and IL-6, which was statistically significant ($p<0.0001$). It was also observed in this study that there were positive relationships between malaria parasite density and IL-1β, between malaria parasite density and IL-10. This finding agrees with previous studies [35].

The positive correlation between malaria parasite density and inflammatory cytokines reported in this study suggests that the level of *P. falciparum* malaria parasite load in peripheral blood may induce corresponding plasmatic levels of inflammatory cytokines such as IL-Iβ, IL-6 and IL-1.

On the contrary, the present study did not observe any significant association between malaria parasite density and TNF-α in *falciparum* malaria-infected pregnant women. Though, the reason for this observation is not clear, however, it may suggest that parasite load dose not only determine the induction of inflammatory cytokine such as TNF-α. It is possible that other confounding factors such as genetic polymorphism and host immunity may probably influence the concentration of inflammatory cytokines in peripheral circulation.

Conclusion

The present study has demonstrated that pro-inflammatory cytokines such as TNF-α, IL-Iβ, IL-6 and anti-inflammatory cytokine, IL-10 are useful parameters for the prognosis and diagnosis of *falciparum* malaria infection in pregnant women. Furthermore, in the present study, the significantly elevated levels of IL-1β, IL-6 and IL-10 among pregnant women infected with *P. falciparum* parasites implicate these cytokines as the major mediators in the host responses to systemic moderate and severe parasitaemia of *P. falciparum* malaria in our locality. The high levels of IL-10 in this study suggest that IL-10 modulates pro-inflammatory cytokines which makes it significant in immune-regulatory role during pregnancy by impeding inflammatory responses. This study demonstrates a weak relationship between TNF-α and *P. falciparum* parasite density but a linear relationship between IL-6 and parasite density, among pregnant women infected with *P. falciparum* as observed and reported in our study.

Acknowledgement

We are grateful to the participants and their relatives for their active participation in this study. We will also acknowledge Alwell S. John, Levi B. Baakel, and other staff of Rivers State Teaching Hospital Port Harcourt formally known as Braithwaite Memorial Hospital and Rivers State college of Health Science and Technology Health Centre, Port Harcourt, for their collaboration and assistance during sample collection and laboratory analysis.

Conflict of Interest

We are not aware of any conflict of interest among authors.

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