

Red Cell and Platelet Indices in Women with Preeclampsia in Federal Medical Centre, Owerri

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Abstract:

Preeclampsia is a serious and life-threatening pregnancy complication. It is universally defined as hypertension and significant proteinuria developed at or after 20 weeks of pregnancy in an otherwise normotensive woman. The aim of this study was to determine the levels of haematological parameters in pregnant women with preeclampsia in Federal Medical Centre Owerri. A total of 120 pregnant women aged 18-45 years at 28-40 weeks of pregnancy were recruited; 60 were women with preeclampsia while 60 were pregnant women with normal blood pressure. Haematological parameters were analyzed using haematology auto-analyser by Sysmex® KX-21N. The median value of RBC ($3.20 \times 10^{12}/l$), Hb (9.60 g/dl), PCV (29.00 %) and MCV (86.00 fl) were significantly lower ($p < 0.05$) in women with preeclampsia compared with normotensive pregnant women RBC ($3.66 \times 10^{12}/l$), Hb (10.60 g/dl), PCV (33.70 %) and MCV (91.30 fl) while the median value of MCH (30.00 pg) and MCHC (34.00 g/dl) were significantly higher ($p < 0.05$) in women with preeclampsia compared with normotensive pregnant women MCH (29.45 pg) and MCHC (32.85 g/dl). The median value of platelet ($136 \times 10^9/l$), MPV (9.20 fl) and PCT (0.14 %) of women with preeclampsia decreased significantly ($p < 0.05$) when compared to Normotensive pregnant women platelet ($199 \times 10^9/l$), MPV (9.40 fl) and PCT (0.16 %) while the median value of PDW (15.70 %) and PLCR (21.00 %) of women with preeclampsia increased significantly ($p < 0.05$) compared to normotensive pregnant women PDW (14.75 %) and PLCR (18.75 %). In this study, red cell and platelet indices were altered in preeclampsia. Thrombocytopenia was observed in most of our study population. Therefore, assessing these markers may be useful in the diagnosis and management of preeclampsia.

Key words: pregnancy; preeclampsia; haemoglobin; red cell indices; platelet indices

Introduction

Preeclampsia is universally defined as hypertension and significant proteinuria developed at or after 20 weeks of pregnancy in an otherwise normotensive woman [1]. Gestational hypertension is the presence of new hypertension (usually systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg) occurring in the second half of pregnancy, while preeclampsia is the combination of gestational hypertension in the new proteinuria [2]. In the absence of proteinuria, hypertension together with evidence of systemic disease such as thrombocytopenia or elevated levels of liver transaminase is required for diagnosis [3]

Recently, the definition of preeclampsia has been broadened. Now the internationally agreed definition of preeclampsia is the one proposed by the International Society for the Study of Hypertension in Pregnancy (ISSHP). According to the ISSHP, preeclampsia is defined as systolic blood pressure at ≥ 140 mmHg and/or diastolic blood pressure at ≥ 90 mmHg on at least two occasions measured 4 hours apart in previously normotensive women and is accompanied by one or more of the following new-onset conditions at or after 20 weeks of gestation: Proteinuria (i.e. ≥ 30 mg/mol protein: creatinine ratio; ≥ 300 mg/24 hour; or $\geq 2+$ dipstick); Evidence of other maternal organ dysfunction,

including: acute kidney injury (creatinine $\geq 90 \mu\text{mol/L}$; 1 mg/dL); liver involvement (elevated transaminases, e.g. alanine aminotransferase or aspartate aminotransferase $>40 \text{ IU/L}$) with or without right upper quadrant or epigastric abdominal pain; neurological complications (e.g. eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, and persistent visual scotomata); or hematological complications (thrombocytopenia—platelet count $<150\,000/\mu\text{L}$, disseminated intravascular coagulation, hemolysis); or Uteroplacental dysfunction (such as fetal growth restriction, abnormal umbilical artery, Doppler wave form analysis, or stillbirth) [4]

Preeclampsia is a multisystem disorder unique to human pregnancy. It is a common complication of pregnancy associated with high maternal morbidity, mortality and uterine fetal growth restriction [5]

Worldwide, preeclampsia affects estimated 2-10% pregnant women [6]. More than 4 million women across the world develop this disorder every year and an estimated 50,000 - 76,000 women and 500,000 infants die of this condition every year [7]. In Nigeria, it is estimated that 3-10% are complicated by Hypertensive Disorder in Pregnancy (HDP) [8]

Risk factors for preeclampsia include nulliparity, multifetal gestations, previous history of preeclampsia, obesity, diabetes mellitus, vascular and connective tissue disorders like systemic lupus erythematosus and antiphospholipid antibodies, age >35 years at first pregnancy, smoking, and African American race [9]. The symptoms of preeclampsia may include edema of the hands and face/eyes and weight gain. Severe preeclampsia may present headache, abdominal pain, agitation, decreased urine output, nausea, vomiting and vision changes.

The pathogenesis of preeclampsia is unknown. Most theories on the etiology of preeclampsia suggest that the disease is a cascade triggered by combination of endothelial cell activation/damage, defective placental vascular remodeling in pregnancy (presumably secondary to impaired trophoblast invasion), reduced uteroplacental perfusion, immune intolerance (rejection of the placenta by the maternal immune system), and exaggeration of inflammatory response [10].

During pregnancy, changes occur in the haematological indices such as red blood cell count, haemoglobin concentration, platelet count, and white blood cell count [11]. Preeclampsia also results in a variety of haematological aberrations [12]. Thrombocytopenia is the most common haematological abnormality found in pre-eclampsia. It is a strong indicator of severity of the disease [13]. The pathogenesis of thrombocytopenia in preeclampsia is not clear. Although it is suggested that low platelet count in the disease are associated with abnormal activation of coagulation system and accelerated platelet consumption. Haemoconcentration manifested with increased haematocrit due to increased endothelial permeability has also been reported in preeclampsia [14]. On the contrary, in a study by [15], observed a significant decrease in the haemoglobin level in preeclampsia with increase in severity of the disease when compared to normotensive pregnant women.

However, most of these studies were done in countries outside of Africa. There were limited published reports on the generalization of these findings to women in Nigeria. Therefore, this study will be done to provide data on the level of haematological indices, in pregnant women with preeclampsia in Owerri, Imo State Nigeria.

Materials And Methods

Study Area:

This study was carried out in Federal Medical Centre Owerri in Imo State, Nigeria. Imo state is one of the 36 states of Nigeria.

Study Design:

Cross-sectional study design was used in the study. A total of 120 subjects all pregnant women aged from 18-45 years in their third trimester (28-40 weeks) were recruited for the study. The subjects were group into two.

The first group consists of 60 pregnant women clinically diagnosed as having preeclampsia (test group) while the second group consists of 60 gestational age-matched normotensive pregnant women which served as control subjects. An oral consent was gotten from the patients after; a structured questionnaire was administered to all respondents who were also part of clinical study.

Sample Size Determination

The sample size was obtained using the formula by Naing *et al.*, (2006). Prevalence rate of preeclampsia is 3.4% (Onoh *et al.*, 2019).

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

Where

n = Sample size

p = prevalence rate 3.4%

z = confidence interval 95% - 1.96

d = Degree of accuracy- 0.05

$$N = 1.96^2 \times 0.034(1-0.034)/0.05^2 = 50$$

Therefore, the minimum sample size was 50. Considering 10% attrition, a sample size of 60 was used for the study.

Sampling Technique

Purposive sampling technique was employed in selecting the participants based on the inclusion criteria.

Sample Collection

After obtaining informed consent, 8mls of venous blood was collected from the fore arm of each subject using a disposable syringe; 2.5mls was dispensed into a sterile EDTA vacutainer for determination of haematological parameter. The collected samples were analysed immediately for haematological tests

Ethical Approval:

Ethical approval was sought and obtained from the Ethics committee of Federal Medical Centre Owerri before the commencement of this study (Reference: FMC/OW/ETHICAL/VOL.1/9316)

Inclusion Criteria

Participants included in this study were pregnant women who were diagnosed of preeclampsia aged 18-45 years in their third trimester attending antenatal care at Federal medical Centre Owerri and normotensive pregnant women who gave their consent.

Exclusion Criteria

Those pregnant women who have evidence of chronic infection like HIV, chronic renal disease, tuberculosis and subjects who are using any kind of anticoagulant drugs were excluded from the study with those less than 18 years and above 45 years. Pregnant women in-active labour, in need of emergency care or having an at-risk pregnancy such as gestational diabetes; gestational hypertension was also excluded from the study.

Laboratory Diagnosis

Hematological Parameter Estimation

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

Principle of Test

The Kx-21N employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC 38 detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the haemoglobin concentration, using the non-cyanide haemoglobin method. Blood is aspirated from the sample probe into the sample rotor valve. Six microlitres (6µl) of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994 ml of diluents. At the same time, 1.0 ml of WBC/HGB lyse fluid is added to prepared 1: 500 dilution sample. When the solution is made to react for approximately 10 seconds, the RBC is haemolyzed and platelets shrink, with WBC membrane maintained as they are. At the same time, haemoglobin is oxidized to methaemoglobin (Sysmex Corporation, 2006). Of the diluted/haemolyzed sample in the WBC transducer chamber, approximately 1.0 ml is transferred to the HGB flow cell. Then, 500µl of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection system. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is directed to the sample in the HGB flow cell. Concentration of the sample is measured as absorbance. The absorbance is compared with that of the diluents alone that was measured before addition of the sample, thereby quantifying HGB (haemoglobin value) (Sysmex Corporation, 2006).

Procedure

An EDTA anticoagulated blood was well mixed, and inserted into the probe. The button was pressed and 0.02ml of blood was aspirated. After

a period of 1 minute the haematological result was displayed in the screen and printed with the aid of the printer.

Results

Table 4.1 showed the comparison of the levels of some haematological variables in the test and control group. From the result obtained, the median values of WBC and basophil did not differ significantly in test group when compared with control group ($p>0.05$). The median value of neutrophil, monocyte and eosinophil were significantly higher in test group when compared with the control group ($p<0.05$). However, the median value of lymphocyte was significantly lower in test group when compared with control group ($p<0.05$).

Table 4.2 shows the comparison of the levels of red cell indices in the test group and control group. From the result of the findings, the median value of MCH and MCHC were significantly higher in test group when compared with the control group ($p<0.05$). The median value of RBC, Hb and PCV were significantly lower in test group when compared with control group ($p<0.05$). However, the median values of RDWC and RDWS did not differ significantly in test group when compared with control group ($p>0.05$).

Table 4.3 showed the comparison of the levels of platelet indices of test and control group. The median value of platelet, MPV and PCT of test group were significantly decreased ($p<0.05$) when compared to control group while the median value of PDW and PLCR showed a significant increase ($p<0.05$) in test group when compared to control group.

Parameters	Preeclampsia	Control	Mann-Whitney U	p-value
WBC ($\times 10^9/l$)	7.20	6.65	1484.500	0.980 ^{ns}
Lymphocyte (%)	25.00	32.00	993.500	<0.001 ^{**}
Neutrophil (%)	72.50	65.00	1021.500	<0.001 ^{**}
Eosinophil (%)	1.00	1.00	878.00	<0.001 ^{**}
Basophil (%)	0.0	0.00	1760.000	0.750 ^{ns}
Monocyte (%)	1.00	0.00	1165.500	<0.001 ^{**}

Significant level- * $P<0.05$, ** $P<0.001$, *** $P<0.0001$ ns-Not significant ($P>0.05$)

Table 4.1: Levels of some Haematological Variables (WBC and Differentials) in Test and Control Groups (Median Values)

Parameters	Preeclampsia	Control Group	Mann-Whitney U	p-value
RBC ($\times 10^{12}/l$)	3.20	3.66	792.000	<0.001 ^{**}
HB (g/dl)	9.60	10.60	1066.000	<0.001 ^{**}
PCV (%)	29.00	33.70	883.500	<0.001 ^{**}
MCV (fl)	86.00	91.30	777.500	<0.001 ^{**}
MCH (pg)	30.00	29.45	1372.500	0.024 [*]
MCHC (g/dl)	34.00	32.85	833.000	<0.001 ^{**}
RDWC (%)	15.50	15.10	1432.500	0.054 ^{ns}
RDWS (μm^3)	51.00	52.15	1529.500	0.155 ^{ns}

Significant level- * $P<0.05$, ** $P<0.001$, *** $P<0.0001$ ns- Not significant ($P>0.05$)

Table 4.2: Levels of some Haematological Variables (Red Cell Indices) in Test and Control Groups (Median Values)

Parameters	Preeclampsia	Control group	Mann-Whitney U	p-value
Platelet ($\times 10^9/l$)	136.00	199.00	568.000	<0.001 ^{**}
MPV (fl)	9.20	9.40	1643.000	0.409 ^{ns}
PCT (%)	0.14	0.16	1127.000	<0.001 ^{**}
PDW (%)	15.70	14.75	1018.000	<0.001 ^{**}
PLCR (%)	21.00	18.75	1310.000	0.010 [*]

Significant level- * $P<0.05$, ** $P<0.001$, *** $P<0.0001$ ns-Not significant ($P>0.05$)

Table 4.3: Levels of some Haematological Variables (Platelet Indices) in Test and Control Groups (Median Values)

Discussion

Physiological changes occur in pregnancy to nurture the developing foetus and prepare the mother for labour and delivery. Some of these changes influence normal biochemical values while others may mimic symptoms of medical disease. During pregnancy, changes occur in the haematological indices such as red blood cell count, haemoglobin concentration, platelet count, and white blood cell count [16]. Preeclampsia also results in a variety of haematological aberrations. In this study, there was a significant lower level of red blood cell and haemoglobin value in preeclampsia compared to normotensive pregnant women. This finding is in agreement with studies done by [17]. They observed a significant lower haemoglobin level in preeclamptic women with increase in severity of the disease when compared to normotensive pregnant women. The anemia is most frequently associated with HELLP syndrome and it is due to microangiopathic intravascular haemolysis – physical destruction of erythrocytes in the microcirculation affected by disseminated microthrombosis. Haemoconcentration manifested with increased haematocrit due to increased endothelial permeability has also been reported in preeclampsia [18]. According to the World Health Organisation, anaemia is defined as haemoglobin values of <12 g/dl in women. This is based on average haemoglobin values in healthy individuals.

In our study, the values of the red cell indices, MCH, MCHC and RDWC were higher while MCV was lower though within normal level in preeclampsia than the control group. The finding is in accordance with the work done by [18], who found an increase in RDW, MCH, and MCHC with a decrease in MCV. It was discovered an increase in MCV, MCH and RDW but no significant change in MCHC. Despite the differences in MCV, MCH, and MCHC values in preeclampsia versus normotensive pregnant women, an increase in RDW in preeclampsia was found in all the studies. Increased RDW values had been linked to inflammation in hypertensive non-pregnant women; inflammatory theory has also been blamed for increased RDW values in preeclampsia. In a study by [19], a positive correlation between high-sensitivity C-reactive protein and increased RDW levels was determined in preeclampsia. In the present study, the normal values of MCV, MCH and MCHC in preeclampsia might be due to satisfactory taking of iron replacement therapy given to pregnant women on ante-natal visit. From the questionnaire, most participants indicated an adequate attendance of ante-natal care in the pregnancy. In addition, the study population was living in the southern part of Nigeria and the research was conducted during the period of rainy season in which vegetable nutrition is common. Nutritional status (e.g., iron, folate, and vitamin B12 deficiency) and eating habits may affect red blood cell indices [10].

White blood cell count is increased in pregnancy with the lower limit of the reference range being typically $6.0 \times 10^9/l$. Leucocytosis, occurring during pregnancy is due to the physiologic stress induced by the pregnant state [12]. In this study, there was non-significant increase in total white cell count in preeclampsia compared with normal pregnant controls. Differential analyses further demonstrated that the increased neutrophil, not monocyte or lymphocyte numbers account for the total leukocyte increase in preeclampsia. The data obtained in this study is consistent with the study by [20] in which an increase in neutrophil count in preeclampsia was found. There is an absolute monocytosis during pregnancy, especially in the first trimester, but decreases as gestation advances. Monocytes help in preventing fetal allograft rejection by infiltrating the decidual tissue (7th–20th week of gestation) possibly, through PGE2 mediated immunosuppression [13]. In this study, the data obtained showed that

monocyte was significantly higher in preeclamptic patients when compared to the control, while lymphocyte and basophil numbers were significantly lower when compared with normotensive pregnant women. This finding is in accordance with the work done by [11]. Lymphocyte count decreases during pregnancy through the first and second trimesters and increases during the third trimester [18].

Leukocytosis is considered to be evidence of an increased inflammatory response during normal pregnancy and in preeclampsia. It was also found the total leukocyte count was even higher in preeclampsia with hemolytic anemia, elevated liver enzymes, and low platelet count (HELLP syndrome) than those without HELLP syndrome, suggesting an association between increased leukocytes and worsening thrombocytopenia in those patients [19].

In this study, platelet count was significantly lower in pregnant women with preeclampsia compared with normotensive pregnant women. This is in agreement with the study done by various researchers [20]. Preeclampsia results to reduced platelet production and lifespan, and reduced antithrombin, leading to activation of the clotting cascade and the fibrinolytic system, which leads to low platelet counts, fibrin deposition and increased fibrin degradation products. As the disease progresses, small fibrin strands cause microangiopathic haemolytic anaemia. Thrombocytopenia is the most common haematological abnormality found in preeclampsia. It is a strong indicator of severity of the disease. The pathogenesis of thrombocytopenia in preeclampsia is not clear. Although it is suggested that low platelet count in the disease are associated with abnormal activation of coagulation system and accelerated platelet consumption [21]. Increased plasma levels of platelet activation markers (β -thromboglobulin and platelet factor-4) and increased expression of activation markers on the surface of platelets in preeclampsia confirm platelet activation in this disease. Besides, an impaired endothelial synthesis of prostacyclin and nitric oxide has been related in preeclampsia [22].

Platelet indices made available as part of the data obtained from full blood count in this study was statistically analyzed. They include mean platelet volume (MPV), which reflects the marrow bone function, platelet distribution width (PDW), which corresponds to size distribution of platelets; plateletcrit (PCT), which corresponds to the volume that platelets have in 100 mL of total blood; and platelet large cell ratio (PLCR), which reflects the percentage of platelets larger than 12 fL. The statistical analysis of these platelet indices in this study showed lower values of MPV and PCT in preeclampsia. MPV showed no significant difference while PCT was significantly lower in preeclampsia when compared to the normotensive pregnant women. In preeclampsia, PDW and PLCR were significantly higher than that of normotensive pregnant women. The result obtained in this study was in keeping with the work of [23]. Some studies have evaluated the applicability of platelet indices, particularly MPV for the clinical and pathophysiological understanding of vascular diseases, including preeclampsia, but their value is not established yet. It has long been known that platelet volume is a direct indicator of increased platelet synthesis [24].

Conclusion

The results obtained from this study also showed alterations in the levels of parameters in preeclamptic women. Anaemia and severe thrombocytopenia were found in these women.

Moreso, the demographic data obtained in this study showed that nulliparity, advanced maternal age and family history of high blood pressure were the most dominant risk factor of preeclampsia.

A keen observation and monitoring of the haematological parameters in preeclamptic women is vital. Therefore, assessing these parameters can be a reliable and competent marker to diagnose and manage preeclampsia thereby improving pregnancy outcome in this disorder.

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