International Journal of Medical Science and Dental Research

Assessment of Some Inflammatory Cytokines in Malaria Infected Pregnant Women in Imo State Nigeria

Okorie, H.M.[1, 2], Obeagu, E.I.[1, 3],Obarezi, H.C.[4] and Anyiam A.F.[5]

1Deparment of Medical Laboratory Science, Imo State University, Owerri, Nigeria
2Department of Medical Laboratory Science, Rivers State University, Nkpoluroworukwo, Port Harcourt
3Department of Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[4]Department of Obstetics and Gynaecology,Federal Teaching Hospital Abakaliki,Ebonyi State,Nigeria.

[5]Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

ABSTRACT: A cross-sectional prospective study was carried out on malaria infected pregnant women attending FMC Owerri , Imo state, Nigeria with the aim of assessing some, inflammatory cytokines. A total of 300 subjects within the age range of 18-45 years:100 infected pregnant women , 100 non- infected pregnant women and 100 non-infected non pregnant women (were recruited) for this study. Blood samples were collected from the subjects using standard method. These were analysed for inflammatory cytokines using ELISA kits. Also malaria parasite infection was screened using rapid test kit (Bio) and confirmed microscopically. In addition, questionnaires were administered to the subjects to elicit demographic information about the consequences of malaria infection. The ages of the participants were analysed using percentages. All statistical analysis was performed using statistical package SAS version 9.4.The results were expressed as mean ± standard error of mean. Two tailed ANOVA and student t-test were used for comparison of differences in various groups and the level of significant was set at $P<0.05$.Pearson correlation was used for test of association of the various groups. The various results were represented graphically using overlay plot, box plot. The data showed the mean age of the participant 29+ 5.2 (40%), followed by 31-35 age range (26%). Most of the participants (78%) were in the third trimester at the time of study, while 14 and 8 were in the second and first trimester respectively. About three quarter of the participants (46%) were self employed, (16.7%) were civil servants and establishment, while the rest were workers in private, traders, students and unemployed..*Plasmodium falciparum* was the only specie identified. The results of the inflammatory cytokines indicated significant increase in TNF-α and IL-6, significant decrease in IL-4 ($P<0.05$). Inflammatory cytokines (TNF-α, IL-4 and IL-10) are implicated in immunopathogenesis of malaria infection in pregnancy.

Keywords: *Inflammatory Cytokines, Malaria, Pregnant Women, Imo State Nigeria*

I. INTRODUCTION

Malaria, a condition caused by infestation with Plasmodium parasite specie, is a major public health problem globally especially in developing countries causing considerable morbidity and mortality especially in sub Saharan Africa where it accounts for up to 1 million death per annum (Murray *et al*., 2012). Nigeria contributes the highest burden to global malaria morbidity and deaths, recording 25% of global malaria cases and about 30% of global malaria deaths (Tolu, 2016). Pregnant women are vulnerable to malaria infection. Malaria during pregnancy is a substantial public health problem in endemic tropical countries, especially sub Saharan Africa. It has been estimated that approximately 125 million pregnant women live in malaria endemic areas in sub Saharan Africa and 32 million of these pregnant women are at risk of malaria (Dellicour *et al*., 2010; Desai *et al*., 2007).

Pregnant women are at high risk of being infected with malaria owning to the ability of the parasite to adhere to trophoblastic villous epithelium and sequester in the placenta which could eventually lead to poor pregnancy outcome (Suguitan *et al*., 2003). It is estimated that over 200,000 infants die annually in sub-Saharan Africa as a result of their mother becoming infected with malaria during pregnancy (Steketee *et al*., 2001). Malaria during pregnancy can lead to maternal and foetal adverse effects, mainly anaemia, cerebral malaria, hemorrhage and low birth weight.

Cytokines are low molecular weight regulatory proteins that are secreted by many cells of the immune system in response to a number of stimuli. They are involved in virtually all physiological responses in the body and are key players in coordinating immune responses between cells, by binding to a variety of receptors and to induce cell-specific immune responses. They are secreted by many cells of the immune system in response to a number of stimuli. During successful pregnancies, fetal trophoblasts and maternal leukocytes secrete predominantly T-helper 2 type cytokines to prevent initiation of inflammatory and cytotoxic type responses that might damage the integrity of the materno-fetal placental barrier (Bennett *et al.*, 1999). In response to invading malaria parasites, however it has been documented that Th-1 type cytokines are produce to reverse the Th-2 type bias within the placenta (Rogerson *et al*., 2003). Inconsistence reports on the response of some pro-inflammatory interleukins to peripheral and placental malaria have been documented (Diouf *et al*., 2007; Ismaili *et al*., 2003). Both pro and anti inflammatory cytokines are found at significantly increased levels in the peripheral blood and in the intervillous spaces of placentas of malaria infected woman. Productions of these cytokines is responsible for the resulting Th-1:Th-2 imbalance observed in Plasmodium falciparum infected placentas (Kabyemela *et al*., 2008; Suguitan *et al*., 2003).

Severe malaria has long been associated with high circulating levels of inflammatory cytokines such as tumour necrosis factor (TNF-a), IL-1, IL-6. Studies have demonstrated a link between TNF-a, IL-6, IL-10 and the severity of the disease in human malaria (Akanmori *et al*., 2000). Anti inflammatory cytokines has also been found to have important roles in the immune response against Plasmodium. IL-10 has an important role as an immunoregulator during plasmodium falciparum infection, neutralizing the effect of the other cytokines produced by Th-1 and CD8 cells (Couber *et al*., 2008; Langhorne *et al*., 2008). Additionally, IL-10 and granulocyte colony stimulating factor (G-CSF) have been found to be elevated and correlated with parasitemia in asymptomatic pregnant women in Ghana (Wilson *et al*., 2010), suggesting that these cytokines may act to reduce symptoms.

II. AIM

The study was done to determine inflammatory cytokines in malaria infected pregnant women in Imo State, Nigeria.

Specific objective

1) To compare the difference between the cytokine parameters (TNF-a, IL-6, IL-10 and IL-in malaria infected pregnant women, non infected pregnant women, nonpregnant women in Imo State of Nigeria.

2) To correlate cytokine parameters with haemostatic parameters in malaria infected pregnant women.

III. Materials and Method

Study area

This study was carried out in Federal Medical Centre Owerri in Imo State, Nigeria.

Study population and sample size

A total of 300 subjects between the age of 18-45 years were recruited for the study. 200 pregnant women attending maternity clinic at Federal medical Centre Owerri and 100 non pregnant women were eligible for the study.

The sample size was obtained using the formula by Naing *et al.*, 2006. Prevalence rate of malaria infected pregnant women is 74.6% (Ohalete *et al.*, 2011).

$n = z^2 \times P(1-P)/d^2$

Where

n = Sample size

p = prevalence rate 74.6%

z = confidence interval 95% - 1.96

d = Degree of accuracy- 0.05

$N = 1.96^2 \times 0.746(1-0.746)/0.05^2$

$= 288$

Experimental design

A cross sectional prospective study was carried out on 3 groups.
Group 1 =100 Malaria Infected Pregnant Subjects,
Group 2 =100 Non Malaria Infected Pregnant Subjects,
Group 3 =100 Non Malaria non Pregnant Subjects.
An oral consent was gotten from the patients after whom a structured questionnaire was administered to all respondents who were also part of clinical study.

Ethical consideration

A letter of introduction was secured from the Head of Department, Medical Laboratory Science of River State University. This letter was submitted to the ethical committee of Federal Medical Centre Owerri to seek for ethical approval to carry out the study. After all considerations the Ethical committee approved my request.

Informed consent

Participants were recruited among pregnant women booked for antenatal care. Thereafter demographic information which includes age, parity, place of residence, education, socio-economic status and medical and obstetrical history was collected using a questionnaire. The second group comprising of non infected pregnant women and uninfected malaria non pregnant woman randomly selected from the workers of the hospital.

Inclusion criteria

- Pregnant women who have no evidence of infection, other inflammatory or chronic diseases.
- Pregnant women who presented symptoms of malaria.
- Pregnant women between the age of 18-45 years.
- Pregnant women in all trimesters

- All women of the same age which are not pregnant and do not present any evidence of inflammatory disease condition will serve as the control

Exclusion criteria

Those excluded from the study were:

- Pregnant women with evidence of chronic infection like HIV, tuberculosis and inflammatory disease;
- Women who did not give their informed consent;
- Pregnant women in need of emergency care or having an at-risk pregnancy such as gestational diabetes, pre-eclampsia and eclampsia;
- Non pregnant women with evidence of chronic infection.

Sample collection

A bout 3.5mls venous blood was collected and dispensed into a plain container to obtain serum. The sample in the plain test tube was allowed to clot at room temperature and centrifuged to separate the serum and the sera for biochemical tests were stored at -20^{O}C prior to use.

Laboratory procedures

All reagents were commercially purchased and the manufacturer's Standard Operating Procedures (SOP) were strictly followed.

A) Malaria Estimation Using Rapid Test kit (Iqbal *et al*., 2000)

As modified by SD BIO LINE One Step Malaria antigen P.F (HRP-II) rapid kit was used.

Test Procedure

The kit was allowed to equilibrate at room temperature. The test device was opened for and labeled for each patient. The specimen was collected with the aid of capillary pipette provided and then transferred into the round specimen well. Four drops of assay diluents was dispensed into the diluents well. The kit was left on a flat bench for a period of 15 minutes before taking result.

B) Malaria Parasite Identification using Giemsa Staining Technique (Cheesbrough, 2010).

Procedure

A drop of blood was placed on the slide to cover the diameter 15-20mm. The blood was smeared evenly on the slide to obtain a thick film and then allowed to air dry with the slide in a horizontal position. Before staining, the stock giemsa stain was diluted in 1:10 dilution using phosphate buffer at pH 7.2. The working solution of the giemsa stain was used to cover the dried thick film for 30 minutes and at the end of the staining period, water was used to gently flush the stain off the slide. The slide was rinsed briefly in gently running tap water and the under surface of the slide blotted dry to remove excess stain. It was left to air dry in a vertical position and then viewed microscopically using x40 and x100 objectives.

Alpha Tumour Necrosis Factor (TNF-α) Assay

Human Alpha Tumour Necrosis Factor Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0110

Procedure

Dillutions of standard was prepared to get a concentration of 80 pg/mL, 40png/mL, 20 pg/mL, 10 pg/mL, 5pg/mL and 0pg/mL. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37^{o}C. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37^{o}C. 50uL of stop solution was added to each well. Optical density of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

IV. Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

Interleukin 1 (1L-6) assay

Human Interleukin 6 Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0102

Procedure

Dillutions of standard was prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37^{oC}. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37^{oC}. 50uL of stop solution was added to each well. Optical density of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

Interleukin-10 (1L-10) assay

Human Interleukin 10 Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-1035

Procedure

Dillutions of standard was prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37oC. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37oC. 50uL of stop solution was added to each well. Optical density of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

Interleukin-4 (1L-4) assay

Human Interleukin 4 Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0065

Procedure

Dillutions of standard was prepared to get a concentration of 300 ng/L, 200 ng/L, 100 ng/L, 50 ng/L, and 25ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37oC. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed

incubated for 10 minutes at 37oC. 50uL of stop solution was added to each well. Optical density of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

V. Statistical analysis

All statistical analysis was performed using Statistical Package SAS VERSION 9.4. The results were expressed as mean plus or minus standard error of mean in tabular form. Analysis of variance (ANOVA) and student t- test were used for comparism of differences in various groups.

All test performed were two tailed and the level of significant was set at $p<0.05$. Tests of association were performed using Pearsons correlation. Results were represented graphically using box plots and overlay plot to show nature of association.

VI. RESULTS

Table 1: Demographic Characteristics of Study Subjects and Mosquito Control Methods Used

Characteristics	n	%	95% Confidence Interval
Age Group (years)			
18 – 24	30	20.0	14.4-27.1
25 – 30	60	40.0	32.5-48.0
31 – 35	39	26.0	19.6-33.6
36+	21	14.0	9.3-20.5
Mean ± SD (years)	150	29.5±5.2	28.7-30.4
Trimester			
1st	8	8.0	4.1-15.0
2nd	14	14.0	8.5-22.1
3rd	78	78.0	69.9-84.9
Parity			
Prime	9	9.0	4.8-16.2
Second	35	35.0	26.4-44.7
Multi	56	56.0	46.2-65.3
Occupation			
Civil Servant	25	16.7	11.6-23.4
Worker in Private	10	6.7	3.7-11.8
Establishment	25	16.7	11.6-23.4
Trader	5	3.3	1.4-7.6
Self-employed	69	46.0	38.2-54.0
Student	13	8.7	5.1-14.3
Unemployed	3	2.0	0.0-5.7
Other			

Percentages may not add up to a 100 due to rounding

Table 1 shows the demographic characteristics of study subjects and control method used. The mean age of the participants was 29.5±5.2. Majority of the pregnant women were in the range 25-30 years of which accounted to 40% followed by 31-35 years (26%). The pregnant women were grouped according to trimester. Majority (78%) of the participant were in their third trimester and the least (8%) in their first trimester. With respect to parity, 9% of the pregnant women were primegravidae followed by secondigravidae (35%) and the highest was the multigravidae (56%).

46% of the pregnant women were self employed, 16.7% civil servant, 8.7% students, 6.7% workers in private, 3.3% traders and 2.0% unemployed. Out of 150 participants, 51.3% used bed nets, 31.3% window net and 17.3% mosquito repellants.

Table 2: Comparison of Mean ± SEM Cytokine Parameters by Treatment

Parameters	**MP+ve Preg Women (n=100)**	**Mp-ve Preg Women (n=100)**	**Control (n=100)**	**P-value**
Tumour Necrosis Factor (TNF-α)	13.31 ±0.27[a]	10.93 ±0.23[b]	10.65 ±0.21[c]	0.0001***
Interleukin-10 (IL-10)	26.04 ±0.6[a]	23.94 ±1.54[a]	29.09 ±2.54[a]	0.116[ns]
[	25.51 ±2.88[a]	14.72 ±3.91 [b]	44.16 ±5.47[c]	0.0001***
Interleukin-4 (IL-4)	24.86 ±0.61[a]	20.06 ±0.48 [b]	18.16 ±0.44 [b]	0.0001***
Interleukin-6 (IL-6)				

Within parameter, mean ± SEM with different superscript are significantly different at p<0.05

Significant level = *P < 0.05; **P < 0.001; P < 0.0001

ns = not significant P > 0.05

Table 2 shows the mean concentration of cytokines. Tumour necrotic factor alpha (TNF-a) concentration is significantly increased among the malaria infected and noninfected women (13.31 ±0.27) when compared with the control group (10.65 ±0.21) (P<0.01). Interleukin-10 is not significantly different in the pregnant women when compared with the control. Interleukin-4 concentration of infected women is 25.51 ±2.88; noninfected women are 14.72 ±3.91 and control is 44.16 ±5.47. The difference among the groups is significant. Interleukin-6 concentration was significantly increased among malaria infected and noninfected pregnant women (24.86 ±0.61 and 20.06 ±0.48 respectively) when compared with the control (18.16 ±0.44).

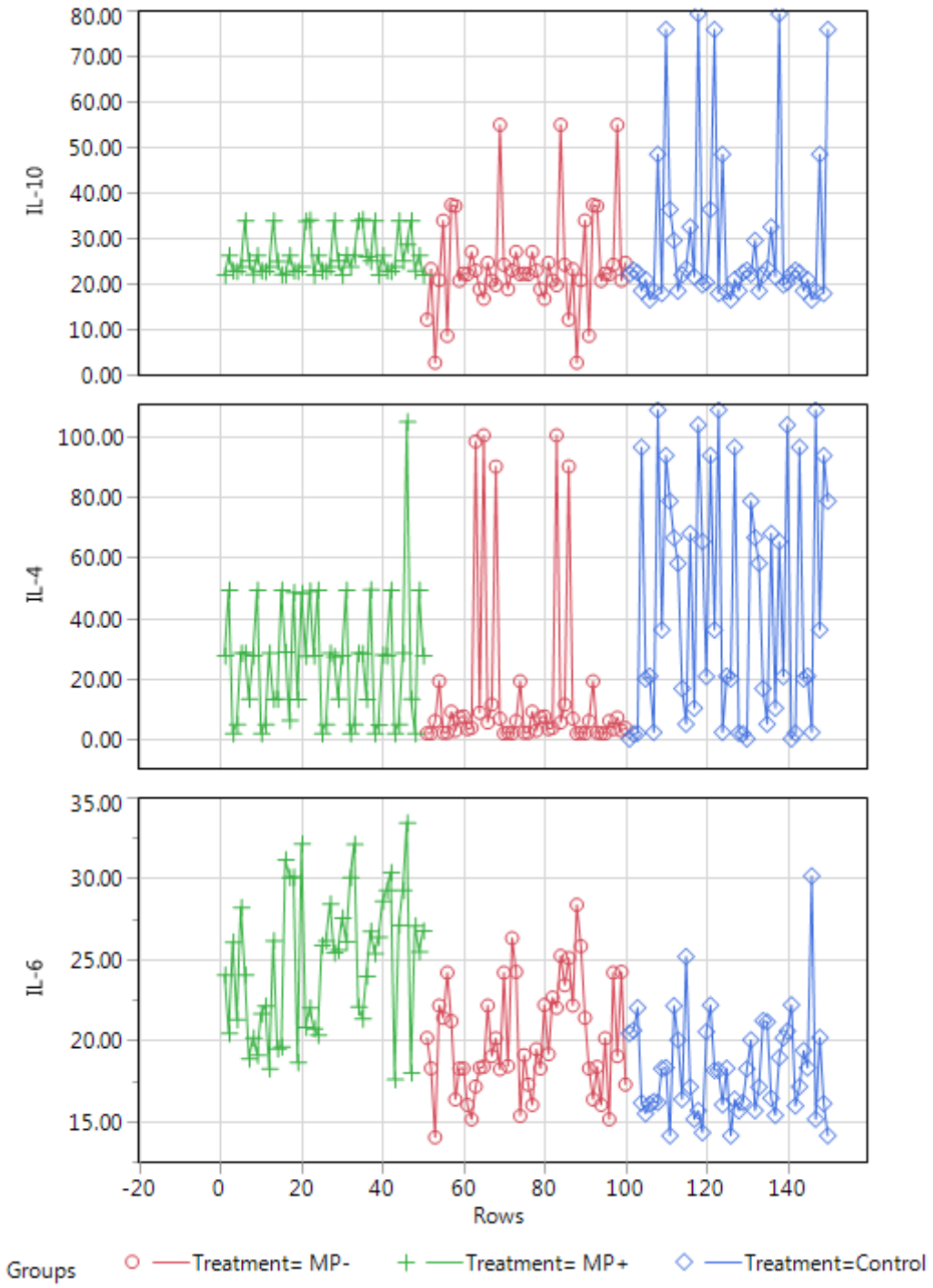

Figure 1: Overlay Plot of Cytokine Parameters by Treatment

Figure 1 shows the overlay graphical plot of some white blood cell parameters and cytokines respectively. Total lymphocyte ranges were quite lower compared to control.The decreased concentration of IL-10 in MP+ pregnant women, decrease IL-4 concentration in MP+ pregnant women and increased concentration of IL-6 in MP+ pregnant women against malaria negatively and controls were well demonstrated graphically in fig 1.

Discussion

The age-related prevalence of malaria infected pregnant women showed a decrease in infection with increase in age from 60% in women in the age group 25-30 years to 21% in those in the 35-above years (Table 1). This

observation agrees with the findings of previous workers (Opara,2008 and Ohalete *et al*., 2011) who stated that age and sex have no bearing on the incidence of malaria.

It was observed that women in their first trimester (8%) had lesser prevalence than those in their second (14%), and third (78%) trimesters respectively. This finding is supported by other findings which reported that over 70% of the infections were in the third trimester (Frank *et al*., 2016). In contrast, other findings observed that the prevalence of infection was higher during the first trimester of pregnancy and decreased steadily during the second and third trimesters. The reason may be because pregnant women generally do not attend antenatal clinic early in pregnancy and a large proportion of them might have unrecognised and untreated malaria infection as most infections are asymptomatic (Agomo *et al*., 2009).

In relation to parity, the prevalence of parasitaemia, was higher among the multigravidae (56%) than the primigravidae (9%) and Secondigravidae (35%). These results were not in accordance with the findings from similar studies conducted in many other malarious areas of the tropics. This is because while those findings are of the view that parasitaemia was significantly higher in primigravidae than in multigravidae (Rogerson *et al*., 2000), indicating a strong relationship between parity and malaria infection with mean parasite density levels decreasing as the number of gestation increased thus confirming that the African primigravidae remain unquestionably the most susceptible (Rogerson *et al*., 2000, WHO 2003) but this is contrary to this particular work which showed that the multigravidae are the most susceptible group which inversely agrees with (Odikamnoro *et al*., 2014) that the protective immunity in pregnancy is not a function of parity. This is further explained by (WHO 2002) that in the first and second pregnancies, women are especially vulnerable to *P. falciparum* parasitaemia.

There was difference in the prevalence of malaria between the different occupational groups surveyed with the highest prevalence among the self employed women. This finding agrees with similar reports (Usip and Opara, 2004), who reported highest prevalence of infection amongst peasant farmers. This may be related to exposure to arthropod vectors, which transmit malaria parasites. Civil servants stay mostly in offices often provided with electric fan which keep away mosquito vectors. Traders and artisans spend most of their time in open places such as shops, open shade etc which exposes them to vector bites and transmission of malaria parasite than occupational groups.

Among the three types of mosquito prevention method practiced by our study subjects, 51.3% uses bed net, 31,3% uses window net and 17.3% uses mosquito repellant. This could be as a result of cost effectiveness of mosquito bed net when compared with that of repellant. Bed net has treated ones which even kill the mosquito once it gets in contact with it. The level of environmental sanitation has been remarkably high over the years until the long period of military intervention in national politics and governance. The urban centers in particular Owerri, the capital city was characterized by unsightly refuse dumps, over filled and blocked gutters and drainages and consequently denied Owerri the beauty and glory of being the cleanest city in the Federation. Stagnant water bodies, over grown bushes and fields even around homes and offices were easily noticeable in both urban and rural communities in the state. These changes in the environment increased vector breeding sites and consequently increased transmission of the malaria parasites in the area.

In this study, exposure to malaria altered the balance of maternal cytokines. Samples from malaria exposed women were markedly different: pro-inflammatory cytokines like TNF-a, appeared in abundance; IL-10 concentrations were decreased. In studies of mice, IL-10 and the inflammatory cytokines play counter-regulatory roles in the placenta (Chaouat *et al*., 1995), a finding consistent with our observations. TNF-a is believed to have a role in parturition, which may explain our detection of this cytokine in some uninfected women. However, exposure to malaria was associated with significant elevations of TNF-a. Malaria infection has long been associated with high circulating levels of inflammatory cytokines such as tumour necrosis factor (TNF-a), IL-1, IL-6. Studies have demonstrated a link between TNF-a, IL-6, IL-10 and the severity of the disease in human malaria (Akanmori *et al*., 2000). Anti inflammatory cytokines has also been found to have important roles in the immune response against Plasmodium. IL-10 has an important role as an

immunoregulator during plasmodium falciparum infection, neutralizing the effect of the other cytokines produced by Th-1 and CD8 cells (Couber *et al*., 2008; Langhorne *et al*., 2008).

We reported an elevated level of IL-6 in the sera from the peripheral blood of infected pregnant women than their uninfected counterparts and control. This therefore implicates IL-6 in the immunopathogenesis of malaria infection in the peripheral blood of pregnant women in our locality. It has been documented that low-density parasitaemia and its treatment induced a mild increase in IL-6 concentration with a sharp fall of haemoglobin content of reticulocyte, implying reduced-capacity of the haemoglobin to incorporate iron which could result in anaemia (Nmorsi *et al*., 2010). Malarial anaemia has been associated with poor pregnancy outcomes (Quirijin *et al*., 2009).

VII. Conclusion

The elevated TNF-α among the malarous pregnant women implicate this cytokine as the major mediator in the host responses to systematic *P. falciparum* malaria in our locality. There is elevated level of IL-6 and IL-4 in the sera from the peripheral blood of uninfected pregnant women than their infected counterparts. This implicates IL-6 and IL-4 in the immunopathogenesis of malaria infection in the peripheral blood of pregnant women in our locality.

Interleukin 10 (IL-10) and IL-4 modulates TNF alpha, they are significant in immunoregulatory role during pregnancy by impeding inflammatory response. Cytokines have effective role in the pathogenesis of malaria and their levels can be useful as diagnostic markers for malaria and for monitoring the severity of the disease.

REFERENCES

[1.] Cheesbrough, M. (2005). *District Laboratory Practice in Tropical Countries, Part 2.* Second edition. Cambridge University Press. Cambridge. pp454.

[2.] Steketee, R. W., Nahlen, B. L., Parise, M. E., and Menendez, C. (2011). The burden of malaria in pregnancy in malaria endemic areas. *American Journal of Tropical Medicine and Hygiene,* 64, 28–35.

[3.] Suguita, L.A., Rose, G.F., Genevieve, F., Ainong Z. & Lucy, T. (2013). Changes in the level chemiokines and cytokines in the placentas of women with plasmodium falciparum malaria. *Journal of Infectious Disease,* 188(7), 1074 - 1082

[4.] Rogerson, S. J., Mkundika, P.C. & Kanjala, M.K. (2013). Diagnosis of plasmodium falciparum malaria at delivery. Comparison of blood film preparation methods and of blood films with histology. *Journal of Clin*

[5.] Rogerson, S. J., Mkundika, P.C. & Kanjala, M.K. (2013). Diagnosis of plasmodium falciparum malaria at delivery. Comparison of blood film preparation methods and of blood films with histology. *Journal of Clinical Microbiology*, 41, 1370 - 1373

[6.] Opara, K.N., (2008). Longitudinal study of malaria infection in uyo Urban Nigeria. Book of abstract proceedings of parasitology and public heath society of Nigeria.

[7.] Ohalette, C.N., Dozie, I.N., Nwachukwu, M.I. & Obiukwu, C.E (2011). Socieconomic consequences of malaria in pregnancy women in Imo State Nigeria. *World Rural Organization,*3(3), 144 - 149.

[8.] Quirigin, C., Sato, .E. & Klein, J. (2009). Evidence for convergent evolution of A and B blood group antigens in primates. *Human Genetics*.,101, 141 - 148.

[9.] Odikamnoro, O., Angela inganga, Ozowara, N.I.& Ngozie Okoh (2014). Prevalence of malaria among pregnant mothers and possible relationship to parity in Abakaliki, South East Nigeria. *European Journal of experimental biology* 4(4), 15 - 19.

[10.] Nnorsi, D.P., Isaac, C., Ohaneme, B.A. & Obiazi, H. A. (2013). Proinflammatory cytokines profile in Nigeria woman infected with plasmodium malaria. *Asian Journal of Tropical Medicine,* 22, 731 - 733.

[11.] Murray, C.J., Rosenfeld, L.C. & Lim, S. S. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet,* 379(9814), 413 - 431

[12.] World Health Organisation. (2010). World Malaria Report 2010.

[13.] World Health Organization (1993). A global Malaria control strategy. *World Health Organization,* Geneva Switzerland. Pp. 30.

[14.] World Health Organization (1998). Malaria. *Fact Sheets,* 94 Geneva.

[15.] World Health Organization (1999). Malaria Initiative Gains.*Bulletin of the World Health Organization.*

[16.] Langharne, F., Ndugu F.N., Sponaas A.M.& Marsh K., (2008). Immunity to malaria. More questions then answers. *Nature Immunology* 9, 725 - 732.

[17.] Kabyemela, E.R., Midial Fried., Jonathan, D.F. & Patrick, E. (2008). Fetal Responses during placental malaria modify the risk of low birth weight. *American society of microbiology* 76 (4) 1527.

[18.] Iqbal,S.S, Mayo'M.W, Bruno, J.G, Bronk,, B.V, Batt, C.A. &Chambers, J.P. (2000). A review of molecular recognition technologies for detection of biological threat agents. *Biosensors and Bioelectronics,* 15(11), 549 - 578.

[19.] Ismaila, J., Van Der Sande M., Holland M.J., & Sambou, (2003) . Plasmodium Fal. Infection of the placenta affects new born immune responses. *British Society of Immunology,* 133(3), 414 - 421.

[20.] Diouf, N. F., Souleymann, D M., JaneFranciols, T. & Plullipine, D. (2007). IL12 producing monocytes and IFN8 and FNFand producing Tlymphocytes are in placenta infected by *P. falciparium. Journal of Reproductive Immunology,* 74(1), 152 - 162.

[21.] Deliciour,S., Andrew, J., Tetem Cartos, A., Guerra S., Robert W.& Fako, R. (2016). Quantifying the number of pregnancies at risk of malaria. *Lancet,* 2(5), 314 - 319.

[22.] Desai, M., Ter Kulle, F.O., Nosten, F.& Asa Moa K., (2017) Epidemiology and burden of malaria in pregnancy. *Lancet,* 7, 931 - 948.

[23.] Couber, K.N., Bolnut, D. G. & Riley, E.M. (2008). The master regulator of immunity to infection. *Journal of immunology,* 180, 5771 - 5777.

[24.] Agomo, C.O., Wellington, A.O., Rose, I.A. & John, U.A, (2009). Prevalence of malaria in pregnant woman in Lagos, South West NigeriaKorean. *Journal of Parasitology;* 47(2), 179 - 183.

[25.] Akanmon, B.D., Kurtzhal, J.A.& Goka, B.Q.,(2010). Plama antibodies from malaria exposed pregnant women recognized variant surface antigens of *P. Falciparum*infected erythrocys in a parity dependent manner and black parasites. To chondroitin sulfate. *The Journal of Immunology,* 165(6), 3301 - 3315.

[26.] Bennet, WA,Lagoo-Deenadayalan S, Whitworth N. S, Stopple, J. A, & Barber, W. H (1999). first trimester human chorionic villi express both immunoregulatory and inflammatory cytokines a role for interleukin 10 in regulating the cytokines network of pregnancy. American journal of reproductive immunology 41: 70-78.

[27.] Wilson , N.O, Bythwood T., Solomon W.,Jolly P,Yatich N & Anderson W. (2010). Elevated level of interleukin 10 and G-CSF associated with asymtomatic malaria in pregnant women. Infectious Disease of obstetics and gyneocology pg 317-340

[28.] Usip, L. P. E. & Opara, K N. (2008), Longitudinal study of malaria infection in Uyo urban Nigeria. Book of abstract, proceedings of parasitilogy and public health society of Nigeria 24- 27,p25.