PREGNANCY - ASSOCIATED PLASMA PROTEIN-A AND PREDICTION OF PREECLAMPSIA AMONG ANTENATING PREGNANT WOMEN IN LAGOS, NIGERIA

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Abstract:

Background: Preeclampsia is a leading cause of maternal morbidity and mortality which accounts for 5%-10% of deaths worldwide. Several studies have attempted to effectively predict preeclampsia early in pregnancy. The effective method of detection and treatment are yet to be determined. Early identification of women at risk would enhance prompt monitoring and treatment of both mother and fetus. International Federation of Gynaecology and obstetrics (FIGO) recommends that the use of risk factors along with biomarkers would be beneficial in predicting the disease among pregnant women. Thus, the discovery of a sensitive and specific biomarker would reduce the unwanted effect of preeclampsia. Several biomarkers have been studied but efforts to find an effective one for the prediction of preeclampsia is still elusive.

The study evaluated the relationship between pregnancy-associated plasma protein-A (PAPP-A) and preeclampsia as a biomarker in predicting preeclampsia.

Objectives: To determine the relationship between the levels of pregnancy-associated plasma protein-A and the onset of preeclampsia among pregnant women with risk factors for preeclampsia and those without known risk factors (control).

Materials and Methods: A prospective cohort study of consenting patients who presented at the maternity unit of Ifako-Ijaiye Mother and Child Centre between 11-15 weeks gestation with risk factors for preeclampsia (cases) and those without known risk factors for preeclampsia (controls). Blood samples were obtained and sent to Lagos State University Teaching Hospital Medical Research Laboratory for analysis of Pregnancy Associated Plasma Protein-A (PAPP-A).

Results: No significant correlation was found between the onset of preeclampsia and the levels of Pregnancy Associated Plasma Protein-A in pregnant women with risk as well as those without risk factors who later on developed the disease ($p>0.05$). Among women without identifiable risk of preeclampsia, median Pregnancy Associated Plasma Protein-A was significantly higher in pregnant women who did not develop Preeclampsia compared to those who subsequently developed preeclampsia ($p=0.004$).

There was a statistically significant difference in the median PAPP-A levels between patients at risk of preeclampsia (cases) compared with those without risk (controls). $p<0.05$.

Conclusion: There was a significantly lower median level of PAPP-A among patients with risk factors for preeclampsia when compared with low-risk patients. Meanwhile, there is no significant correlation between the levels of PAPP-A and onset of preeclampsia among those who subsequently developed the disease in those at risk and those without.

Introduction:

Preeclampsia (PE), a pregnancy-specific syndrome in humans, has a worldwide incidence of approximately 2–8%.¹ It is a life-threatening multi-systemic disorder of unknown aetiology and a leading cause of maternal and fetal morbidity and mortality worldwide.^{2,3,4} Preeclampsia accounts for more than 50,000 deaths worldwide each year.² According to the World Health Organization, its incidence is seven times higher in developing countries (28%) than in developed countries (4%).² It is a major cause of maternal and perinatal mortality and morbidity in the United Kingdom and responsible for 24% of all maternal deaths in India.^{2,3} It is one of the top three causes of maternal deaths in Nigeria accounting for about 30% of all maternal deaths.⁴ Studies carried out in Benin City and Sokoto, both in Nigeria and Dessie, in Ethiopia revealed a prevalence of 5.6%, 6% and 8.4% respectively.^{4,5,6}

A major challenge in modern obstetrics is the early identification of pregnancies at risk of early onset of preeclampsia.⁷ Identification of such pregnancy would necessitate timely and prompt measures to improve placentation and reduce the prevalence of the disease and its complications.^{1,8,9} In addition, it may reduce the health and financial burden of preeclampsia.¹⁰ According to FIGO, a combination of biomarkers and the presence of maternal risk factors could promptly identify early onset of the disease thus allowing enough time for appropriate intervention.^{1,11,12}

Pregnancy-associated plasma protein-A is produced by syncitio-trophoblast and has hydrolytic activity for insulin-like growth factor binding protein.⁸

It increases the bioavailability of insulin-like growth factor, which in turn mediates trophoblastic invasion and modulates glucose and amino acids transport in the placenta.^{12,13,14} Hence, a high serum level of PAPP-A should lead to a high level of free insulin-like growth factors with its benefits which include adequate trophoblastic invasion.^{13,14} Insulin-like growth

factor seems to play a significant role in trophoblastic invasion, affecting early development, vascularization of the placenta with the placental bed and activating downstream signaling pathways.^{15,16,17} Thus, high PAPP-A concentrations lead to enhanced growth factor bioactivity which invariably leads to enhanced growth; while low PAPP-A concentrations lead to low growth factor bioactivity with poor placental invasion hence preeclampsia.^{17,18,19}

This study may provide a sound scientific basis for using Pregnancy Associated Plasma Protein-A combined with other known risk factors, as a screening method for preeclampsia in the pregnant population in our environment. It may help with risk evaluation and close early monitoring to reduce the perinatal and maternal morbidity and mortality due to preeclampsia; particularly among those with features identified as high risk for preeclampsia.

Material and Methods

The study was conducted at Mother and Child Centre (MCC), the obstetric arm of Lagos State University Teaching Hospital, located at Ifako Ijaiye, Lagos State, Nigeria. This study was a prospective case-control study, in which pregnant women whose gestational ages were between 11 and 15 weeks with risk factors for preeclampsia, who were recruited using convenient sampling method as cases in group A. An equal number of pregnant women without risk factors for preeclampsia and at the same gestational age range was also recruited, using a convenient sampling method, as controls and assigned group B. Patients in at-risk group A (cases) were given daily prophylactic low dose (75mg) aspirin, which was commenced at presentation until term.²⁰

Inclusion criteria for group A participants include; nulliparous woman, previous history or family history of preeclampsia or hypertensive disease irrespective of parity, history of alcohol ingestion, renal disease, antiphospholipid syndrome, thrombophilia, diabetes, index

pregnancy with new husband, multiple gestation and BMI of 30 and above. Pregnancy with gestational age between 11 and 15 weeks with no identifiable risk for preeclampsia and those with risk but not consenting were excluded from the study.

The control group B included consenting pregnant women, whose gestational ages were between 11 and 15 weeks with no identifiable risk factor for preeclampsia.

The proposed sample size for the study was calculated using the formula for cohort study.²¹

$$n = \frac{[z\alpha\sqrt{2pq} + z\beta(\sqrt{p_1q_1 + p_0q_0})]}{(P_1 - P_0)^2}$$

A total of 180 pregnant women were recruited, divided into two groups with 90 pregnant women in each group; Group A (Cases) were those who were between gestational ages 11 and 15 weeks with a risk factor for preeclampsia and group B (Control) were those healthy pregnant women within the same gestational age range with no risk factors for preeclampsia.

Ethical consideration.

The study protocol was approved by the Health Research and Ethics Committee of Lagos State University Teaching Hospital. Samples were collected from subjects who had given their written consent for inclusion in the study.

Data and Sample collection

Data were collected from eligible participants using well-structured questionnaires administered by the investigator to obtain participants' personal information on socio-demographic details, obstetrics history, screening for preeclampsia risk factors and results of Pregnancy Associated Plasma Protein-A. It also contained a section for the outcome of pregnancy. The proformas were filled by the researcher and trained assistants.

5ml of venous blood was collected from the antecubital vein of each of the participants into plain specimen bottles, both for the study and control groups.

Pregnancy-associated plasma protein A was assayed using Eagle Bioscience PAPP-A ELISA Assay Kits. Eagle Biosciences PAPP-A ELISA Assay Kit is an immune-enzymatic colorimetric method for quantitative determination of PAPP-A (Pregnancy Associated Plasma Protein A) concentration in human serum.

Data were entered into Microsoft excel 2013 and analyzed using Statistical Package for Social Science (SPSS IBM 23). Results are presented as frequency tables and cross tables. Normal distribution assumption was assessed using Kolmogorov Smirnov test.

The association between categorical variables were tested using Chi-square and Fischer exact test. Mean comparison between two groups was carried out using Independent Student t test while the Man Whitney U test was used to compare two median. Cases and Control were matched for age. Linear relationship between variables was assessed using Spearman correlation. Receiver operating characteristics (ROC) was used to determine cut-off of PAPP-A. A confidence level of 95% was used with error margin of 5% and the level of significance set at a p-value of <0.05.

Results

A total of 180 pregnant women were recruited for the study comprising 90 with risk factors for preeclampsia (Group A) and 90 with no identifiable risk factor for preeclampsia (Group B) within 11-15 weeks of gestation. Age distribution of the two group was comparable statistically ($p=0.843$) with majority of subject within the age range of 25-34 years. There was no significant difference statistically in marital status distribution of subjects with risk factors and those without ($p=0.560$).

Table 1: Socio-demographic characteristics of subjects

	Group A (n=90)	Group B (n=90)	Total	X ²	p-value
Age group (Years)				4.108	0.392
20-24	11(12.2)	9(8.9)	19(10.6)		
25-29	29(32.2)	32(35.6)	61(33.9)		
30-34	31(34.4)	32(35.6)	63(35.0)		
35-39	19(21.1)	15(16.7)	34(18.9)		
≥40	0(0.0)	3(3.3)	3(1.7)		
Mean ± SD	30.16±4.7	30.70±4.5		0.124**	0.843
Marital status				0.339	0.560
Single	1(1.1)	2(2.2)	3(1.7)		
Married	89(98.9)	88(97.8)	177(98.3)		

**Independent t-test

Less than half 40(44.4%) of the subjects at risk of preeclampsia were primigravida while none of the subjects with no risk for preeclampsia was a primigravida. This difference was statistically significant ($p < 0.001$). Also, more than half 52(57.8%) of all subjects (at risk) were nulliparous, while all subjects with no risk had at least one baby. The difference in parity pattern of

the two groups was statistically significant, $p < 0.05$

Median Pregnancy Associated Plasma Protein-A in pregnancies at risk of preeclampsia was 4,000mIU/L with interquartile range of 2300-4,600 mIU/L. Minimum and maximum level of PAPP-A among subjects at risk was 1,000mIU/L and 10,000mIU/L respectively.

Figure 1: Pregnancy associated plasma protein-A among the cases

Median Pregnancy Associated Plasma Protein-A in pregnancies with no identifiable risk factor was 6400.0mIU/L with interquartile range of (4000- 9400)mIU/L. Minimum and maximum level of PAPP-A among subjects with no risk was 2,000mIU/L and 10,000mIU/L respectively.

Figure 2: Pregnancy Associated Plasma Protein-A among control group

Median Pregnancy Associated Plasma Protein-A (PAPP-A) were lower among pregnant women with risk factors compared to pregnant women with no identifiable risks. There was statistically significant difference in the median PAPP-A levels between the cases and controls, $p < 0.001$

Table 2: Median comparison of Pregnancy Associated Plasma Protein-A in study and control subjects

	Group A Median (Q1, Q3)	Group B Median (Q1, Q3)	U-value	p-value
Pregnancy associated plasma A	4000.0 (2300, 4600)	6400.0 (4000, 9400)	-4.017	<0.001

U: Man Whitney U test

Almost one-third 27(30.0%) of pregnant women with risk factors for preeclampsia subsequently developed preeclampsia while 8(8.9%) pregnant women with no risk factor also developed the outcome. There was statistically significant difference in number of subjects who developed preeclampsia among risk group compared to no risk group ($p < 0.001$).

$\chi^2 = 12.804$, $p < 0.001$

Figure 3: Association between study group and development of preeclampsia

Twenty-seven (27) women developed preeclampsia in the high-risk group, 7 developed it after 37 weeks of gestation while 20 developed it before 37 weeks of gestation. Among 8 subjects who developed preeclampsia in subjects with no identifiable risk, 5 developed preeclampsia at ≥ 37 weeks of gestation while 3 developed the disease at < 37 weeks of gestation

T-value= -2.616, $p=0.013$

Figure 4: Comparison of Mean gestational age at onset of preeclampsia in pregnant with risk factors for preeclampsia and those without.

One fifth 18(20.0%) of patients in the high risk group had preterm delivery while almost all patients in the control group were term delivery ($p=0.004$). Almost one third 29(32.2%) of subjects with risk of preeclampsia had caesarean section as mode of delivery while less than one tenth 5(5.6%) of subjects without risk delivered through Caesarean section ($p<0.05$). No significant difference was found in gender distribution of babies in the two groups ($p=0.435$)

Table 3: Table showing cut-off, sensitivity and specificity and AUC of Pregnancy Associated Plasma Protein-A in subjects identified with preeclampsia in group A and group B

Pregnancy Associated Plasma Protein-A	Cut-offs	Sensitivity	Specificity	AUC (95% CI)
Group A	1700.0	0.631	0.550	0.643(0.539, 0.738)
Group B	3500	0.636	0.800	0.779(0.703, 0.854)

Discussion

Preeclampsia still contributes largely to maternal morbidity and mortality in Nigeria. It is associated with life-threatening conditions especially in environments where there are limited or poor facilities for antepartum and intrapartum care. It has also been found to be

associated with increased perinatal morbidity and mortality.

This study was conducted to compare the levels of PAPP-A assayed between 11 and 15-weeks gestation in patients with risk factors and those without risk factors for preeclampsia and also to determine the association between the marker in

those who subsequently developed the disease in the two groups.

The age distribution of the two groups was comparable ($p=0.843$) with the majority of the subjects within the ages of 25-34 years. There was no significant difference in marital status distribution of subjects at risk and those without risk ($p=0.560$). Less than half 40(44.4%) of patients at risk were primigravida while none of the patients without risk is primigravida. This difference was statistically significant ($p<0.001$). This is mainly because 'primigravida' is one of the inclusion criteria for cases being a risk factor for preeclampsia.

The median Pregnancy Associated Plasma Protein-A in pregnancies at risk for preeclampsia was significantly lower than those without risk factors. This agreed with several studies establishing a correlation between low levels of the biomarker with the onset of preeclampsia.^{7,8,14,12,13,15,22,23} However, this study did not show any correlation between the levels of PAPP-A and the onset of preeclampsia between the cases and controls. Nonetheless, when the levels of PAPP-A were compared in those who did not develop the disease, to those who did, higher levels were found in those who did not develop the disease. This was the case in both subjects and the controls. This finding was similar to that of Brown et al in Accra, Ghana who established in their study that an average level of Pregnancy Associated Plasma Protein-A between gestational ages of 8weeks to 13weeks among Africans was higher than the level among Caucasians. The levels among Africans from his study who were pregnant women without risk factors for preeclampsia was 2.34 MoM interquartile range (1.24-3.97) MoM and he further suggested different levels among different ethnic groups in Ghana.¹³

Decreased levels of maternal serum Pregnancy Associated Plasma Protein-A (PAPP-A) is associated with an increased risk of adverse pregnancy outcome.^{22,23,24,25} In this study, the combination of risk factors with a low level of PAPP-A below 1700mIU/L (0.8MoM) was shown to have good specificity and sensitivity in

predicting development of preeclampsia in a pregnant woman. This value was higher than the 0.4MoM in various studies across Europe which was recommended as the cut off for PAPP-A by Alain G following a research conducted in Canada.¹² This is in keeping with the findings of Brown et al in Ghana where he affirmed an average higher PAPP-A among Africans compared with Caucasians.¹³ Hence there is a need for a different reference value for Africans if it would be considered a viable screening tool to curtail the menace of preeclampsia

Regarding pregnancy outcomes, one fifth 18(20.0%) of all group A delivery were preterm while almost all Group B deliveries were term deliveries ($p=0.004$). Almost one third 29(32.2%) of subjects with risk of preeclampsia had Caesarean section as mode of delivery while less than one-tenth 5(5.6%) of subjects without risk delivered through caesarean section ($p<0.05$). There was no significant difference in the gender distribution of babies in different groups ($p=0.435$). Babies of mothers with no risk of preeclampsia have significant higher birth weight, APGAR score at 1 minute and APGAR score at 5 minutes compared to babies of mothers at risk of preeclampsia ($p<0.005$). However no significant difference in mean placental weight of babies with risk and those without risk of preeclampsia ($p>0.05$).

Receiver operating characteristics show AUC (Area Under Curve) value of 0.643 {1700mIU/L(0.8MoM)} for Pregnancy Associated Plasma Protein-A in discriminating between preeclampsia and no preeclampsia subjects in group A. This means PAPP-A cut off of 1700mIU/L has a sensitivity of 63.1%, specificity of 55.0% and accuracy of 64.3% in predicting preeclampsia among the high-risk population.

Receiver operating characteristics show an AUC value of 0.779{350mIU/L(0.20MoM)} for Pregnancy Associated Plasma Protein-A in discriminating between preeclampsia and no preeclampsia subjects in group B. This means PAPP-A cut off of 3500mIU/L has a sensitivity of

63.6%, specificity of 80.0 % and accuracy of 77.9% in predicting preeclampsia among no risk population.

Conclusion

Biomarkers are increasingly essential in the prediction and early diagnosis of preeclampsia. The absence of both an adequate mode of prevention and treatment of preeclampsia potentiates the need for an effective biomarker to assist in the early detection of the disease and its complications. In this era of prevention, the discovery of an effective biomarker would be beneficial in combating preeclampsia worldwide.

Recommendations

This study invalidates the use of the levels of Pregnancy Associated Plasma Protein-A as a screening tool in low risk and high risk patients for preeclampsia. Prospective, possibly a multi-centre studies with consideration of ethnicity could be done to validate the findings of this study and determine if early measurement of serum Pregnancy Associated Plasma Protein-A among patients with high risk for preeclampsia can be a useful tool in predicting onset of preeclampsia among patients in order to triage for closer monitoring to avert maternal and perinatal morbidity and mortality.

Limitations of the study

The study was institution based with a small sample size which may not be representative of the general population. A larger study, possibly multi-centre study may be more representative. Furthermore, quantitative assessment of proteinuria in each patient would have been preferable as against the dip stick used in this study for improved quality of testing. Lastly The use of low dose aspirin as a preventive intervention among high risk patients for preeclampsia which could not be withheld because of non-maleficent ethical consideration.

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