

## Peripheral Blood Assessment of Impact of Malaria on Haematological Parameters of Pregnant Women Attending Ante-natal in Two Tertiary Hospitals in Port Harcourt, Nigeria

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### Abstract

### Original Research Article

In pregnancy and malaria, changes in haematological parameters could be due to normal physiological adaptation to pregnancy-induced stress on the haematological system or it could be a result of response to pathological changes due to malaria infection. The cross-sectional study aimed at using venous blood to assess the effect of malaria infection on the levels of haematological parameters in malaria-infected pregnant women attending ante-natal clinics of two tertiary hospitals in Port Harcourt and compared it with non-infected subjects to ascertain the extent of the influence of malaria coexisting with pregnancy. Ethical clearance was obtained from the Ethical Board of the two tertiary Hospitals. About 3mls of venous blood was drawn from 240 subjects comprising 80 malaria-infected pregnant women, 80 non-infected pregnant women and 80 apparently healthy non-pregnant women. Sysmex KX21 auto analyzer was used to analyze haematological parameters. Malaria parasite was diagnosed by Microscopy, pregnancy test done with Rapid Diagnostic Kit and data analyses with Statistical Analysis Software (SAS version 9.4). ANOVA was used for comparison of mean differences and level of significance set at  $P < 0.05$ . Results in this study showed that RBC  $3.94 \pm 0.04 \times 10^6/\mu\text{L}$ , Hb  $10.96 \pm 0.10$  g/dl, MCHC  $33.18 \pm 0.17$  g/dl, lymphocyte  $1.94 \pm 0.05 \times 10^3/\mu\text{L}$  and platelet  $202.43 \pm 5.31 \times 10^3/\mu\text{L}$ , of the malaria-infected and RBC  $3.80 \pm 0.04 \times 10^6/\mu\text{L}$ , Hb  $10.68 \pm 0.10$  g/dl, MCHC  $32.38 \pm 0.11$  g/dl, lymphocyte  $1.99 \pm 0.07 \times 10^3/\mu\text{L}$  and %, platelet  $214.83 \pm 5.54 \times 10^3/\mu\text{L}$  of non-infected pregnant women were significantly reduced ( $p = 0.0001$ ) compared to the values of RBC  $4.35 \pm 0.04 \times 10^6/\mu\text{L}$ , Hb  $11.76 \pm 0.12$  g/dl, MCHC  $34.53 \pm 0.13$  g/dl, lymphocyte  $2.39 \pm 0.50 \times 10^3/\mu\text{L}$  and platelet  $247 \pm 6.19 \times 10^3/\mu\text{L}$  of the non-pregnant women. Packed cell volume  $33.00 \pm 0.30\%$  of infected pregnant women,  $33.09 \pm 0.24\%$  of the non-infected women were also significantly reduced ( $p = 0.10$ ) compared to the non-pregnant values  $34.17 \pm 0.30\%$ . WBC and Neutrophil values of infected-women  $7.24 \pm 0.20 \times 10^3/\mu\text{L}$  and  $4.72 \pm 0.17 \times 10^3/\mu\text{L}$  respectively, and  $7.22 \pm 0.15 \times 10^3/\mu\text{L}$ , and  $4.41 \pm 0.14 \times 10^3/\mu\text{L}$  respectively of non-infected women were significantly increased ( $p = 0.0001$ ) compared to the values WBC  $5.37 \pm 0.10 \times 10^3/\mu\text{L}$  and neutrophil  $2.36 \pm 0.09 \times 10^3/\mu\text{L}$  of non-pregnant. White blood cells and neutrophil counts were significantly increased in the *Plasmodium falciparum* infected-pregnant women compared to the non-infected pregnant values in the study, Anaemia was present, however, some inadequacies in the alterations of haematological parameters in the study could be due to combined impairment of the immune system hence pregnancy often causes impaired immunity as do malaria. The use of venous blood in place of placental histology for examination of malaria infection in pregnancy is still very reliable.

**Keywords:** Haematological parameters, Malaria Parasite. Pregnant, Non pregnant, infected and Non-infected, *Plasmodium falciparum*, Tertiary and Hospital.

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## INTRODUCTION

Changes in Haematological parameters occasioned by the presence of malaria or Pregnancy are normal occurrence observed in pregnant women or in individuals suffering from malaria parasitaemia. These

changes in pregnant situation could be due to normal physiological adaptation to pregnancy induced stress on the haematological system; it could also be a result of response to pathological change. Malaria however, has been described to have pathological effect on haematological parameters [1]. The alterations which

occur in haematologic system during pregnancy is a vital tool for fetal homeostasis and because of these significant changes with its associated risks, appropriate measurement, observations, data gathering and evaluation are essential. Thus, haematological parameters are useful tools for the assessment of the status of health condition of a person in situation of environmental, nutritional and pathological stress. As a result of malaria infection, pregnant women suffer disproportionately from severe anaemia and maternal mortality, and maternal anaemia is the commonest consequence of *Plasmodium falciparum* malarial infection [2]. It was reported [3] that haemodilution is a common occurrence during pregnancy in which hemoglobin level falls as a result of increased production of blood plasma than red blood cell, therefore it is expected that pregnant women with underlying illness such as malaria which also causes reduced haemoglobin should have a more severe situation especially that malaria in pregnancy is named as one of the poor health conditions that causes anemia [3]. Haematological parameters were described by [4] to be related to blood and blood forming organs; hence many recent studies involving haematological changes in malaria and in pregnancy were conducted using placental histology due to the accumulation of infected red blood cells in the maternal blood spaces of the placenta. But there has been conflicting reports on the variation in some haematological parameters in malaria yet malaria continues to remain a complex challenge in pregnant women and children [5]. Another report states that when the parasite is transmitted into the blood stream, the major blood cells like red blood cells, leucocytes and the thrombocytes are affected while the life cycle of the malaria progresses. The change in haematological parameters affects the haematopoietic system causing complications of malaria such as fever, reduced oxygen transport, still birth in pregnancy, pre term delivery and miscarriages [5]. In another report, they associated malaria with anaemia and non anaemia, mild and severe thrombocytopenia, leucopenia and leucocytosis. Since malaria is a key factor in maternal anaemia and mortality, unraveling the damage caused by the presence of malaria in pregnancy has not been fully exhausted, especially in Port Harcourt which is located in the temperate and humid region of Nigeria favoring the breeding of mosquitoes. This study was considered, using peripheral blood samples of the subjects in place of placental histology to create room for expanded understanding of how peripheral malaria in pregnancy affects haematological parameters which are very essential for the general health of mother and child during the period of pregnancy.

## MATERIALS AND METHODS

### Study Area

The study was conducted at University of Port Harcourt Teaching Hospital, Port Harcourt (UPTH) and Rivers State University Teaching Hospital, (RSUTH), the both are two different tertiary hospitals which serves

as reference centers for Port Harcourt metropolis Rivers State, Nigeria.

### Subjects

A total of two hundred and forty subjects comprising of eighty malaria infected-pregnant women, eighty non-infected pregnant women and eighty non-pregnant women aged between 18- 50 years of age were recruited into the study. Pregnant subjects who tested positive and negative for malaria parasite and non-pregnant women who tested negative for malaria were included. All non-pregnant subjects whose blood samples showed positive for parasitaemia microscopically and those subjects who refused to give informed consent were excluded from the study.

### Blood Sampling

Venous blood sample of 3 milliliters were aseptically drawn from the forearm vein using standard venepuncture technique according to the method described by [6]. The samples were dispensed into EDTA (ethylene diamine tetra acetic acid) bottles that were provided for the estimation of the levels of the haematological parameters and preparation of malaria parasite films.

### Laboratory Analysis

The analyses of haematological parameter (Red Blood Cells, Haemoglobin, Packed Cell Volume, MCV, MCH, MCHC, WBC, Neutrophils, Lymphocytes, Monocytes and platelets) were performed using Sysmex KN21 auto analyzer. Both thin and thick films for malaria parasite examination and confirmation were stained with 3% Giemsa and pregnancy test was done using Rapid Diagnostic Kit.

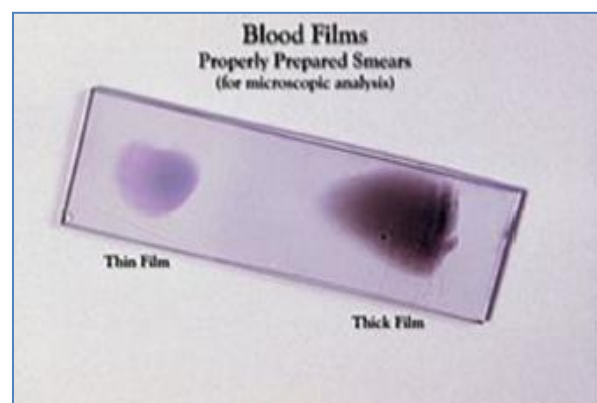


Fig-1: Thick and thin blood films used for examination and identification of malaria parasite.

### Ethical Consideration

The Research Ethics Committees of the University of Port Harcourt Teaching Hospital (UPTH) and that of Rivers State Hospitals Management Board administered the ethical approval. Before the enrollment of participants, a well-structured questionnaire was given to them to capture relevant data which included their demographic and clinical information such as – age, on anti- malaria or malaria

vaccine, anti-inflammatory drugs, HIV drugs, cancer drugs or Hepatitis B surface antigen (HBsAg), Hepatitis C virus (HCV) drugs.

## STATISTICAL ANALYSIS

The results obtained were expressed as mean  $\pm$  standard error of mean. Box plot graphs were used to show the trend of the level of the parameters in the

study groups. The data were analyzed using the statistical analysis software (SAS version 9.4). One-way analysis of variance (ANOVA) was used for comparison of mean difference between the groups and level of significance was set at  $P < 0.05$ .

## RESULTS

**Table-1: Demographic Characteristics of the study subjects**

Characteristic	N (%)	Pregnant-Positive		Pregnant-Negative		Non-Pregnant (Control)	
Overall	240 (100)	80	33.3	80	33.3	80	33.3
<b>Age Group (Years)</b>							
< 25							
25-30	34 (14.2)	11	4.6	12	5.0	11	4.6
31-35	100 (41.7)	30	12.5	36	15.0	34	14.2
36+	75 (31.3)	28	11.7	26	10.8	21	8.8
	31 (12.9)	11	4.6	6	2.5	14	5.8
Age (Mean $\pm$ SD)	30.2 $\pm$ 5.4	30.6 $\pm$ 5.1		29.4 $\pm$ 4.9		30.5 $\pm$ 6.1	

SEM: Standard error of mean within each parameter, means  $\pm$  SEM with different superscripts are significantly different at  $p < 0.05$ . Significance Level: \*= $p < 0.05$ ; \*\*= $p < 0.01$ ; \*\*\*= $p < 0.001$ ; \*\*\*\*= $p < 0.0001$ ; ns=Not significant ( $p > 0.05$ ).

The assessment of haematological parameters was studied on 80 malaria-infected pregnant women, 80 non-malaria infected pregnant women and 80 non-pregnant malaria negative women as controls as shown in Table 1: Majority of the study participants, 100 (41.7%) were within the age range of 25 – 30 years, this

was followed by those within the age range of 31 – 35 years 75 (31.3%). Those below the age of 25 years were 34 (14.2%) and the list proportion of the study participants were those from 36 and above years 31 (12.9%). The mean age of the study participants was 30.2 $\pm$ 5.4 years

**Table-2: Biometric Parameters of Malaria infected Pregnant Women, Malaria Negative Pregnant Women and Non-Pregnant Women (Mean  $\pm$  SEM)**

Parameter	MP Positive Pregnant(n=80)	MP Negative Pregnant(n=80)	Non-Pregnant Control (n=80)	Test Statistics P-Value
BMI (kg/m <sup>2</sup> )	27.43 $\pm$ 0.45	27.00 $\pm$ 0.50	26.49 $\pm$ 0.42	0.340 <sup>ns</sup>
SBP (mmHg)	109.66 $\pm$ 1.20 <sup>a</sup>	108.55 $\pm$ 1.30 <sup>ab</sup>	105.38 $\pm$ 1.05 <sup>b</sup>	0.031*
DBP (mmHg)	66.48 $\pm$ 1.06	66.23 $\pm$ 1.13	66.23 $\pm$ 0.82	0.980 <sup>ns</sup>

SEM: Standard error of mean; <sup>++</sup>Applies only to women were pregnant and positive for Malaria parasites. Within each parameter, means  $\pm$  SEM with different superscripts are significantly different at  $p < 0.05$ . Significance Level: \*= $p < 0.05$ ; \*\*= $p < 0.01$ ; \*\*\*= $p < 0.001$ ; \*\*\*\*= $p < 0.0001$ ; ns=Not Significant ( $p > 0.05$ ).

The mean  $\pm$  SEM of systolic blood pressure (SBP) of malaria infected pregnant women 109.66 $\pm$  1.20mmHg was significantly higher than the control value of 105.38  $\pm$  1.05mmHg ( $P = 0.031$ ). However, the SBP, 108.55 $\pm$  1.30mmHg of the non-infected pregnant women was not significantly higher than the non-pregnant control value of 105.38 $\pm$  1.05 mmHg ( $P > 0.05$ ). The DBP of the malaria positive pregnant women, malaria negative pregnant women and non-

pregnant malaria negative women were 66.48 $\pm$  1.06mmHg, 66.23 $\pm$  1.13mmHg and 66.23 $\pm$  0.82mmHg respectively. No significant variation in the mean of DBP was observed between the groups ( $P > 0.05$ ). The Mean  $\pm$  SEM BMI of all the pregnant women were seen to be higher than that of the non-pregnant women although it was not significantly increased between the groups ( $P > 0.05$ ).

**Table-3: Comparison of WBC, RBC, HB, PCV, MCV, MCH, MCHC, PLT and MXD among the Study Groups (Mean ± SEM)**

Parameter	Pregnant-Positive (n=80)	Pregnant-Negative (n=80)	Non-Pregnant (Control) (n=80)	Test Statistics
				P-Value
WBC (X10 <sup>3</sup> /μl)	7.24±0.20 <sup>a</sup>	7.22±0.15 <sup>a</sup>	5.37±0.10 <sup>b</sup>	<0.0001****
RBC (x10 <sup>6</sup> /μL)	3.94±0.04 <sup>a</sup>	3.80±0.04 <sup>b</sup>	4.35±0.04 <sup>c</sup>	<0.0001****
HB (g/dl)	10.96±0.10 <sup>a</sup>	10.68±0.10 <sup>a</sup>	11.76±0.12 <sup>b</sup>	<0.0001****
PVC (%)	33.00±0.30 <sup>a</sup>	33.09±0.24 <sup>a</sup>	34.17±0.30 <sup>b</sup>	0.010**
MCV (fl)	81.75±1.28	82.29±1.68	78.74±0.70	0.111 <sup>ns</sup>
MCH (pg)	27.93±0.26	28.07±0.29	27.29±0.30	0.119 <sup>ns</sup>
MCHC (g/dl)	33.18±0.17 <sup>a</sup>	32.38±0.11 <sup>b</sup>	34.53±0.13 <sup>c</sup>	<0.0001****
PLT (X 10 <sup>3</sup> /μl)	202.43±5.31 <sup>a</sup>	214.83±5.54 <sup>b</sup>	247.11±6.19 <sup>c</sup>	<0.0001****
MXD (%)	9.01±0.36	8.92±0.34	9.83±0.36	0.130 <sup>ns</sup>

SEM: Standard error of mean; <sup>+</sup> Women were pregnant and positive for Malaria parasites; <sup>-</sup> Women were pregnant but negative for Malaria parasites. Within each parameter, means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: \* = p<0.05; \*\* = p<0.01; \*\*\* = p<0.001; \*\*\*\* = p<0.0001; ns = Not Significant (p>0.05).

The mean ± SEM value of white blood cell (WBC) count of 7.24±0.02 x 10<sup>3</sup>/μl of the malaria infected pregnant women was significantly higher than the non-pregnant control value of 5.37±0.10 x 10<sup>3</sup>/μl (P<0.0001). There was no statistically significant difference in the WBC values between malaria infected pregnant women and non-infected pregnant women (P>0.05). Red Blood Cell (RBC) count of malaria infected pregnant women was 3.94±0.04 x 10<sup>6</sup>/μl, while the non-infected pregnant women had 3.80 ±0.04x10<sup>6</sup>/μl, these values were significantly different (P<0.0001) when compared with the non-pregnant control value (4.35±0.04x10<sup>6</sup>/μl). Haemoglobin (Hb) values of 10.96±0.0 g/dl in the malaria infected pregnant women and 10.68±0.10g/dl in the non-infected pregnant women were both significantly lower than the non-pregnant control value of 11.76±0.12g/dl (p<0.0001). Packed Cell Volume (PCV) demonstrated a similar trend as the Hb values, the values obtained in

the infected pregnant women 33.00±0.30% and non-infected pregnant women 33.09±0.24% were significantly lower when compared with the non-pregnant control group 34.17± 0.30% (p=0.01). The mean value of MCHC of infected pregnant women was 33.18±0.17g/dl. This value was significantly lower when compared with the non-pregnant control value of 34.53±0.13g/dl (p<0.0001). There was a similar reduction in MCHC of 32.38±0.11g/dl value among non-infected pregnant women (p<0.0001). The platelet count of malaria infected pregnant women 202.48±5.31x10<sup>3</sup>/μl was significantly lower than the non-pregnant control value of 247.11±6.19 x 10<sup>3</sup>/μl (p<0.0001). The values of MCV and MCH were not affected significantly by malaria parasites as there was no statistically significant difference in the mean values of these parameters among the study participants (p>0.05).

**Table-4: Comparison of Lymphocyte, Neutrophil, MXD and RDW-SD among the Study Groups (Mean ± SEM)**

Parameter	Pregnant-Positive <sup>+</sup> (n=80)	Pregnant-Negative <sup>-</sup> (n=80)	Non-Pregnant (Control) (n=80)	Test Statistics
				P-Value
Lymphocyte (%)	27.17±0.66 <sup>a</sup>	27.68±0.58 <sup>a</sup>	46.11±0.83 <sup>b</sup>	<0.0001****
Neutrophil (%)	63.79±0.72 <sup>a</sup>	60.68±1.51 <sup>b</sup>	44.76±1.10 <sup>c</sup>	<0.0001****
Lymphocyte (X10 <sup>3</sup> /μl)	1.94±0.05 <sup>a</sup>	1.99±0.07 <sup>a</sup>	2.39±0.50 <sup>b</sup>	<0.0001****
MXD (X 10 <sup>3</sup> /μl)	0.70±0.05 <sup>a</sup>	0.67±0.03 <sup>a</sup>	0.51±0.02 <sup>b</sup>	<0.0001****
Neutrophil (X 10 <sup>3</sup> /μl)	4.72±0.17 <sup>a</sup>	4.41±0.14 <sup>a</sup>	2.36±0.09 <sup>b</sup>	<0.0001****
RDW-SD (FL)	43.12±0.75 <sup>ab</sup>	44.64±0.66 <sup>a</sup>	41.08±0.37 <sup>b</sup>	0.001***

SEM: Standard error of mean; <sup>+</sup> Women were pregnant and positive for Malaria parasites; <sup>-</sup> Women were pregnant but negative for Malaria parasites. Within each parameter, means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: \* = p<0.05; \*\* = p<0.01; \*\*\* = p<0.001; \*\*\*\* = p<0.0001; ns = Not Significant (p>0.05).

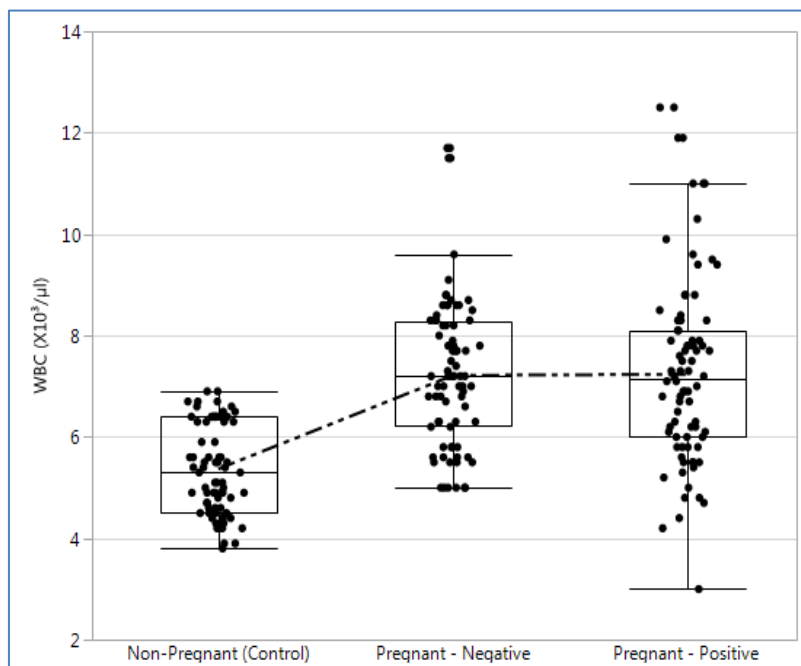
The mean ± SEM value of the lymphocytes in this study as presented on the table shows that the of lymphocytes percentage of malaria infected pregnant women (27.17±0.66%) and non-infected pregnant women (27.68±0.58%) were both significantly lower

than the non-pregnant control value of 46.11±0.83% (p<0.0001). The absolute lymphocyte count demonstrated similar trend in the reduction of the values. The absolute lymphocyte count of infected pregnant women (1.94±0.05 x 10<sup>3</sup>/μl) and non-infected

pregnant women ( $1.99 \pm 0.07 \times 10^3/\mu\text{l}$ ) were significantly lower than the non-pregnant control value of  $2.39 \pm 0.50 \times 10^3/\mu\text{l}$  ( $p < 0.0001$ ). There was a marked elevation of neutrophil percentage of  $63.79 \pm 0.72 \times 10^3/\mu\text{l}$  in the malaria infected pregnant women and  $60.68 \pm 1.57 \times 10^3/\mu\text{l}$  among non-infected pregnant women when compared with the non-pregnant control value of  $44.76 \pm 1.10 \times 10^3/\mu\text{l}$ . Similarly, the absolute neutrophil count of  $4.72 \pm 0.17 \times 10^3/\mu\text{l}$  in the malaria infected pregnant women and  $4.41 \pm 0.14 \times 10^3/\mu\text{l}$  in the non-infected pregnant women were markedly elevated when compared with the non-pregnant control value of  $2.36 \pm 0.09 \times 10^3/\mu\text{l}$  ( $p < 0.0001$ ).

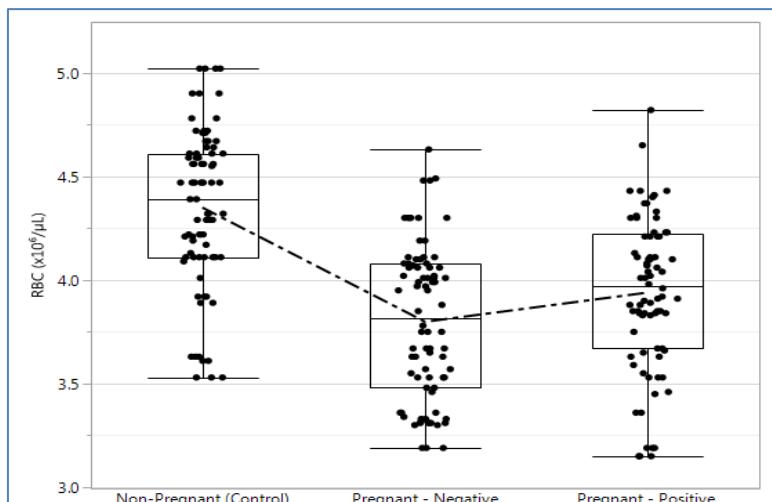
variation in the three study groups, malaria infected pregnant women, malaria non infected pregnant women and non-pregnant controls are graphically compared for parameters of WBC, RBC, Hb, PCV, MCHC and platelets. The box plots presented revealed high level of agreement of the three groups with each other as shown by the distribution and scores. In figure 1, the WBC count increased significantly upward from control values and maintained the same level in malaria infected and malaria non pregnant women. RBC, Hb, PCV and MCHC values decreased from control values to a significant level in the other study groups. The trends were all the same. Platelets showed a steady and significant fall from control value as shown in figure 7.

Box plot representation of some haematological parameters showing the trend of

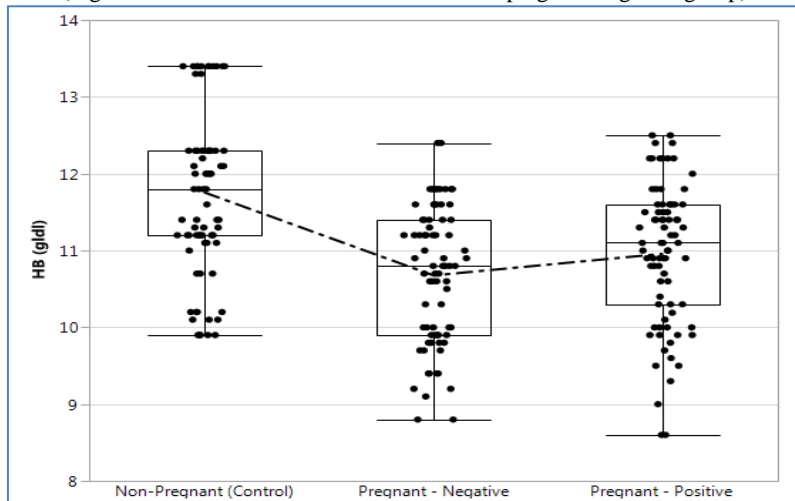


**Fig-2: Boxplot of WBC ( $\times 10^3/\mu\text{l}$ ) by Study Group**

(The WBC values of pregnant negative and pregnant positive values are both higher than non-pregnant controls, malaria could not have been a cause of increase).

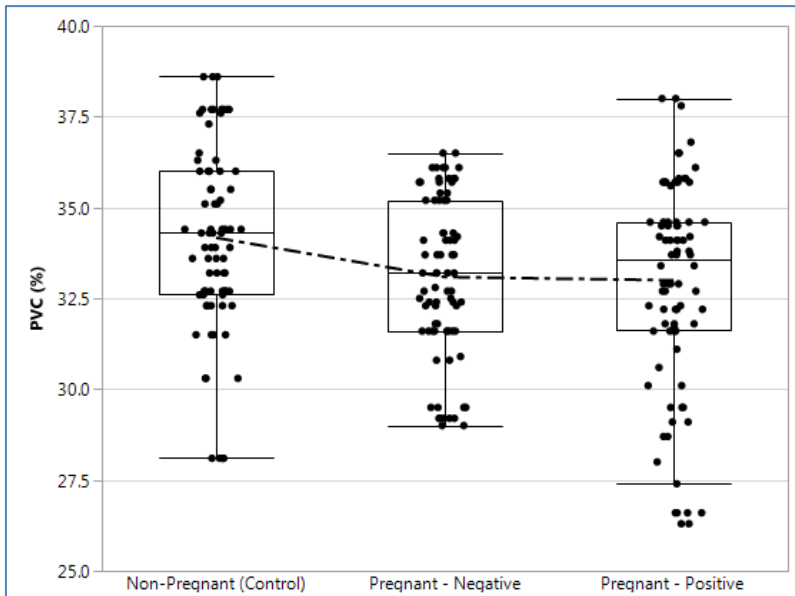


**Fig-3: Boxplot of RBC ( $\times 10^3/\mu\text{L}$ ) by Study Group**  
(Significant reduction occurred more with the pregnant negative group)

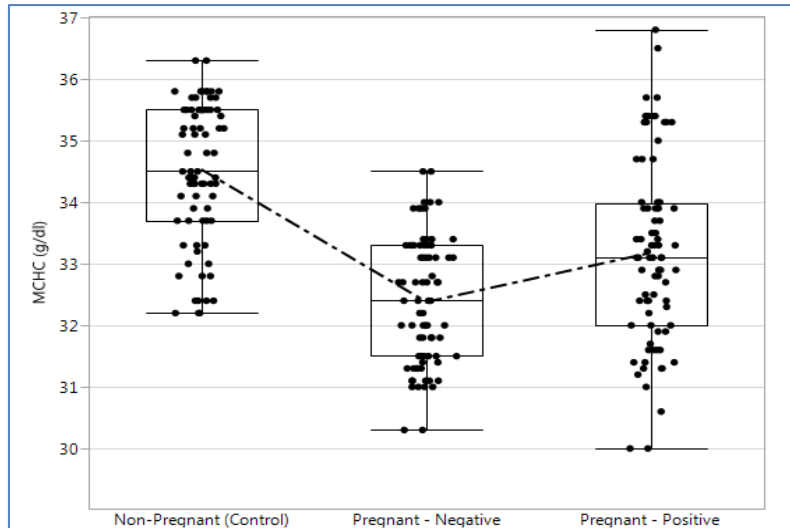


**Fig-4: Box plot of HB (g/dl) by Study Group**

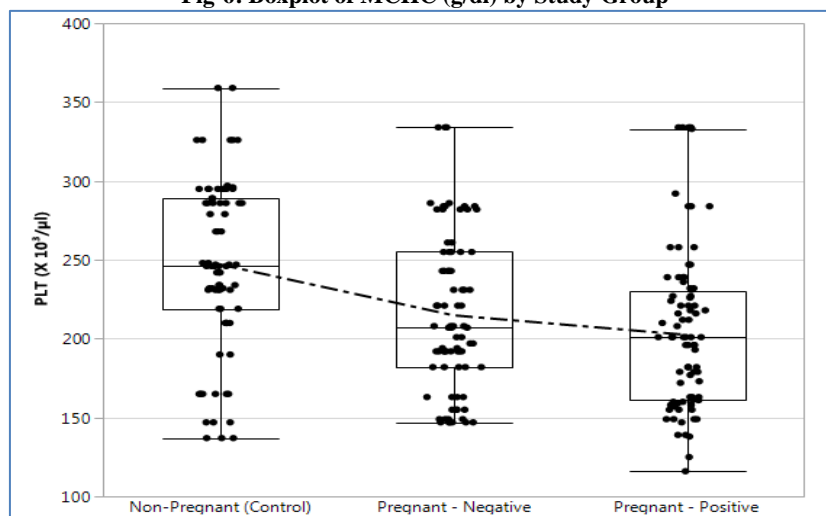
(Pregnancy induced haemoglobin reduction .not much difference between malaria negative and malaria positive groups)



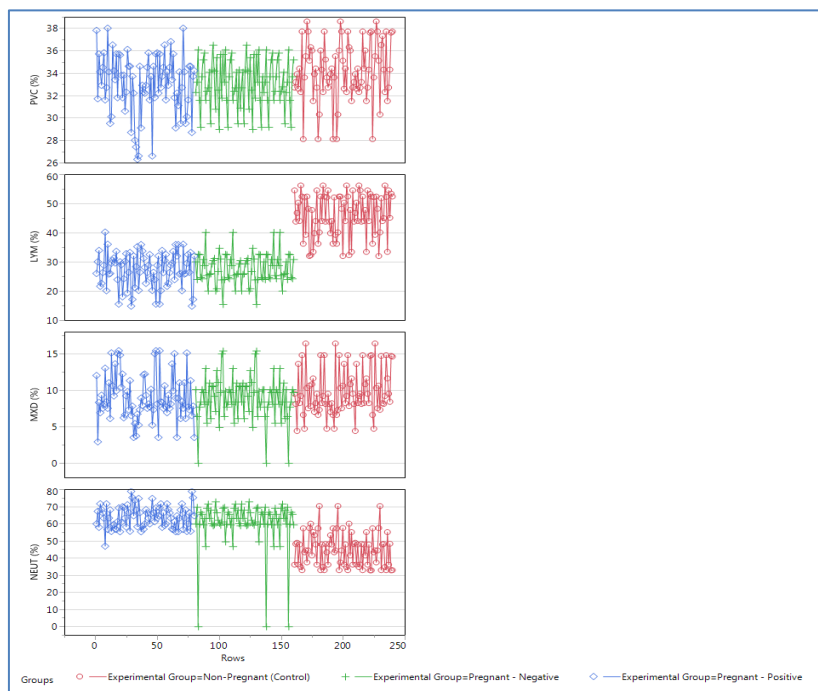
**Fig-5: Boxplot of PVC (%) by Study Group**



**Fig-6: Boxplot of MCHC (g/dl) by Study Group**



**Fig-7: Boxplot of PLT (X 10<sup>3</sup>/µl) by Study Group**



**Fig-8: Overlay plots of Neutrophil (%), Lymphocyte (%), MXD (%) and PVC (%) graphically comparing the trend of influence of malaria and pregnancy in the parameters to show the difference in the groups**

## DISCUSSION

There was significant elevation of leucocytes count in malaria infected pregnant women in this study which contradicts the finding of [7] and [8] where malaria parasitaemia especially in *plasmodium falciparum* was explained to induce low white blood cell count (leucopenia). Our finding correlates with the finding of [9] which reported increase in mean values of leucocytes in malaria parasitaemia in pregnancy. This finding is also similar to previous studies done by [10], and [11], the different reports stated that during pregnancy, WBC value usually increases with lower limit values and may remain elevated throughout the period of puparum. The elevation in the mean value of leucocytes in this study above the values from previous studies on pregnant women with malaria, may be related to the body system adjusting to the physiological stress and pathological stress induced by pregnancy and malaria infection at the same time. It could also be due to the fact that white blood cells are known to be responsible for the body's defense, and the number of WBC's in the blood is often an indicator of the disease or health condition of the individual thus the WBC count is seen as an important subset of the complete blood. The observed increased leucocytes count could also be due to marked increase in the value of neutrophil count in this study (a subset of the WBCs that are phagocytes). Phagocytosis is one of the ways that neutrophils play a role in the clearance of malaria parasites and the observed neutrophilia could also be due to impaired neutrophilic apoptosis [12].

The marked elevation in the mean value of the neutrophil count observed in this study in malaria infected pregnant women above the count seen in non-infected pregnant women and non-pregnant control corroborates with a previous work done by [12]. They reported elevated neutrophil count in malaria parasite infection, their report stated that the neutrophils are effector cells in malaria pathogenesis, as they interact with coagulation, proteins and plasma elastase increases in patients suffering malaria infection due to *P. falciparum*. The neutrophil elastase when they are released by the activated neutrophils, cleave the infected red blood cells (iRBC) antigens involved in *Plasmodium falciparum* iRBC cytoadherence which result in the phagocytosis of the iRBC in vivo [13]. In this study, the increase in neutrophil count may be related to activation of neutrophil production from the marrow or suppressed peripheral removal otherwise known as apoptosis which results to increased number of neutrophils in the peripheral.

The observed reduction in lymphocyte count in this study followed a reducing pattern in the study groups. The absolute lymphocyte count of the infected pregnant women was lower than that of the pregnant

women not infected with malaria and lower than the non-pregnant control value. This finding was similar to previous studies reported by [14] that in *P. falciparum* malaria, low lymphocyte count is a well-established fact but usually replaced by lymphocytosis after a few days of treatment with appropriate drug and it normalizes in couple of weeks. The level of lymphopenia seen in the non-infected pregnant women compared to the non-pregnant control value could be associated to normal adaptation to pregnancy probably the body is responding to the altered immunity which often occur during pregnancy. And pregnancy is a well-established cause of altered immunity particularly cellular immunity [15].

A common cause of altered immunity is under nutrition, the body lacks protein and other nutrients that are necessary for the production of lymphocytes and it is a known fact that most pregnant women do not feed well on balanced diet to get the required nutrient meant to keep them healthy and develop their baby adequately. This is the reason why pregnant women are given vitamin supplements during the course of pregnancy. Altered immunity may also occur as a relatively nonspecific response to various forms of stress such as acute infection. The lymphopenia observed in malaria infected women, therefore could be due to the malaria and pregnancy acting synergistically to impair the cellular immunity thereby reducing the lymphocyte count and potentially increasing susceptibility to malaria infection. Red blood cell count, Haemoglobin (Hb) concentration and percentage of Packed Cell Volume (PCV) in this study were significantly reduced in the malaria infected pregnant women compared to the non-pregnant experimental group. Also the non-infected pregnant women had significantly lower values compared to the non-pregnant control. This observation was similar to the finding of [9] who reported low red blood cell count, low haemoglobin concentration and low haematocrit in pregnant women infected with malaria compared to their non-infected counterpart. It also agrees with the reports of [16] where Hb and haematocrit levels were recorded to be low in the first and third trimesters of pregnancy. The other previous reports which our finding in this study agrees with are the findings of [17] and [18], though their result did not show significance, yet there was decrease in the levels of PCV, red blood cell count and Hb concentration. The significant reduction of red blood cell count, Hb concentration and PCV observed in malaria infected pregnant women in this study reveals the presence of anaemia in pregnancy contributed by malaria parasite infection. This observed anaemia is a known complication of pregnancy on these haematological parameters. It has also been reported in malaria parasite infections. The parasites as they penetrate the red blood cells, stick to the linings of the



small blood vessels and this leads to the parasites providing obstruction to tissue perfusion. Therefore patients with anaemia experience dehydration and hypovolaemia.

Red cell destruction is also an inevitable part of malaria infection, as anaemia further compromises oxygen delivery. The pathogenesis of anaemia in malaria infection following the destruction of red blood cells is thought to arise from a combination of haemolysis of parasitized red blood cells, onward removal of both parasitized and unparasitized red blood cells, depressed and infected erythropoiesis together with dyserythropoietic changes. Red blood cells, in this study, were seen to be significantly reduced in the non-infected pregnant women compared to the mean value observed in the infected pregnant women. While the reduction in the mean value of red blood cell, PCV and Hb in pregnancy compared to the non-pregnant values could be associated to normal occurrence from haemodilutional effect in pregnancy, the reason for the slight increase in malaria infected women above that of the non-infected women in this study cannot be explained. But anaemia is known to be Multifactorial in origin. Although malaria is an important contributor of anaemia when it is present, we suggest that proper nutritional and antenatal care must have played a role in maintaining proper erythropoiesis giving good red cell indices values in these subjects. This suggestion is inference from the suggestion of [19], who reported that impaired erythropoiesis is responsible for bone marrow dysfunction and few of the different mechanism through which malaria could cause anaemia. The values of MCHC, MCH and MCV were also considered in this study. The MCHC values among the infected and non-infected pregnant women were lower than the mean values of the non-pregnant control. This finding was in consonance with the previous report by [20] who reported that in severe malaria, there was marked reduction in the deformability of uninfected red blood cells leading to low MCHC. Our finding however was at variance with the report of [9] and [1] who reported high MCHC, MCV and MCH in malaria infection with *plasmodium*. It was therefore suggested in this study that the low MCHC could be as a result that when red blood cells are attacked by the *plasmodium* parasites, invasion by the parasite occur, which leads to alteration of the host red cells. These alterations in the red blood cells which includes loss of normal shape, increased rigidity of the cell membrane, increased permeability to a wide variety of ionic and other species, increased adhesiveness, mostly to the endothelial surfaces bring about enhanced parasite survival within the red cells thereby increasing the manifestation of the disease which anemia is one of them [21]. Also the low MCHC could be associated to low Hb in the infected subjects because MCHC is known as an index of Hb concentration per red cell, indicating the oxygen carrying capacity of each red cell and it is affected therefore by haemodilution in pregnancy. MCV and

MCH values conversely were not significantly affected by malaria in pregnancy. This observation contradicts the findings by [1] and [9] the two different findings reported high MCV and MCH in malarious women. But our finding is similar to the findings of [22] who reported slightly low values in MCH, MCV and MCHC. These observations could be associated to poor in-take of iron supplements during their pregnancy period. It could also be the outcome of the presence of few red blood cells and not just haemodilution or deformability of the cell but also increase destruction of infected and non-infected red blood cells and also premature cells [3] and [22].

Reduction in platelet count observed in this study occurred among the infected pregnant women and non-infected pregnant women compared to the non-pregnant control. This observed thrombocytopenia correlates with the reports of [23] and [24], who in their different studies stated that in pregnancy, platelet count does decrease. Also it is similar to the report of [25] who reported that in patients with *P.falciparum* infection, there was significantly lower platelet count. But our finding however is at variance with the result of a study by [26] which states that in normal pregnancy state, platelet behavior if at all changes, the change is usually very little compared to the non-pregnant state. From our finding in this study, we suggested that the thrombocytopenia observed in malaria infected pregnant women may be due to immune-mediated destruction of circulating platelets which has been postulated as a cause of thrombocytopenia seen in malaria infection [1]. They summarized that malaria infected Patients have elevated levels of specific IgG in the blood which binds to platelets-bound malaria antigens and this possibly lead to accelerated destruction of the platelets. We also suggest that the thrombocytopenia observed in this study may be an influence by the presence of *P.falciparum* infection resulting to reduced platelet survival from peripheral destruction occasioned by factors such as splenic uptake or sequestration and destruction of platelet production. This suggestion was drawn from the reports of [27] that thrombocytopenia is a common feature of *P.falciparum* and *plasmodium vivax* infection.

## CONCLUSION

The findings of this study showed significantly increased levels of white blood cells and neutrophil counts in the *Plasmodium falciparum* infected-pregnant women compared to the non-infected pregnant values. Hb, PCV, MCHC, platelets, red blood cells and lymphocytes showed significantly reduced levels in the malaria infected pregnant women, an indication of presence of anaemia. This shows that actually malaria parasite infection coexisting with pregnancy influences alterations in the haematological parameters. Furthermore, it is clear that the use of venous blood in place of placental histology for examination of malaria infection in pregnancy is still reliable. Some of the

inadequacies observed as the haematological alteration in this study could be associated to combined impairment of the immune system since pregnancy often causes impaired immunity as do malaria. MCV and MCH were not significantly affected in malaria infected pregnant women in spite the anaemia that was noticed. It is thus recommended that further studies be carried out to investigate the inverse relationship which existed between the MCV and MCH in this study.

## REFERENCES

- Kotepui M, Piwkhram D, PhunPhuech B, Phiwklam N, Chupeerach C, Duangmano S. Effects of malaria parasite density on blood cell parameters. *PLoS One*. 2015;10(3).
- Uneke CJ. Impact of placental *Plasmodium falciparum* malaria on pregnancy and perinatal outcome in sub-Saharan Africa: part III: placental malaria, maternal health, and public health. *The Yale journal of biology and medicine*. 2008 Mar;81(1):1.
- Avery JW, Smith GM, Owino SO, Sarr D, Nagy T, Mwalimu S, Matthias J, Kelly LF, Poovassery JS, Middii JD, Abramowsky C. Maternal malaria induces a procoagulant and antifibrinolytic state that is embryotoxic but responsive to anticoagulant therapy. *PloS one*. 2012;7(2).
- Ross JS, Wilson KJ. *Ross and Wilson Anatomy and Physiology in health and illness*. Churchill Livingstone; 1987.
- Babalola AS, Idowu OA, Sam SO, Fabusoro E. Risk factors associated with occurrence of placental malaria in a population of parturients in Abeokuta, Ogun State, Nigeria. *Malariaworld J*. 2015;6(8).
- Sood R. Clinical haematology, In: *Textbook of Medical laboratory technology*. Jaypee Brothers Medical Publishers, New Delhi. 2006; 1<sup>st</sup> edn, 196-200
- Mckenzie FE, Prudhomme WA, Magill AJ, Forney JR, Permpnich B. White blood cell counts and malaria. *Journal of Infectious Disease*. 2005; 192: 323-330.
- Malik AM, Zaffer N, Ali N, Malik M, Khan R. Haematological findings and endemicity of malaria in Gadap region. *Journal of the College of Physicians and Surgeons, Pakistan*. 2010; 20(2): 112-116.
- Adesina KT, Balogun OR, Babatunde AS, Sanni MA, Fadeyi A, Aderibigbe S. Impact of parasitaemia on haematologic parameters in pregnant women at booking in Ilorin, Nigeria. *Trends in Medical Research*. 2009; 4: 84-90.
- Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. *The Lancet infectious diseases*. 2007 Feb 1;7(2):105-17.
- Nnaemeka AM, Okonkwo EC, Anyim C, Uchenna IU. Haematological profile of pregnant women infected with malaria parasite at Federal Teaching Hospital Abakiliki, Ebonyi State. *American Journal of Microbiology*. 2014; 5(1): 11-17
- Francischetti IMB, Seydel KB, Monteiro RQ. Blood coagulation, inflammation and malaria. *Microcirculation*. 2008; 15(2): 81-107.
- Aitken EH, Alemu A, Rogerson SJ. Neutrophils and Malaria: *Frontiers Immunology*. 2018; 9 (305):1-11
- Hviid, L, Kemp K. What is the cause of lymphopenia in malaria: *Infection and Immunology?* 2000; 68(10) 6087-6089
- Mandale WL, Msefula CL, Gondwe EN, Drayson MT, Molyneux ME, MacLennana CA. Cytokine profiles in Malawian Children Presenting with uncomplicated malaria, severe malarial anemia, and cerebral malaria. *Clinical and Vaccine Immunology*. 2017; 24(4): 1-11
- Kalaivani K. Prevalence and consequences of anaemia in pregnancy. *Indian Journal of Medical Research*. 2009;130 (5):627-633.
- Sukrat B, Wilasrusmee C, Siribumrungwong B, McEvoy M, Okascharoen C, Attia J, Thakkestian A. Hemoglobin concentration and pregnancy outcomes: A systematic review and meta-analysis. *Biomedical Research International*. Article ID 769057.
- Ozougwu JC, Obimba KC, Obiukwu CE, Elom MO. Studies of the effect of malaria parasite on haematological profile of pregnant malarious women. *World Journal of Medical Sciences*. 2015;12(4): 383-386
- Shankar AH. Nutritional modulation of malaria morbidity and mortality. *Journal of Infectious Disease*. 2000; 182: 37-53.
- Ifukor PC, Jacobs J, Ifukor RN, Ewrhe OL. Changes in haematological indices in normal pregnancy. *Physiology Journal*. 2013; 1:4
- Cook DG, Cappuccio FP, Atkinson RW, Wicks PD, Chitolie A, Nakandakare ER, Sagnella, G.A, Humphries SE. Ethnic differences in fibrinogen levels: The role of environmental factors and the fibrinogen gene. *American Journal of Epidemiology*. 2001; 158 (8): 799-805
- Goswami MT, Patel VN, Pandya NH, Amita K, Mevada AK, Desai K, Solank KB. Maternal anaemia during pregnancy and its impact on perinatal outcome *International Journal of Biomedical and Advance Research*. 2014; 5(2): 99-102
- Chandra S, Tripathi AK, Mishra S, Amzaru M, Vaish Angchaisukiri P. Coagulopathy in malaria. *Thrombosis Research*. 2014; 133: 5-19
- Prisco D, Ciuti G, Falciani M. Haemostasis and pregnancy: Haemostatic changes in normal pregnancy. *Journal of Hematological Report*. 2005;1(10): 1-3
- Ladhani S, Lowe B, Cole AO, Kowuondo K, Newton CR. Changes in white blood cells and platelets in children with *falciparum* malaria:

- relationship to disease outcome. British Journal of Haematology. 2002; 19: 839–847.
26. Howie P. W Blotting and fibrinolysis in pregnancy. Post Graduate Medical Journal. 1979; 55: 362-366.
27. Angchaisukiri P. Coagulopathy in malaria. Thrombosis Research. 2014; 133: 5-19.