

## Research Article

# Comparative evaluation of platelet indices in Preeclamptic and Non-Preeclamptic patients in the Enugu Metropolis

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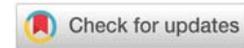
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## Abstract

Preeclampsia (PE) is one of the most common causes of maternal mortality and morbidity. It complicates 5% - 6% of all pregnancies globally and up to 15% of pregnancies in Sub-Saharan Africa. The present study was designed to determine the values of platelet indices in preeclamptic patients compared to non-preeclamptic controls. A total of 70 patients comprising 35 preeclamptic cases and 35 non-preeclamptic controls aged 18-40 years were recruited for the study. Blood samples (3mls) were collected from patients for the estimation of platelet indices using the Mindray 530 BC automated hematology analyzer, Mindray, Japan. The data were analyzed by SPSS version 22 using *T*-test and Pearson correlation. The level of significance was set at  $p < 0.05$  and the result was presented as mean + SD. The result revealed a significant increase in the MPV (13.5 + 0.18 vs. 09.4 + 0.20), PDW (16.2 + 0.037 vs. 06.0 + 0.19) and PLCR (38.0 + 1.19 vs. 26.2 + 1.19) between the preeclamptic patients and non-preeclamptic controls. There was a significant decrease in the PLT (174.1 + 15.5 vs. 231.1 + 15.05), PCT (1.9 + 0.14 vs. 3.3 + 0.75), and PLCC (60.7 + 3.24 vs. 69.4 + 3.17) for the preeclamptic cases compared to non-preeclamptic control. These findings may be useful in the management of the adverse outcomes of preeclampsia for the Enugu population.

## Introduction

Preeclampsia (PE) is one of the most common causes of maternal mortality and morbidity [1]. It complicates 5-6 of all pregnancies as well as 25% in women with pre-existing hypertension [2]. It is a multi-system disorder observed as gestational hypertension usually after the 20<sup>th</sup> week of gestation with systolic blood pressure > 140 mmHg and diastolic blood pressure > 90 mmHg accompanied by significant proteinuria (> 300 mg/L or 500 mg/24 hours urine or 2+ or more proteinuria on dipstick) with or without edema [1,2]. According to the classification of the American College of Obstetrics and Gynecology (ACOG), preeclampsia can be categorized into mild (140-159/90-109 mmHg) and severe (> 160/110 mmHg) [2]. Mild preeclampsia occurs in approximately 15% of pregnancies while severe preeclampsia occurs in about 1% to 2% [3]. Platelets are small, anucleated, discoid-shaped cells that emanate from precursor cells known

as megakaryocytes which are part of the hemopoietic cell line [4,5]. Studies have shown that platelets play an important role in the pathogenesis of preeclampsia [6,7]. Platelet indices involving the Plateletcrit (PCT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and the Platelet Large Cell Ratio (PLCR), and the Platelet Concentration (PLCC) are a group of derived platelet parameters obtained as a part of the automated complete blood count. There is currently a paucity of data on the values of platelet indices in preeclampsia patients in the Enugu metropolis. The present study was therefore designed to determine the values of platelet indices in preeclamptic subjects and non-preeclamptic controls in the Enugu metropolis.

## Materials and methods

### Study area

This study was carried out in Enugu State, South East,

Nigeria. Enugu State is made up of three senatorial zones namely Enugu East, Enugu West, and North. The senatorial zones are divided into seventeen Local Government Areas comprising 450 communities. The State takes its name from its capital and largest city, Enugu. It has an area of 7,161 km<sup>2</sup> with a population of 3,267,837 comprising mainly the Igbo tribe of South Eastern Nigeria; about 50% of which lives in rural areas. It lies between longitudes 6°30'E and 6°55'E and latitudes 5°15'N and 7°15'E [8].

### Study design

This was a case-control study carried out on patients attending antenatal clinic and/or admitted in the maternity wards in four different hospitals in the Enugu metropolis namely the Enugu State University of Science and Technology Teaching Hospital, Parklane, the University of Nigeria Teaching Hospital, Ituku-Ozalla, Mother of Christ Specialist Hospital and the Annunciation Specialist Hospital. The subjects were divided into two groups. Group A comprised 35 cases of preeclampsia patients defined by blood pressure < 140/90 mmHg and Proteinuria in 24 hours < 300 mg and edema after 20 weeks of gestation while group B involved 35 normal pregnant women after their 20 weeks of gestation.

### Ethical considerations

Ethical approval was obtained from the respective institutions' Ethical Management Committee as well as signed informed consent from the subjects.

### Sample size

The sample size for the study was calculated using the Leslie Kish formula [9].

$$n = \frac{Z^{\alpha 2} PQ}{D^2}$$

Where

n = minimum required sample size when the population is greater than 10,000.

Z<sup>α</sup> = the α level of the coefficient interval or the standard normal deviation set at 1.96 corresponding to the 95% confidence interval.

P = the proportion in the target population estimated to have preeclampsia 3.3% [10].

D = The width of the confidence interval set at 0.05

D = (1-P); the proportion of non-occurrence.

Substituting into the formula

$$n = \frac{1.96 \times 1.96 \times 0.033(1 - 0.033)}{0.05 \times 0.05} = 49$$

But an estimated 68 registered preeclampsia patients attended the four different clinics in the last year. Since this

is less than 10,000, the sample size was adjusted using the formula.

$$Nf = \frac{n}{1} + \frac{(n)}{N} = \frac{49}{1 + 49/68} = 28$$

Considering a response rate of 90%, the sample size was further adjusted to accommodate attrition using the formula.

$$N_s = nf/r$$

Where N<sub>s</sub> = adjusted sample size for a response rate

N<sub>f</sub> = calculated sample size

r = the anticipated response rate of 90% (0.9)

Substituting into the formula

$$N_s = \frac{28}{0.9} = 31$$

Therefore, a total of 70 subjects involving 35 cases of preeclampsia and 35 non-preeclampsia control were recruited for the study.

### Subject recruitment

Subjects were recruited by convenient sampling.

**Inclusion criteria:** Both the cases and controls were in the age group of 18-40 years.

**Exclusion criteria:** Pregnant women with a major systemic disease that may affect the patient's blood pressure or the platelet indices e.g. liver disease, hepatitis infection, diabetes mellitus, and cardiovascular disease were excluded. Patients using any drug that affect liver function, platelet function, or platelet count were also excluded.

### Sample collection

About 10 milliliters of clean catch urine were collected in clean dry containers for the determination of proteinuria and 3 milliliters of blood were drawn aseptically by venipuncture and dispensed into an EDTA bottle for estimation of platelet indices.

### Determination of proteinuria

Proteinuria was determined using the dipstick method for urinalysis [2]. This is a semi-quantitative test aimed at detecting abnormal levels of chemical substances (in this case, protein) in urine. The combi-9 test strips impregnated with tetra bromophenol blue for protein detection were used.

**Principle:** Tetrabromophenol blue is yellow at PH 3.0, but changes to bluish green in the presence of protein at the same PH. The intensity of the color change is proportional to the amount of protein present in the urine.

**Procedure:** The test end of the strip was dipped into freshly voided urine for about 1 second, the excess urine was drained along the container and the color on the strip was compared

with the color chart on the test strip container. Presence and/or absence of protein was noted and the degree of proteinuria scored subjectively as Nil, 1+(30mg/dl), 2+(100mg/dl), 3+(300mg/dl) and 4+(more than 300mg/dl).

### Determination of platelet indices

Platelet indices were determined by performing an automated full blood count using Mindray 530 BC autoanalyzer, Japan [11].

**Principle:** The Mindray 530 BC automated analyzer works on the principle of electrical impedance (colter principle). The principle of impedance technology is electrical resistance (or impedance) in which a known dilution of cells in suspension passes through a small orifice. The electrolyte-containing diluents serve as a conductor of a constant electrical current between two electrodes. As cells pass through the orifice, they impede the electrical current which is detected as an increase in resistance. Each cell causes a resistance pulse, thus allowing for cell counting.

**Procedure:** The sample was aspirated by letting the machine sample probe into the blood sample and then pressing the probe button. Approximately 20  $\mu$ l of blood was aspirated by the machine. The result of platelet indices was displayed on the screen after 30 seconds as part of the full blood count result. A printout copy of the results was released on thermal printing paper.

### Statistical analysis

Statistical analysis was done using the statistical package for social sciences version 22 (SPSS Inc, Chicago). The Shapiro-Wilk test was carried out to ensure that the continuous variable was normally distributed. Differences in mean among the two groups were compared by unpaired *T* - test. Determinations of correlation between variables were done by Pearson correlation test. The level of significance for all the inferential statistics was set at  $p < 0.05$ .

### Results

There was a significant increase ( $p < 0.05$ ) in both the systolic and diastolic blood pressure as well as the Platelet Distribution Width (PDW), Mean Platelet Volume (MPV) and Platelet Large Cell Ratio (PLCR) for the preeclamptic cases compared to the non preeclamptic control while there was a significant decrease ( $p < 0.05$ ) in the values of the Platelet Count (PLT), Platelet Crit (PCT) and Platelet Large Cell Concentration (PLCC) for the preeclamptic cases compared to the non-preeclamptic controls (Table 1).

There was a significant positive association ( $p < 0.05$ ) between the systolic blood pressure and platelet indices for the preeclamptic cases while a non-significant ( $p > 0.05$ ) positive association in the systolic blood pressure and the platelet indices were observed for the non-preeclamptic control (Table 2).

There was a significant positive association ( $p < 0.05$ ) between the diastolic blood pressure and platelet indices for the

preeclamptic cases while a non-significant ( $p > 0.05$ ) positive association in the diastolic blood pressure and the platelet indices were observed for the non-preeclamptic control (Table 3).

### Discussion

Conflicting results have been published regarding changes in platelet indices in preeclamptic and non-preeclamptic patients. Some researchers report the difference in the platelet indices while others report no difference [12]. Kasliwal, et al. [13], Majumder, et al. [14] and Halder and Barui [15] reported a significant decrease in the platelet count, platelet crit, and the platelet large cell concentration which had a positive correlation with the patient blood pressure for preeclamptic patients compared to non-preeclamptic control. This is consistent with the findings of the present study which recorded a significant decrease in these parameters for the preeclamptic patient

**Table 1:** Blood Pressure and Platelet indices in case and controls.

	Preeclampsia (n = 35) (mean + SD)	Non-preeclampsia (n = 35) (mean + SD)	<i>p</i> value
SBP (mmHg)	169.56 $\pm$ 20.02	117.42 $\pm$ 6.01	0.006
DBP (mmHg)	107.45 $\pm$ 8.14	75.36 $\pm$ 8.20	0.002
PLT ( $\times 10^9/L$ )	174.1 $\pm$ 15.15	231.1 $\pm$ 15.05	0.014
PDW (FL)	16.2 $\pm$ 0.037	06.0 $\pm$ 0.037	0.011
MPV (FL)	13.5 $\pm$ 0.18	09.4 $\pm$ 0.20	0.000
PCT (%)	1.9 $\pm$ 0.14	3.3 $\pm$ 0.75	0.002
PLCR (%)	38.0 $\pm$ 2.14	26.2 $\pm$ 1.19	0.001
PLCC ( $\times 10^9/L$ )	60.7 $\pm$ 3.24	69.4 $\pm$ 3.17	0.001

PLT: Platelet Count; PDW: Platelet Distribution Width; MPV: Mean Platelet Volume; PCT: Plateletcrit; PLCR: Platelet Large Cell Ratio; PLCC: Platelet Large Cell Concentration; SBP:Systolic Blood Pressure; DBP:Diastolic Blood Pressure

**Table 2:** Correlation of systolic blood pressure with the platelet indices.

	Preeclampsia		Non-preeclampsia	
	<i>r</i> value	<i>p</i> value	<i>r</i> value	<i>P</i> value
PLT	0.211	0.004	0.616	0.976
PDW	0.471	0.001	0.013	0.601
MPV	0.129	0.008	0.059	0.134
PCT	0.189	0.002	0.305	0.403
PLCR	0.224	0.000	0.216	0.482
PLCC	0.146	0.031	0.255	0.180

PLT: Platelet Count; PDW: Platelet Distribution Width; MPV: Mean Platelet Volume; PCT: Plateletcrit; PLCR: Platelet Large Cell Ratio; PLCC: Platelet Large Cell Concentration; *r*: Coefficient of Correlation;  $\alpha$ : Significant at  $p < 0.05$ .

**Table 3:** Correlation of diastolic blood Pressure with the platelet indices.

PLT	0.880	0.004	0.317	0.269
PDW	0.159	0.006	0.698	0.073
MPV	0.462	0.001	0.051	0.116
PCT	0.369	0.001	0.301	0.812
PLCR	0.777	0.002	0.547	0.440
PLCC	0.638	0.009	0.171	0.269

PLT: Platelet Count; PDW: Platelet Distribution Width; MPV: Mean Platelet Volume; PCT: Plateletcrit; PLCR: Platelet Large Cell Ratio; PLCC: Platelet Large Cell Concentration; *r*: Coefficient of correlation.

compared to the non-preeclamptic control. The reason for this decrease may be explained by the continuous consumption and activation of platelets. However, our present finding is not consistent with the findings of Thalor, et al. [16] who reported no significant difference in the platelet count and platelet crit for preeclamptic patients compared to non-preeclamptic control but suggested that a decrease in these platelet indices for preeclamptic patients may be due to gestation itself rather than preeclampsia. Majumder, et al. [14], Halder and Barui [15], Thalor, et al. [16], Kumar and Singh [17], Orgul, et al. [18] and Karateke, et al. [19] reported a significant increase in the platelet indices involving the mean platelet volume, platelet distribution width and platelet large cell ratio which correlates positively with the blood pressure for preeclamptic patients compared to non-preeclamptic control. This is also consistent with the findings of a significant increase in the same parameters for the preeclamptic cases in the present study. The reason for this could be explained by increased platelet turnover by the bone marrow in response to a low-grade inflammation due to an increase in blood pressure which supports the idea that platelet survival time is decreased resulting in increased destruction of platelets. However, the present findings are not consistent with the findings of Sheeha, et al. [20] who reported no difference in the mean platelet volume and platelet distribution width for preeclamptic patients compared to non-preeclamptic controls. Preeclampsia could trigger an adverse maternal immune response to the fetal allograft [21,22]. During the alloimmune response, platelets act as inflammatory mediators to induce the activation of coagulation proteins, causing a decrease in natural anticoagulant proteins and a decrease in fibrinolytic activity culminating in a prothrombotic condition [23,24]. The outcome of this is a failure of microvasculature circulation which may result in fetal growth restriction, placental abruption, or spontaneous abortion [25,26]. Platelet indices such as the mean platelet volume, platelet distribution width, platelet large cell ratio, and platelet large cell concentration are a group of platelet parameters derived from the routine automated full blood count estimation which has been identified as good markers for early diagnosis and prognosis of preeclampsia [27,28]. This eases the labor-intensive and costly methods of measuring the rate of platelet production and activation by calculating the indices from the results of the routine automated full blood count. The utilization of these simple markers could therefore facilitate early detection of maternal and fetal complications thereby playing a role as a prognostic tool in the management of preeclampsia [29,30].

## Conclusion

The findings of the present study suggest that the estimation of platelet indices could be considered an early, simple, rapid, and cost-effective diagnostic and prognostic marker for preeclamptic patients.

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