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Assessment of some haematological parameters in malaria infected pregnant women in Imo state Nigeria

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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 haematological parameters, 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 haematological parameters using automated sysmex machine. Also malaria parasite infection was screened using rapid test kit (Bio line) 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 and correlation matrix to show nature of association. 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, Statistically, the result of this study revealed that malaria parasites deceased in PCV, RBC and Hb of pregnant malaria subjects compared to non-pregnant malaria negative women ($P<0.05$) among treated means. Similarly, there was significant reduction in platelet count of infected pregnant women and non-infected pregnant women ($P<0.05$). On the other hand, there is significant increase in WBC count of malaria pregnant women when compared to non-malaria non-pregnant women ($P<0.05$). From the present study, it can be inferred that haematological parameters are reliable and competent measures in diagnosing severity of malaria infection, even from the early stages.

Keywords: malaria, pregnant women, sysmex machine, *Plasmodium falciparum*, haematological parameters.

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).

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. 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.

The platelet count decreases in normal pregnancy, possibly due to increased destruction and hemodilution, with a maximal decrease in the third trimester (O'Riordan and Higgins, 2003).

Aim

The study is aimed at assessing some haematological parameters in malaria infected pregnant women in Imo State, Nigeria.

Specific objective

To compare the difference between haematological parameters (full blood count, Red cell indices and platelet count) in pregnant women infected with malaria, non-infected and non pregnant women in Imo State of Nigeria.

Materials and Methods

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 which a structured questionnaire was administered to all respondents who was 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.

Sample collection

Eight milliliters (8ml) of venous blood was drawn from each participants using standard veno puncture techniques.

2.5mls was dispensed into EDTA container for determination of haematological parameters and malaria detection, 2.0mL dispensed into 0.25mL of 3.2% trisodium citrate anticoagulated container for coagulation studies and 3.5mls dispensed into a plain container to obtain serum. The sample in the citrate anticoagulated test tube was centrifuged for 5 minutes at 3000 rpm to separate the plasma. The sample in the plain test tube was allowed to clot at room temperature

and centrifuged to separate the serum. The collected samples were analysed immediately for haematological and coagulation test while 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

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).

Methodology

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.

C) Hematological parameter estimation

The EDTA blood was measured on a fully automated haematological analyser, a five part auto analyser able to test 19 parameters per sample using the Sysmex® KX-21N autohaematological analyser. Standardization, calibration of the instrument and processing of the sample was done according to the manufactures instruction.

Procedure

An EDTA anticoagulated blood was well mixed, inserted into the probe. The button was pressed and 0.02ml of blood was aspirated. After a period of 1 minute the heamatological results were displayed in the screen and printed with the aid of the printer.

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 $p<0.05$. A test of association was performed using Pearsons correlation. Results were represented graphically using box plots and overlay plot to show nature of association.

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: Comparisons of Mean ± SEM of Hematological Parameters by Treatment

Parameter	Treatment			Test Statistics
	MP$^+$(n=100)	**MP $^-$**(n=100)	**Control**(n=100)	**P-Value**
HB (g/dl)	10.11±0.14^a	10.49±0.13 ^{a}	12.09±0.19^b	<0.0001***
PCV (%)	30.50±0.41 ^{a}	31.57±0.40 ^{a}	36.39±0.57^b	<0.0001***
RBC (x 10^{12}/L)	4.04±0.05 ^{a}	4.16±0.06 ^{a}	4.60±0.07^b	<0.0001***
MCV (fl)	75.62±0.82 ^{a}	76.57±1.28 ^{a}	79.43±0.90^b	0.025*
MCH (pg)	21.33±0.51	25.11±4.02	19.21±0.86	0.212^{ns}
MCHC (g/dl)	33.19±0.02	33.19±0.03	33.19±0.01	0.987^{ns}
RDW	17.02±0.10 ^{a}	17.20±0.10^a	17.53±0.16^b	0.011*
Platelet	164.94±6.34 ^{a}	172.86±6.14 ^{a}	236.64±9.36 ^{b}	<0.0001***
PCT (ng/mL)	0.15±0.01 ^{a}	0.13±0.01 ^{a}	0.19±0.01^b	<0.0001***
MPV	7.18±0.09 ^{a}	7.12±0.10 ^{a}	7.90±0.26^b	0.002*
PDW	8.48±0.25	8.61±0.27	9.35±0.49	0.177^{ns}
Total WBC (x10^9/l)	4.82±0.29 ^{a}	5.45±0.23 ^{a}	3.84±0.22^b	<0.0001***
Total Lymph	0.90±0.06 ^{a}	1.04±0.06 ^{a}	1.34±0.14 ^{b}	0.005*
Total Mono	0.34±0.02 ^{a}	0.33±0.01 ^{a}	0.42±0.02 ^{b}	0.001**
Total Grand	3.70±0.23 ^{a}	4.01±0.18 ^{a}	2.06±0.16 ^{b}	<0.0001***
Lymphocyte (%)	22.70±1.22 ^{a}	25.02±1.01 ^{a}	41.12±1.31^b	<0.0001***
Monocyte (%)	0.68±0.45	0.62±0.10	0.54±0.39	0.6602^{ns}
Neutrophil (%)	74.46±1.57 ^{a}	74.24±1.09 ^{a}	58.14±1.34^b	<0.0001***
Eosinophil (%)	1.94±0.20 ^{a}	0.26±0.06^b	0.34±0.07^c	<0.0001***
Basophil (%)	0	0	0	---

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

a, b, c - Indicates significant differences
PCV - Packed cell volume
Hb - Haemoglobin
Significant level - *P < 0.05, **P < 0.001, ***P<0.0001
ns - Not significant (P > 0.05)

Table 2 shows the comparisons of mean and standard error of mean (SEM) of haematological parameters according to the three groups. Haemoglobin level of 11.1± 0.14 g/dL in the malaria positive group and 10.49± 0.13 g/dL in the malaria negative pregnant women were significantly lower than the control (12.09 ±0.19g/dl) (P<0.05). A similar trend was observed in the mean concentration of packed cell volume and red blood cell counts. Mean cell volume (MCV) of 75.62±0.82 in the MP+ group was significantly lower than the control. No significant differences were recorded in the red blood cell indices of MCH and MCHC. Platelet value of infected women (164.94 ±6.34 x 10^9) and uninfected women (172.86 ±6.14 x 10^9) was significantly different when compared with the control (236.64 ±9.36 x 10^9) (P=0.001).

Total white blood cell when compared among the groups was significantly higher in the infected women and uninfected women (4.82 ±0.29 and 5.45 ±0.23 x 10^9 respectively) than the control (3.84 ±0.22 x 10^9). There was a lower value in infected women (22.70 ±1.22%) and uninfected women (25.02 ±1.01%) when compared with the control (41.12 ±1.31%) and their differences were significant. Neutrophil percent of malaria infected pregnant women (74.46 ±1.57%) and malaria negative women (74.24 ±1.09%) were significantly higher than the control (58.14 ±1.34%). Eosinophil percentage of infected women (1.94 ±0.20%) was higher than control (0.34 ±0.07%); while that of uninfected women (0.26 ±0.06) was lower than that of the control. Differences among the three groups were significant.

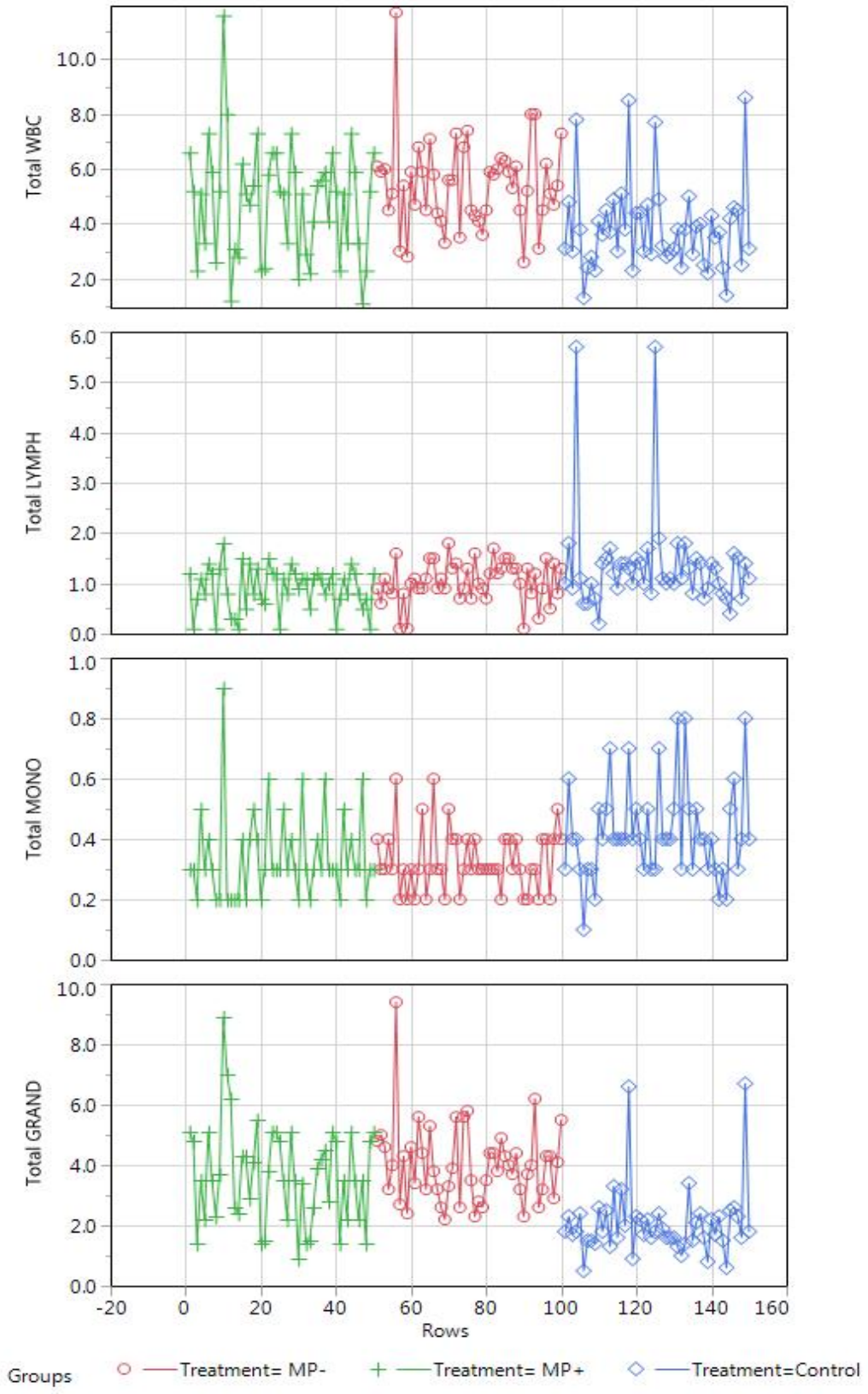

Figure 1: Overlay plot of Total WBC, Total Lymphocytes, Total Monocytes and Total Grand

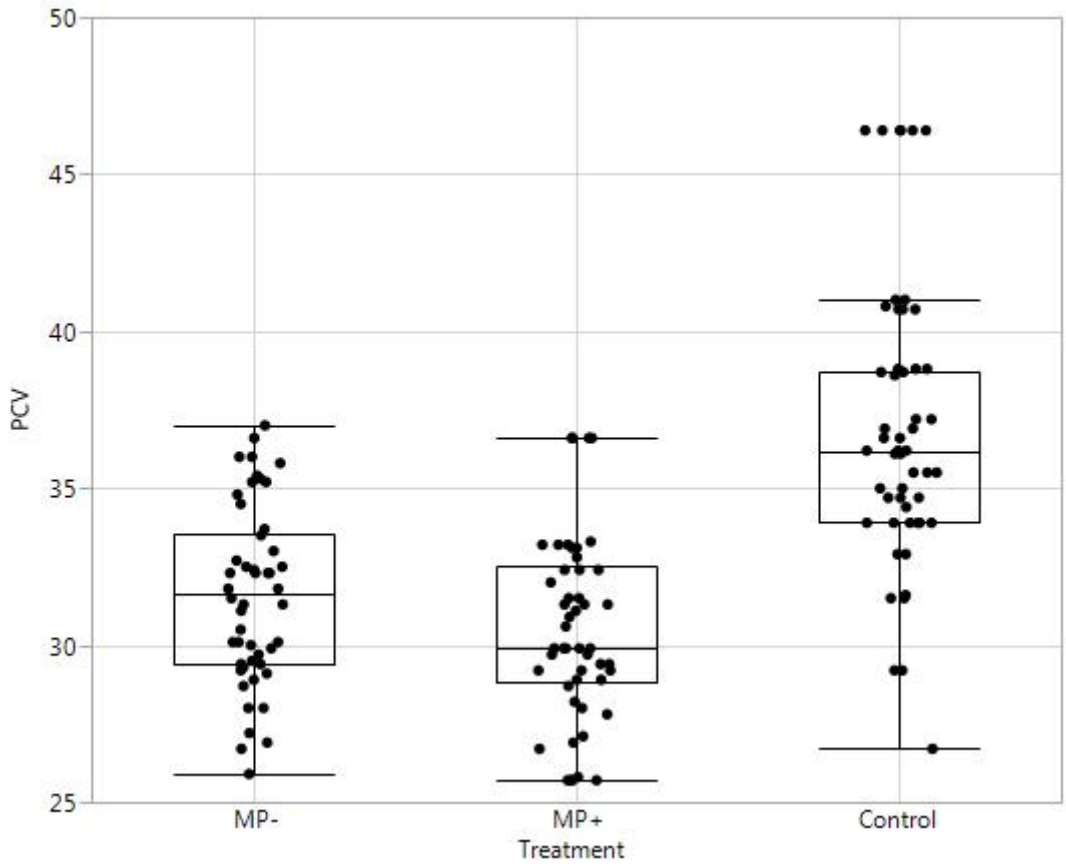

Figure 2: PCV(%) by Treatment

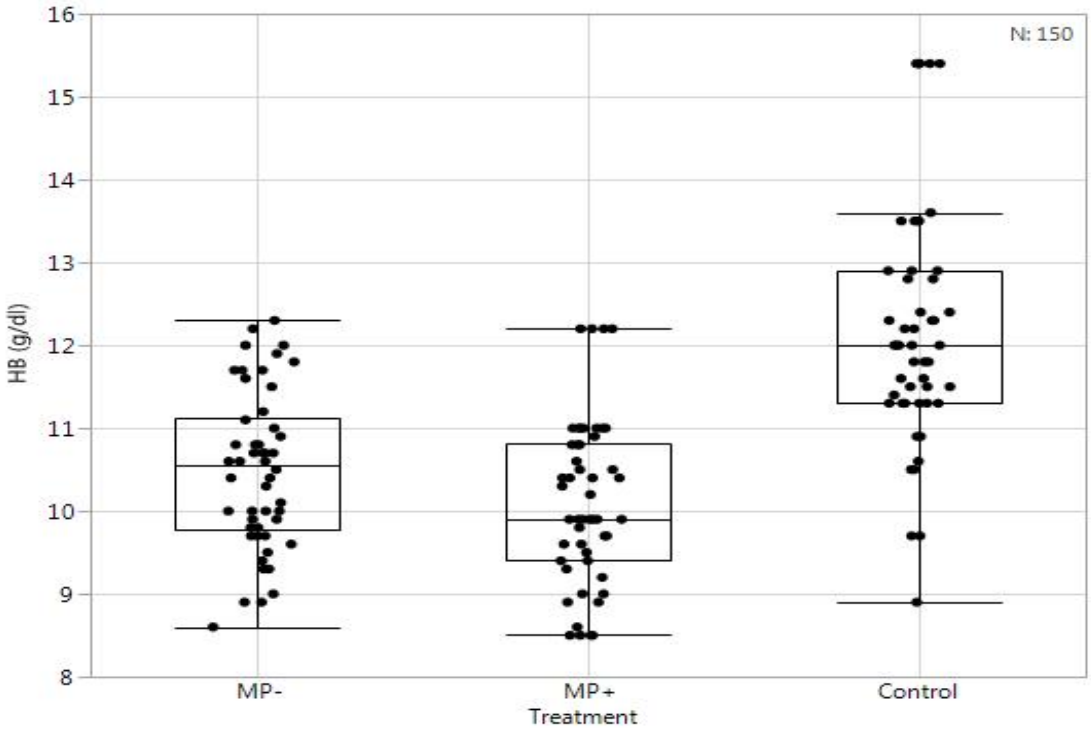

Figure 3: Hemoglobin (g/dl) by Treatment

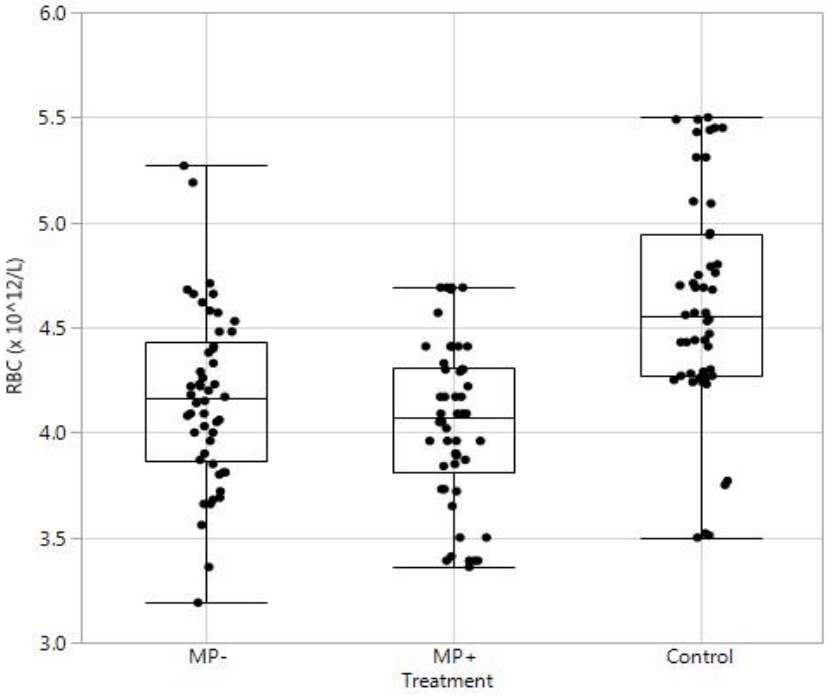

Figure 4: RBC (x 10^{12}/L) by Treatment

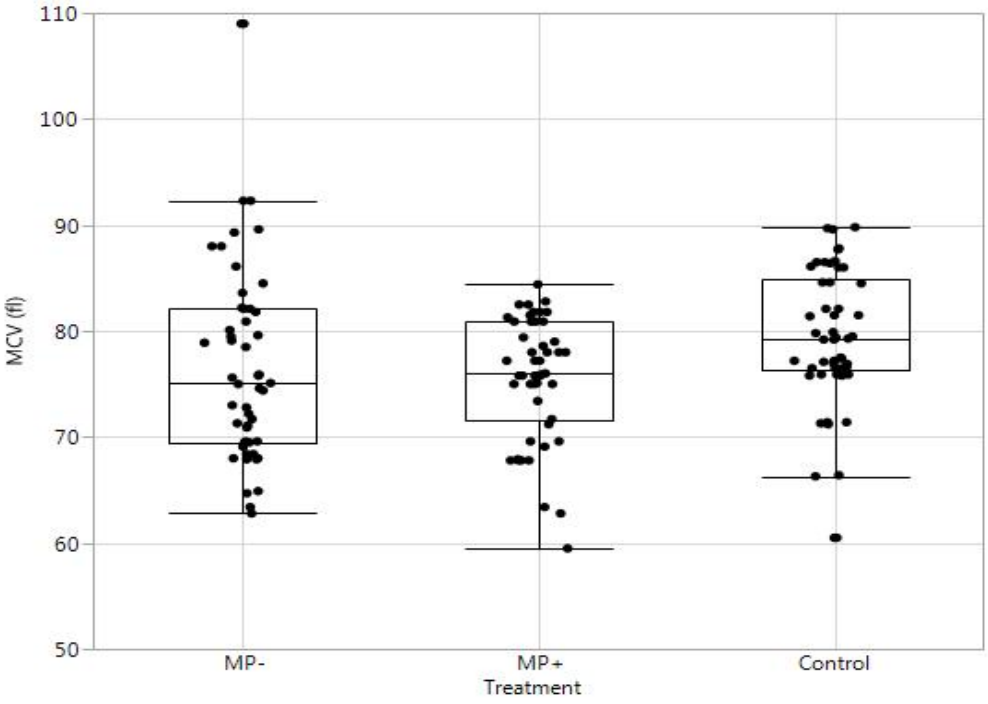

Figure 5: MCV (fl) by Treatment

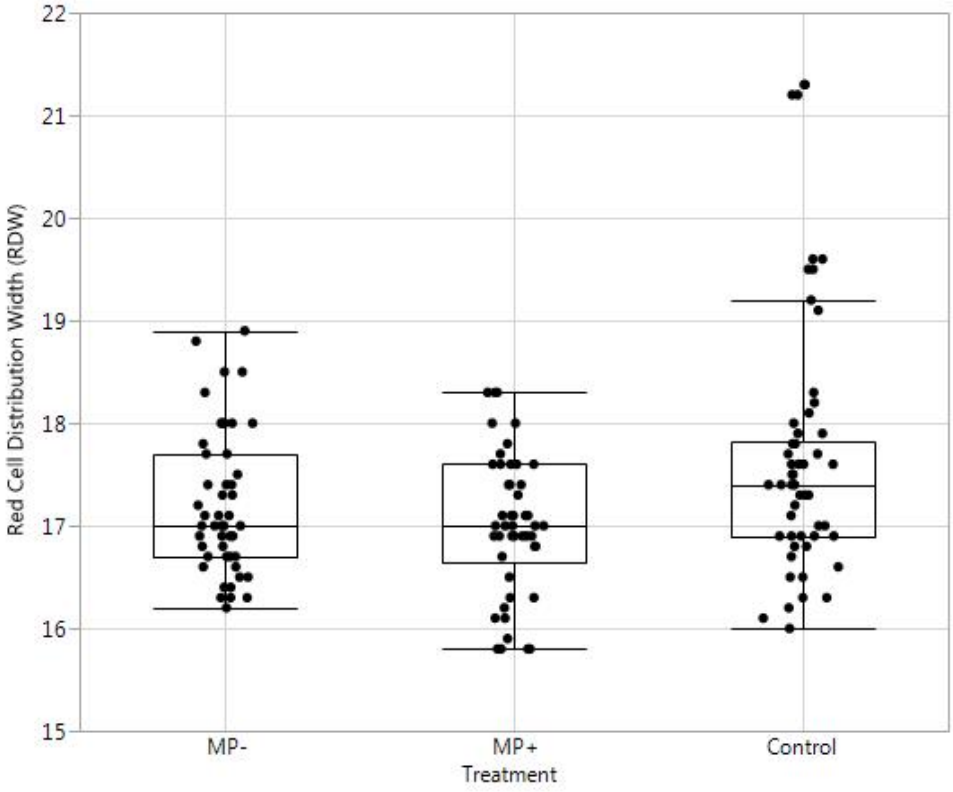

Figure 6: Red Cell Distribution with (RDW) by Treatment

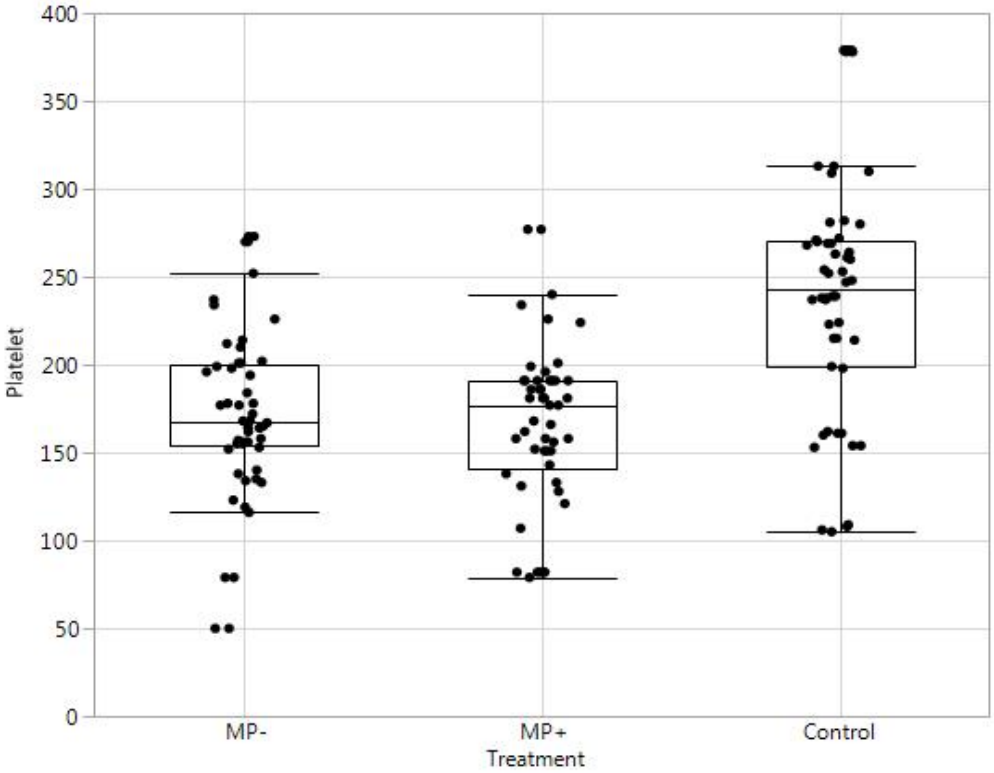

Figure 7: Platelet by Treatment

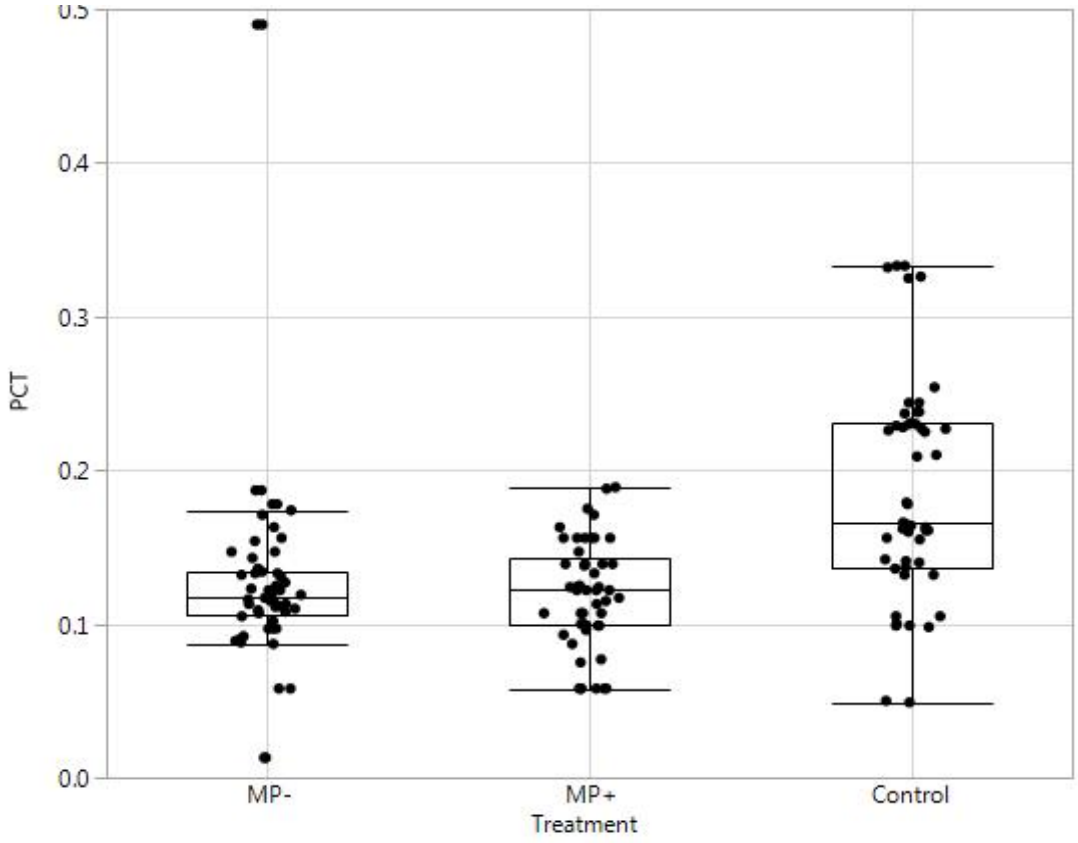

Figure 8: PCT by Treatment

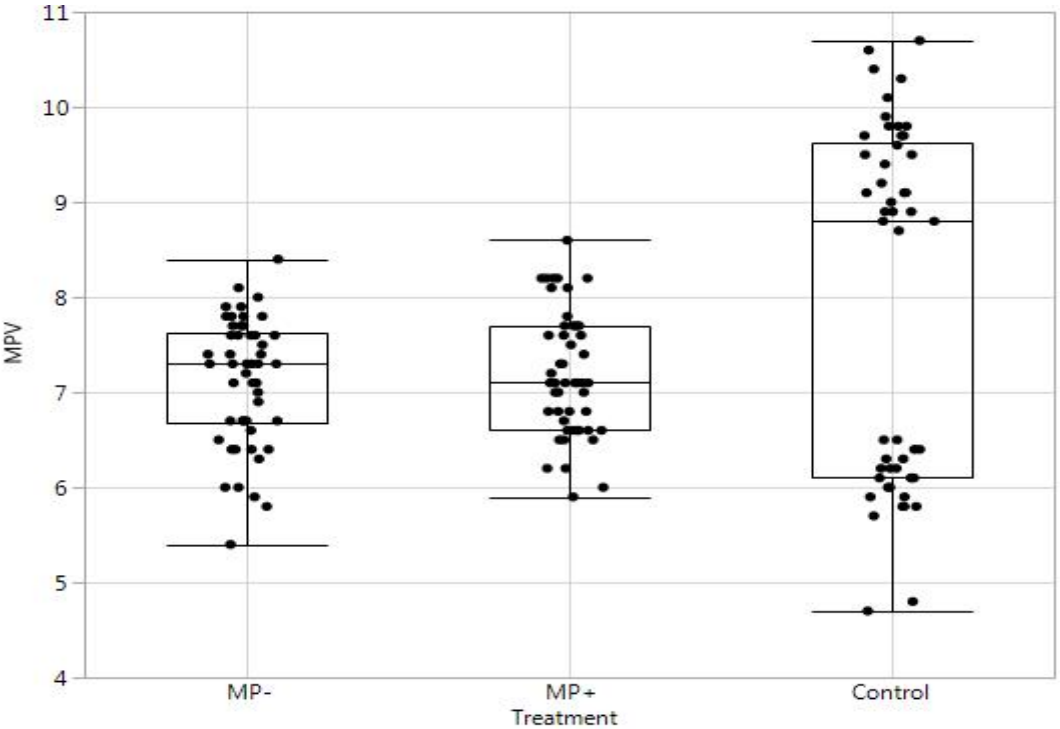

Figure 9: MPV by Treatment

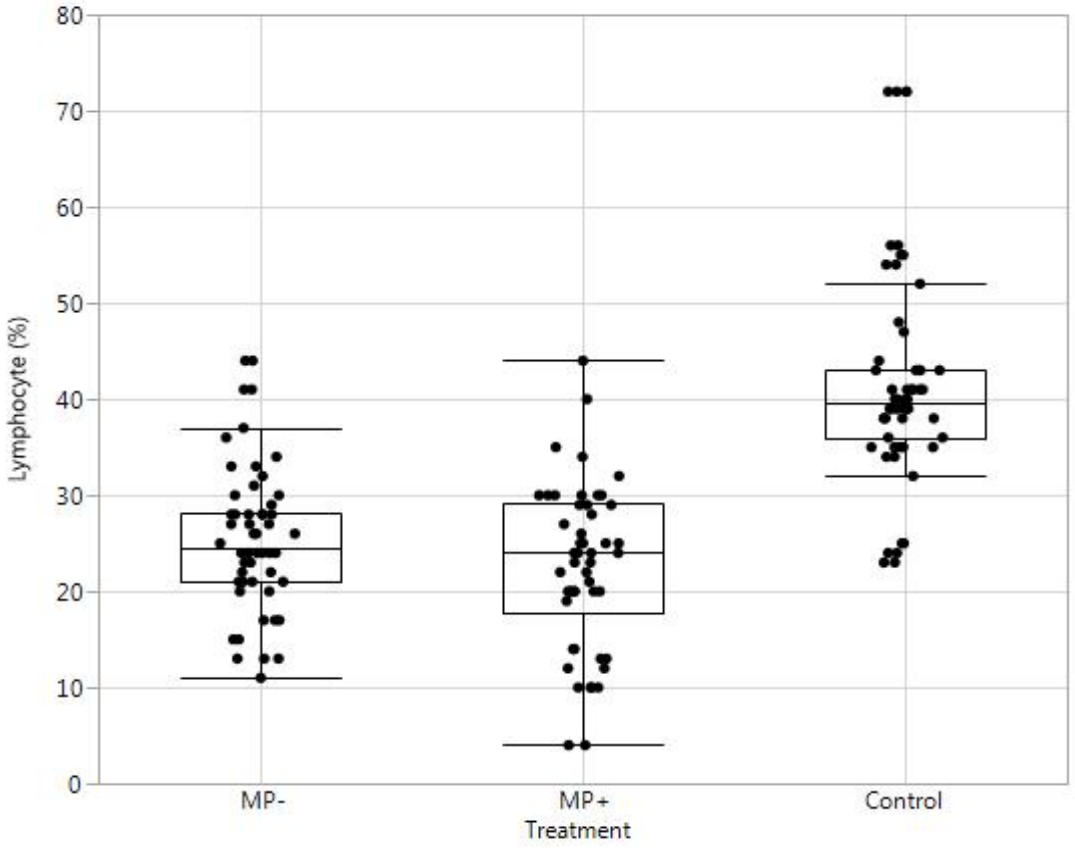

Figure 10: Lymphocyte (%) by Treatment

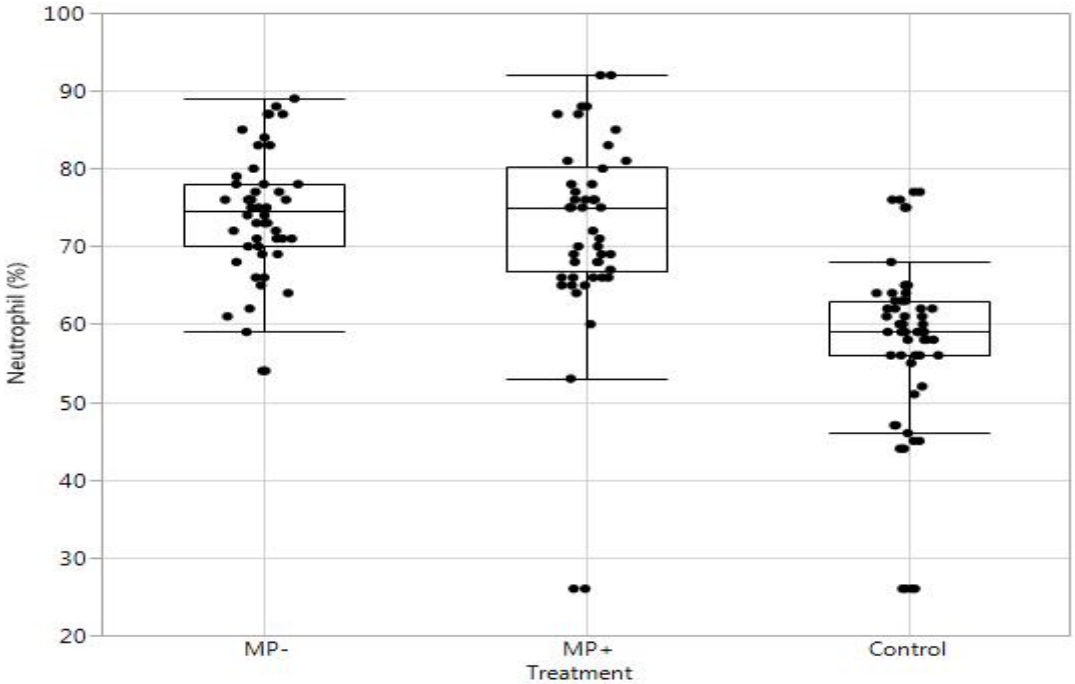

Figure 11: Neutrophil (%) by Treatment

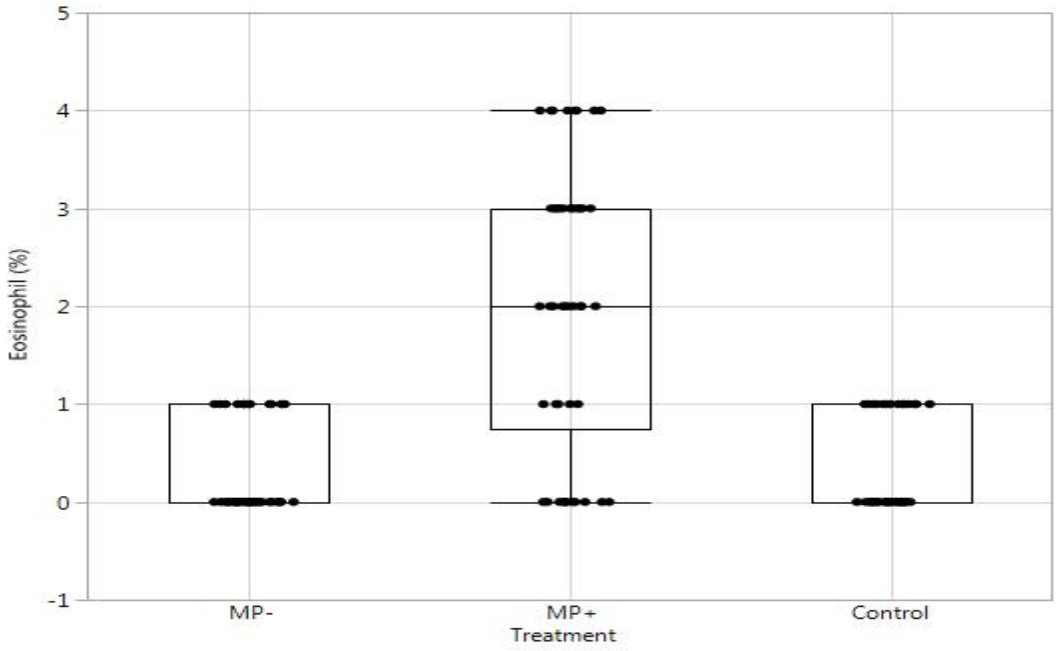

Figure 12: Eosinophil (%) by Treatment

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.

It was observed that women in their first trimester (8%) had lesser prevalence than those in their second (14%), and third (78%) trimesters respectively. 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 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, 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 (WHO 2003) but this is contrary to this particular work which showed that the multigravidae are the most susceptible group which inversely agrees with 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 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.

There is a decrease in red blood cell and haemoglobin in pregnant women compared to non pregnant women. This is due to haemodillution as a result of some hormonal changes that occur in pregnancy. During pregnancy, plasma renin activity tends to increase and aterial natriuretic peptide levels tend to reduce, though slightly. This suggests that, in pregnant state, the elevation in plasma volume is in response to an underfilled vascular system resulting from systemic vasodilatation and increase in vascular capacitance, rather than actual blood volume expansion, which would produce the opposite hormonal profile instead (i.e., low plasma renin and elevated aterial natriuretic peptide levels). Red cell mass (driven by an increase in maternal erythropoietin production) also increases, but relatively less, compared with the increase in plasma volume, the net result being a decrease in hemoglobin concentration. Thus, there is dilutional anemia.

In our study there is a significant decrease in RBC and Haemoglbin level of malaria infected pregnant women.

In our study there was a decrease in MCV, MCH and MCHC values in pregnant women than the control with more decrease in malaria infected pregnant women. The relationship between MCHC and malaria parasitaemia is expected knowing that anaemia is the commonest complication of *Plasmodium falciparum* infection..

There is a reduction in platelet count in pregnant women compared with non pregnant women (Control). The platelet count decreases in normal pregnancy possibly due to increased destruction and haemodilution with a maximal decrease in the third trimester (Boehlen & Hohlfeld, 2000). Pregnancy is associated with changes in haemostasis, including an increase in the majority of clotting factors, a decrease in the quantity of natural anticoagulants and a reduction in fibrinolytic activity (O'Riordan & Higgins, 2003).

Neutrophils are the major type of leucocytes on differential counts. This is likely due to impaired neutrophilic apoptosis in pregnancy. The neutrophil cytoplasm shows toxic granulation. Neutrophil chemotaxis and phagocytic activity are depressed, especially due to inhibitory factors present in the serum of a pregnant female (Jessica *et al*., 2007). There is also evidence of increased oxidative metabolism in neutrophils during pregnancy. Immature forms as myelocytes and metamyelocytes may be found in the peripheral blood film of healthy women during pregnancy and do not have any pathological significance (Karalis, 2005). They simply indicate adequate bone marrow response to an increased drive for erythropoesis occurring during pregnancy.

Lymphocyte count decreases during pregnancy through the first and second trimesters and increases during the third trimester. There is an absolute monocytosis that is decrease in monocyte count during pregnancy, especially in the first trimester, but decreases as gestation advances.

We found a decrease value in white blood cell of infected pregnant women when compared with both uninfected pregnant women and non pregnant women. This finding of decrease in the white blood cell count was as a result of depressed cellular immunity from the combined effect of malaria and pregnancy.

There was increase in neutrophil, monocyte and eosinophil; and decrease in lymphocyte count of infected pregnant women when compared with the uninfected pregnant women. Neutrophil and lymphocyte counts were the most important leukocytic changes associated with malaria infection. The increase in neutrophil count associated with malaria infection was in line with previous studies which suggested that it might be activated neutrophil production or release from the marrow or suppressed peripheral removal (Maina *et al*., 2010).

Conclusion

The present study has demonstrated that the haematological parameters are reliable and competent measures to diagnose severity of malaria infection, even at the early stages. Haematological parameters could be good and reliable adjunt in the early diagnosis of malaria in severely infected patients.

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