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Assessment of Haemostatic Parameters on Preeclampsia Subjects in Aba, Abia State

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Abstract

The present study assessed the haemostatic parameters in preeclampsia subjects in Aba, Abia state Nigeria. A total of sixty subjects between the ages of eighteen to forty – four years were used for this study. Thirty were preeclampsia subjects who were medically diagnosed while thirty were apparently healthy individuals who served as control subjects. Platelet count (PLC), prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen were determined. The results were analyzed using SPSS version 20.0. Probability value $P<0.05$ was considered statistically significant. There was lower mean SD in platelet count (235.17±32.99 $\times 10^9$/L versus 260.27± 35.82 $\times 10^9$/L for control), and fibrinogen levels **(**224.47±16.95 mg/dl versus 214.37 ± 14.63 mg/dl) in the preeclampsia subjects in comparison with their control values in this study. There was no significant difference between the prothrombin time and activated partial thromboplastin time of the preeclampsia subjects and the control. The mean values of platelets count significantly decreased with increased pregnancy duration, 269.40±36.66 $\times 10^9$/L, 222.70±11.93 $\times 10^9$/L and 213.40±4.50 $\times 10^9$/L for first, second, and third trimesters respectively. There was a significant decrease in the platelet count as the ages of the preeclampsia subjects increased (268.00±13.54, 233.94±24.49 and 203.57±17.30 $\times 10^9$/L for 18-24 years, 25-34 years and 35-44 years respectively). Low levels of these haemostatic parameters can lead to ineffective hemostasis, inability to control hemorrhage, inhibited blood supply to vital organs and severe blood loss (haemorrhage) which can result to increased bleeding time, hemophilia, thrombocytopenia and may also lead to death.

Keywords*: haemostasis, preeclampsia, haemorrhage, platelets*

Introduction

According to the American College of Obstetrics and Gynaecology[1], Preeclempsia is defined as the presence of hypertension and proteinuria occurring after 20 weeks of gestation in a previously

normotensive subject. It is usually referred with subjects with elevated blood pressure of systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg in not less than two (2) occasions, four hours apart. It is accompanied by proteinuria of ≥ 300 mg/24 hours and other features such as oedema.[2-9]
Pre-eclempsia is a potentially dangerous pregnancy complication characterised by high blood pressure. Pre-eclampsia usually begins after 20 weeks of pregnancy in a woman whose blood pressure had been normal. It can lead to serious, even fatal, complications for both mother and baby.[10] There may be no symptoms. High blood pressure and protein in the urine are key features. There may also be swelling in the legs and water retention, but this can be hard to distinguish from normal pregnancy.[11]

Hemostasis is the term given to a group of mechanisms which prevent the outflow of blood from blood vessels. It is also defined as a state of dynamic equilibrium between anti- and procoagulation reactions, which may be modulated by various factors, including oxidative stress. Many systems take part in hemostasis, including the wall of the blood vessel, the clotting process with its various factors, including fibrinogen, and the fibrinolytic and phagocyte systems. Blood platelets are also very important element of hemostasis.[12-30]
The study was done to assess the haemostatic parameters in preeclampsia subjects in Aba, Abia state, Nigeria.

Materials And Methods

Study Area

The study was done in Aba is a city in the southeast of Nigeria.

Advocacy Mobilization and Pre - survey Contacts

A formal letter of introduction was obtained from the Head of Department, Medical Laboratory Science of Imo State University, Owerri. The letter with the thesis proposal was submitted to the ethical committee of Abia State University Teaching Hospital, Aba. An ethical approval letter was obtained from the hospital to collect samples from the study participants. Questionnaires and informed consent were obtained from the subjects after several meeting on their clinic days. Also, a demographic data was obtained and a day was fixed for collection of blood samples.

Study Population

The sample size was calculated according to Aronye (2004). A total of sixty subjects between the ages of eighteen to forty – four years were used for this study. Thirty were preeclampsia subjects who were medically diagnosed while thirty were apparently healthy individuals who served as control subjects. The preeclampsia subjects were further grouped according to trimester, first trimester (n = 10), second trimester (n = 10) and third trimester (n = 10). Also, they were grouped according to age, 18 – 24, 25 – 34, 35 – 44 years.

Inclusion Criteria

The study participants were preeclampsia individuals who had been attending antenatal clinic at the Abia State University Teaching Hospital, Aba Nigeria for the past two to three months.
They were between the ages of 18 to 44 years. Subjects that were apparently healthy individuals served as control subjects.
Subjects agreed to be given informed written consent.

Exclusion Criteria

The study excluded:
Subjects below 18 years and above 44 years of age.
Subjects who have severe complications.
Individuals whom informed written consent were not obtained.

Blood Sample Collection

The blood samples were collected using standard venipuncture technique described by Kapil *et al*. (2017). Seven milliliters of blood were collected from each subject; 2.5 ml of blood was dispensed into 10% w/v dipotassium EDTA bottle for determination of platelets count while 3.6 ml of blood was dispensed into 0.4 ml of 3.8% of trisodium citrate container for the determination of prothrombin time, platelet count, activated partial thromboplastin time and fibrinogen.[31]

Laboratory Procedures

All reagents were commercially purchased and the manufacturer's Standard Operating Procedures (SOP) were strictly followed.

Determination of Platelet Count

The test was done using, a five - part haematology Sysmex KX-21N auto analyzer according to Lewis *et al*. (2006).

Procedure

The sequenstrene blood sample was placed in the spiral mixer and allowed to mix well. The whole blood was activated in the LCD screen; the sample number (code) was input by pressing enter. The sample was mixed well. The cap was removed and the sample inserted into the probe and the stand switch was pressed. The LCD screen displayed the analysis. The sample was removed and re - capped. The unit executed the analysis automatically and the result was displayed on the screen.

Normal range

150 - 400 cells $x10^9$ /L

Determination of Prothrombin time

The test was done by manual quick method according to Hirch *et al*. (1992) as modified by Giesse Diagnostics, USA. Catalogue number: Ref.1001, 1016

Procedure

The reagent vial was reconstituted with 4mL of distilled water, mixed very well and allowed to stay for 15 minutes. It was prewarmed by incubation at 37^{o}C. In a clean plastic tube, 100μL of trisodium anticoagulated blood was added. It was incubated at 37^{o}C for 2 minutes. Then, 200μL prewarmed PT reagent was added. It was examined intervally and the time for the formation of clot was recorded

Normal value

11 - 15 Seconds

Determination of Activated Partial Thromboplastin Time Estimation

This is done by manual method according to Brandt *et al*., (1981) as modified by GIESSE Diagnostics, USA. Catalogue number: 1002/12, 1006, 1009

Procedure

The APTT reagent vial was pre warmed by incubation at 37^{o}C. In a clean plastic tube, 100μL supernatant of trisodium citrate anticoagulated blood was added. Also, 100μL of prewarmed APTT reagent was added, mixed and incubated for 3 minutes. Then, 100Lμ of calcium chloride reagent was added. It was observed for clot formation and the time was recorded.

Normal value

20 - 30 Seconds

Determination of fibrinogen

The test was done by turbidimetric method according to Clauss (1957) as modified by Giesse Diagnostic, Italy. Catalogue number: 1004, 1005

Procedure

A semi - automated Rayto 22^{0}C coagulometer was used. In a clean dry tube, 25μL of plasma was pipette into it and 225μL of imidazole buffer was added to make 1: 10 dilutions. A test curvette was added into the holder and there was a display prompted to add sample displayed on the screen. Then 100μL of diluted sample was pipette into the test curvette and it was incubated for five minutes and a display showed sample warming. There was another display in the screen prompted to add reagent, 50μL of thrombin was added with a display showing on the screen testing. The result was displayed on the screen and was recorded.

Normal Range

150 - 350 mg/dL

Statistical Analysis

All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21. The results were expressed as mean and standard deviation in tables. Comparison of mean values among different groups was expressed using Student Independent T - test and one way analysis of variance (ANOVA). The probability at Level of $p < 0.05$ was considered statistically significant.

Results

Table 1: The Mean ± Standard Deviation values of Haemostatic Parameters in Preeclampsia Subjects of the Study Population

Parameter	Preeclampsia Subjects (n=30)	Control Subjects (n=30)	t - value	P- value
Platelet Count (×10^{9}/L)	235.17 ± 32.99	260.27± 35.82	2.823	0.007*
Prothrombin Time (s)	13.10 ± 0.20	13.23 ± 0.26	0.582	0.301
APTT (s)	28.27± 0.57	28.30 ± 0.71	0.736	0.060
Fibrinogen (mg/dl)	224.47 ± 16.95	214.37 ± 14.63	2.471	0.016*

Key: APTT: Activated Partial Thromboplastin time;

n: Sample Size.

***: Statistically significant at P < 0.05.**

Table 1 shows the mean ± standard deviation values of haemostatic parameters in preeclampsia subjects of the study population. The result showed that the mean value (235 ± 32.99×**10^9/L** of platelet count in preeclampsia subjects was lower which was not statistically significant (P = 0.007) when compared with the mean value (260.27 ± 35.82×**10^9/L**) of the control subjects. There was no statistical difference (P = 0.301) in the mean value (13.10 ± 0.20s) of prothrombin time in preeclampsia subjects when compared with the mean value (13.23 ± 0.26s) of the control subjects. Also, that there was no statistical significant difference (P = 0.06) in the mean value (28.27 ± 0.57s) of activated partial thromboplastin time in preeclampsia subjects when compared with the mean value (28.30 ± 0.71s) of the control subjects. There was statistically significantly (P = 0.016) higher mean value of fibrinogen (224.47 ± 16.95 **mg/dL**) in preeclampsia subjects when compared with the mean value (214.37 ± 14.63 **mg/dL**) of the control subjects.

Table 2: The Mean ± Standard Deviation Values of Study subjects according to Trimester

Parameter	1st Trimester (n=10)	2nd Trimester (n=10)	3rd Trimester (n=10)	F-value	P-value
Platelet Count(×10^9/L)	269.40 ± 36.66	222.70 ± 11.93	213.40 ± 4.50	17.936	0.001*
PT (s)	12.70 ± 0.67	12.50 ±0.7	12.60 ± 0.97	0. 159	0.854
APTT (s)	27.30 ± 1.06	27.10 ±0.99	27.30 ± 1.25	0.109	0.897
Fibrinogen (mg/dL)	222.90 ± 24.17	225.50 ±14.32	225.00 ±11.47	0.062	0.940

Key:

N: sample size

APTT: Activated Partial Thromboplastin time;

PT: Prothrombin Time;

***: Statistically significant at P < 0.05.**

The result from haemostatic parameters showed that there was progressive statistically decrease (P = 0.001) in the mean values (269.40 ± 11.93×**10^9/L**, 222.70 ± 11.93×**10^9/L**, 213.40 ± 4.50×**10^9/L**) platelet count is preeclampsia subject across all trimesters. There was no significant difference (P = 0.854) in the mean values (12.70 ± 0.67, 12.50 ± 0.7, 12.60 ± 0.97**s**) of prothrombin time in preeclampsia subjects in the first, second and third trimesters. Similarly, there was no significant difference (P = 0.897) in the mean values (27.30 ± 1.06, 27.10 ± 0.99, 27.30 ± 1.25**s**) of activated partial thromboplastin time in preeclampsia subjects in first, second and third trimesters respectively. In the mean values (222.80 ± 24.17 **mg/dL**, 225.50 ± 14.32 **mg/dL**, 225.00 ± 11.47 **mg/dL**) of fibrinogen in preeclampsia subjects, there was non- progressive statistical increase (P = 0.940) across all the trimester.

Table 3: The Mean ± Standard Deviation values of studied subjects in relation to age

Parameter	18 - 24 Years (n = 10)	25 - 34 Years (n = 10)	35 - 44 Years (n =10)	F-value	P-value
Platelet count(×10^9/L)	268.00 ±13.54	233.94± 24.49	203.57 ±17.30	19.984	0.001*

PT (Sec)	**12.80 ±0.45**	**12.50 ±0.86**	**12.71 ±0.76**	**0.380**	**0.687**
APTT (Sec)	**27.20± 1.10**	**27.17 ±1.10**	**27.43± 1.13**	**0.144**	**0.866**
Fibrinogen (mg/dl)	**221.00±26.37**	**222.00 ±21.71**	**226.44 ±11.08**	**0.295**	**0.747**

Key:

N: Sample size

APTT: Activated Partial Thromboplastin time;

PT: Prothrombin Time;

***: Statistically significant at P < 0.05.**

The result from haemostatic parameters showed that there was progressive decrease in the mean values (268.00 ±13.54×**10^9/L**, 233.94 ± 24.45×**10^9/L**, 203.57 ± 17.30×**10^9/L**) of platelet count which was statically significant (p = 0.001) across all the ages in preeclampsia subjects. There was no significant difference (P = 0.687) in the mean values (12.80 ± 0.45s, 12.80 ± 0.86s, 12.71 ± 0.76s) of prothrombin time across all ages. There was no significant difference (P = 0.68) in the mean values (27.20 ± 1.10s, 27.17 ± 1.10s, 27.43 ± 1.13s) of activated partial thromboplastin time in preeclampsia subjects across all the ages. There was progressive increase in the mean values (221.14 ±26.37mg/dL,222.00 ±21.71, 226.44 ±11.08) of fibrinogen which was not statistically significant (p = 0.747).

Discussion

Preeclampsia is a multisystem disorder of unknown etiology and is unique to pregnant women after twenty weeks of gestation. It is a progressive disease with a variable mode of presentation and rate of progression.[32]

T**he platelet count, prothrombin time, and activated partial thromboplastin time in the preeclampsia subjects were all lower than the control** subjects, thereby suggesting no risk of hypercoagulability tendency that could result from alteration of thrombo–haemorrhagic stability in support of thrombosis. Despite the decline in the test group, the values are not too low to cause major cardiovascular and thrombotic events among the various stages of pregnancy. These results are similar to the work done by Rui *et al.*[33] Also, the fibrinogen level was higher in the preeclampsia subjects than the control subjects; raised fibrinogen concentration is well established as risk factor for thrombotic episodes in the general population. In pregnancy, fibrinogen concentration is raised naturally, but the upper limit, above which the rise may be considered pathological, is uncertain.

The result showed **that the** values of activated partial thromboplastin time, platelets count, prothrombin time decreased with increased pregnancy duration. Seo *et al*. [34]also reported similar results. Low activated partial thromboplastin time is a measure of hyper–coagulable conditions that could predispose patients to thrombotic events. There was a decline in platelets counts, but the values are within normal reference range. This suggests no alteration in metabolic body chemistry due to platelets counts. The highest values for serum iron and fibrinogen level were observed amongst subjects in the third trimester and lowest value amongst subjects in the first

trimesters of pregnancy. T**he decrease in the platelet count as the ages of the preeclampsia subjects increases is in conformation with the study carried out by Linkins *et al*.** [35]

Conclusion

In conclusion, a significant decline in platelets counts as well as an increase in fibrinogen level in preeclampsia subjects was seen in this study. Also, the study showed that as pregnancy duration increases, the platelets count decline. Low levels of haemostatic parameters can lead to ineffective hemostasis and inability to control hemorrhage which can lead to severe blood loss.

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