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■ Original Research Article

Association of Heat Stable Placental Alkaline Phosphatase with Severe Preeclampsia Among Pregnant Women at The Federal Teaching Hospital Abakaliki, Southeast, Nigeria.

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Abstract

Background: So far, there is no accurate diagnostic or predictive investigation for pre-eclempsia. Placental alkaline phosphatase (PLAP) in preeclamptic pregnancies appears to show a trend which may be useful in prognostication.The aim of this study was to evaluate the correlation of placental alkaline phosphatase with severe preeclampsia and pregnancy outcome. **Methodology:** This was a case-controlled study conducted at the Federal Teaching Hospital Abakaliki. A total of 214 pregnant women consisting of 107 women in the study group and 107 women in the control group. Each patient with preeclampsia was matched with a healthy for gestational age and parity. Data was analyzed using SPSS. Findings were presented in tables using descriptive statistics. Relationships were assessed using Pearson's correlation and a p-value < 0.05 was statistically significant. **Results:** The mean values of serum PLAP for the study and control groups were respectively 126.5IU/L and 51.1IU/l, and this was statistically significant (p-value < 0.01). The association between serum PLAP and neonatal birth weight was not statistically significant. The performance of PLAP showed a sensitivity and specificity of 80.0% and 82.0% respectively.
Conclusion: There result showed that Serum PLAP maybe a potential use as an early marker of disease onset and prognostication.

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Keywords: Adverse pregnancy outcome, correlation, Placental alkaline phosphatase, Severe preeclampsia

Introduction

Pregnancy is a physiological state associated with significant alteration in virtually all the systems in the body[1]. In an uncomplicated pregnancy, the parturient tolerates these changes after which there is reversal to the pre-pregnancy state in most cases within six weeks of delivery[2,3]. Preeclampsia complicates 5-10% of all pregnancies[4,5]. Hospital based studies in Nigeria report an incidence of between 1.2-16%[6-8]. Preeclampsia is reported to be the second most common cause of iatrogenic prematurity and perinatal deaths. They account for about 50,000-75,000 of maternal deaths per annum, but this figure might be an underestimation of the actual disease burden because of widespread underreporting in developing countries[9]. The high burden of preeclampsia and eclampsia in developing countries is thought to be due to limited use of magnesium sulphate, lack of well-defined protocols for case management, poverty, and poor health seeking behaviour[10]

Understanding the clinical characteristics and maternal biomarkers associated with normal and abnormal pregnancy remains a major goal of obstetrics. It is impossible to manage pregnancy and undertake appropriate interventions without such knowledge. In preeclamptic women, the serum placenta (heat stable) alkaline phosphatase (PLAP) has been reported to constitute about 55% of total alkaline phosphatase[11]. Numerous studies have reported that variations in serum levels of PLAP may be associated with disease progression in preeclampsia, but these findings are inconsistent[11-17]. In evaluating a patient for pre-eclampsia, several tests have been extensively studied to screen for and possibly predict onset, but no single candidate test has shown unequivocal accuracy in this regard[2,3]. Since the placenta is a rich source of enzymes and is at the centre of the disease pathogenesis, assessment of placental function by evaluating placental specific enzymes may play a role in detection and management of the disease.

Placental (heat-stable) alkaline phosphatase (PLAP) is an isoenzyme of serum total alkaline phosphatase which is specific to the placenta[18]. Total Serum placental alkaline phosphatase begins to rise at about the 16th week of pregnancy and peaks at about 38 week's gestation and is completely cleared during the puerperium[7,12,13]. In some pregnancies complicated by preeclampsia, there is a rise in serum PLAP (yes, there is a rise in all pregnancies but higher when compared, if there is tissue disruption like in placenta diseases e.g., pre-eclampsia and hepatic obstruction) which is thought to be due to the compensatory activities of syncytiotrophoblast cells of the placenta following ischaemic damage to placental tissue that occurs in preeclampsia. This attribute of the placenta may offer a window into understanding the pattern of disease progression using PLAP as a biomarker in disease monitoring and prognostication[13,14] (justified by above statement). Some studies carried out outside Nigeria have reported an association between PLAP and preeclampsia. Only one study was identified in Northern Nigeria but none in the Southeast. Determination of this association using a cheap and effective biomarker like PLAP will help in the initiation of policies that will incorporate assessment of serum PLAP as a routine investigation in preeclampsia case management. In Abakaliki, the application of this parameter would be invaluable due to paucity of resources for the more advanced diagnostic tools, especially given that serum PLAP is easy to determine, affordable and tissue specific.

Methodology

Study design

This was a case-controlled study. The study group consisted of women diagnosed with preeclampsia while the control group consisted of healthy pregnant women matched for parity and gestational age. Deliveries included in the study were from 28weeks, which is the age of viability in our environment. Pregnant women diagnosed with preeclampsia were first evaluated at the antenatal clinic or the Emergency unit of the hospital from where they were admitted into the antenatal ward for stabilization and delivery depending on the disease severity and/or gestational age of the parturient. Patient recruitment followed a systematic sampling method where every 3rd patient was recruited.

The blood pressure was gotten using the Acuson® brand mercury sphygmomanometer with appropriate cuff size covering at least 2/3rd of the length of the patients left arm. Blood pressure was measured with the patient in reclined position.

Before taking measurements, the patient is allowed 30 minutes rest. In taking the measurement, the cuff was inflated while simultaneously palpating the radial pulse. Inflation was continued for a further 20mmHg beyond the point where the radial pulse becomes impalpable. Pressure was slowly released at a pace of 2mmHg until the radial pulse becomes palpable again and this indicates the systolic blood pressure. The cuff is then re-inflated, and a stethoscope applied to the cubital fossa and the pressure slowly released as in the previous fashion. The phase 1 Korotkoff sound is recorded as the systolic blood pressure (SBP) while the phase 5 Korotkoff sound was recorded as the diastolic blood pressure (DBP) measurement. Severe hypertension was diagnosed if the patients' blood pressure was recorded as systolic or diastolic blood pressure of equal to or more than 160mmgHg or 110mmHg respectively. Urinalysis was done by dip stick testing of clean catch midstream urine. The participants were given a wide bore clean universal bottle and asked to collect a clean catch midstream specimen of urine. Prior to taking the sample, the patients were asked to wash their perineum using clean water provided in the toilet. They were educated to stand astride, open the sample bottle, and collect a mid-stream specimen of urine. After specimen collection, urinalysis was performed to test for proteinuria. A proteinuria of 2+ or more was considered significant. Severe hypertension with significant proteinuria confirms a diagnosis of severe preeclampsia.

After blood pressure measurement, a flexible tourniquet was applied about 10 cm above the ante-cubital fossa of either upper limb to make the veins distend with blood. The skin over the site for venipuncture was cleaned with a cotton wool soaked with 70% Alcohol and allowed to dry. A sterile 21G size hypodermic disposable needle was used to draw 5 ml of blood. The blood collected was gently emptied into a sterile plain specimen bottle containing no anticoagulant and immediately sent to the chemical pathology laboratory for processing.

Ethical consideration

Ethical approval for this study was obtained from the Research and Ethics Committee of the Federal Teaching Hospital Abakaliki.

Inclusion criteria

1. Women who give an informed consent to participate in the study.
2. Women diagnosed with preeclampsia at a gestational age of 28 weeks or more.
3. Healthy normotensive pregnant women who met the matching criteria.

Exclusion criteria

1. Women with chronic medical diseases such as renal, liver or heart diseases
2. Multiple pregnancies
3. Gestational trophoblastic diseases.
4. Urinary tract infection and malaria in pregnancy.
5. Pregnant women with malignancy.
6. Women who smoke.
7. Anaemia in pregnancy.
8. Over-weight and obese women

Blood Sample Analysis and Extraction of PLAP

Reagents were manufactured by Quimica Clinica Applicada (QCA) Laboratories limited, Spain[20]. It is stable to expiry when stored at +2 to +8^{o}C.It had three components:

1. Solution A: This is a ready-to-use 25ml solution of 65mM Chromogenic substrate (Phenolphthalein monophosphate in 7.8M of 2-amino-2-methyl-1-propanol at pH of 10.4).
2. Solution B: Two colour developers (containing 0.1M phosphate, Na_3PO_4, buffer).
3. Solution C: Standard. Ready-to-use.

At the laboratory, serum extraction was done by centrifugation at 8000 rpm for 5 minutes. The extracted serum was stored in a regulated freezer at -20^{o}C until a total sample of 24 (12 for each of study and control groups) was reached and then analysed.

- Each batch of specimen was analysed using reagents from the same pack after a pre-analysis was conducted using the commercially procured control sera to ensure precision within batches. Batched analysis ensured consistency, optimal use of reagent and made for efficient quality control.
- After analysis, the serum PLAP was documented for each sample. The expiry date, batch number and NAFDAC number for each batch reagents were documented.

Technique

The Phenolphthalein Monophosphate Substrate Method (Chromogenic method) of estimation of placental alkaline phosphatase was used for this study. This method is based on the principle that serum alkaline phosphate (ALP) hydrolyzes a colourless substrate of Phenolphthalein Monophosphate giving rise to phosphoric acid and phenolphthalein which at an alkaline PH of 10.4 (using 2-Amino-2-methyl-1-propanol buffer) turns into a pink colored solution whose absorbance can photometrically be determined at 546nm wavelength[21]. The rate of increase in absorbance is directly proportional to the alkaline phosphatase activity in the serum (concentration).

To determine the level of placental alkaline phosphatase, 0.5ml of serum was put into a thin-walled glass tube and immersed in a thermostatically controlled water bath (Incubator) which was maintained at 65 ^{0}C for 30 minutes. PLAP remains stable at this temperature while alkaline phosphatases from other sources are denatured. The serum was cooled in an ice bath for 3 minutes before returning it to room temperature. To estimate the concentration of serum PLAP in the sample, a drop (0.04ml) of the chromogenic substrate was diluted in 1ml of deionized water and incubated at 37^{o}C, and then 0.1ml of the cooled serum was added to the mixture and allowed to stand for 20 minutes. Thereafter, 5ml of the colour developer was added to the mixture and read spectrophotometrically. The value of serum PLAP was documented in the proforma.

Quality Control

To ensure precision within batch analysis, control sera were procured from QCA laboratories (Serriscann Normal and Seriscann Anormal)[20] which were used to test for accuracy of each batch analysis. Also, each batch was analysed by the same laboratory scientist using reagents constituted from the same reagent pack.

Labour And Delivery

For those who had vaginal delivery, their labour was managed using the departmental protocol for management of preeclampsia. This entailed the use of partographs and Cardiotocographs for monitoring of labour events. Those that were delivered abdominally had the procedure performed by a senior registrar or consultant in the Obstetrics and Gynaecology department. All the deliveries were attended by the neonatology team.

Post Delivery

Baby: Following the delivery of the baby, the umbilical cord stump left on all the neonates were reduced to a length of 5cm. This was to avoid over estimation of the neonatal birth weight. All the babies were weighed unclad, using the same weighing scale which was checked daily for zero error.

Placenta: The placenta and membranes were severed from the umbilical cord at the point of attachment. The placenta was examined under clear running water and a bright light source to remove blood clots and examined for completeness. Thereafter, the placenta was placed in a bowl of known weight and weighed using a zero-error electronic weighing scale. The actual placental weight was determined by subtracting the weight of the empty bowl (predetermined) from the final weight of the bowl with placenta. The same weighing scale and bowl were used for all the patients.

Outcome Measures

Primary/main outcome measures

Mean serum level of PLAP in preeclamptic and normotensive pregnant women.

Secondary outcome measures

1. Mean neonatal and placental birth weights.
2. Number of participants who suffered maternal and neonatal complications.
3. Gestational age at delivery

Cofounders

1. Maternal age
2. Gestational age
3. Parity

Data Collection and Analysis

The study lasted for six months between 1st of September, 2017 to 28th February, 2018. Samples were collected at delivery and matched. A structured proforma was used to collect data. Analyzed was done using International Business

Machine-Statistical Package for Social Sciences Version 22 (IBM-SPSS 22, 2015, Atlanta Georgia). Data was presented in tables, means and standard deviation. Means were compared using the Z-test for continuous variables and Chi square test for categorical variable. Associations between variables were compared using Pearson's correlation and statistical significance was at P< 0.05. Receiver Operating Curve Characteristics (ROC) was plotted to elicit the cut off, sensitivity and specificity of PLAP.

Results

In Table 1, The mean maternal age of 29.4±6.9years was significantly higher in the study group compared to the mean age of the control group, 27.3±5.5 (p-0.019). The mean systolic and diastolic blood pressures of preeclamptic women was 179.2±17.6mmHg and118.6±13.1mmHg respectively which were significantly higher

Table 1: Comparison of the clinical parameters of the study and control groups

Variables	Study (pre-eclamptic)	Control (normotensive)	z-test score	p-value
Mean maternal age (years)	29.4±6.9	27.3±5.5	2.366	*0.019
Mean GA at delivery (days)	37.1±1.1	38.1.9±2.9	0.402	*0.023
Mean birth weight	2.9±0.7	3.2±0.7	1.89	0.061
Mean SBP(mmHg)	179.2±17.6	114.1±8.1	53.77	*0.000
Mean DSP(mmHg)	118.6±13.1	71.5±7.5	21.95	*0.000
Mean MAP(mmhg)	138.9±13.4	85.7±7.0	31.49	*0.000
Mean PLAP(IU/L)	126.5	51.1	105.3	*0000

*Significant

Table 2: Correlation Between Blood Pressure, Proteinuria and PLAP in the Study Group

Blood pressure	Mean Blood pressure	Mean PLAP (IU/L)	Correlation coefficient (r)	p-value
SBP	-	-		*0.000
140-149				
150-159	151.1	80.0		
≥160	181.2	129.9	0.366	
DBP			0.418	*0.000
90-99	93.0	109.3		
100-109	103.3	117.6		
≥110	121.7	128.4		
MAP	138.9	126.5	0.433	*0.000
Proteinuria	1+	78.3	0.478	*0.000
	2+	106.9		
	3+	145.2		
	4+	167.1		

compared to the normotensive controls (118.6+13.1 and 71.5 + 7.5 mmHg), p-value of 0.000. The mean values of serum PLAP for the study and control groups were respectively 126.5IU/L and 51.1IU/L and the p-value was 0.0000.

In Table 2, there was a positive correlation between the serum PLAP of women in the study group with their blood pressure and degree of proteinuria, p-value 0.000. There was a significant positive correlation between the serum PLAP and gestational age of the study group (Table 3).

Table 3: Gestational age at delivery and PLAP

Group	Mean GA (weeks)	Mean PLAP (IU/L)	Coefficient of correlation (r)	P-Value
Study				
28-33	31.8	65.4	0.215	0.030
34-36	35.1	124.8		
37-41	38.9	133.0		
≥42	42	155.0		
Control				
28-33	29.4	58.1	-0.127	0.198
34-36	35.4	59.5		
37-41	39.3	49.7		
≥42	42.6	42.0		

Table 4: Correlation between neonatal birthweight and PLAP

Group	Birth weight	Mean Birth Weight	Mean PLAP (IU/L)	Correlation coefficient	p-value
Study	<2.5	1.72	91.0	0.066	0.512
	2.5-3.99	3.27	137.8		
	≥4.0	4.10	95.0		
Control	<2.5	1.87	65.8	-0.103	0.303
	2.5-3.99	3.12	49.8		
	≥4.0	4.30	41.5		

Table 5: Correlation between mean placental weights and PLAP

Group	Mean Placental Weight (kg)	Mean PLAP (IU/L)	Correlation coefficient	P-value
Study	0.63	126.5	0.463	0.000
Control	0.78	51.1	0.085	0.391

Table 6: Pregnancy outcome of the study and control groups

Variable	Study group	Percentage	Control group	Percentage
Mode of delivery				
Caesarean section	66	64.7	24	23.1
Spontaneous vertex delivery	32	31.4	76	73.1
Vacuum delivery	4	3.9	4	3.8
Indications for caesarean section	2	2.0	-	-
Reduced fetal movement	24	23.5	6	5.8
Fetal distress	32	31.4	-	-
Severe pet +unfavourable cervix	4	3.9	2	1.9
Two previous caesarean sections	4	3.9	2	1.9
Cephalopelvic disproportion	2	2.0	12	11.5
Poor progress	2	2.0	-	-
Fetal macrosomia	-	-	2	1.9
Cord prolapsed				
Maternal complications				
Eclampsia	12	11.8	-	-
Abruptio placentae	6	5.9	-	-
Acute renal failure	2	2.0	-	-
Stroke	2	2.0	-	-
PPH	4	3.9	-	-
HELLP Syndrome	4	3.9	5	4.8
Maternal mortality	2	2.0	-	-
APGAR Scores				
Normal (≥7)	63	61.8	94	90.8
Mild (4-6)	20	19.6	6	5.8
Severe (≤3)	17	16.7	4	3.8
Neonatal complications				
NICU admission	53	52.0	13	12.5
Low birth weight	2	2.0	-	-
Perinatal death	11	10.8	2	1.9

Table 4 shows that there was no statistically significant correlation between the neonatal birth weights of participants in the study and control groups. Even though the mean placental weight was lower for the study group participants (0.63kg versus 0.78kg), there was a significant (p-value 0.000) positive correlation (0.463) between the mean placental weights and serum PLAP of the study group. Correlation was weak (0.085) and not significant for the control group, p-value 0.391 (Table 5).

There were more caesarean sections in women in the study group compared to the control group (64.7% versus 23.1%). The commonest indication for caesarean section in the study group was unfavorable cervix (31.4%) followed by fetal distress, 23.5%. Instrumental deliveries were similar in both arms, 3.8 and 3.9%. Eclampsia was the commonest complication among the study group. There was no maternal death in the control, but it was 2.0% in the study group. Mild and severe birth asphyxia were recorded in 37% of the study group participants but this was observed in only 9.6% of the control group. NICU admission was the commonest adverse neonatal outcome in the study group. Perinatal death among babies born in the study group was almost five-fold that of those born in the control group, 10.8% versus 1.9% (Table 6).

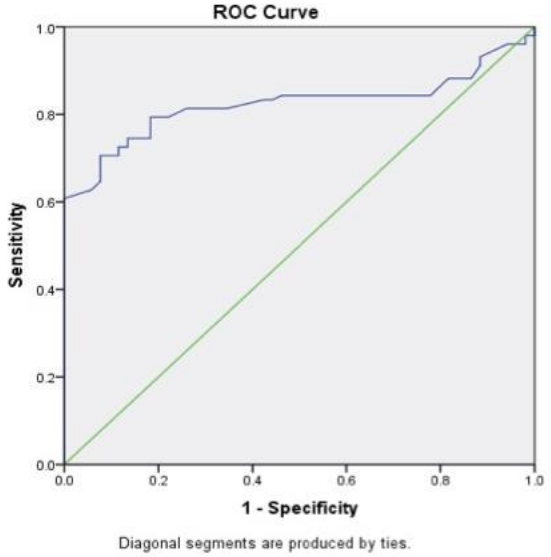

Figure1: Receiver Operating Curve Characteristics (ROC)

Table 7: Key

Parameter	Optimum cut off	Sensitivity	Specificity	AUC
PLAP	54.5	80%	82%	0.823

The performance of serum PLAP was evaluated using the receiver operating characteristics curve (ROC) which shows that the association of serum PLAP and disease severity had a sensitivity and specificity 80.0% and 82.0% respectively while the optimum cut off value was 54.5 IU/L (Figure 1 and Table 7). They presented with the complication of HELP syndrome and were recruited as such.

Discussion

Many biomarkers have been tried in a bid to predict and prognosticate this disease, but findings remain inconclusive[13]. The role of placental alkaline phosphatase in the aetiopathogenesis of preeclampsia has been a subject of considerable interest but findings are inconsistent[22-28]. In this study, the mean serum Placental Alkaline Phosphatase (PLAP) level in the study group was higher than that of the control group (126.5IU/L versus 51.1IU/L), although these values are within the normal reference range (24-161IU/L)[13]reported by Aliyu et al in their study population, the difference was statistically significant in our study. This is likely due to the ethnic variation between the study participants. While the predominant ethnic group in the Aliyu study was Hausa/Fulani, the Ibos make up much of our study population, study group and control alike. PLAP has been reported to have ethno-racial variation[11-17].

Interestingly, the blood pressure variation followed a similar trend re-enforcing the fact that serum PLAP rose with disease severity. This agrees with the findings by Aliyu et al[13], Rajagambeeram et al[5] and Mangal et al[22]. The rise in serum PLAP among preeclamptics is thought to be due to compensatory increase in syncytiotrophoblast cell activity in response to placental dysfunction occasioned by preeclampsia. PLAP plays an important role in fetal metabolism and wellbeing[22,29]. Although Sindu et al[23] and Nahar et al[25] found a significantly lower PLAP level among preeclamptic women in their study, this was attributed to severe widespread placental damage in the face of severe preeclampsia which overwhelms the compensatory response mounted by the remaining functional trophoblastic tissues.

We found a positive correlation between the serum PLAP and disease severity which was based on patients' blood pressure and the level of proteinuria. This observation is remarkable for its

potential use in disease prognostication as was reported by Rajagambeeram et al[12] and Aliyu et al in Zaria[13]. Since the aetio-pathogenesis of preeclampsia is in the placenta, a significant rise in the serum PLAP reflects the extent of placental dysfunction and should serve as a tool to alert the Obstetrician of possible fetomaternal compromise if timely intervention is not initiated. The association observed in the study group with advancing gestational age can be attributed to not only the presence of placental disease but also due to the natural history of PLAP in pregnancy which typically rises with advancing gestational age even in normal pregnancies[24,30]. A similar correlation between serum PLAP and gestational age was also reported by Mangal et al[22] who found an increased PLAP activity with advancing gestational age in pregnancies complicated by preeclampsia while Sulaiman[31] found a significantly reduced PLAP levels in women who had miscarriages, implying that serum PLAP activity was low at early gestational age and failing pregnancies[31].

Overall, the serum PLAP showed a positive but weak correlation with the neonatal birth weight of the study group participants. This correlation was not statistically significant (p-value of 0.512) and was rather negative for the control group. The finding of a negative correlation for the control group is surprising because reduced levels of PLAP in normal pregnancy may suggest intrauterine growth restriction[32] which is apparently not the case here and calls for further research. Onyesom et al found a significant positive correlation between serum PLAP level and cord blood glucose, albumin, and neonatal birth weight among healthy pregnant controls[26,28]. Strongly in support of the fact that the placenta is the source of PLAP, and its serum level is a reflection of syncytiotrophoblast activity in response to pregnancy stressors, we found a positive correlation between the mean placental weight and serum PLAP which was statistically significant. Conversely, the correlation was weak and not significant for the control group. This comparison is a major strength of this study as other scholars did not evaluate it[11-13,22].

Caesarean section rate was high for the study group compared to the control group (64.7% versus 23.1%). The commonest indication for caesarean section in the study group was unfavorable cervix (31.4%) followed by fetal distress, 23.5%. This was because a high proportion of the patients did not present in labour, and when they did, intrapartum fetal distress in some cases necessitated emergent delivery to avert adverse fetal outcomes. Even when delivery was timely, prior mismanagement before arrival to the unit in the cases of referred patients, contributed to adverse outcomes reported in this study. For instance, Eclampsia was the commonest complication among the study group. Delayed treatment of preeclampsia may result in Eclampsia[6,7]. There was no maternal death in the control group but 2% of women in the study group died because of their disease. Birth asphyxia was recorded in 37% of the babies born to the study group participants but this was observed in 9.6% of the control group. An association between serum PLAP and fetomaternal complications was not assessed for which is a weakness of this study.

The Receiver operating characteristics curve (ROC) of serum PLAP showed a serum cut-off value of 54.5IU/L with a sensitivity of 80.0% and specificity 82.0% and a significant area under the curve (AUC-0.823) for the study group. At this cut-off value, patients with severe preeclampsia should come under close surveillance and stable patients should be delivered to avert potential fetomaternal compromise should the pregnancy be allowed to continue.

In conclusion, this study has shown that heat stable alkaline phosphatase may have a role in the monitoring of women diagnosed with severe preeclampsia and would come handy in delivery planning. It is cheap and easy to of process which makes it suitable for developing economies.

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Conflict of interest: There was no conflict of interest

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