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EVALUATION OF *Plasmodium falciparum* PARASITAEMIA AND ASSOCIATED INFLAMMATORY CYTOKINES AMONG PREGNANT WOMEN IN GOMBE STATE, NIGERIA

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ABSTRACT

Either the balance between or absolute levels of inflammatory cytokines may be important in malaria presentation and its outcome. In malaria-infected pregnant women, the relationships between, plasma components, development of immunocompetence and disease severity is poorly understood. A total of 725 blood samples were collected from the study subject. Malaria examination was carried out using standard parasitology and haematological techniques and blood plasma was analysed for cytokines using Th1/Th2 human commercial standard enzyme linked immunosorbent assay kit (Elabscience®, USA). Pregnant women within the age of 21-25 years had the highest infection rate of 38.1% with the mean parasitaemia levels of 5872.29±3079.29 parasites/µl while those within the age group 46-50 years had the lowest infection rate of 0.43% with parasitaemia levels of 143.00±0.00 parasites/µl. The difference between infection and age range were not statistically significant (F = 691.90, P = 0.00), but there was a significant difference between age range and parasiteaemia (F = 0.435, P = 0.86). The concentrations of IFN-γ, TNF-α, IL-6 and IL-10 were elevated among *P. falciparum* infected pregnant women than the un-infected counterparts (P > 0.05). The co-relations between malaria parasitaemia and cytokine levels was very low. TNF-α (P = 0.398, r = -0.10), IFN-γ (P = 0.837, r = 0.03), IL-10 (P = 0.704, r = 0.05) and IL-6 (P=0.595, r= -0.06). It is therefore concluded that, cytokines could be used as prognostic and diagnostic biomarkers for *falciparum* malaria progression or regression outcome.

Keywords: *Plasmodium-falciparum*, Parasitaemia, Inflammatory-cytokines, Pregnant woman, Gombe State

INTRODUCTION

Globally, over two thirds of malaria deaths cases occur among under five years old children and pregnant women (WHO, 2018). Almost half of the world's population is at the risk of getting malaria infection. Malaria infection can occur in all ages and sexes but morbidity and mortality related to malaria are more usual in pregnant women (Goshu and Azeb, 2019; CDC, 2017; WHO, 2011). Pregnancy reduces women immunity making them to suffer from malaria infection because of the changes that take place in their immune systems during pregnancy and the presence of placenta which harbours parasites (WHO, 2017a; WHO, 2017b). The rate of malaria cases is rising as a result of increased risks factor of transmission in places where malaria control and management has become less, there is development of drug-resistant strains of the parasite, and in some cases, massive movement from one locality to another (Foghi, *et al*., 2021). Under five years' children and pregnant women are more susceptible to malaria infection because of their immature and low level of immunity (Omang *et al.,* 2020; Wogu and Onosakponome, 2020). Malaria infection during pregnancy is a measure treat for the woman, her foetus, and the new born baby (Omang *et al.,* 2020). Malaria contributes to high morbidity, anaemia, decrease birth weight, miscarriage and stillbirth (Foghi, *et al*., 2021). Malaria can be asymptomatic in regions where the level of immunity acquired is high and this can lead to pregnancy induce anaemia (Acquah *et al*., 2020). Malaria still remains a major public health treat in many countries, despite the accomplishment recorded in its prevention and control (Wogu and Nduka, 2018). Immunity is leisurely developed toward plasmodium infection and protection on malaria parasite appears subsequently than against malaria symptoms Response to immunity is different with blood stages and liver because various antigens of plasmodium are expressed at the liver and blood stages (Langhorne, 2008).

Cytokine plays a significant role in malaria pathology and protection. Pro-inflammatory cytokines are associated with protective cell-mediated immunity by their ability to induce parasite killing through monocytes/macrophages and neutrophils while, anti-inflammatory cytokines inhibit the production and possible cytopathic effects of pro-inflammatory cytokines and may thus be associated with the malaria susceptibility (Nnaemeka *et al.,* 2009). Little is contributed on the role of cytokine in regulating immune response to malaria, but it's believed that, the relative balance between Th1 and Th2 cytokines determined the possibility that a response will be protective or pathologic (Ho *et al.,* 2000; Perkins *et al.,* 2000; Nnaemeka *et al.,* 2009). The early recreation of inflammatory reaction induced by IFN-γ and IL-12. TNF- α appears to be essential for parasite clearance and parasitemia control in malaria infection (*Barkat et al.,* 2019). The high production of pro-inflammatory cytokines is associated with severe malaria and these may increase adherence of parasitized red blood cells to the endothelium through upregulation of adhesion molecules in *P. falciparum* infections (Barkat *et al.,* 2019; Malaguarnera & Musumeci, 2002). The production of Th2-type cytokines by maternal leukocytes prevent initiation of inflammatory and pathologic dissolution that may possibly detriment the integrity of the pregnancy outcome. (Nana *et al.,* 2010; Nmorsi *et al*., 2010). The invading of parasites by pro-inflammatory cytokines response induce the production of Th1-type cytokines which reverses the Th2-type bias, leaving the pregnant woman vulnerable to Th1-dependent infections such as malaria (Nana *et al.,* 2010). In spite of the role Inflammatory cytokines in the establishment and maintenance of pregnancy as well as protecting the foetus from infections, information on pro-

inflammatory cytokine levels of malaria-infected pregnant women is limited in our locality. The present study examined the level of some cytokines and its correlation with malaria parasitaemia among pregnant women in Gombe State Nigeria.

MATERIALS AND METHODS

Study Area

Six Local Government Areas randomly selected from the three geopolitical zones of Gombe State were used for this study. Gombe State is located in the Northeastern part of Nigeria.

Ethical Clearance

An approval with the reference number MOH/ADM/621/VOL1/211 was obtained from the Ministry of Health ethical committee Gombe State before the commencement of research.

Inclusion/exclusion Criteria

During the data collection, the subjects were informed about the purpose of the study, Verbal or written consent was obtained from relevant authorities and the study participants at the Primary Health cares in the study area, after briefing them about the purpose of the research. Confidentiality, and the right not to participate or withdraw at any time in the study was assured. Laboratory investigations were free of charge for the purpose of this study. All women who lived in the communities where the health facility is located, self-identified as pregnant, and give their consent were allowed for the study.

Sample size Determination

The sample size was determined using the standard formula for calculation of minimum sample size (Osaro *et al.,* 2019).

Study Subjects

A total of seven hundred and twenty-five (725) consented volunteer's pregnant women who are attending antenatal at primary health care facilities in the study area were used as study subjects for research.

Sample Collection

Random blood sample collection of 725 blood samples was collected from pregnant women of varying age ranging from 18 to 50 years, attending Clinics in study area.

Specimen collection/ Microscopy

Venous blood samples were obtained from the selected pregnant women with the help of a trained laboratory scientist. The blood samples were store in EDTA bottles. Care was taken while using syringes to avoid excessive bleeding.

Determination of malaria parasites and parasitaemia

A thick blood smear was prepared from the blood samples collected and stained with 10% Giemsa to determine malaria parasites according to earlier published protocol (Cheesbrough, 2010). The stained slides were examined by microscope using 100 power fields under oil immersion. Parasitaemia was calculated using an assumed white blood count of 8000/µL of blood (WHO, 2010a).

Determination of cytokine concentration in plasma

The concentrations of IL-6, IL-10, TNF-α and IFN- γ in plasma were determined using Th1/Th2 ELISA kits according the manufacturers' instructions (Catalog Numbers: E-EL-H0108, E-OSEL-H0001, E-EL-H0109 and E-EL-H6154. Elabscience®, USA).

Data Analysis

The data collected were subjected to statistical analysis using the statistical package for Social Sciences version 23 to determine any significant relationship between: Age, Gravidity and Trimester stages of pregnancy. Analysis of Variance, student's t-test and Pearson's correlation coefficient was used for tests of association and differences of means were considered significant at a 95% confidence limit i.e. when P values are below 0.05.

RESULTS AND DISCUSSION

Result

Out of the 725 examined pregnant women, 231(31.9%) were positive for *P. falciparum* malaria. Table 1, shows the age related mean parasiteamia levels of *P. falciparum*-infected pregnant women. Pregnant women within the age group 21-25 years has the highest infection rate of 38.1% and mean parasitaemia levels of 5872.29 ± 3079.29 parasites/µl, whereas, those within the age group 46-50 years has the lowest infection rate of 0.43% and parasitaemia levels of 143.00±0.00 parasites/µl. There was significant difference between *P. falciparum* infection and age group of the pregnant women (F = 691.90, P = 0.00), but there was no significan difference between parasite density and age range (F = 0.435, P = 0.86).

Table 2 shows the cytokines level of *P. falciparum* infected and uninfected pregnant women. The mean concentration of IFN-γ was higher in *P. falciparum* infected pregnant women compared with the mean concentrations of TNF-α, IL-10 and IL-6 in the uninfected. Mean concentrations of cytokines were generally elevated among the *P. falciparum* infected pregnant women than their uninfected counterpart. However, the difference was not statistical significant (P > 0.05).

The mean level of Interleukin 6 (IL-6) was highest in moderate parasitaemia (168.90±1.41 pg/ml) and least in severe parasitaemia (151.10±20.37 pg/ml) the difference was not statistically significant (P = 0.595). Interleukin 10 (IL-10) had a higher mean level in severe (198.65±1.38 pg/ml) parasitaemia than that in moderate and mild parasitaemias (190.51 ± 8.02pg/ml and 187.18 ± 5.57pg/ml), but the difference was not statistically significant (P = 0.704). The mean level of Tumour Necrosis Factor-alpha (TNF-α) was slightly higher in mild parasitaemia (945.68±2.75pg/ml) than of moderate and severe parasitaemia (945.13±4.64pg/ml and 936.88 ± 9.89pg/ml) the difference was not statistically significant (P = 0.398). More also the mean level of Interferon-gamma (IFN-γ) was slightly higher in severe parasitaemia (1834.50 ± 0.50pg/ml) and lower mild parasitaemia (1806.72±27.43pg/ml). The difference was not statistically significant (P = 0.837). However, Pearson correlation shows a very low relationship between malaria parasite density and the cytokine's levels (Table 3).

Table 1: Comparison of Age related Parasitaemia levels among *P. falciparum* infected pregnant women (N=231).

Age groups (Years)	No. Infected(%)	Mean±SEM Parasitaemia
15-20	29(12.6)	2835.17±1526.53
21-25	88(38.1)	5872.29±3079.29
26-30	42(18.2)	1922.88±887.49
31-35	54(23.4)	1323.44±458.72

36-40	15(6.49)	3089.26±2263.95
41-45	2(0.86)	200.50±63.50
46-50	1(0.43)	143.00±0.00
F	691.90	0.435
P-value	0.00*	0.86

Mean±SEM = mean standard error mean of the age groups (N=231).

Table 2: Cytokines level of *P. falciparum* infected and un-infected pregnant women (N=94)

Parameters	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)	IFN-γ (pg/ml)
Infected n = 73	167.04±1.82	188.36±4.53	945.11±2.33	1811.93±21.41
Un-infected n = 21	164.70±5.37	178.10±12.94	855.44±62.10	1648.33±119.66
P-value	0.599	0.301	0.826	0.787

Mean±SEM = mean standard error mean of *P falciparum* infected and uninfected pregnan women (N=94).

Table 3: Comparisons of inflammatory cytokine levels and parasitaemia levels among *P. falciparum* infected pregnant women (N=73).

	Parasitaemia in pregnant women				
Cytokines	**Mild (1-999 Parasites)**	**Moderate (1000-9999 Parasites)**	**Severe (>10,000 Parasites)**	**P.value**	**r-value**
IL-6 (pg/ml)	167.77±1.86	168.90±1.41	151.10±20.37	0.595	-0.06
IL-10 (pg/ml)	187.18±5.57	190.51±8.02	198.65±1.38	0.704	0.05
TNF-α (pg/ml)	945.68±2.75	945.13±4.64	936.88±9.89	0.398	-0.10
IFN-γ (pg/ml)	1806.72±27.43	1829.17±3.36	1834.50±0.50	0.837	0.03
TOTAL (73)	n =57	n = 12	n =4	Not Sig.	Very Low

Mean±SEM = mean standard error mean of parasitaemia levels (N=73).

Discussion

The prevalence rate of *P. falciparum* infection observed among the pregnant women in this study is 31.9%, which is lower than the rates of 40%, 60%, 78.4%, and 92% reported in Abia and Gombe State Nigeria, respectively (Ali, 2022; Ejike *et al*., 2017; Shaibu *et al*., 2019; Yoriyo and Hafsat, 2014). Additionally, the prevalence rate is higher than the rates of 18.5% and 19.5% reported in Sudan and Lagos Nigeria, respectively (Barkat et al., 2019; Olukosi and Afolabi, 2018). The difference in the prevalence rate in this study compared to the previous studies could be due to the difference in regions, time and season in which the studies were conducted and the environmental conditions which harbours the breading site of the intermediate host in the study area. Although this research was conducted in both the dry and rainy seasons from June 2021 to February 2022. The average value of the mean parasitaemia 3454.00±18465.77 parasites/µl recorded in this study was higher than the 685.56±484.55 parasites/µl reported in Abia, Nigeria (Ifeanyichukwu *et al*., 2017) and lower than that reported among pregnant women 5226 parasites/µl in Rivers, Nigeria (Wokem *et al.,* 2021). The differences in mean parasitaemia levels observed could be due to the, pattern of transmission which differs with regions, methods of examination and solitude adherence of parasite to brain and other internal organs such as placenta, endothelial cells in the spleen and kidney tissues may decrease parasite load in blood circulation (Ifeanyichukwu *et al*., 2017). The age group 21-25 pregnant women recorded a higher prevalence rate of *P. falciparum* infection at 38.1% with a mean parasitemia level of 5872.29±3079.29, while the age group of 46-50 recorded the lowest prevalence of infection with a mean parasitemia level of 143.00 ±0.00 . The finding is similar to those of Muhammad *et al.,* 2021; Hadiza *et al.,* 2018; Yoriyo and Hafsat, 2014 who also recorded the higher prevalence of infection among the lower age group than the higher age group in a study conducted among pregnant women in Gombe and Zamfara respectively. This may be due to existence of natural immunity (pregnant women acquires as the age increases) to infectious disease, such as malaria (Yoriyo and Hafsat, 2014).

The findings of the present study have shown an increase in levels of cytokines evaluated among infected pregnant women. This finding corroborates those of earlier researchers in Nigeria (Wokem *et al*., 2021; Barikuura *et al.,* 2019; Ifeanyichukwu *et al*., 2017; Boston *et al*., 2012; Nmorsi *et al*., 2010) who showed that TNF-α, IFN-γ**,** IL-10 and IL-6 were elevated in infected pregnant women than in uninfected ones. Therefore, these cytokines can be predictive biomarkers of malaria infection during pregnancy. However, Bayoumi *et al.,* 2008 reported that both ati and pro-inflammatory cytokines were higher among uninfected pregnant women than their infected counterparts. The investigation also contradicts the report in which the levels of IFN-γ, was unchanged between the infected and uninfected patients (kabyemela *et al*., 2008). The variation in cytokine's concentration could be due to the difference, in sample size, and study areas. The study showed that inflammatory cytokine such as IL-10 and IFN-γ evaluated were more in severe parasitaemia than that in moderate and mild parasitaemia, IL-6 evaluated was more expressed in moderate and mild parasitaemia than severe parasitaemia and TNF-α, evaluated was elevated in mild and moderate pareasitaemia than in severe parasitaemia. The increased levels of IL-10 in severe malaria observed is as a result of low effective clearance of parasites due to its down-regulatory activity of antiparasite inhibitory immune function (Nnaemeka *et al.,* 2009; Hugosson *et al.,* 2004) IL-10 inhibits the production of IL-6 from monocytes and severe malaria is associated with low level of IL-6 (Pestka *et al.,* 2004; Luty *et al*., 2000). Treatment of low parasite density induced a mild secretion of IL-6 concentration with a sharp decline in haemoglobin content of reticulocyte, this reduces the haemoglobin capacity to incorporate iron which could lead to anaemia during pregnancy (Nmorsi, *et al.,* 2010). This finding agrees with that of Wokem *et al.* (2021) and Ifeanyichukwu *et al.* (2017) who assert that IFN-γ and IL-10 was significantly

high in moderate and severe infection than in mild infection. However, the investigation contradicts the report where the levels of IL-6 was elevated in severe and moderate parasitaemia than in mild, and TNF-α, elevated in moderate and severe than in mild (Wokem *et al*., 2021; Ifeanyichukwu *et al*., 2017). Difference was not significant between parasitaemia and evaluated cytokine levels. However, the result supports the suggestion that increase in parasitaemia could not be a major determinant in the secretion of both anti-inflammatory and proinflammatory cytokines. Other factors like genetic and difference in individual levels of immunity may determine the amount of the respective cytokine's secretion (Ifeanyichukwu *et al*., 2017).

CONCLUSION

This study has shown that malaria in pregnancy induces increased secretion of cytokines. The increased levels of cytokines observed amomg the infected pregnant women, represent a normal level response to malaria infection, even though excessive production of IL-6 and IL-10 may lead to severe malaria and fatal outcome. Therefore, evaluation of these cytokines could serve as diagnostic biomarkers in malaria infected pregnant women.

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