Sokoto Journal of Medical Laboratory Science 2021; 6(3): 56 - 64

SJMLS - 6 (3) - 008

Altered Haematological Variables of Pre-Eclamptic Patients in University of Calabar Teaching Hospital, Nigeria

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https://dx.doi.org/10.4314/sokjmls.v6i3.8

Abstract

Pre-eclampsia is a gestational complication with immense outcomes on foetal/ infant and maternal health. This study assessed haematological variables of pre-eclamptic pregnant women receiving antenatal care at University of Calabar Teaching Hospital (UCTH), Calabar. Following due ethical considerations, 90 subjects aged 18-45 years were enrolled. They comprised 30 pre-eclamptic patients admitted into the antenatal ward, UCTH; 30 pregnant women with no medical condition attending antenatal clinic, UCTH and 30 apparently healthy non-pregnant women of same age range as control subjects. Weight, height and blood pressure were measured while a pre-tested structured questionnaire was used to obtain demographic data. Packed cell volume (PCV), haemoglobin (HB), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC) with differentials, platelet count and mean platelet volume (MPV) were determined using Sysmex Haematology autoanalyzer (Sysmex, Japan). Significantly lower ($p<0.05$) PCV, HB, MCV and platelet count were recorded among pre-eclamptic patients compared to the pregnant and non-pregnant controls. Conversely, body mass index, blood pressures, MCHC, WBC and MPV of pre-eclamptic patients were significantly higher ($p<0.05$) compared to pregnant and non-pregnant controls. There were also significant differences ($p<0.05$) in RBC and differential white cell count among the three groups. Also, we observed appearance of protein in urine associated with increased blood pressure. It is concluded that haematological changes occur in pre-eclampsia and these changes reflect the body's response to systemic inflammation induced by pregnancy and amplified by the presence of hypertension.

Keywords: Pre-eclampsia, haematological variables, pregnant women

Introduction

Pregnancy is the physiological situation of carrying a developing embryo or foetus within the female body in the uterus. Physiological changes in pregnancy include cardiovascular, metabolic, renal, respiratory and haematological transformations (Guyton and Hall, 2011). Pregnancy is sometimes complicated with an underlying medical condition one of which is pre-eclampsia. Pre-eclampsia (previously called toxemia of pregnancy), is a medical situation in which hypertension is present in pregnancy in association with high concentrations of protein in the urine. It usually occurs in the second or third trimesters of pregnancy and if not arrested on time, leads to eclampsia. Pre-eclampsia is diagnosed when a pregnant woman develops high blood pressure; that is, two separate readings taken at least six hours apart of 140 or more in systolic blood pressure and 90 or more in diastolic blood pressure in addition to 300mg of protein in a 24-hour urine sample (proteinuria) (O' Brien *et al*., 2012). Symptoms of pre-eclampsia include swelling in the feet, legs and hands, abdominal pain, severe headaches, reduced urine output, dizziness, nausea and vomiting and rapid weight gain due to a

significant increase in body fluids (Roberts and Gammill, 2005). Apart from the presence of pregnancy-induced hypertension (PIH) and pregnancy-induced proteinuria arising after 20 weeks gestation, other findings in pre-eclampsia include renal insufficiency, hepatocellular dysfunction, convulsions (eclampsia), severe headaches as well as haematological disturbances which comprise thrombocytopenia, disseminated intravascular coagulation and haemolysis (Duckitt, 2005). Pre-eclampsia mostly occurs in pregnant teens and in women over forty years and first-time pregnancies. Other risk factors include a mother or sister who had pre-eclampsia, a history of high blood pressure prior to pregnancy, having history of diabetes or obesity, multiple pregnancies, kidney disease, lupus and rheumatoid arthritis. Women who experience pre-eclampsia are at risk for poor birth outcomes, preterm delivery and infant death. Pre-eclampsia and eclampsia can affect liver and kidney function often leading to haemolysis, elevated liver enzymes and low platelets (HELLP syndrome) and in rare cases, decreased blood flow to the brain which could result in a stroke (Sheikh and Venyo, 2012; Maybury and Waugh, 2004).

Of the two hundred and thirteen million pregnancies documented globally in 2012, nineteen percent (19%) occurred in Africa with 293,000 pregnancy-related deaths recorded in 2013. Common etiology included but was not limited to maternal bleeding and sepsis, complications of abortion, high blood pressure and obstructed labor (Sedgh *et al*., 2014; Global Burden of Disease, 2013). Globally, pre-eclampsia complicates about two to ten percent of pregnancies. According to the World Health Organization (WHO), the incidence of pre-eclampsia is seven times higher in developing countries than in developed countries (Osungbade and Ige, 2011; Dolea and Abouzahr, 2003). Indeed, a prevalence of 1.2% has been reported in the University of Calabar Teaching Hospital, Calabar where the current study is sited (Kooffreh *et al*., 2014). The focus of the study is to assess haematological variables of pregnant women with pre-eclampsia admitted in the antenatal ward of University of Calabar Teaching Hospital (UCTH), Calabar with a view to provide information which may be useful in the management and care of pregnant women generally and those with pre-eclampsia specifically.

Materials and Methods

Study design/Ethical approval

The study site is University of Calabar Teaching Hospital (UCTH) located in Calabar, Southern Nigeria. Cross-sectional experimental study design was employed; follow-up was not required. Ethical clearance was obtained from the Health Research Ethics Committee of UCTH before commencement of the study and informed consent was obtained from all participants.

Selection of subjects

A total of 90 subjects aged 18-45 years were recruited for the study. They comprised of 30 pregnant women with pre-eclampsia (blood pressure > 140/90) admitted in the antenatal ward of UCTH, 30 pregnant women without any complications attending antenatal clinic in UCTH and 30 non-pregnant apparently healthy women drawn from staff of UCTH who served as control subjects. Pregnant women with known malignancies and any other disease condition as well as participants below the age of 18 and those who refused consent were excluded from the study.

Collection of demographic information and samples

A structured questionnaire was administered to obtain demographic information while blood pressure, weight and height were measured using sphygmomanometer and body mass index (BMI) health scale respectively. Subjects were given universal containers to collect urine for urinalysis. Three milliliters (3ml) of venous blood were collected from each subject with minimum stasis and dispensed into ethylene diamine tetra-acetic acid (EDTA) to a final concentration of 2 mg/ml. The samples were kept at room temperature until utilized within three hours of collection.

Sample analysis

Body mass index (BMI) was calculated by dividing weight by the height squared. Qualitative detection of protein in urine was done using COMBI 2 strip.

The strip was dipped into the urine sample up to the designated mark; excess urine was drained off and the color change on the strip was compared to the standard included in the kit within one minute and interpreted according to the chart. Packed cell volume (PCV), Haemoglobin (Hb), red blood cell count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), total white blood cell count (TWBC), platelet count (PLT) and mean platelet volume (MPV) were determined using Sysmex Automatic Haematology Analyzer XP-300 (2012) (Sysmex, Japan). Differential white cell count was determined by manual visual technique using a well-made and stained blood film read microscopically at x100 (oil immersion objective).

Statistics

Data generated from this study were analyzed using one-way analysis of variance (ANOVA), Tukey HSD Post Hoc and student's t –test on statistical package for social sciences (SPSS) version 21 software. A p-value <0.05 was considered to be statistically significant. Results are expressed as mean ± standard deviation.

Results

Haematological variables of 30 pre-eclampsia patients were compared with 30 pregnant and 30 non-pregnant control subjects. Table 1 presents demographic parameters of pre-eclamptic patients and their controls. The mean ages were 29.20±4.72, 31.53±4.83 and 32.27±4.79 years respectively for pre-eclamptic, normal pregnant and non-pregnant subjects. The systolic and diastolic blood pressures as well as the body mass index of the pre-eclamptic patients were observed to be significantly higher ($p<0.05$) than the values for the controls. In figure 1, the appearance of protein in the urine of pre-eclamptic patients obtained from urinalysis using dipstick which is a semi-qualitative method shows that majority (53.4%) had one plus (+) protein in their urine. Figure 2 express strong association between systolic and diastolic blood pressures and levels of protein in urine. Table 2 shows some haematological parameters of pre-eclamptic patients and control subjects. The packed cell volume and haemoglobin concentration, RBC and MCV of the pre-eclampsia patients were significantly lower ($p<0.05$) than values obtained for normal pregnant and non-pregnant controls while the mean cell haemoglobin concentration and total white cell count was significantly higher ($p<0.05$) for pre-eclamptic patients when compared to their controls. The neutrophil, lymphocyte and eosinophil count of pre-eclamptic patients were similar to values obtained for the normal pregnant control but differed significantly from that obtained for non-pregnant control subjects. While the neutrophil and eosinophil count of pre-eclamptic patients were significantly raised ($p<0.05$). The lymphocyte count was significantly reduced ($p<0.05$) when compared with the control subjects. The platelet count of the pre-eclamptic patients was observed to be lower than that of the normal pregnant subjects and significantly lower ($p<0.05$) when compared with non-pregnant controls. Conversely, the mean platelet volume (MPD) of the pre-eclamptic patients was significantly higher than that obtained for the normal pregnant and non-pregnant control subjects. Table 3 presents some haematological parameters of pre-eclamptic patients and normal pregnant controls based on gestational age with fifteen subjects each in second and third trimesters. The absolute neutrophil count was significantly lower ($p<0.05$) while the lymphocyte count was significantly higher ($p<0.05$) for the pre-eclamptic patients in the second trimester when compared with third trimester. All other parameters studied were not affected by gestational age.

Table 1: Demographic data, blood pressure and body mass index of pre-eclamptic patients and control subjects

Parameters	Pre-Eclamptic Patients (N=30)	Normal Pregnant Control (N=30)	Non-Pregnant Control (N=30)
Age (Years)	29.20±4.72	31.53±4.83	32.27±4.79
Marital Status			
Married	29 (96.7%)	30 (100%)	30 (100%)
Single	1 (3.3%)	-	-
Educational Level			
Tertiary	23 (76.7%)	25 (83.3%)	30 (100%)
Secondary	4 (13.3%)	4 (13.3%)	-
Primary	3 (10.0%)	1 (3.3%)	-
SBP (mmHg)	156.67±20.73*	114.33±8.17	116.33±6.15
DBP (mmHg)	95.77±6.72*	72.33±9.71	72.33±8.58
BMI (Kg/m^2)	36.17±5.20*	30.74±5.74	26.69±3.95

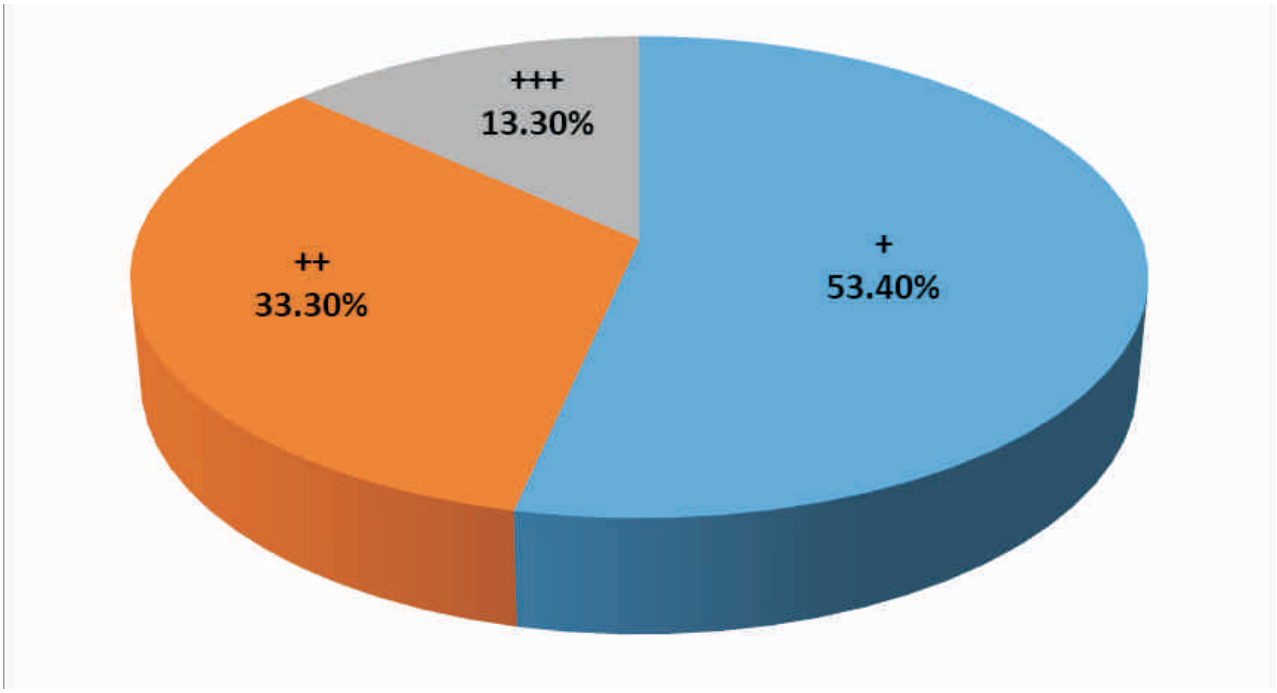

Figure 1: Appearance of protein in urine of pre-eclamptic patients

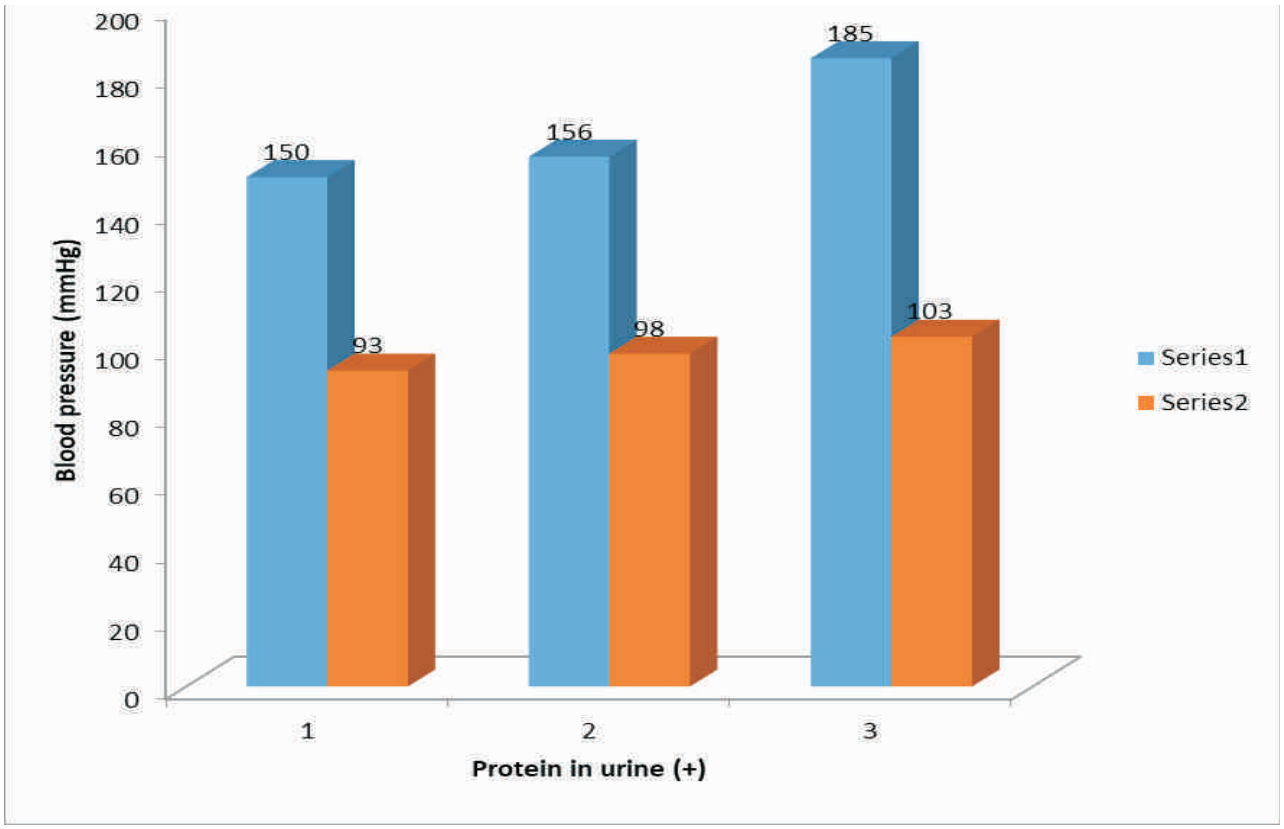

Figure 2: Systolic and diastolic blood pressures and levels of protein in urine of pre-eclamptic patients
Series 1: Systolic blood pressure
Series 2: Diastolic blood pressure

Table 2: Altered haematological variables of pre-eclamptic patients and control subjects

Variables	Pre-Eclamptic Patients (N=30)	Normal Pregnant Control (N=30)	Non-Pregnant Control (N=30)	Remark
PCV (L/L)	0.32±0.04**	0.35±0.03*	0.38±0.02	S
HB (g/L)	107.0±17.0*	108.0±11.0*	118.0±7.0	S
RBC ($x10^{12}$/L)	3.91±0.55	3.74±0.41*	4.11±0.37	S
MCV (fl)	84.60±7.98**	95.36±7.66	94.26±9.52	S
MCH (pg)	27.74±2.49	28.72±2.50	28.82±2.39	NS
MCHC (g/dl)	32.22±2.64**	30.14±0.96	30.63±1.57	S
TWBC ($x10^9$/L)	8.02±2.28*	6.72±1.28*	5.32±1.62	S
NEUTROPHIL ($x10^9$/L)	5.07±0.19*	4.56±0.11*	2.69±0.14	S
LYMPHOCYTE ($x10^9$/L)	2.23±0.18*	1.66±0.09*	2.15±0.11	S
EOSIN0PHIL ($x10^9$/L)	0.75±0.04	0.58±0.04*	0.39±0.03	S
PLT ($x10^9$/L)	202±55*	244±68	264±56	S
MPV (fl)	10.40±1.26**	8.64±0.49	9.01±1.00	S

S = Significant difference (P<0.05); NS=No difference
Post Hoc *Different from non-pregnant control; **Different from normal-pregnant and non-pregnant control

Table 3: Haematological variables of pre-eclamptic patients and normal pregnant control based on gestational age

Variables	Pre-Eclamptic Patients N=30		Normal Pregnant Control N=30	
	Second Trimester N=15	Third Trimester N=15	Second Trimester N=15	Third Trimester N=15
PCV (L/L)	0.32±0.06	0.33±0.04	0.35±0.04	0.36±0.03
HB (g/L)	105.00±19.00	109.00±13.00	108.00±13.00	107.00±9.00
RBC ($x10^9$/L)	3.98±0.66	3.83±0.41	3.79±0.45	3.67±0.37
MCV (fl)	82.45±8.56	86.73±7.01	93.31±7.72	98.05±6.97
MCH (pg)	27.24±2.91	28.23±1.96	28.41±2.56	29.12±2.47
MCHC (g/dl)	31.79±3.42	32.65±1.53	30.18±0.84	30.10±1.14
TWBC ($x10^9$/L)	7.99±2.22	8.04±2.42	6.77±1.19	6.66±1.46
NEUTROPHIL ($X10^9$/L)	4.75±0.17*	5.38±0.19	4.70±0.09	4.39±0.13
LYMPHOCYTE ($X10^9$/L)	2.51±0.16**	1.94±0.16	1.61±0.09	1.73±0.12
EOSINOPHIL ($X10^9$/L)	0.73±0.09	0.65±0.06	0.45±0.02	0.12±0.03
PLT ($X10^9$/L)	199.00±61.00	206.00±51.00	253.00±67.00	233.00±70.00
MPV (fl)	9.99±1.31	10.81±1.10	8.52±0.47	8.77±0.50

*Significantly lower ($P<0.05$) than third trimester
**Significantly higher ($P<0.05$) than third trimester

Discussion

Pre-eclamptic patients that constituted test subjects in this research were mostly well educated (76.7% of tertiary education) and married (96.7%) with reasonably high socio-economic status in similarity with the control groups. The mean age of patients with pre-eclampsia was observed to be lower than that of the control groups; an observation somewhat at variance with earlier views that the incidence of hypertension in pregnancy was driven by increase in maternal age (Chames and Sibal, 2001). It is interesting to note that gestational hypertension and in fact, pre-eclampsia is currently occurring in younger women as well, hence the need to be adherent to blood pressure monitoring during pregnancy irrespective of maternal age. High systolic and diastolic blood pressures as well as proteinuria recorded for the pre-eclamptic patients in this study were the premise for categorizing the subjects as such and is attributable to increase in arterial pressure sequel to leakage of protein into urine. This is further confirmed by the strong association observed (figure 2) between blood pressure and protein in urine which is also consistent with previous literature (Gifford, 2000). More so, the body mass index (BMI) of pre-eclamptic patients was higher than the control group; possibly reflecting weight gain as a result of salt and water retention as also documented by earlier reports (Hall, 2011; Porth, 2004; Guyton). Haematological variables are known to vary during pregnancy. It has been reported that red cell mass increases within this period due to an increase in maternal erythropoietin production but is relatively less when compared with the increase in plasma volume. This results in a fall in haemoglobin concentration (Guyton and Hall, 2011; Faupel-Badger *et al.*, 2007). There is also a small increase in mean corpuscular volume (MCV) due to increased production of RBCs to meet the demands of pregnancy coupled with a higher proportion of young RBCs which are larger in size (Faupel-Badger *et al.*, 2007). However, significantly lower ($p<0.05$) MCV mean value was recorded for the pre-eclamptic patients in the current study, possibly pointing to

derangement in erythropoietic response as a complication of pre-eclampsia. This may have given rise to the observed significantly lower ($p<0.05$) mean value of PCV among the same study subjects compared to both pregnant and non-pregnant controls (table 2). It is also possible that this change is partly a consequence of magnified haemodilution due to excessive water retention as earlier mentioned, especially as the HB of the pre-eclamptic patients varied significantly ($p<0.05$) with only the non-pregnant controls. Conversely, the increased RBC and MCHC of the pre-eclamptic patients when compared with normal pregnant and non-pregnant controls can be attributed to a pseudo increase in red cell mass as a consequence of haemodilution which is further aggravated by retention of water in pre-eclamptic condition.

The total WBC count of pre-eclamptic patients was significantly higher ($p<0.05$) than control values. A higher white cell count implies an immunologic response by the body to the challenges including systemic inflammation associated with hypertension in pregnancy (Choi and Pai, 2002). Similarly, the differential white cell count showed significantly higher values of neutrophils and lymphocytes for pre-eclamptic patients when compared with non-pregnant controls with similar values for normal pregnant controls. The eosinophil count of all pregnant subjects was comparable however, it was significantly higher ($p<0.05$) when comparison was made between the normal pregnant and non-pregnant controls. Also, the eosinophil count obtained for pre-eclamptic patients ($0.75\pm0.04 \times 10^9$/L) is above the reference range of 0.66×10^9/L (Dacie and Lewis, 2010). White blood cell count has been reported to be increased in pregnancy due to the physiologic stress induced by the pregnant state. Increase in neutrophil count is probably due to mobilization of neutrophils to site of endothelial injury as seen in pre-eclampsia; a necessary inflammatory response, although actual neutrophil chemotaxis and phagocytic activity are reportedly depressed despite the foregoing (Edlestam *et al.*, 2001). Although literature suggests that lymphocyte count decreases during pregnancy while eosinophil count does not change significantly (Kline *et al.,* 2005), the present study observed that whereas lymphocyte count is lower in pregnancy, eosinophil count could be higher. A possible explanation is that pre-eclampsia is associated with both the modulation of immune response and defective trophoblast invasion. The syndrome of pre-eclampsia is described as an excessive maternal inflammatory response directed against foreign foetal antigens that induce a chain of events including surface trophoblast invasion, defective spiral artery remodeling, placental infarction and release of pro-inflammatory cytokines and placental fragments in the systemic circulation (Matthiesen *et al*., 2005). Furthermore, Guyton and Hall (2011) observed that pre-eclampsia is induced by allergy or autoimmune condition and this may account for the slight eosinophilia observed in this study.

In this study, the platelet count for the pre-eclamptic patients was significantly lower while the mean platelet volume was significantly higher ($p<0.05$) than for the control groups. A lower platelet count may have resulted from mobilization and involvement in addressing endothelial injury induced by hypertension and in fact conforms to thrombocytopenia in toxemia of pregnancy as reported by Porth (2004). However, the increase in MPV indicates that although the platelets are fewer in number, they are larger in size implying a state of platelet activation and consumption which may result in hypercoagulability. On its own, pregnancy induces a state of inflammation and is a factor of hypercoagulability, as a physiologically adaptive mechanism to prevent *post-partum* bleeding. When complicated with an additional underlying condition such as pre-eclampsia, the changes observed in normal pregnancy may be amplified with possible risk of developing thrombosis (Gresele, 2008). Haematological parameters of normal pregnant subjects were not affected by gestational age (table 3) however, for pre-eclamptic patients, the neutrophil count increased from second to third trimesters while the lymphocyte count decreased. This observation is different from previous report that lymphocyte count decreases during pregnancy through the first and second trimesters and increases during the third trimester (Kline *et al.,* 2005). It was also observed in this study that the

pre-eclamptic subjects and the matched normal pregnant subjects were in their second and third trimesters of pregnancy. The fact that no subject was in the first trimester implies that pregnant women in the study area do not register early for antenatal care.

Conclusion and Recommendation

Packed cell volume, haemoglobin, mean corpuscular volume and platelet count are significantly lower while mean corpuscular haemoglobin concentration, total white blood cell count, neutrophil and eosinophil count and mean platelet volume are significantly higher in pre-eclamptic patients when compared to normal pregnant and non-pregnant control subjects indicating the body's response to the state of systemic inflammation induced by pregnancy and amplified by the presence of hypertension. Early attendance to antenatal clinic could significantly reduce the occurrence of pre-eclampsia and other pregnancy complications.

Conflict of interest: None declared

Acknowledgements

The authors wish to acknowledge pre-eclamptic patients who consented to participate in this study and the clinicians in charge of their care and management who assisted in the collection of blood samples.

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Citation: Stella B. Egbe, Patience A. Akpan, Euphoria C. Akwiwu, Josephine O. Akpotuzor. Altered Haematological Variables of Pre-Eclamptic Patients in University of Calabar Teaching Hospital, Nigeria. ***Sokoto Journal of Medical Laboratory Science; 6(2): 56- 64.***