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CHANGES IN HAEMATOLOGICAL PARAMETERS IN MALARIA PARASITAEMIA AMONG PREGNANT WOMEN WITH VARYING HAEMOGLOBIN GENOTYPES

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ABSTRACT
Malaria parasitemia has been known to drastically affect haematological parameters. But little is known of its interaction with various haemoglobin genotypes and how they conbine to cause changes in haematological indices. The aim of this study was to determine the changes in haematological parameters in haemoglobin variants in pregnant women with sub-clinical malaria. Five millilitres of venous blood was collected aseptically into 1% diamine tetraacetic acid bottle and carefully mixed for the analysis. Determination of haemoglobin variant was done using cellulose acetate membrane electrophoresis with Tris-EDTA-borate buffer (pH 8.9). Haematological parameters were determined using Mindray BC-6800, an auto Haematology analyzer system, Mindray BC-6800. Statistical Analysis System (SAS) version 12 was used. Descriptive statistics mean separation using one-way analysis of variance (ANOVA). Quantitative data were presented as percentages, while continuous variables such as age were expressed as mean ±standard deviation and a level of significance set at $P < 0.05$. A total of eight hundred and twenty-eight (828) participated in the study. The mean age of the study participant was 29.5±5.31 years. Majority of the participants 612(73.9%) had haemoglobin electrophoretic pattern of AA. The abnormal haemoglobin variant of SS constituted 2(0.24%) in this study population. Those with AS were 214(25.8%). The mean PCV among HbAA, HbAS and HbSS was 33.98±0.25% 33.39±0.24% and 25.20±1.80% at P-value (0.043) respectively. Neutrophil percentage was not affected by any of the characteristics above ($P > 0.05$). Lymphocyte percentage was slightly significantly elevated among participated age <21 years ($P < 0.02$). There was a significant elevation in the mean monocyte percentages during the second trimester of pregnancy ($P < 0.05$). No significant changes in the MCHC and MCH values were observed when compared with the measured characteristics ($P > 0.05$). The platelet count was remarkably reduced among participants aged 21-25years and above. Mean reticulocyte count was found to be higher among the HbSS genotype with mean value of 4.45±0.05 as compared with HbAS (1.93±0.05) and HbAA (1.97±0.08). In conclusion, Haemoglobin variants were observed to suffer malaria infection but at varying frequencies and changes were observed in haematological parameters as influenced by malaria and variants of haemoglobin genotypes, and due to the interaction between both factors.

KEYWORDS: HbAA, HbAS and HbSS.

INTRODUCTION
Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoa belonging to the *Plasmodium* type such as *vivax*, *ovale*, *malariae*, and *falciparum* (Caraballo, 2014). Of the four malaria species infecting human, *P. falciparum* caused the bulk of severe disease and complications, partly because of its ability to adhere to endothelium and sequester in the vascular cells (Francis *et al.*, 2012), and *P. falciparum* is the predominant species in the tropical Africa due to rare case of negative Duffy blood group which has affinity to *P. vivax* and *P. ovale*. The disease is most commonly transmitted by an infected female Anopheles mosquito (Francis *et al.*, 2012). This transmission may be influenced by climatic conditions that may enhance mosquito breeding and malaria incidence, such as temperature, humidity and rainfall. Malaria remains a major health problem and it is estimated to affect from 350 to 500 million people annually and accounts for 1 to 3 million deaths per year, where Nigeria accounts for roughly 25% of the malaria burden in sub Saharan Africa (Jayleen *et al.*, 2015) with 11% maternal mortality (WHO, 2010). Malaria causes symptoms that typically include fever, tiredness, vomiting, and headaches, in severe cases it can cause yellow skin, seizures, coma or death which usually begin ten to fifteen days after being bitten (Caraballo, 2014).

Malaria is a life threatening infection causing a high fever and chills as a result of a bite from mosquito

infected with parasites (Caraballo, 2014). This can result in high mortality rate in immunocompromised subjects such as pregnant women and infants (Oliver *et al*., 2014). The understanding of how malaria specifically affects pregnant women is crucial in our efforts to improve maternal and perinatal health and curb the spread of this preventable infectious disease. Some genetic disorders have been suggested to protect against malaria infection. Mutations in the structural activity of the beta globin chain of haemoglobin molecule results in accumulation of the toxic molecules (ROS) (Piel *et al*., 2010). When oxygen tension is reduced, the deoxygenated sickle cell haemoglobin (HbS) molecules undergo polymerization that leads to the formation of long fibers which cause the red blood cell to assume a sickle-like shape (Elliot *et al*., 2008). The formation of polymers leads to changes in red cell HbSS. This leads to poor solubility of HbS resulting in the formation of long fibers (polymers) (Elliot *et al.*, 2008, Vaidya and Mather, 2009). The formation of polymers leads to changes in red cell membrane and metabolism, causing the cells to lose its elasticity and distorted with a sickle shape and shortens its life span. The sickled cells adhere to vascular endothelium and to one another blocking small blood vessels. They become trapped in the spleen and haemolyzed easily; haemolysis is further increased when there is infection like malaria parasite. As a response, erythropoiesis increases and lyzed cells are replaced resulting in an increased proportion of reticulocytes with increase transferrin receptor on the plasma membrane of the reticulocyte as one of the final steps of erythroid differentiation and additional ribosomal RNA (Prchal, 2010), compared with the old blood cell in HbAA with 120 half-life without sickle cell trait (Wood *et al*., 2008).

The RBC reoxygenation phase is a major source of free radical production in SCD. During this period, normal RBCs can generate a significant amount of superoxide due to an electron transfer between the heme iron and oxygen. In the presence of oxygen, heme auto-oxidizes inducing methemoglobin and superoxide formation. Although both haemoglobin A (HbA), and HbS blood have a tendency to autoxidize into methemoglobin and superoxide (Aslan *et al*., 200), unlike HbA, which can counter this reaction to form harmless byproducts, HbS can become overwhelmed by the continual source of superoxide and, via its dismutation, H_2O_2. The formation of H_2O_2, when exposed to methaemoglobin, decomposes haemoglobin and releases iron. This iron can then react with remaining H_2O_2 to further produce OH, the most reactive and harmful of the reactive species (Aslan *et al*., 2000). Sickle cells ultimately generate about twofold greater quantities of superoxide, H_2O_2, and OH than HbA. This reaction ultimately triggers the function of G6PD and hence its accumulation in the SCD subjects. Under normal circumstances, H_2O_2, which is either produced through a two electron transfer or due to sickling, is removed by two methods: GPX or catalase (Aslan *et al*., 2000) the endogenously produced H_2O_2 cannot be sufficiently cleared (Aslan *et al*., 2000). GSH, a cofactor for GPX, is easily oxidized to glutathione disulfide (GSSG) through its reduction of free radicals and ROS and is an essential element for GPX to reduce H_2O_2. The aim of this study was to determine the changes in haematological parameters in haemoglobin variants among pregnant women with sub-clinical malaria.

MATERIALS AND METHODS

Study Area

The study was carried out in Port Harcourt, the capital city of Rivers State Nigeria. Port Harcourt is situated within geographical co-ordinates 4°′49′27″N 7°2′1″E.

Study Population

A total of eight hundred and twenty eight (828) subjects were randomly selected. This comprised of 507 infected with malaria parasite and three hundred and twenty one (321) non-infected with the malaria parasite (controls). These subject whose ages ranged from<21 to 36 years and above were sub-clinical pregnant women registered in the antennal clinic of University of Port Harcourt Teaching Hospotal, UPTH. Consent was obtained from each participant prior to blood collection. Their demographical information were collected using a questionnaire.

Sample Size

Purposive sampling and randomized method were used in the selection of subjects, taking into consideration, the total number of patient attending the antenatal clinic in University of Port Harcourt Teaching Hospital, Rivers State.

The sample size was calculated by the Cochran's sample size formula as shown below (Cochran, 1977).

The sample size was obtained using a prevalence of malaria in pregnant women in Nigeria as 72.5% (Nzeako *et al*., 2013) and the sample size was calculated using this formula;

$$\mathbf{N} = \frac{Z^2 pq}{d^2}$$

Where N = The desired sample size
Z = The Standard Normal deviate usually set at 1.96 corresponding to the 95%.
Confidence level
p = The prevalence of target population
q = 1- p
d = degree of accuracy desired set at 0.05

Therefore, $N = \frac{(1.96)^2 \times 0.725 \times (1-0.725)}{(0.05)^2}$ p = 72.5% or 0.725
N = 306.4
By adding 10% of non-respondent, the sample size was 337.

Study Design

This cross sectional study research was carried out in 828 pregnant women of which 507 were infected with malaria parasite (subjects) and 321 not infected with the malaria parasite (control). These pregnant women were recruited from the Department of Obstetrics and Gynaecology, in the University of Port Harcourt Teaching Hospital after approval from institutional Ethical Clearance Committee from 11th May 2018 to July 31st 2018. Antennal women were enrolled in the study at first visit irrespective of the gestational age. The demographical data of the subjects were obtained. These included age, parity, and trimester. The women were of varying age ranging from 18 to 36 years and above.

Sample Collection and Handling

All recruited patients were given study numbers which was used for all data collection /laboratory processes. Blood sample (5ml) was withdrawn with minimum stasis under aseptic conditions from the ante-cubital vein (Epidi *et al*., 2008).

Experimental Analyses

Determination of Haemoglobin electrophoretic pattern

The haemoglobin genotype was determined by cellulose acetate membrane electrophoresis (CAME) as describe by (Awah and Uzoegwu, 2006). Which is based on the principle that at alkaline pH haemoglobin is a negatively charge protein and in an electric field will migrate towards the anode. Structural variants with surface charge differences will separate from HbA, those without a change in charge will not.

Determination of Malaria Parasitemia

The method that involves the use of light microscope of thick and thin Giemsa stained blood smears remains the standard method for diagnosing malaria, and was used (WHO, 2010). It is based on the principle of use of Romanowsky stains. These stains contain eosin which is an acidic anionic dye and methylene blue (azure) which are basic cationic dyes. When diluted in buffered water at pH of 7.2, ionization occurs. Eosin component stains the parasite nucleus red, while the methylene blue components stain the cytoplasm blue. At the end of the experiment, the parasite density count of parasites/µl of blood was accomplished by enumerating the number of parasites in relation to absolute leukocytes/µl with the formula below.

$$\frac{\text{Parasite count X Absolute WBC Value}}{\text{Number of leucocytes (WBC)counted (200)}} = \text{Parasite/µl}$$

Determination of Haematological Indices

Haematological parameters were determined using Mindray BC-6800, an auto Haematology analyzer system, Mindray BC-6800 (Shenzhen, 2017). This is based on a combination of light scatter, electrical impedance, fluorescence, light absorption, and electrical conductivity methods to produce complete red blood cell, platelet, and leukocyte analyses. All the widely used automated instruments analyze cell in flow and are essential highly specialized flow cytometers.

Statistical Analysis

Data collected for this study were registered in the computer by creating a spreadsheet and subjected to statistical analysis using statistical package Statistical Analysis System (SAS) version 12, 2013. Descriptive statistics mean separation using one-way analysis of variance (ANOVA). The effect of G6PD deficiency was determined on parasite density, Haemoglobin variant and Haematological indices using pairwise correlation for association between parameters. Comparisons were assessed using mean and chi-square test. Quantitative data are presented as percentages, while continuous variables such as age are expressed as mean ±standard deviation and a level of significance set at $P < 0.05$.

RESULTS

This study was aimed at determining the changes in hematological parameters among different haemoglobin variants in pregnant women with malaria parasitemia. A total of eight hundred and twenty-eight (828) participated in the study. Of this, five hundred and seven (507) representing 61.2% were infected with malaria parasite while the remaining three hundred and twenty-one (321) representing 38.8% served as controls (uninfected subjects). The mean age of the study participant was 29.5±5.31 years. Majority of the participants 612(73.9%) had haemoglobin electrophoretic pattern of AA. The abnormal haemoglobin variant of SS constituted 2(0.24%) in this study population. Those with AS were 214(25.8%). The mean PCV among HbAA, HbAS and HbSS was 33.98±0.25% 33.39±0.24% and 25.20±1.80% at P-value (0.043) respectively.

Table 4.1 shows the mean ± SEM of some haematological parameters according to the experimental group, age, trimester, parity and haemoglobin electrophoretic pattern. The overall mean parasite density of the infected subjects was 741.835±309.8/µl. The difference in the parasite density among different age groups were not statistically significant (P>0.05). Similarly, no statistical significant difference was observed when the mean values among different trimesters were compared as a well as the haemoglobin pattern of the subjects (P>0.05). The mean parasite density of 5147.78±356.79/µl in the primigravida group was significantly higher than 4131.02±294.11/µl observed in the multigravida group (P = 0.028).

Table 4.2 shows the mean ±SEM of some white blood cell parameters by experimental group, age, trimester parity and haemoglobin electrophoretic pattern. Neutrophil percentage was not affected by any of the characteristics above (P > 0.05). Lymphocyte percentage was slightly significantly elevated among participated age <21 years (P < 0.02). There was a significant elevation in the mean monocyte percentages during the second trimester of pregnancy (P < 0.05). The other

white blood cell parameters of Eosinophil and basophil percentages did not show any statistical difference when compared with the measured characteristics of the participants (P>0.05).

Table 4.3 shows the mean ± SEM of the red cell indices of the red cell indices of MCHC, MCH, MCV, platelet and RBC. No significant changes in the MCHC and MCH values were observed when compared with the measured characteristics (P > 0.05). There was a significant elevation in MCH value among participants aged 21-25 years. The platelet count was remarkably reduced among participants aged 21-25years and above. There was no statistical significance difference in the mean RBC value (P > 0.05).

Table 4.4 is a continuation of the previous table showing the mean± SEM of the reticulocytes percentage. No statistical significant difference was observed when compared with the measured characteristics mentioned above (P > 0.05), although it was found to be higher among the HbSS genotype with mean value of 4.45±0.05 as compared with HbAS (1.93±0.05) and HbAA (1.97±0.08).

Table 4.5 shows the pairwise Pearson Correlation between haematological parameters among the infected group. Lymphocyte percentages correlated negatively with TWBC (r = -0.1511, P = 0.0006) and positively with PCV (r= 0.1486, P = 0.0008). Monocytes was significantly and negatively correlated with lymphocytes and basophils (r = -0.1116, P = 0.0119 and r = -0.1100, P = 0.0132) respectively. MCHC was found to be negatively and significantly correlated with total white blood cell count (TWBC), (r = -0.0934, p = 0.0358).

Table 4.6 shows the pairwise correlations between the red cell indices and the measured parameters of parasite density. MCV was found to significantly relate positively with parasite density (r = 0.1475, p = 0.0009). Similar positive correlation between MCV, lymphocyte and basophil and MCH were noted while eosinophil related negatively.

Table 4.7 shows Pairwise Correlations Between Hematological Parameters Among Non-Infected Group (control). There was a positive correlation coefficient between PCV and Age (r =0.1218; p<0.0291), between PCV and Hb (r =0.3519; p<0.0001) and between Neutrophil and TWBC(r =0.2131; p<0.0001). There was a negative correlation coefficient between lymphocytes and TWBC (r =-0.1586; p<0.0044), between lymphocytes & neutrophils (r =-0.7305; p<0.0001), between eosinophils and neutrophils (r =-0.1429; p<0.0104), between monocytes and TWBC (r = -0.1424; p<0.0106) and between monocyte & Neutrophils (r =-0.2146; p<0.0002). There was a positive correlation coefficient between monocyte and lymphocyte (r =0.1408; p<0.0116) and between Basophil and Age (r =0.1373; p<0.0138). There was a strong negative correlation coefficient between Basophils and Neutrophils (r =-0.1875; p<0.0007), and a strong positive correlation coefficient between Basophils and lymphocyte (r =0.1832; p<0.0010) and between basophil and monocyte (r =0.1239; p<0.0264).

Table 4.8 shows Pairwise Correlation Between Haematological Parameters Among Non-Infected Group (Control). A strong negative correlation coefficient between MCHC and Hb (r =-0.1802; p<0.0012), between MCHC and PCV (r =-0.3293; p<0.0001) and between MCH. The result shows a very strong positive correlation coefficient between MCH and MCHC (r =0.3212; p<0.0001) at a significant value of <0.0001 and between platelet and TWBC (r =0.1387; p<0.0128). There was a strong negative correlation coefficient between platelet & monocyte (r =-0.1661; p<0.0028) and between platelet & MCV (r =-0.1709; p<0.0021). Also there was a very strong positive correlation coefficient between RBC and Hb(r =0.3230; p<0.0001) and between RBC and PCV (r =0.2950; p<0.0001). A very strong negative correlation coefficient between RBC & MCHC(r =-0.2971; p<0.001), between RBC and MCH (r =-0.1823; p<0.0010), between RBC & MCV (r =-0.1573; p<0.0066) and between Reticulocytes and Hb (r =-0.1103; p<0.0483) was observed.

Table 4.1: Mean ± SEM of Hematological Parameters by Experimental Group, Age Group, Trimester, Parity and Genotype.

Characteristic	N	Parasite Density (Parasites/µl)		Hb (g/dL)		PCV (%)		TWBC(109/L)	
		Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value
Experimental Group									
Infected	507	741,835±309.8	-----	11.26±0.18	0.139^{ns}	33.99±0.22	0.245^{ns}	7.17±0.10	0.100^{ns}
Uninfected (Control)	321	0.0±0.0		10.91±0.08		33.52±0.37		6.93±0.11	
Age Group (Years)									
< 21	41	4484.95±1107.50	0.997^{ns}	12.83±2.13^{a}	0.022*	32.90±0.68	0.505^{ns}	7.11±0.27	0.954^{ns}
21-25	159	4695.85±446.60		10.98±0.11^{b}		33.29±0.36		7.09±0.16	
26-30	328	4454.06±347.80		11.08±0.09^{b}		33.91±0.32		7.14±0.12	
31-35	185	4543.42±544.10		10.99±0.10^{b}		33.97±0.42		6.99±0.14	
36+	115	4600.99±638.90		11.06±0.13^{b}		34.26±0.67		7.01±0.16	
Trimester									
1	56	5467.29±930.13	0.165^{ns}	11.44±0.22	0.638^{ns}	34.69±0.98	0.288^{ns}	6.81±0.19	0.050^{ns}
2	292	4006.59±294.21		11.01±0.09		33.47±0.31		6.88±0.10	
3	480	4760.44±331.43		11.15±0.19		33.91±0.26		7.23±0.11	
Parity									
Primigravida	335	5147.78±356.79^{a}	0.028*	11.33±0.27	0.136^{ns}	34.04±0.32	0.325^{ns}	7.13±0.11	0.579^{ns}

Multigravida	493	4131.02±294.11[b]		10.98±0.06		33.64±0.25		7.05±0.09	
Genotype									
AA	612	4533.37±272.70	0.989[ns]	11.19±0.15	0.352[ns]	33.98±0.25[a]	0.043*	7.05±0.08	0.787[ns]
AS	214	4573.54±409.10		10.97±0.10		33.39±0.24[a]		7.16±0.14	
SS	2	3970.00±1095.00		8.30±0.80		25.20±1.80[b]		7.51±1.48	

SEM: Standard error of mean;
Within each Characteristic, means ± SEM with different superscripts are significantly different at p<0.05.
Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ns=Not Significant (p>0.05).

Table 4.2: Mean ± SEM of Hematological Parameters by Experimental Group, Age Group, Trimester, Parity and Genotype (Cont'd).

Characteristic	N	Neutrophil (%)		Lymphocyte (%)		Eosinophil (%)		Monocyte (%)		Basophil (%)	
		Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value
Experimental Group											
Infected	507	67.64±2.04	0.251[ns]	26.79±0.35	0.441[ns]	2.66±0.09	0.865[ns]	6.18±0.11	0.312[ns]	0.32±0.07	0.2978[ns]
Uninfected (Control)	321	64.67±0.45		26.37±0.41		2.70±0.24		6.36±0.16		0.24±0.01	
Age Group (Years)											
< 21	41	60.11±1.81	0.393[ns]	29.13±1.19[a]	0.0.021*	2.55±0.24	0.575[ns]	6.25±0.29	0.549[ns]	0.24±0.02	0.245[ns]
21-25	159	63.26±0.75		27.69±0.72[ab]		2.60±0.18		6.01±0.19		0.45±0.21	
26-30	328	66.86±1.80		25.91±0.37[b]		2.68±0.12		6.27±0.13		0.25±0.01	
31-35	185	69.80±4.19		26.94±0.62[b]		2.49±0.14		6.48±0.25		0.26±0.01	
36+	115	66.82±3.04		25.82±0.69[b]		3.10±0.62		6.15±0.21		0.25±0.01	
Trimester											
1	56	62.03±1.42	0.206[ns]	28.09±1.25	0.292[ns]	2.53±0.24	0.646[ns]	6.32±0.30[ab]	0.005**	0.27±0.01	0.845[ns]
2	292	69.32±3.30		26.73±0.43		2.56±0.12		6.63±0.17[a]		0.26±0.01	
3	480	65.28±0.81		26.40±0.36		2.76±0.17		6.01±0.11[b]		0.31±0.07	
Parity											
Primigravida	335	65.10±1.81	0.367[ns]	26.92±0.44	0.368[ns]	2.76±0.13	0.488[ns]	6.18±0.13	0.508[ns]	0.34±0.06	0.303[ns]
Multigravida	493	67.42±1.73		26.43±0.34		2.61±0.16		6.30±0.12		0.26±0.05	
Genotype											
AA	612	66.21±1.48	0.929[ns]	26.83±0.32	0.286[ns]	2.75±0.14	0.477[ns]	6.24±0.10	0.953[ns]	0.25±0.01	0.263[ns]
AS	214	67.23±2.42		26.14±0.48		2.45±0.14		6.27±0.19		0.40±0.16	
SS	2	70.30±0.10		20.40±0.60		3.20±0.70		5.75±0.05		0.35±0.05	

SEM: Standard error of mean;
Within each Characteristic, means ± SEM with different superscripts are significantly different at p<0.05.
Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ns=Not Significant (p>0.05).

Table 4.3: Mean ± SEM of Hematological Parameters by Experimental Group, Age Group, Trimester, Parity and Genotype (Cont'd).

Characteristic	N	MCHC (g/dl)		MCH (pg)		MCV (fl)		Platelet (10^9/l)		RBC (10^{12}/l)	
		Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value
Experimental Group											
Infected	507	33.00±0.20	0.191[ns]	28.44±0.16	0.527[ns]	84.88±0.45	0.324[ns]	205.95±2.57	0.069[ns]	4.10±0.09[a]	0.337[ns]
Uninfected (Control)	321	32.65±0.11		28.62±0.27		85.79±0.90		198.48±3.17		3.98±0.03[b]	
Age Group (Years)											
< 21	41	33.15±0.58	0.484[ns]	28.20±0.66	0.093[ns]	81.38±2.22[b]	0.019*	219.42±10.78[a]	0.024*	3.95±0.08	0.697[ns]
21-25	159	32.87±0.25		29.20±0.39		87.68±1.62[a]		193.05±4.81[b]		3.95±0.05	
26-30	328	32.88±0.20		28.22±0.15		84.26±0.58[b]		207.50±3.14[b]		4.16±0.15	
31-35	185	32.50±0.15		28.27±0.24		85.75±0.63[ab]		203.72±4.05[b]		4.01±0.03	
36+	115	33.28±0.55		28.89±0.61		85.16±0.90[ab]		197.30±4.88[b]		4.03±0.05	
Trimester											
1	56	32.51±0.23	0.297[ns]	28.81±0.39	0.280[ns]	87.66±0.88	0.339[ns]	212.50±7.17	0.155[ns]	3.93±0.06	0.268[ns]
2	292	33.12±0.27		28.77±0.27		85.02±0.49		206.23±3.39		3.95±0.03	
3	480	32.74±0.15		28.32±0.17		85.08±0.70		200.02±2.63		4.14±0.10	
Parity											
Primigravida	335	33.04±0.29	0.267[ns]	28.67±0.30	0.366[ns]	84.99±0.52	0.655[ns]	206.44±3.21	0.163[ns]	3.99±0.03	0.330[ns]
Multigravida	493	32.74±0.09		28.40±0.13		85.40±0.66		200.75±2.55		4.10±0.10	
Genotype											
AA	612	32.75±0.14	0.324[ns]	28.44±0.16	0.684[ns]	85.45±0.55	0.668[ns]	204.41±2.30	0.464[ns]	3.98±0.02	0.072[ns]
AS	214	33.19±0.29		28.71±0.33		84.64±0.69		199.40±4.09		4.28±0.22	
SS	2	33.20±0.40		27.60±0.50		81.55±1.75		179.50±9.50		3.30±0.10	

SEM: Standard error of mean;
Within each Characteristic, means ± SEM with different superscripts are significantly different at p<0.05.
Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ns=Not Significant (p>0.05).

Table 4.4: Mean ± SEM of Hematological Parameters by Experimental Group, Age Group, Trimester, Parity and Genotype (Cont'd).

Characteristic		Reticulocyte (%)	
	N	Mean ± SEM	P-value
Experimental Group			
Infected	507	1.96±0.07	0.985^{ns}
Uninfected (Control)	321	1.97±0.10	
Age Group (Years)			
< 21	41	1.69±0.09	0.413^{ns}
21-25	159	1.89±0.05	
26-30	328	2.10±0.14	
31-35	185	1.89±0.05	
36+	115	1.91±0.08	
Trimester			
1	56	2.30±0.55	0.288^{ns}
2	292	1.93±0.11	
3	480	1.95±0.03	
Parity			
Primigravida	335	2.10±0.14	0.063^{ns}
Multigravida	493	1.88±0.03	
Genotype			
AA	612	1.97±0.08	0.105^{ns}
AS	214	1.93±0.05	
SS	2	4.45±0.05	

SEM: Standard error of mean;
Within each Characteristic, means ± SEM with different superscripts are significantly different at p<0.05.
Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ns=Not Significant (p>0.05).

Table 4.5: Pairwise Correlations Between Hematological Parameters Among Infected Group.

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
PCV (%)	Hb (g/dl)	0.1271	507	0.0405	0.2119	0.0041*
Lymphocyte (%)	PCV (%)	0.1486	507	0.0623	0.2326	0.0008*
Lymphocyte (%)	TWBC(10^9/L)	-0.1511	506	-0.2352	-0.0648	0.0006*
Monocyte (%)	Lymphocyte (%)	-0.1116	507	-0.1968	-0.0247	0.0119*
Monocyte (%)	Eosinophil (%)	0.1589	507	0.0728	0.2426	0.0003*
Basophil (%)	Monocyte (%)	-0.1100	507	-0.1952	-0.0231	0.0132*
MCHC (g/dl)	TWBC(10^9/L)	-0.0934	506	-0.1791	-0.0063	0.0358*
MCH (pg)	Monocyte (%)	-0.1082	507	-0.1935	-0.0213	0.0148*
MCH (pg)	Basophil (%)	0.6668	507	0.6155	0.7125	<.0001*

Table 4.6: Pairwise Correlations Between Hematological Parameters Among Infected Group.

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
MCV (fl)	Parasite Density (Parasites/µl)	0.1475	507	0.0612	0.2316	0.0009*
MCV (fl)	Lymphocyte (%)	0.1067	507	0.0198	0.1920	0.0163*
MCV (fl)	Eosinophil (%)	-0.1171	507	-0.2021	-0.0303	0.0083*
MCV (fl)	MCH (pg)	0.2318	507	0.1477	0.3125	<.0001*
Platelet (10^9/l)	Hb (g/dl)	0.0891	507	0.0020	0.1748	0.0449*
Platelet (10^9/l)	MCV (fl)	0.0911	507	0.0041	0.1768	0.0402*
RBC (10^12/l)	PCV (%)	0.0937	507	0.0067	0.1793	0.0350*
RBC (10^12/l)	TWBC(10^9/L)	-0.1045	506	-0.1899	-0.0175	0.0187*
Reticulocyte (%)	Eosinophil (%)	0.0918	507	0.0048	0.1775	0.0387*

Table 4.7: Pairwise Correlations Between Hematological Parameters Among Non-Infected Group (Control)

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
PCV (%)	AGE	0.1218	321	0.0125	0.2282	0.0291*
PCV (%)	Hb (g/dl)	0.3519	321	0.2521	0.4442	<.0001*
Neutrophil (%)	TWBC(10^9/L)	0.2131	321	0.1061	0.3152	0.0001*
Lymphocyte (%)	TWBC(10^9/L)	-0.1586	321	-0.2635	-0.0500	0.0044*
Lymphocyte (%)	Neutrophil (%)	-0.7305	321	-0.7778	-0.6750	<.0001*
Eosinophil (%)	Neutrophil (%)	-0.1429	321	-0.2484	-0.0339	0.0104*
Monocyte (%)	TWBC(10^9/L)	-0.1424	321	-0.2480	-0.0335	0.0106*
Monocyte (%)	Neutrophil (%)	-0.2046	321	-0.3072	-0.0974	0.0002*
Monocyte (%)	Lymphocyte (%)	0.1408	321	0.0318	0.2464	0.0116*
Basophil (%)	AGE	0.1373	321	0.0282	0.2431	0.0138*
Basophil (%)	Neutrophil (%)	-0.1875	321	-0.2910	-0.0796	0.0007*
Basophil (%)	Lymphocyte (%)	0.1832	321	0.0752	0.2869	0.0010*
Basophil (%)	Monocyte (%)	0.1239	321	0.0146	0.2302	0.0264*

Table 4.8: Pairwise Correlations Between Hematological Parameters Among Non-Infected Group (Control)

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
MCHC (g/dl)	Hb (g/dl)	-0.1802	321	-0.2841	-0.0722	0.0012*
MCHC (g/dl)	PCV (%)	-0.3293	321	-0.4235	-0.2280	<.0001*
MCH (pg)	MCHC (g/dl)	0.3212	321	0.2195	0.4161	<.0001*
Platelet (10^9/l)	TWBC(10^9/L)	0.1387	321	0.0297	0.2445	0.0128*
Platelet (10^9/l)	Monocyte (%)	-0.1661	321	-0.2706	-0.0577	0.0028*
Platelet (10^9/l)	MCV (fl)	-0.1709	321	-0.2752	-0.0626	0.0021*
RBC (10^12/l)	Hb (g/dl)	0.3230	321	0.2214	0.4177	<.0001*
RBC (10^12/l)	PCV (%)	0.2950	321	0.1917	0.3918	<.0001*
RBC (10^12/l)	MCHC (g/dl)	-0.2971	321	-0.3937	-0.1939	<.0001*
RBC (10^12/l)	MCH (pg)	-0.1823	321	-0.2860	-0.0743	0.0010*
RBC (10^12/l)	MCV (fl)	-0.1513	321	-0.2565	-0.0426	0.0066*
Reticulocyte (%)	Hb (g/dl)	-0.1103	321	-0.2171	-0.0008	0.0483*

DISCUSSION

This study was conducted to assess the changes in haematological indices in varying haemoglobin variants in sub-clinical malaria infected pregnant women attending the antenatal care of Port Harcourt Teaching Hospital. In this study, a prevalence of malaria burden in pregnant women was 61.2% as against the prevalence of 72.5% (Nzeako *et al*., 2013). This may be due to the method of analysis used to estimate malaria parasitaemia. The plus sign is a semi-quantitative method that is less accurate and can be misinterpreted since the density of parasitaemia cannot be accurately quantified base on the volume of blood used, unlike the quantitative method of count against absolute TWBC (Hammamil *et al*., 2013). This gives approximate density of parasites per microliter of blood. Also in Nzeako *et al*., (2013) study, all the four *Plasmodium* species (*P. ovale, P. malaria, P. vivax and P. falciparum*) were estimated to the prevalence of 72.5%. Moreso, the prevalence of HbAS (25.8) was lower in malaria endemic area (Port Harcpourt) than HbAA (73.9) in this study, which has a relative protection against malaria parasite (Nzeako *et al*., 2013).

In this study, there was a significant level of anaemia among sickle cell haemoglobin in pregnant women according to the baseline value stated by WHO, that define anaemia in pregnant women as a haemoglobin level <11g/dl (WHO, 2001). Haemoglobin and haematocrit generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced. This report is corroborated by the findings of (Elliot *et al.*, 2008, Vaidya and Mather, 2009). The anaemia may be due to increased destruction of red blood cells and decreased production of red blood cells in response to anaemia due to dyserythropoiesis (Haldar and Mohandas, 2009). Mutation in the globin chain when valine replaces glutamic acid at the 6^{th} amino acid position (Kohne, 2011), result in sickle cells that is liable to lysing under unfavourable conditions. Globin gene mutation impairs the function of the red blood cells that has detrimental effect on the individual red blood cells causing anaemia as a result of haemolysis. The sickle RBC often ruptures during its transport through the blood vessels. Haemolysis, along with the consequence of repeated sickling and unsickling, causes the premature destruction of erythrocytes (Conran *et al*., 2009). Imbalance in the production of alpha and beta-globin chain results in increased apoptosis during erythroblast maturation. As a result, there exists an imbalance between the amount of iron molecules that are incorporated into the erythroblast by transferrin during erythropoiesis and the amount of iron released into the haemoglobin contained within each erythrocyte (Centis *et al*., 2000). Sickle erythrocytes produced have a decreased half-life survive only for 10 days compared

with 120 days in normal RBCs (Wood *et al*., 2008). This result to a rapid turnover of RBCs leading to increase proportion of reticulocytes which are rich in arginase (biomarker of haemolysis) that can be release into the plasma during haemolysis as well as haemoglobinand iron (Moris *et al*., 2008).

Accumulation of ROS may also result in anaemia (Manganelli *et al*., 2013; McDade *et al*., 2008; Valiaveedan *et al.*, 2011). As a result of the increase production of free radical superoxide, the generation of nitric oxide is decreased that function to regulate vascular tone, blood flow, and adhesion. Furthermore, the amount available for use is limited as well. The interaction between superoxide and nitric oxide is more detrimental than their individual actions. In normal systems superoxide can easily be dismutased by superoxide dismutase (SOD), the increase production of O_2^- seen in SCD overwhelms the defenses of body and reacts at diffusion-limited rates with NOresults in the generation of peroxynitrite ($ONOO^-$). $ONOO^-$is favoured over spontaneous superoxide dismutation and NO autoxidation. The formation of $ONOO_2$ produces more reactive free radical OH and nitrogen dioxide (NO_2) (Aslan *et al.*, 2000) further increasing the anaemia.

The increase level of cell-free haemoglobin resulted to the consumption and decreased bioavailability of NO (Morris *et al*., 2005). Concentration of plasma haemoglobin due to haemolysis, allows for the reaction of both deoxyl-HbS and oxy-HbS with NO causing its decrease in the cell. The binding of NO to deoxygenated haemoglobin results in the formation of a stable Fe^{2+}Hb-NO complex, which can readily react in Fenton reactions. The reaction between NO and oxygenated haemoglobin can form methamoglobin and nitrate (Belcher *et al*., 2010).

NO is further reduced due to the consequence of eNOS (eNOS is an enzyme made up of a reductase domain and an oxygenase domain which produces NO) uncoupling. This occur when the conversion of L-arginine into NO and L-citruline is reversed for the production of superoxide due to reduction of the cofactors L-arginine and tetrahydrobiopterin (BH_4). L-Arginine concentrations are decreased in SCD, and this interrupts the electron flow through the eNOS domains and favours the generation of superoxide over NO (Wood *et al.*, 2006). Arginase, a product of haemolysis can reduce the concentration of Arginine which is a major target in haemolysis resulting in a significant decrease in NO bioavailability. A deficiency of BH4 can lead to the production of superoxide via the uncoupling of eNOS (Wood *et al*., 2006). BH_4can be inactivated by $ONOO^-$ (Wood *et al.*, 2006). Under normal circumstances, circulating blood cells loosely come into continuous contact with the endothelial cells of blood vessels.

In SCD, there is an increase in adherence to the vessel walls. Endothelia dysfunction is modulated by the interaction between blood cell and molecular components of in the endothelium. Blood cells adherence to the endothelium can be modulated by factor such as decrease NO bioavailability and ROS. Hypoxia regulates the productions of VCAM-1 and ICAM-1, although VCAM-1 is solely responsible for the adherence of sickle cells to the endothelium (Belcher *et al*., 2010). Vascular cell adhesion molecul-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) are associated with decreased NO bioavailability (Kato *et al.*, 2005) and increase haemolysis (Vilas-Boas *et al.*, 2010). Blood cell adherence to the endothelium can be modulated by factors such as decrease NO bioavailability, ROS and inflammation (Wood *et al*., 2008) thereby causing anaemia.

Furthermore, marked splenomegaly during acute infection reflects extensive sequestration of red blood cells by the spleen resulting in anaemia (Safeukui *et al*., 2008). Infection with malaria parasite enhanced the breakdown of the red blood cells the more in sickle cells haemoglobin as the infection increases the unstable nature of the sickle cell (Francis, 2010). A glycolipid material is released from the rupture of schizont. This parasite product induces activation of the cytokine cascade. Cells of the macrophage monocyte series and endothelium are stimulated to release cytokines. Initially tumour necrosis factor (TNF) and interleukin -1 (IL-1) are produced and these in turn induces the release of other cytokines including IL-6 and IL-8. The presence of the parasite and resulting cytokines (host inflammatory) are associated with many of the symptoms and signs of the infection, particularly anemia, fever and malaria (Dondorp *et al.*, 2008). The reticuloendothelia system of the spleen selectively destroy the digested red blood cells fragments as a result splenomegaly developed due to increase in the number of phagocytes resulting in anaemia.

Interestingly, a significant thrombocytopenia related to age was observed between the age group of 21-25 years in this study, which is consistence with the age group of between 20-25 years (Multigravida) (Ezhil *et al.*, 2017; Huparikar *et al.*, 2016). Platelet count falls slightly due to hemodilution and increased turnover as gestation progresses. Women with multiple pregnancies, have a larger increase in blood volume than those with single pregnancy resulting in further drop of platelets that is prevalence in age group 20 -25 years multigravida. Haemodilution is associated with Disseminated Intravascular Coagulation (DIC) that is characterized by systemic activation of blood coagulation. Which result in general deposition of fibrin, leading to microvascular thrombi in various organs and contribution to multiple organ dysfunction syndromes (MODS) (Levin, 2007). DIC develop in the course of the HELLP syndrome (haemolysis, elevated liver enzymes and low platelets), which complicate pregnancy induced hypertension due to increase platelet aggregation. The massive and ongoing activation of coagulation may result in depletion

of platelets and coagulation factors. Hypertension is defined as repeated BP measurements ≥ 140/90 mmHg that result in abnormal vasoconstriction. In preeclampsia, derangement of endothelial-derived vasoactive factors are the predominance substances which are vasoconstrictors (endothelin, thromboxane A_2) over vasodilators (NO, prostacyclin) (Fujiyama *et al*., 2001). Congestion due to portal hypertension results in hypersplenism that also caused reduction in number of circulating platelets (Saboo *et al*., 2012).

Thrombocytopenia also resulted from platelets destruction due to the multigravida status of pregnant women. Multigravida demonstrates higher immunity may not present symptoms of malaria infection as a result of high placental inflammation (Lafayette *et al*., 2007). This also results in the aggregation of platelets thereby reducing the lifespan. Cytoadherence and related phenomenon, rosettes with uninfected erythrocytes platelets-mediated clotting of infected erythrocytes (Rowe *et al.,* 2009). Hypersplenomegaly thought to occur as an immune response to antigenic stimuli resulting in further destruction of platelets.

Platelets have central role in innate immunity, initiating and participating in multiple inflammatory processes, directly binding pathogens and even destroying the platelets (Jenne *et al*., 2013). Infection such as malaria parasite that is increase in pregnancy due to increase in cortisol hormone (Bouyou-Akot *et al.,* 2005; Elekov, 2004) also leads to accumulation of ROS that initiate haemolysis of red blood cell either extravascularly or intravascularly thereby attracting platetelets. Malaria parasites infection accelerates the release of proinflammatory cytokines (tumor necrosis factor-alpha (TNF-a) and interleukins (IL-3, IL-6, and IL-11) 103. The various cytokines are important regulators of inflammatory, cell growth, and maturation; they have key roles in thrombopoiesis and are elevated during infection (Jenne *et al*., 2013). This action leads to the stimulation of B cell activity and production of IgM, including PAIgG, which increases the removal of platelets from the circulation (Kalambokis and Tsianos, 2011).

Subjects with serious infections suffer from thrombocytopenia, thus reducing their contribution to inflammation. The amebocytes ability of platelets facilitates hemostatic function as well as encapsulation and phagocytosis of the pathogens by the means of exocytosis of their intracellular granules containing bactericidal defense molecules. Blood clotting supports the immune function by trapping the pathogenic bacteria within (Levin, 2007), leading to obstruction of lumen of blood vessel and subsequent hypoxic tissue damage. The thrombosis is directly in agreement with platelets, neutrophils and monocytes. The process is initiated either by immune cells *sensu stricto* (Cox *et al*., 2011; Jenne *et al*., 3013) by activating their pattern recognition receptors (PRRs), or by platelet-bacterial binding. Platelets can bind to bacteria either directly through thrombocytic PRRs (Jenne *et al.,* 2013) and bacterial surface proteins, or via plasma proteins that bind both to platelets and bacteria (Jenne *et al*., 2013). Monocytes respond to bacterial pathogen-associated molecular pattern (PAMPs) or damaged-associated molecular pattern (DAMPs) by activating the extrinsic pathway of coagulation. The thrombosis is directly in agreement with platelets, neutrophils and monocytes. They also turn on the negative phospholipid surface by activating the intrinsic pathway of coagulation XII.

Regulatory defects in immunothrombosis are suspected to be major factor in causing pathological thrombosis in many forms, such as disseminated intravascular coagulation (DIC) or deep vein thrombosis. DIC is a prime example of both dysregulated coagulation process as well as undue systemic inflammatory response resulting in numerous microthrombi (Gaertner and Massberg, 2016). TFN-α, and other inflammatory cytokines suppress hepatic production of TPO (Kalambokis *et al*., 2012), inhibit the growth and differentiation of megakaryocytes and induced apoptosis. TNF-α reduces vascular nitric oxide production, (Kato *et al*., 2005) which is the main mediator for the development of portal hypertension, and suppresses TPO production. HELLP syndrome is a severe type of preeclampsia that results in thrombocytopenia due to an acceleration of the normal increase in platelets destruction that occurs during pregnancy (Alexander *et al.*, 2004). The massive and ongoing activation of coagulation may result in depletion of platelets and coagulation factors.

Furthermore, ROS also resulted from depression of plasma oncotic pressure in the glomerular capillaries. This depression then results in hypervolemia and hyper fusion of glomeruli (Berger *et al*., 2009). This action lead to increased ventricular ejection rate and hence hypertension thereby causing glomerular lesion (glomerular endotheliosis) (Lafayette *et al.,* 2007; Yuan *et al*., 2004). Once the endothelia cell gets damage and blood flows out of the blood vessels, the platelets react immediately and get attracted by the tough fiber which surround the walls of the the blood vessels. The platelet then stick to the fiber and start changing their shape thereby making a type of mesh which stops the blood from flowing out of the body. This is as a result of the predominance of substancesthat are vasoconstrictors (endothelin, thromboxane A2) over vasodilators (NO, prostacyclin) (Fujiyama *et al.,* 2001). Primarily, platelets adhere to the site of endothelial disruption, leading to platelet plug (Cheesbrough, 2009). Collagen exposure of thrombin action results in the secretion of platelet granules content,which is characterized by release of granules containing von Willebrand factor, adenosine 5' –diphosphate (ADP), serotonin, fibrinogen, lysosoma enzymes, beta thrombo globulin and heparin neutralizing factor. Collagen and thrombin activate platelet prostaglandin synthesis. This leads to membrane release

of diacylglycerol, inositol triphosphate and arachidonate resulting to the formation of a labile substance, thromboxane A_2 which lower platelet cyclic adenosine monophosphate (CAMP) levels and initiate the release reaction. This serves to recruit other platelets into the growing platelet plug, which acts to stop the bleeding. Simultaneously, the synthesis of thromboxane A_2 and release of serotonin and adenosine diphosphate leads to vasoconstriction to reduce blood loss at the site of vascular injury (Fujiyama *et al*., 2001).

Thrombocytopenia also occurred as as result of platelet destruction via endothelial dysfunction which exposes the surface adhension molecule, P-selectin an essential component of platelet aggregation and as such associated with thrombocytopenia (Maeda *et al*., 2010). Interaction of platelet with endothelial cells leads to activation of factor X, this facilitate the formation of the thrombin-activating prothrombinase complex, activate the extrinsic pathway of coagulation by releasing von Willebrand Factor (vWF) (Berger *et al.*, 2009). vWF acts as a carrier for coagulation factor VIII resulting in platelets consumption.

Increased shear stress or the level of fluid stress applied to platelets leads to an increased rate of vWF-platlet aggregation and promote cleavage of vWF by ADAMTS13. Under the conditions of excessive high fluid shear stress, vWF undergoes a conformational transition from a globular state to an extended chain conformation that is more adhesive to platelets (Arya *et al*., 2002). This leads to aggregation complexes within the vasculature and thrombus formation. ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif 13) is a shear-dependent metalloproteinase produced by hepatic cells, which cleaves unusually large vWF (Lian, 2005), high levels of shear stress both enhanced vWF-platelet aggregation and promote cleavage of vWF by ADAMTS13.

Immune destruction of platelet is another factor resulting in thrombocytopenia. Immune development due to successive pregnancies, result in placental sequestration and consequently platelet consumption (Machteld *et al.*, 2012). Increased pooling of platelet in the spleen enlarged by congestive splenomegaly secondary to portal hypertension (Saboo *et al.*, 2012) the sequestrated platelets are still capable of removing TPO from the circulation. They further contribute to the development of thrombocytopenia by lowering TPO levels (Rios *et al*., 2005), depression of megakaryocyte due to hormone suppression (thrombopoitin, TPO) and direct bone marrow suppression. TPO is primarily made in liver by both parenchymal cells and sinusoidal endothelia cells and is secreted into the circulation at a constant rate. Bone marrow suppression due to iron overload also suppresses TPO.

The liver plays a pivotal role in the fibrinolytic system and is responsible for sustaining a balance between bleeding and thrombosis to maintain homeostasis. The liver is important in both the production of multiple factors involved in the process and clearance of breakdown products. Under normal circumstances, deposition of fibrin within the vascular system triggers the conversion of plasminogen into the active enzyme plasmin, which then degrades fibrin and liberates fibrin and fibrinogen degradation products into the circulation (Cesarman-Maus and Hajjar, 2005). This plasminogen-t-plasmin conversion is driven by tissue plasminogen activator (t-PA) and opposed by plasminogen activator inhibitor (PAI) (Cotran *et al*., 2005). Alpha-2-antiplasmin is among the major inhibitors of plasmin and fibrinolysis (Cotran *et al*., 2005). Thrombin-activatable fibrinolysis inhibitor (TAFI) inhibits recruitment of plasminogen to thrombi slowing fibrinolysis (Cotran *et al*., 2005). Fibrinolysis is increased in cirrhosis. There is a reduced production of clotting and inhibitory factor, as well as decrease clearance of activated factors, leading to accelerated intravascular coagulation. There is also decreased clearance of t-PA and PAI-1 from the circulation (Cotran et al., 2005) and decrease hepatic synthesis of alpha-2-antiplasmin and TAFI (Van *et al*., 2001). This hypercoagulation state with excessive platelet consumption plays a role in the development of thrombocytopenia and increase thrombosis consumes platelets (Ikura *et al.*, 2013). Also deficiency in endothelial NO synthase (eNOS) as a result of deficiency of the cofactor tetrahydrobiopterin (BH4) result in low NO (Kato *et al.*, 2005).

The difference in the parasite density among defferent genotype was statistically difference been lower in HbSS, followed by HbAS and then HbAA in this study, supported by the report of Elliot *et al*., (2008); Foller *et al.*, (2009); Taylor *et al*., (2013). This may be due to the point mutation at the 6^{th} position of beta globin chain where valine is substituted for glutamic acid resulting in polymerization HbS cell (Elliot *et al*., 2008), this leads to the generation of ROS.

Generally, oxidative stress caused damage by inducing endothelial cell dysfunction, apoptosis and angiogenesis (Erica *et al*., 2011). The uncoupling of the mitochondrial electron in HbS leads to excessive superoxide production which may stimulate several adhesion molecules such as ICAM-1(Amer *et al*., 2006; Kato *et al.*, 2005).

Heme oxygenase-1 (SOD-1) which has significant antioxidative and anti-inflammatory effects is increased in HbS (Belcher *et al*., 2010) and thereby decreased the expressions of levels of hypoxia inducing factors such as MiRNA, vascular endothelial growth factor (VEGF) and neutralized free peroxynitrite, decreased the activation of NF-kB and the transcription of NF-kB-dependent molecules such as ICAM-1, PfEMP-1 and blood vessels from angiogenesis. The altered cytoskeletal properties of HbS-containing RBC have been suggested to impact parasite protein trafficking to the RBC surface due to short halflife compared with HbAA (Cyrklaff *et al*.,

2011; Klian *et al*., 2015) thereby reducing cytoadhension. Also, HbAS has an effect on the immune system. It was suggested that the HbAS variant favourably alters the immune response directly by increasing phagocytosis of infected RBCs or through the influence of inflammatory cytokines following endothelial activation as a result of altered cytoadhesion properties (Cholera *et al*., 2008; Manjurano *et al*., 2015; Taylor *et al.*, 2013).

In case of HbAA increased levels of gamma globulin were found thereby preventing sequestration of malaria parasite properties (Cholera *et al*., 2008; Mangano *et al*., 2015; Taylor *etal*., 2013). Spleen clearance does not prevent malaria parasites as more and more are continually pumped into the ststem through the cycle exoerythrocytic phase in the liver to the erythrocytic phase in the blood stream. When the liver cells rupture, the merozoites are release into the bloodstream and invade red blood cells where they initiate a second phase of asexual multiplication (erythrocytic schizogony) resulting in the production of about 8-16 merozoites which invade new red blood cellscausing fever, body weakness, vomiting, just to mention a few (Francis, 2010). The spleen clearance only alleviates the complications posed by placenta sequestration on the baby.

CONCLUSION

It was observed that Lymphocyte percentage was elevated among participated age <21 years. Mean monocyte percentages during the second trimester of pregnancy were elevated. Significant changes were not observed in MCHC. The platelet count was remarkably reduced among participants aged 21-25years and above. Reticulocyte count was found to be higher among the HbSS genotype as compared to HbAS and HbAA in infected subjects. Haemoglobin variants were observed to suffer malaria infection but at varying frequencies.

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